
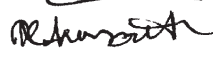
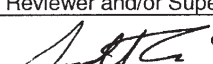



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Karnoup, Anton (AS) (U369292)		 08/24/2010
Kuppannan, Krishna (K) (U386368)		 8/24/2010
Reviewer Name(s)		Reviewer and/or Supervisor Signature(s)/Date
Young, Scott (SA) (U289561)		 8/24/2010
Deshmukh, Balasaheb (BK) (U099523)		 8/24/2010
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Descriptive Summary and Conclusions

2m-EPSPS is a recombinant herbicide tolerance enzyme, similar to the wild-type EPSPS, except that amino acids 102 and 106 have been modified from a T (Thr) to I (Ile), and P (Pro) to S (Ser), respectively (TIPS mutation). A bacterially-expressed 2m-EPSPS sample from DAS Regulatory Labs was examined by peptide mass fingerprinting (using both MALDI-TOF MS and ESI LC/MS), with the purpose of detailed analysis of the protein's primary structure.

The sample of 2m-EPSPS was examined for purity and integrity using SDS-PAGE, and for intact MW by ESI LC/MS. The sample of 2m-EPSPS was subsequently reduced and alkylated, and digested by 4 proteolytic enzymes (as 4 separate digests: Trypsin, Asp-N, Arg-C, and Glu-C at pH 8.0). The resulting proteolytic digests were examined by MALDI-TOF and ESI LC/MS mass-spectrometry techniques in order to obtain confirmation of the protein's amino-acid sequence, with the focus on terminal sequences and the sites of TIPS mutations.

SDS-PAGE resulted in an apparent molecular weight for 2m-EPSPS of approximately 45 ± 2 kDa. LC/MS analysis of the intact 2m-EPSPS sample resulted in an accurate MW = 47285.8 ± 0.5 Da for the principal component of the 2m-EPSPS sample. This result is within 0.0024% of the theoretical average MW for the 2m-EPSPS protein (47284.68 Da). No N-terminal Met¹ was present, as expected (the protein was expressed without Met¹ in the coding sequence). The presence of the TIPS-mutation, and the absence of the non-mutated variants were also confirmed.

Peptide mass fingerprinting of 2m-EPSPS, based on four proteolytic digests, resulted in 100% sequence coverage for its 444- amino-acid-long sequence. The accurate mass of the main sample component measured by LC/MS also confirmed the identity of 2m-EPSPS.

To further confirm the details of the primary structure of 2m-EPSPS sample, N- and C-terminal proteolytic fragments, as well as proteolytic fragments containing the TIPS mutation were subjected to MS/MS fragmentation analysis. The following N-terminal sequence was confirmed by MS/MS analysis: ¹AGAEIIVLQPIK¹². The following C-terminal sequences were confirmed by MS/MS analysis: ⁴³⁶DVLSTFVKN⁴⁴⁴, and ⁴²⁹KTFPDYFDVLSTFVKN⁴⁴⁴. No other variants of the N- and C-terminal sequences were detected. Amino acid sequences of the following fragments containing TIPS mutation were confirmed: ¹⁰⁶SLTAAVTAAGGNATYVLDGVPR¹²⁷, and ⁹¹EEVQLFLGNAGIAMR¹⁰⁵. No fragments representing the absence of the TIPS mutation or partial TIPS mutation were detected.

DISTRIBUTION LIST

Schafer, Barry (BW) (U097380)
Harpham, Nicholas (NJ) - U407575
Young, Scott (SA) - U289561
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Schafer, Barry (BW) - U097380

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INTRODUCTION

A sample of purified recombinant 2m-EPSPS (also known as Double Maize Mutant Gene (DMMG) protein) herbicide tolerance protein (Batch TSN033171-0001) was submitted by Barry Schafer of Dow AgroSciences for analytical characterization. In conjunction with Dow AgroSciences characterization, Analytical Sciences Laboratory was requested to provide analytical data on full protein sequence coverage, N- and C-terminal sequencing, and the TIPS mutation sites. TIPS mutation is a mutation, in which, relative to the wild-type protein, Thr residue is replaced with Ile residue, and Pro residue is replaced with Ser residue. In 2m-EPSPS, Thr¹⁰² was replaced with Ile¹⁰², and Pro¹⁰⁶ was replaced with Ser¹⁰⁶.

Original experimental data are stored in the raw data archive MD-2010-007963¹.

EXPERIMENTAL

Samples

Sample of 2m-EPSPS protein, lot no. TSN033171-0001, was received in the form of dry powder from Barry Schafer of Dow AgroSciences, LLC. A stock solution at 1 mg/mL (by dry weight) in PBS buffer, pH7.4, was prepared and stored at 4 °C.

SDS-PAGE analysis

Equipment:

- a) Centrifugal evaporator (Centrivap), Labconco, cat. no. 7812013, S/N 051146935 A
- b) Bio-Rad Criterion Cell, cat. no. 165-6001
- c) Bio-Rad PowerPac 1000, cat. no. 165-5054
- d) Traceable Digital Thermometer, model NEW 15-078J
- e) Fisher brand Heating Block
- f) Eppendorf Centrifuge, model 5415D, serial no. 5425 17645
- g) Eppendorf adjustable pipettes: 2-20 µL, and 10-100 µL
- h) Aros 160 Orbital Shaker
- i) Fisher Vortex Genie 2, serial no. 2-156856
- j) Eppendorf safe-lock microfuge tubes 1.5 mL, cat. no. 22 36 332-8
- k) Bio-Rad gel loading tips, cat. no. 223-9917
- l) Parafilm
- m) Graduated cylinder, 1000 mL
- n) Fluor-S Multimager, Bio-Rad, cat. no. 170-7700; Quantity One Version 4.2 software

Reagents and Standards:

1. Laemmli Sample Buffer, Bio-Rad, cat. no. 161-0737, lot no. 310008426

2. β -mercaptoethanol, Fisher, Certified lot no. 004508
3. 4-20% Tris-HCl Criterion Precast Gel, Bio-Rad, lot no. C072410A1
4. Tris/Glycine/SDS Running Buffer, Bio-Rad, cat no. 161-0732, lot no. 68199A
5. Coomassie Stain Solution, Bio-Rad, lot no. 68198A
6. Destaining solution I: 45% methanol/ 45% Milli-Q water/ 10% acetic acid
7. Destaining solution II: 5% methanol/ 88% Milli-Q water/ 7% acetic acid
8. Certified Precision Plus Unstained Protein Standards, Bio-Rad, cat. no. 161-0363, lot no. 300000271
9. Bovine serum albumin (BSA), Sigma, Cat. no. A1900, Lot no. 036K7575 (prepared at 1.0 mg/mL in PBS buffer, pH7.4, and stored at +4 °C)
10. Wt-EPSPS, lot no. TSN032933-003: provided by B. Schafer (DAS)

Analytical Procedure:

The apparent molecular weight of 2m-EPSPS was determined by high-resolution SDS-PAGE analysis.

Preparation of reagents, samples, and standards are shown below:

- a. Aliquots of the sample (1, 2, 4, 5 μ L of sample solution, approximately 1mg/mL based on dry weight) were dried to completeness in a centrifugal evaporator.
- b. Final Laemmli sample buffer was prepared by adding 50 μ L of β -mercaptoethanol to ~950 μ L of Bio-Rad Laemmli buffer. The sample buffer was thoroughly mixed by a vortex.
- c. The dried 2m-EPSPS samples were dissolved in a final Laemmli sample buffer (10 μ L). After briefly mixing 2m-EPSPS samples in the Laemmli buffer, the microfuge tubes were sealed with Parafilm, and placed in a pre-heated heat block set at 95 °C for ~1.5 minutes. The microfuge tubes were removed from the heating block and briefly centrifuged. The entire 2m-EPSPS sample was loaded on an SDS-PAGE gel in one lane.
- d. Known amounts of wt-EPSPS reference material (batch TSN032933-0003²) were also prepared alongside 2m-EPSPS samples, using identical procedures.
- e. Known amounts of BSA protein standard were also prepared (from a 1 mg/mL stock solution). Aliquots of 0.5, 1, 2, 3 μ L (0.5, 1, 2, 3 μ g) of BSA were loaded onto separate SDS-PAGE gel lanes.

Instrument Conditions:

A 4 - 20 % Tris-HCl Criterion pre-cast gel was removed from the storage container, the comb removed from the gel, the wells thoroughly rinsed with deionized water, and the tape removed from the bottom of

the cassette. The Criterion gel cassette was inserted into one of the slots in the Criterion electrophoresis tank. The upper buffer chamber of the Criterion gel was filled with approximately 1X Tris/Glycine/SDS (100 mL of 10X Tris/Glycine/SDS mixed with 900 mL of deionized water) premixed running buffer. The remaining running buffer was added to the lower buffer chamber. Approximately 12 μ L of certified unstained precision protein standards, Bio-Rad, was loaded into far right and far left wells with a pipette using gel-loading tips. After applying the samples and reference materials to the gels, the lid was placed on the tank, the electrical leads were plugged into the power supply, and the power was turned on. Constant current of 50 mA was applied to the Criterion cell for ~1.5 hours, until the dye reached the bottom of the gel. After the electrophoresis was complete, the power supply was turned off and the electrical leads were disconnected. The gel was removed from the Criterion gel cassette, transferred to Coomassie Stain Solution (Bio-Rad) in the gel cassette tray to cover the gel (~40-mL), and placed on an orbital shaker at 35 rpm for approximately 30 minutes. The Coomassie Stain Solution was discarded and replaced with ~40-mL of Destaining Solution I (45% deionized water, 45% methanol, 10% acetic acid), and placed on an orbital shaker at 35 rpm for ~30 minutes. The Destaining Solution I was replaced with Destaining Solution II (88% deionized water, 5% methanol, 7% acetic acid) and the gel was destained for approximately 16 hours.

Methods for determining protein's apparent molecular weight: After destaining of the SDS-PAGE gel was complete, a gel image was acquired using the Bio-Rad Fluor-S Multimager, as specified by the manufacturer. The captured image was then analyzed using Quantity One version 4.2 software utilizing tools for determining molecular weight. The molecular weight value was determined relative to the certified Bio-Rad protein standards defined for the gel, and the band's position in the lane. To estimate the quantity of the 2m-EPSPS material on the SDS-PAGE gel, a calibration curve was obtained from the measured band densities and the known μ g amounts of BSA reference material on the same gel. The calibration curve was applied to the lanes containing 2m-EPSPS material to estimate the amount of 2m-EPSPS on the gel.

In-solution protein processing and enzymatic digests

Equipment:

- a) Mettler AE168 analytical balance serial no. F00518
- b) Eppendorf Centrifuge, Model 5415D, serial no. 5425 17645
- c) Eppendorf, Thermomixer R, serial no. 5355 20846
- d) Centrifugal evaporator (Centrivap), Labconco, cat. no. 7812013, S/N 051146935 A
- e) Eppendorf adjustable pipettes: 2.5 μ L serial no. 296447, 2-20 μ L serial no. 286820, 10-100 μ L serial no. 289560, and 1000 μ L serial no. 33165

- f) Fisher Vortex Genie 2, serial no. 2-156856
- g) Siliconized microcentrifuge tubes, 1.5mL, Fisher, cat no. 02-681-320
- h) NAP-5 SEC gravity cartridge, GE Healthcare, Cat. no. 17-0853-02, Lot no. 354677
- i) Parafilm
- j) Eppendorf pipette tips (epTips) 10µL
- k) Fisher brand Reditip General Purpose, 200µL and 1000µL

Reagents and Standards:

- 1. Acetonitrile, Fisher Scientific, Cat. no. A998-1, Lot no. 094014
- 2. Ammonium bicarbonate, Sigma, cat no. A-6141
- 3. Dithiothreitol (DTT), Pierce, cat no. 20290
- 4. Iodoacetamide (IAA), Sigma, cat no. I-1149
- 5. Guanidinium Hydrochloride (Gu:HCl), Pierce, Cat. no. 24110, Lot no. DH54867
- 6. Trypsin, Roche, cat no. 11-418-025-001, Lot no. 11366139
- 7. Asp-N, Roche, cat no. 11-054-589-001, Lot no. 11210921
- 8. Glu-C, Roche, cat no. 11-047-817-001, Lot no. 14530520
- 9. Arg-C, Roche, cat no. 11-370-529-001, Lot no. 10960720
- 10. Formic Acid (FA) (98%), Fluka, Lot no. 1255194
- 11. Trifluoroacetic Acid (99+%) (TFA), Aldrich, Lot no. 00339JD
- 12. Milli-Q deionized water

Reagent Solution Preparation:

- a. 25 mM Ammonium Bicarbonate buffer: dissolved 98.83 mg NH_4HCO_3 in 50 mL of Milli-Q water; filtered through 0.22 µm sterile syringe filter.
- b. 100 mM Tris buffer: dissolved 121.1 mg Tris in 10 mL of Milli-Q water; adjusted pH to 8.04 with HCl; filtered through 0.22 µm sterile syringe filter.
- c. Protein dissolution buffer (6M guanidine hydrochloride (Gu:HCl)/ 400 mM ammonium bicarbonate, pH 7.8): to 316 mg of ammonium bicarbonate, 7.5 mL of 8M Gu:HCl solution and 2.5 mL of water were added. pH was adjusted to 7.8 with NaOH. Buffer was filtered through 0.22 µm sterile syringe filter.
- d. DTT solution (100 mM; prepared fresh): dissolved 15.4 mg DTT in 1 mL of water.
- e. Alkylating reagent (IAA) (200 mM; prepared fresh): dissolved 37 mg IAA in 1 mL of water.

- f. Trypsin solution: Dissolved 50 µg of dried trypsin (contents of 2 vials) in 200 µL of 100 mM Tris buffer immediately prior to digestion procedure.
- g. Arg-C solution: Dissolved 15 µg of dried Arg-C (contents of 3 vials) in 150 µL of Milli-Q deionized water immediately prior to digestion procedure. Reconstituted activation solution concentrate in 100 µL of Milli-Q deionized water.
- h. Asp-N solution: Dissolved 8 µg of dried Asp-N (contents of 4 vials) in 200 µL of Milli-Q deionized water immediately prior to digestion procedure.
- i. Glu-C solution: Dissolved 150 µg of dried Glu-C in 150 µL of Milli-Q deionized water immediately prior to digestion procedure.

In-solution Protein Processing Procedure (Reduction/ alkylation/ digestion):

- a. Two 560-µL aliquots of 2m-EPSPS protein stock solution were dispensed in 1.5-mL siliconized microcentrifuge tubes and dried in a centrifugal evaporator to completeness.
- b. Reduction and carboxyamidomethylation (alkylation) of protein: approximately 180-µL of protein dissolution buffer, 6M Gu: HCl/ 0.4M ammonium bicarbonate, pH 7.8, was added to each dry 2m-EPSPS [Batch TSN033171-0001] aliquot, and the samples were mixed by pipette action. Twenty microliters of 100 mM DTT (reducing reagent) solution was added to each microfuge tube. The microfuge tubes were sealed, vortexed, and incubated at 65 °C for 30 min in a thermomixer at 1100 rpm. The microfuge tubes were cooled to room temperature, centrifuged for 30 sec, and 40 µL of 200 mM IAA (alkylating reagent) solution was added to each tube. The microfuge tubes were incubated in the dark at room temperature for 1 hour. Eighty microliters of DTT solution was added to each tube to consume unreacted IAA, and the tubes were allowed to incubate for 20 min at room temperature.
- c. Desalting of the reduced/alkylated protein sample was performed using a NAP-5 gravity cartridge (Sephadex G-25) as per the manufacturer's procedure. NAP-5 cartridges were pre-equilibrated with 100 mM Tris buffer, pH 8.04, and protein elution was performed with the same buffer (final volume: 1-mL). The reduced and alkylated protein sample from each tube was split into 2 equal parts (500 µL each in a separate tube; final total number of tubes = 4) prior to digestion with various enzymes.
- d. In-solution Tryptic digestion of reduced/alkylated protein: 20 µL of trypsin solution was added to the 500-µL of reduced/alkylated 2m-EPSPS protein sample in 100 mM Tris buffer, pH8.04. The digest was incubated for 16 hours (overnight) at 37 °C in a Thermomixer, shaking at 800 rpm.

- e. Arg-C digestion of reduced/alkylated protein: 75 μ L of Arg-C solution was added to the 500- μ L of reduced/alkylated 2m-EPSPS protein sample, followed by addition of 25 μ L of Arg-C activation solution. The digest was incubated for 16 hours (overnight) at 37 °C in a Thermomixer, shaking at 800 rpm.
- f. Glu-C (pH8) digestion of reduced/alkylated protein: 75 μ L of Glu-C solution was added to the 500- μ L of reduced/alkylated 2m-EPSPS protein sample. The digest was incubated for 16 hours (overnight) at room temperature (~23 °C), shaking at ~800 rpm.
- g. Asp-N digestion of reduced/alkylated protein: 100 μ L of Asp-N solution was added to the 500- μ L of reduced/alkylated 2m-EPSPS protein sample. The digest was incubated for 16 hours (overnight) at 37 °C in a Thermomixer, shaking at 800 rpm.
- h. Prior to mass-spectrometry analyses, digested samples were concentrated in a centrifugal evaporator: dried to completeness and reconstituted in 100 μ L of 0.1% aqueous TFA. The samples were centrifuged, and 80 μ L of each sample were taken for LC/MS and LC/MS/MS analyses. The remaining 20 μ L of each sample were purified for MALDI-TOF MS analysis using C18 zip-tips (procedure is described separately under “MALDI-TOF MS analysis of 2m-EPSPS proteolytic digests”).

MALDI-TOF MS of 2m-EPSPS proteolytic digests:

Reagents and Materials:

1. Acetonitrile, Fisher, cat no. A998-1, Lot no. 080757
2. Milli-Q deionized water (18 M Ω cm⁻¹, TOC 30-20 ppb)
3. Siliconized 0.6-ml microcentrifuge tubes, Fisher, cat no. 02-681-330
4. Pipette tips 0.2-10 μ L, Eppendorf
5. Zip Tips C18, Millipore, cat no. ZTC18S096
6. α -cyano-4-hydroxycinnamic acid (CHCA), Fluka, cat no. 28480
7. Trifluoroacetic acid (TFA), Fisher, cat no. 04902-100
8. ProteoMass MALDI-MS calibration kit, Sigma, cat. no. MS-CAL2

Analytical Procedure:

MALDI-TOF MS: The in-solution proteolytic digests were purified using C18 ZipTips, according to the manufacturer's procedure. For each sample, 4- μ L fractions eluting off C18 ZipTips in 10 %, 25 %, 50 % and 70 % acetonitrile/ 0.1 % TFA were deposited onto a MALDI plate, mixed with 1 μ L of CHCA matrix solution (prepared as saturated solution in 50% ACN/ 0.1% TFA, and centrifuged), air-dried, and

analyzed by MALDI-TOF MS. All mass spectra were acquired on a Voyager DE STR MALDI-TOF mass spectrometer (S/N 4260). The following mass spectrometer settings were used:

Mode of operation: reflector
Extraction mode: delayed
Polarity: positive
Acquisition control: manual
Accelerating voltage: 20000 V
Grid voltage: 62%
Mirror voltage ratio: 1.12
Extraction delay time: 200 nsec
Acquisition mass range: segments 300-1000 Da, 600-4000 Da, and 1900-6000 Da
Number of laser shots: 500/spectrum
Laser intensity: 1600 – 2000 (varied)
Low mass gate: 500-1900 Da (varied)
Timed ion selector: off

External mass calibration was performed with peptide standards utilizing a Sigma mass calibration kit (cat. no. MS-CAL2), consisting of the calibration mixture (monoisotopic $[M+H]^+$ m/z values used): Bradykinin (fragment 1-7), m/z 757.3997; Angiotensin II, m/z 1046.5423; P14R synthetic peptide, m/z 1533.8582; ACTH (fragment 18-39), m/z 2465.1989; and Insulin oxidized B chain (bovine), m/z 3494.6513.

The proteolytic fragments for all digests were assigned using theoretical ion tables generated using Micromass BioLynx and GPMAW (v 7.01a) software packages.

ESI-LC/MS of intact 2m-EPSPS protein

Reagents and Standards:

1. Acetonitrile (HPLC grade, 99.9%, J.T. Baker), Lot no. 10827
2. Isopropanol (HPLC grade, EMD), Lot n. LH44299
3. Formic acid (Sigma), Lot no. 12026KD
4. 0.1 % formic acid in water (HPLC grade, J.T. Baker), Lot no. GI6503
5. Deionized water, 18.2 M Ω cm⁻¹, MilliQ gradient A10, Millipore, freshly drawn
6. Poly-DL-Alanine, Sigma, Catalog no. P9003, Lot no. 97H5912
7. Ribonuclease A (RNase A), Sigma, Catalog no. R5000, Lot no. 122K1319
8. Bovine serum albumin (BSA), Sigma, Catalog no. A3059, Lot No. 034K0598
9. β -Galactosidase (from *E.coli*), Sigma, Catalog no. G8511, Lot no. 105K6020

Analytical Procedure:

ESI-LC/MS: The sample preparations were analyzed directly by mass spectrometry. All mass spectra were acquired on a Waters Q-ToF Micro MS system (S/N YA137). The mass spectrometer was calibrated prior to use in the mass range 700-2300 amu using 0.1 mg/mL Poly-DL-Alanine solution (in acetonitrile). A mixture of proteins with known molecular masses (RNase A, BSA, β -Galactosidase; solutions in deionized water at 20 mg/mL were used) was run as a test standard. The following mass spectrometer settings were used.

LC : Acquity UPLC system
 Mobile Phase A : 0.1 % TFA in water
 Mobile Phase B : 0.1 % TFA in isopropanol (IPA)
 Post-LC/ Pre-MS : addition of 7% glycerol solution at 40 μ L/min
 Column : 2.1x100 mm BEH C4 1.7 μ 300 Å, P/N 186004496, Lot no. 0105191051
 Flow rate : 300 μ L/min
 Column temperature : room temperature (~23 °C)
 Injection volume : 10 μ L
 Injection loop : 20 μ L
 UV detection : 215 and 280 nm, 2 points/sec

Gradient table:

Time, min	Flow rate, mL/min	%A	%B
Initial	0.3	95	5
22.5	0.3	50	50
25	0.3	50	50
25.1	0.3	95	5

Waters Q-ToF Micro MS system: MS Parameters:

Capillary : 2850 V
 Desolvation Gas : 600 L/hr
 Desolvation Temperature: 410 °C
 Source Temperature : 100 °C
 Sample Cone : 35 V
 MCP : 2350 V
 Mode : ESI-TOF-MS +
 Scan Range : 700 – 2300 amu
 Scan Cycle Time : 0.98 sec/scan

The Micromass-supplied electrospray maximum entropy algorithm (MAXENT 1) was used to transform the spectra to a mass axis and to resolution enhance the transformed spectra. The maximum entropy algorithm was set to optimize the spectra with a resolution of 2 Da/channel. The resulting resolution-enhanced spectral peaks were centered and integrated to display the accurate mass for intact molecular mass analysis.

ESI-LC/MS and MS/MS of 2m-EPSPS proteolytic digests*Reagents and Materials:*

1. Acetonitrile, HPLC grade, Fisher Scientific, Lot no. 094014
2. Milli-Q deionized water
3. 98% Formic Acid (Fluka), Lot no. 1255194
4. Poly-DL-Alanine, Sigma, cat. no. P9003, Lot no. 97H5912
5. Leucine Enkephalin acetate salt, Sigma, cat. no. L-9133, Lot no. 095K5109
6. Waters polypropylene plastic HPLC vials, P/N 186002640, lot no. 2640500710

Analytical Procedure:

ESI-LC/MS: All mass spectra were acquired on a Waters Q-ToF Micro MS system (S/N YA137). The mass spectrometer was calibrated prior to use in the mass range 80 – 1900 amu using 0.1 mg/mL Poly-D,L-Alanine solution in acetonitrile. The following liquid chromatography and mass spectrometer settings were used:

LC : Acquity UPLC system
Mobile Phase A : 0.1 % formic acid (FA) in water
Mobile Phase B : 0.1 % formic acid (FA) in acetonitrile
Column : 2.1x100 mm Acquity BEH C18 1.7 μ m 130 Å; P/N 186002578,
Lot no.115A1430810
Flow rate : 100 μ L/min
Column temperature : 50 °C
Injection volume : 4 μ L
Injection loop : 20 μ L
UV detection : 214 nm, 10 pts/sec

Gradient table:

Time, min	Flow rate, mL/min	%A	%B
Initial	0.1	95	5
5	0.1	95	5
35.4	0.1	60	40
37	0.3	10	90
40	0.3	10	90
40.1	0.3	95	5
46	0.3	95	5

46.1	0.1	95	5
49	0.1	95	5
51	0	95	5

MS : QTOF-micro mass spectrometer (S/N YA137)

ESI : Micromass lock-spray electrospray interface

Mode : +TOFMS

MS Parameters (peptide mass fingerprinting):

Capillary : 2850 V
 Desolvation Gas : 250 L/hr
 Desolvation Temperature : 250 °C
 Source Temperature : 100 °C
 Sample Cone : 35 V
 Extraction Cone : 1.5 V
 Collision Energy : 10.0 V
 MCP : 2350 V
 Mode : ESI-TOF-MS +
 Scan Range : 80 – 1900 amu
 Scan Cycle Time : 0.90 sec/scan

MS/MS Parameters:

Capillary : 3153 V
 Desolvation Gas : 250 L/hr
 Desolvation Temperature : 120 °C
 Source Temperature : 80 °C
 Sample Cone : 51 V
 Extraction Cone : 1.0 V
 MCP : 2753 V
 Mode : ESI-TOF-MS +
 Scan Range : 80 – 1900 amu

Survey Scan

Collision Energy : 10.0 V
 Scan Cycle Time : 0.98 sec/scan
 Precursor Selection : Included Masses only
 Include Window : +/- 500 mDa
 Include Retention Time : 60 sec
 Peak Detection Window : 3 Da

MS/MS Scan

MS to MSMS Switch Criteria : Intensity
 MS to MSMS Switch Threshold : 1 counts/sec
 MSMS to MS Switch Criteria : Intensity falling below threshold
 Switchback Threshold : 3 counts/sec
 MSMS Switch After Time : 20 sec
 Scan Cycle Time : 1 sec/scan

Methods:

The samples were injected using partial loop configuration. After sample injection, the column was held at 5 % MPB for 5 minutes. The gradient from 5 % MPB to 40 % MPB was then employed. At the end of

the gradient, the MPB concentration was increased to 90% to allow removal of any hydrophobic components. The column was then re-equilibrated to the initial conditions.

The Time of Flight (ToF) analyzer was calibrated daily using a 0.1 mg/mL solution (in acetonitrile) of Poly-D,L-Alanine at 20 μ L/min flow rate. The same instrument parameter file (with the calibration parameters) was used for MS data acquisitions. Data acquisition was performed with cycle times of 1 sec/scan (scan acquisition time: 0.88 sec; interscan delay: 0.1sec). The lock mass data was acquired using 2.5 μ M Leucine-Enkephalin peptide solution (0.1 % formic acid in 50 % acetonitrile was used as the solvent) flowing at 3 – 5 μ L/min. The lock mass channel was sampled every 7 sec during MS analysis and 10 sec during MS/MS analysis. The reference ion used was the singly charged Leucine-Enkephalin ion at m/z 556.2771.

Peptide mass fingerprinting of the UPLC-MS data was performed manually. The spectrum of each chromatographic peak was summed, smoothed (SG, 2x3 channels), centroided (4 channels, top 80 %, by height) and m/z error corrected (lock mass channel: 10 scans, m/z 556.2271 \pm 0.5 Da). In-source fragmentation observed was used to further confirm the identity of the peptides. The mass-spectra of proteolytic fragments, as well as in-source fragments, were analyzed using MassLynx (Micromass) and GPMaw v. 7.01a (Lighthouse Data) software.

The spectra from tandem MS experiments (for N- and C-terminal fragments, and fragments containing TIPS mutation) were also summed, smoothed (SG, 2x3 channels), centroided (4 channels, top 80 %, by height) and m/z error corrected (lock mass channel: 10 scans, m/z 556.2271 \pm 0.5 Da). The fragments were assigned using a theoretical fragmentation ion table generated using Micromass BioLynx and GPMaw v. 7.01a (Lighthouse Data) software packages.

The raw data are archived in a raw data archive MD-2010-007963 ¹.

RESULTS

The sample of 2m-EPSPS was examined for purity and integrity by SDS-PAGE (**Figure 1**). A wild-type reference (wt-EPSPS) and a generic standard protein (BSA) were also loaded on the same SDS-PAGE gel to facilitate analysis.

SDS-PAGE analysis resulted in an apparent molecular weight of 2m-EPSPS of approximately 45 ± 2 kDa (**Figure 1**). Based on known quantities of a BSA standard loaded alongside 2m-EPSPS on the same SDS-PAGE gel, concentration in the original "1 mg/mL" stock solution was determined to be 0.62 mg/mL. The absence of other protein bands on the SDS-PAGE gel indicated high homogeneity and integrity of the 2m-EPSPS sample.

LC/MS analysis of the intact 2m-EPSPS sample (**Figure 2**) resulted in an accurate MW = 47285.8 ± 0.5 Da for the principal component of the 2m-EPSPS sample. This result is within 0.0024% of the theoretical average MW for the 2m-EPSPS protein (47284.68 Da).

The sample of 2m-EPSPS was subsequently reduced, alkylated, and digested by four proteolytic enzymes (as 4 separate digests: Trypsin, Asp-N, Arg-C, and Glu-C at pH8). The resulting proteolytic digests were examined by MALDI-TOF and ESI LC/MS mass-spectrometry techniques in order to obtain confirmation of the protein's amino-acid sequence and sequence details. Peptide mass fingerprinting of 2m-EPSPS based on the four proteolytic digests resulted in 100% sequence coverage (**Figure 3** and **Tables 1** through **4**). The LC/MS chromatograms of the 4 digests are shown in **Figures 4** through **7**.

To further confirm the details of the primary structure of 2m-EPSPS sample, N- and C-terminal proteolytic fragments, as well as proteolytic fragments containing the TIPS mutation were subjected to MS/MS fragmentation analysis. **Tables 5** through **11** list the results of these experiments. The following N-terminal sequence was confirmed by MS/MS analysis: $^1\text{AGAEIVLQPIK}^{12}$ (T1 fragment from tryptic digest, **Table 5**; i.c.1 fragment from Asp-N digest, **Table 6**). The following C-terminal sequences were confirmed by MS/MS analysis: $^{436}\text{DVLSTFVK}^{444}$ (D27 fragment from Asp-N digest, **Table 7**), $^{429}\text{KTFPDYFDVLSTFVK}^{444}$ (R20 fragment from Arg-C digest, **Table 8**). No other variants of the N- and C-terminal sequences were detected.

Amino acid sequences of the following fragments containing TIPS mutation were confirmed: $^{106}\text{SLTAAVTAAGGNATYVLDGVPR}^{127}$ (R5 and T12 fragments from Arg-C and tryptic digests; **Tables 9** and **10**, respectively), $^{91}\text{EEVQLFLGNAGIAMR}^{105}$ (T11 fragment from tryptic digest, **Table 11**). No fragments representing the absence of the TIPS mutation or partial TIPS mutation were detected.

REFERENCES

1. Raw data archive MD-2010-007963
2. MD-2010-007962 (AL report "Confirmation of primary structure of wt-EPSPS protein by MALDI-TOF MS peptide mass fingerprinting"; A. Karnoup)

Table 1. Observed tryptic fragments of 2m-EPSPS. Cys residues were reduced and carboxyamidomethylated.

Fragment #	# of missed cleavages	Start - End	Sequence	Theor. mono. m/z (MH ⁺)	Retention Time, min	Observed m/z, LC/MS *	Observed in-source peptide fragments	% ACN (elution from C18-zt; MALDI)	Observed m/z MALDI-MS	Modification	Comment
T1	0	1-12	(-) AGAEIVLQPIK	1267.73	20.83	1267.74 (1+), 634.36 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1267.58	no Met ¹	major LC peak; sequence confirmed by MS/MS fragmentation
T1 + Met	0	(-)-12	(-) MAGAEIVLQPIK	1398.77	ND	ND		ND	ND		
T2	0	13- 19	EISGTVK	733.41	6.45	733.41 (1+)	in-source fragments match sequence	ND	ND		
T3	0	20- 24	LPGSK	501.30	3.98	501.30 (1+)	some in-source fragments match	ND	ND		coelutes with T39
T4	0	25- 29	SLSNR	576.31	3.21	576.31 (1+)		ND	ND		coelutes with T32, T13, T43
T5	0	30- 60	ILLAAALSEGTTVDNLLNSEDEVHYMLGALR	3340.78	36.33	1670.87 (2+), 1114.26 (3+), 835.94 (4+)	in-source fragments match sequence	50, 70%	3340.47		colutes with T18
incorrect cleavage 1	n/a	33-60	LAALSEGTTVDNLLNSEDEVHYMLGALR	3001.52	31.59	1501.27 (2+), 1001.17 (3+), 751.14 (4+)	some in-source fragments match	ND	ND		incorrect (tryptic/chymotryptic) cleavage
incorrect cleavage 2	n/a	34-60	AALSEGTTVDNLLNSEDEVHYMLGALR	2888.44	30.10	1444.72 (2+), 963.48 (3+), 722.84 (4+)	some in-source fragments match	ND	ND		incorrect (tryptic/chymotryptic) cleavage
T6	0	61- 70	TLGLSVEADK	1032.56	17.42	1032.56 (1+), 516.78 (2+)	in-source fragments match sequence	10, 25%	1032.29		coelutes with T41
T9	0	75- 83	AVVVGCGGK	846.45	9.78	846.46 (1+)	in-source fragments match sequence	10%	846.10		coelutes with T21
T10	0	84- 90	FPVEDAK	805.41	13.18	805.41 (1+), 403.21 (2+)	in-source fragments match sequence	10, 25%	805.20		
T11	0	91-105	EEVQLFLGNAGIAMR	1647.85	25.48	1647.87 (1+), 824.43 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1647.68		sequence confirmed by MS/MS fragmentation
T11 no mut	0	91-127	EEVQLFLGNAGTAMRPLTAAVTAAGGNATYVLDGVPR	3730.92	ND	ND		ND	ND		
T11 no PS-mut	0	91-127	EEVQLFLGNAGIAMRPLTAAVTAAGGNATYVLDGVPR	3742.96	ND	ND		ND	ND		
T11 no TI-mut	0	91-105	EEVQLFLGNAGTAMR	1635.82	ND	ND		ND	ND		
T12	0	106-127	SLTAAVTAAGGNATYVLDGVPR	2104.10	23.87	1052.54 (2+), 702.03 (3+)	in-source fragments match sequence	10, 25, 50, 70%	2103.88		sequence confirmed by MS/MS fragmentation
T13	0	128-129	MR	306.16	3.21	306.16 (1+)		ND	ND		coelutes with T32, T32, T4
T14	0	130-141	ERPIGDLVVLGK	1295.77	20.67	1295.78 (1+), 648.38 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1295.63		
T15	0	142-159	QLGADVDFCLGTDCPPVR	2019.93	23.18	1010.46 (2+), 673.98 (3+)	in-source fragments match sequence	25, 50, 70%	2019.71		
T16	0	160-170	VNGIGGLPGGK	968.55	16.10, 16.71	969.54 (1+), 485.26 (2+)	in-source fragments match sequence	ND	ND	deamidated Asn161	deamidation
T18	0	173-203	LSGSISSQYLSALLMAAPLAGDVEIEIDK	3217.73	36.33	1609.38 (2+), 1073.26 (3+)	some in-source fragments match	ND	ND		coelutes with T5
incorrect cleavage 3	n/a	182-203	LSALLMAAPLAGDVEIEIDK	2295.28	34.76	1148.15 (2+), 765.76 (3+)	some in-source fragments match	ND	ND		incorrect (tryptic/chymotryptic) cleavage; minor LC peak
incorrect cleavage 4	n/a	187-203	MAAPLAGDVEIEIDK	1797.96	28.65	1797.93 (1+), 899.49 (2+)	some in-source fragments match	ND	ND		incorrect (tryptic/chymotryptic) cleavage; minor LC peak
T19	0	204-215	LISIPYVEMTLR	1434.80	27.55	1434.82 (1+), 717.89 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1434.66		
T20	0	216-219	LMER	548.29	4.60	548.29 (1+)	in-source fragments match sequence	ND	ND		
T21	0	220-223	FGVK	450.27	9.78	450.27 (1+)	some in-source fragments match	ND	ND		coelutes with T9
T22	0	224-232	AEHSDSWDR	1102.46	4.85	1102.46 (1+), 551.73 (2+)	in-source fragments match sequence	10, 25, 50%	1102.32		
T23	0	233-236	FYIK	570.33	14.95	570.33 (1+)	some in-source fragments match	10, 25%	570.17		
T27	0	246-285	NAYVEGDASSASYFLAGAAITGTVTVGCGTSLQGDVK	3924.84	ND	ND		50, 70%	3924.38		
T28	0	286-296	FAEVLEMMGAK	1225.60	24.03	1225.60 (1+), 613.29 (2+)	in-source fragments match sequence	25, 50, 70%	1225.46		
T29	0	297-311	VTWTETSTVTGPPR	1630.84	19.55	1630.92 (1+), 815.92 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1630.68		
T30	0	312-316	EPFGR	605.30	10.33	605.31 (1+)	in-source fragments match sequence	10, 25%	605.15		
T32	0	318-320	HLK	397.26	3.21	397.26 (1+)		ND	ND		coelutes with T43, T13, T4
T33	0	321-328	AIDVNMNK	904.46	13.81	904.46 (1+), 452.72 (2+)	in-source fragments match sequence	ND	ND		

Table 1 (continued). Observed tryptic fragments of 2m-EPSPS. Cys residues were reduced and carboxyamidomethylated.

Fragment #	# of missed cleavages	Start - End	Sequence	Theor. mono. m/z (MH ⁺)	Retention Time, min	Observed m/z, LC/MS *	Observed in-source peptide fragments	% ACN (elution from C18-zt; MALDI)	Observed m/z MALDI-MS	Modification	Comment
incorrect cleavage 5	n/a	329-341	MPDVAMTLAVVAL	1330.70	33.28	1330.71 (1+), 665.85 (2+)	in-source fragments match sequence	ND	ND		incorrect (tryptic/chymotryptic) cleavage
T34	0	329-350	MPDVAMTLAVVALFADGPTAIR	2259.19	35.88	1130.10 (2+), 753.73 (3+)	in-source fragments match sequence	50, 70%	2258.96		
T35	0	351-356	DVASWR	733.36	13.88	733.37 (1+)	in-source fragments match sequence	ND	ND		
T38	0	363-367	MVAIR	589.35	13.01	589.35 (1+)	in-source fragments match sequence	10, 25%	589.20		
T39	0	368-372	TELTK	591.34	3.98	591.33 (1+)	in-source fragments match sequence	ND	ND		coelutes with T3
T40	0	373-391	LGASVEEGPDYCIITPEK	2075.00	21.01	1038.00 (2+), 692.33 (3+)	many in-source fragments match sequence	25, 50, 70%	2074.78		
T41	0	392-404	LNVTADTYDDHR	1532.73	17.42	1532.74 (1+), 766.87 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1532.57		coelutes with T6
T42	0	405-422	MAMAFSLAACAEVPTIR	1937.97	ND	ND		50, 70%	1937.77		
incorrect cleavage 6	n/a	405-409	MAMAF	570.23	22.20	570.24 (1+)	in-source fragments match sequence	ND	ND		incorrect (tryptic/chymotryptic) cleavage
incorrect cleavage 7	n/a	410-422	SLAACAEVPTIR	1386.73	20.25	1386.76 (1+), 693.87 (2+)	in-source fragments match sequence	ND	ND		incorrect (tryptic/chymotryptic) cleavage
T43	0	423-428	DPGCTR	705.30	3.21	705.30 (1+)		ND	ND		coelutes with T32, T13, T4
T45	0	430-443	TFPDYFDVLSFVK	1678.84	32.53	1678.86 (1+), 839.90 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1678.64		
T10-11	1	84-105	FPVEDAKEEVQLFLGNAGIAMR	2434.24	28.51	1217.63 (2+), 812.08 (3+)	some in-source fragments match	25, 50, 70%	2433.99		
T38-39	1	363-372	MVAIRTELTK	1161.67	ND	ND		25, 50, 70%	1161.66		
T44-45	1	429-443	KTFPDYFDVLSFVK	1806.93	29.77	903.96 (2+)	some in-source fragments match	50, 70%	1806.73		
T8-10	2	74- 90	RAVVVGCGGKFPVEDAK	1788.94	ND	ND		50%	1788.59		
T9-11	2	75-105	AVVVGCGGKFPVEDAKEEVQLFLGNAGIAMR	3261.68	ND	ND		50%	3261.15		
T36-39	3	357-372	VKETERMVAIRTELTK	1904.06	ND	ND		25, 50, 70%	1903.68		

ND = not detected

Table 2. Observed Arg-C fragments of 2m-EPSPS. Cys residues were reduced and carboxyamidomethylated.

Fragment #	# of missed cleavages	Start - End	Sequence	Theor. mono. m/z (MH ⁺)	Retention Time, min	Observed m/z, LC/MS *	Observed in-source peptide fragments	% ACN (elution from C18-zt; MALDI)	Observed m/z MALDI MS	Modification	Comment
R1	0	1 - 29	(-) AGAEIVLQPIKEISGTVKLPGSKSLNR	3021.69	ND	ND		ND	ND		N-term
R1 + Met	0	(-1) - 29	(-) MAGAEIVLQPIKEISGTVKLPGSKSLNR	3152.73	ND	ND		ND	ND		N-term
i.c. 1	n/a	1 - 19	(-) AGAEIVLQPIKEISGTVK	1982.11	24.92	991.55 (2+), 661.37 (3+)	in-source fragments match sequence	ND	ND		N-term., tryptic-like cleavage; major LC peak
i.c. 2	n/a	1 - 24	(-) AGAEIVLQPIKEISGTVKLPGSK	2464.39	26.08	1232.77 (2+), 822.13 (3+), 616.85 (4+)	in-source fragments match sequence	ND	ND		N-term., tryptic-like cleavage; trace LC peak
i.c. 3	n/a	20 - 24	LPGSK	501.30	4.02	501.30 (1+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
R2	0	30- 60	ILLALLSEGTTVDNLNSEDVHYMLGALR	3340.78	36.34	1670.90 (2+), 1114.26 (3+), 835.94 (4+)	in-source fragments match sequence	25, 50, 70%	3341.02		
R3	0	61- 74	TLGLSEADKAAKR	1458.83	14.52	729.92 (2+), 486.94 (3+)	in-source fragments match sequence	10, 25%	1458.80		
i.c. 4	n/a	75 - 83	AVVVGCGGK	846.44	9.89	846.45 (1+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 5	n/a	84-105	FPVEDAKEEVQLFLGNAGIAMR	2434.24	28.46	1217.63 (2+), 812.08 (3+), 609.31 (4+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
R4	0	75-105	AVVVGCGGKFPVEDAKEEVQLFLGNAGIAMR	3261.68	28.04	1631.35 (2+), 1087.89 (3+), 816.16 (4+)	in-source fragments match sequence	10, 25, 50, 70%	3261.61		major LC peak
R4 no TI mut	0	75-105	AVVVGCGGKFPVEDAKEEVQLFLGNAGTAMR	3249.64	ND	ND		ND	ND		
R5	0	106-127	SLTAAVTAAGNATVYLDGVPR	2104.10	23.85	1052.56 (2+), 702.03 (3+)	in-source fragments match sequence	10, 25, 50, 70%	2104.08		
R4-5 no mut	0	75-127	AVVVGCGGKFPVEDAKEEVQLFLGNAGTAMRPLTAAVTAAGNATVYLDGVPR	5344.75	ND	ND		ND	ND		
R4-5 no PS mut	0	75-127	AVVVGCGGKFPVEDAKEEVQLFLGNAGIAMRPLTAAVTAAGNATVYLDGVPR	5356.78	ND	ND		ND	ND		
i.c. 6	n/a	130-141	ERPIGLVVLGK	1295.76	20.75	1295.75 (1+), 648.38 (2+), 432.59 (3+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 7	n/a	132-141	PIGLVVLGK	1010.62	22.89	1010.63 (1+), 505.81 (2+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 8	n/a	142-159	QLGADVDFLGTDCPPVR	2019.92	23.18	1010.47 (2+), 673.97 (3+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 9	n/a	132-159	PIGLVVLGKLQGLADVDFLGTDCPPVR	3011.53	32.74	1506.25 (2+), 1004.51 (3+), 753.62 (4+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 10	n/a	204-215	LISIPYVEMTLR	1434.80	27.54	1434.82 (1+), 717.90 (2+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
R9	0	216-219	LMER	548.29	4.65	548.29 (1+)	in-source fragments match sequence	10, 25%	548.27		
R10	0	220-232	FGVKAHSDSWDR	1533.71	13.22	1533.69 (1+), 767.36 (2+), 511.90 (3+)	in-source fragments match sequence	10, 25, 50, 70%	1533.69		
i.c. 11	n/a	233-236	FYIK	570.32	14.94	570.33 (1+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 12	n/a	233-240	FYIKGGQK	940.52	11.14	940.51 (1+), 470.76 (2+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 13	n/a	286-296	FAEVLEMMGAK	1225.59	24.07	1225.60 (1+), 613.30 (2+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 14	n/a	297-311	VTWTETSVTVGPPR	1630.84	19.59	1630.83 (1+), 815.93 (2+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
R12	0	312-316	EPFGR	605.30	10.36	605.31 (1+)	in-source fragments match sequence	10, 25%	605.29		
i.c. 15	n/a	317-328	KHLKAIDVNMNK	1410.78	11.64	1410.73 (1+), 705.89 (2+), 470.93 (3+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 16	n/a	329-350	MPDVAMTLAVVLFADGPTAIR	2259.18	35.87	1130.10 (2+), 753.73 (3+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
R14	0	351-356	DVASWR	733.36	13.93	733.37 (1+)	in-source fragments match sequence	10, 25%	733.35		
R15	0	357-362	VKETER	761.42	3.21	761.42 (1+)	some in-source fragments match	10, 25%	761.40		coelutes with R19
R16	0	363-367	MVAIR	589.35	13.01	589.35 (1+)	in-source fragments match sequence	10, 25%	589.33		
i.c. 17	n/a	368-372	TELTK	591.33	4.02	591.33 (1+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 18	n/a	373-391	LGASVEEGPDYCIITPPEK	2074.99	21.04	1038.01 (2+), 692.33 (3+)	most in-source fragments match	ND	ND		tryptic-like cleavage
i.c. 19	n/a	392-404	LNVTADTYDDHR	1532.73	17.43	1532.72 (1+), 766.87 (2+), 511.58 (3+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
i.c. 20	n/a	373-404	LGASVEEGPDYCIITPPEKLNVTADTYDDHR	3588.71	23.46	1794.81 (2+), 1196.91 (3+), 897.93 (4+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
R18	0	405-422	MAMAFSLAACAEVPTIR	1937.96	27.07	969.49 (2+), 646.67 (3+)	in-source fragments match sequence	10, 25, 50, 70%	1937.93		
R19	0	423-428	DPGCTR	705.30	3.21	705.30 (1+)	some in-source fragments match	ND	ND		coelutes with R15
i.c. 21	n/a	429-443	KTFPDYFDVLSTFVK	1806.92	29.70	1806.91 (1+), 903.97 (2+), 602.97 (3+)	in-source fragments match sequence	ND	ND		tryptic-like cleavage
R20	0	429-444	KTFPDYFDVLSTFVK (-)	1920.97	29.18	960.99 (2+), 640.99 (3+)	in-source fragments match sequence	10, 25, 50, 70%	1920.95		C-term; major LC peak

ND = not detected

i.c. = "incorrect" cleavage

Table 3. Observed Asp-N fragments of 2m-EPSPS. Cys residues were reduced and carboxyamidomethylated.

Fragment #	# of missed cleavages	Start - End	Sequence	Theor. mono. m/z (MH ⁺)	Retention Time, min	Observed m/z, LC/MS *	Observed in-source peptide fragments	% ACN (elution from C18-zt; MALDI)	Observed m/z MALDI-MS	Modification	Comment
i.c. 1	2	1-12	(-) AGAEIVLQPIK	1267.72	20.93	1267.73 (1+), 634.36 (2+)	in-source fragments match sequence	ND	ND		N-terminal
i.c. 2	3	1-37	(-) AGAEIVLQPIKEISGTVKLPKSKLSNRILLAAALS	3816.21	30.18	1272.73 (3+), 954.80 (4+), 764.03 (5+)	some in-source fragments match	ND	ND		N-terminal
i.c. 3	0	13-37	EISGTVKLPKSKLSNRILLAAALS	2567.51	26.36	1284.24 (2+), 856.50 (3+), 642.62 (4+)	in-source fragments match sequence	ND	ND		
i.c. 4	1	13-43	EISGTVKLPKSKLSNRILLAAALSEGTTVV	3153.80	27.81	1577.39 (2+), 1051.94 (3+), 789.19 (4+)	in-source fragments match sequence	ND	ND		
D1	0	1-43	(-) AGAEIVLQPIKEISGTVKLPKSKLSNRILLAAALSEGTTVV	4402.52	30.78	1468.12 (3+), 1101.38 (4+), 881.28 (5+)		25, 50, 70%	4402.53	no Met ¹	N-terminal
D1 + Met	0	(-)-43	(-) MAGAEIVLQPIKEISGTVKLPKSKLSNRILLAAALSEGTTVV	4533.56	ND	ND		ND	ND		N-terminal
D2	0	44- 50	DNLLNSE	804.37	16.28	804.36 (1+)	in-source fragments match sequence	ND	ND		
D3	0	51- 68	DVHYMLGALRTLGLSVEA	1945.02	29.36	973.00 (2+), 649.01 (3+)	in-source fragments match sequence	10, 25, 50, 70%	1945.05		
D4	0	69- 87	DKAARKRAVVGCGGKFPVE	1988.08	15.33	994.54 (2+), 663.36 (3+), 499.16 (4+)	in-source fragments match sequence	10, 25, 50, 70%	1988.08		
D5	0	88-122	DAKEEVQLFLGNAGIAMRSLTAAVTAAGGNATYVL	3522.83	36.18	1174.92 (3+)		50, 70%	3522.90		trace LC peak
D5 deamidated	0	88-122	DAKEEVQLFLGNAGIAMRSLTAAVTAAGGNATYVL	3523.83	34.96	1175.24 (3+)	some in-source fragments match	ND	ND		Asn deamidation; trace LC peak
D5 no mut	0	88-122	DAKEEVQLFLGNAGTAMRPLTAAVTAAGGNATYVL	3520.81	ND	ND		ND	ND		
D5 no PS-mut	0	88-122	DAKEEVQLFLGNAGIAMRPLTAAVTAAGGNATYVL	3532.85	ND	ND		ND	ND		
D5 no TI-mut	0	88-122	DAKEEVQLFLGNAGTAMRSLTAAVTAAGGNATYVL	3510.79	ND	ND		ND	ND		
D6	0	123-134	DGVPRMRERPIG	1382.73	13.38	1382.71 (1+), 691.86 (2+), 461.57 (3+), 346.44 (4+)	in-source fragments match sequence	10, 25, 50, 70%	1382.75		coelutes with D14
i.c. 5	0	130-134	ERPIG	571.31	4.88	571.32 (1+)	some in-source fragments match	ND	ND		
D7	0	135-145	DLVVGLKQLGA	1112.67	23.73	1112.66 (1+), 556.83 (2+)	in-source fragments match sequence	50, 70%	1112.67		
D9	0	148-153	DCFLGT	712.30	18.90	712.29 (1+)	in-source fragments match sequence	ND	ND		
D10	0	154-194	DCPPVRVNGIGGLPGKGVKLSGSISSQYLSALLMAAPLALG	4064.20	32.21	1355.73 (3+), 1017.02 (4+)	in-source fragments match sequence	25, 50, 70%	4064.24		Asn deamidation likely
D11	0	195-201	DVEIEII	830.45	26.57	830.44 (1+)	in-source fragments match sequence	10, 25%	830.35		
i.c. 6	0	202-217	DKLISIPYVEMTLRLM	1922.04	31.26	961.52 (2+), 641.34 (3+)	in-source fragments match sequence	ND	ND		
D12	0	202-227	DKLISIPYVEMTLRLMERFGVKAHS	3062.62	ND	ND		25, 50, 70%	3062.61		
i.c. 7	0	218-224	ERFGVKA	806.44	11.39	806.44 (1+), 403.73 (2+)	some in-source fragments match	ND	ND		trace LC peak
i.c. 8	0	218-227	ERFGVKAHS	1159.58	6.70	1159.58 (1+), 580.29 (2+), 387.20 (3+)	in-source fragments match sequence	ND	ND		
D14	0	231-251	DRFYIKGGQKYKSPKNAYVEG	2448.27	13.38	1224.63 (2+), 816.75 (3+), 612.81 (4+), 490.45 (5+)	some in-source fragments match	10, 25, 50, 70%	2448.29		coelutes with D6
i.c.9	1	291-311	EMMGAKVTWTETSVTVTGPPR	2278.11	20.64	1139.57 (2+), 760.04 (3+)	in-source fragments match sequence	ND	ND		
i.c. 10	2	291-322	EMMGAKVTWTETSVTVTGPPREFGRKHLKAI	3554.85	19.28	1777.86 (2+), 1185.62 (3+), 889.46 (4+), 711.77 (5+)	in-source fragments match sequence	ND	ND		

Table 3 (continued). Observed Asp-N fragments of 2m-EPSPS. Cys residues were reduced and carboxyamidomethylated.

Fragment #	# of missed cleavages	Start - End	Sequence	Theor. mono. m/z (MH ⁺)	Retention Time, min	Observed m/z, LC/MS *	Observed in-source peptide fragments	% ACN (elution from C18-zt; MALDI)	Observed m/z MALDI-MS	Modification	Comment
D17	0	323-330	DVNMNKMP	948.43	15.56	948.43 (1+), 474.72 (2+)	some in-source fragments match	ND	ND		coelutes with i.c. 17 fragment
D18	0	331-343	DVAMTLAVVALFA	1320.72	36.32	1320.73 (1+), 660.87 (2+)	in-source fragments match sequence	ND	ND		
D19	0	344-350	DGPTAIR	729.39	10.18	729.39 (1+)	in-source fragments match sequence	10, 25%	729.32		
i.c. 11	0	351-358	DVASWRVK	960.52	14.71	960.52 (1+), 480.76 (2+)	in-source fragments match sequence	ND	ND		
D20	0	351-381	DVASWRVKETERMVAIRTELTKLGASVEEGP	3457.81	24.18	1729.40 (2+), 1153.26 (3+), 865.20 (4+), 692.36 (5+), 577.12 (6+)	in-source fragments match sequence	25, 50, 70%	3457.84		
i.c. 12	2	351-368	DVASWRVKETERMVAIRT	2147.13	18.45	1074.08 (2+), 716.38 (3+), 537.53 (4+), 430.22 (5+)	in-source fragments match sequence	ND	ND		
i.c. 13	1	359-368	ETERMVAIRT	1205.62	14.52	1205.63 (1+), 603.31 (2+)	in-source fragments match sequence	ND	ND		
i.c. 14	4	359-381	ETERMVAIRTELTKLGASVEEGP	2516.30	22.81	1258.65 (2+), 839.43 (3+), 629.81 (4+)	some in-source fragments match	ND	ND		trace LC peak
i.c. 15	0	369-381	ELTKLGASVEEGP	1329.68	17.43	1329.71 (1+), 665.34 (2+)	in-source fragments match sequence	ND	ND		
D21	0	382-397	DYCIITPEKLNVTAI	1846.96	25.77	1846.98 (1+), 923.98 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1846.97		
i.c. 16	1	401-415	DDHRMAMAFSLAACA	1666.71	23.04	1666.73 (1+), 833.86 (2+)	in-source fragments match sequence	ND	ND		trace LC peak
i.c. 17	0	416-422	EVPTIR	813.48	15.56	813.48 (1+), 407.24 (2+)	in-source fragments match sequence	ND	ND		coelutes with D17
D25	0	423-432	DPGCTRKTFP	1178.56	12.38	1178.56 (1+), 589.78 (2+), 393.52 (3+)	in-source fragments match sequence	10, 25, 50%	1178.58		
D27	0	436-444	DVLSTFVKN (-)	1022.55	21.41	1022.55 (1+), 511.77 (2+)	in-source fragments match sequence	10, 25%	1022.46		C-terminal; sequence confirmed by MS/MS fragmentation
D6-7	1	123-145	DGVPRMRERPIGDLVVGLKQLGA	2476.38	ND	ND		10, 25, 70%	2476.29		
D23-24	1	401-422	DDHRMAMAFSLAACAEPVTIR	2461.18	ND	ND		10, 25%	2461.08		

ND = not detected; i.c. = "incorrect" cleavage (for Asp-N, cleavage may occur N-terminal to E, in addition to D)

Table 4. Observed Glu-C (pH8) fragments of 2m-EPSPS. Cys residues were reduced and carboxyamidomethylated.

Fragment #	# of missed cleavages	Start - End	Sequence	Theor. mono. m/z (MH ⁺)	Retention Time, min	Observed m/z, LC/MS *	Observed in-source peptide fragments	% ACN (elution from C18-zt; MALDI)	Observed m/z MALDI-MS	Modification	Comment
A1	0	1-5	(-) AGAEE	476.20	ND	ND		ND	ND	no Met ¹	N-terminal
A1 + Met	0	(-1) - 5	(-) MAGAEE	607.24	ND	ND		ND	ND		N-terminal
A2	0	6-13	IVLQPIKE	939.59	18.39	939.59 (1+), 470.29 (2+)	in-source fragments match sequence	10, 25, 70%	939.61		Coelutes with A33
A3	0	14- 38	ISGTVKLPKSGKSLNRILLALAE	2567.51	25.89	1284.25 (2+), 856.51 (3+), 642.62 (4+)	in-source fragments match sequence	10, 25, 50, 70%	2567.62		
A5	0	45- 50	NLLNSE	689.35	16.32	689.35 (1+)	some in-source fragments match	ND	ND		coelutes with A50
A7	0	52- 67	VHYMLGALRTLGLSVE	1758.96	ND	ND		10, 25, 50, 70%	1759.04		
i.c. 1	n/a	58-67	ALRTLGLSVE	1058.61	20.56	1058.63 (1+), 529.81 (2+)	in-source fragments match sequence	ND	ND		
A12	0	93-123	VQLFLGNAGIAMRSLTAAVTAAGGNATYVLD	3065.61	ND	ND		25, 50, 70%	3065.67		
A12 no TI mut.	0	93-123	VQLFLGNAGTAMRSLTAAVTAAGGNATYVLD	3053.57	ND	ND		ND	ND		
A12 no PS mut.	0	93-123	VQLFLGNAGIAMRPLTAAVTAAGGNATYVLD	3075.63	ND	ND		ND	ND		
A12 no mutations	0	93-123	VQLFLGNAGTAMRPLTAAVTAAGGNATYVLD	3063.59	ND	ND		ND	ND		
A13	0	124-130	GVPRMRE	844.45	9.11	844.44 (1+), 422.72 (2+)	in-source fragments match sequence	10, 25%	844.45		
A23	0	212-218	MTLRLE	893.46	21.05	893.46 (1+), 447.23 (2+)	in-source fragments match sequence	25, 50, 70%	893.49		
A24	0	219-225	RFGVKAE	806.45	9.35	806.45 (1+), 403.73 (2+), 269.49 (3+)	in-source fragments match sequence	10, 25%	806.47		
A27	0	232-250	RFYIKGGQKYKSPKNAYVE	2276.22	ND	ND		10, 25, 50, 70%	2276.31		
A33	0	292-301	MMGAKVTWTE	1153.54	18.39	1153.54 (1+), 577.27 (2+)	in-source fragments match sequence	70%	1153.60		coelutes with A2
A34	0	302-323	TSVTVTGPPREPFGKHLKAID	2406.33	ND	ND		10, 25, 70%	2406.41		
i.c. 2	n/a	302-315	TSVTVTGPPREPFG	1444.74	19.08	1444.76 (1+), 722.87 (2+)	in-source fragments match sequence	ND	ND		
A37	0	345-351	GPTAIRD	729.39	ND	ND		50, 70%	729.46		
A38	0	352-359	VASWRVKE	974.54	12.61	974.55 (1+), 487.77 (2+), 325.52 (3+)	in-source fragments match sequence	10, 25, 50, 70%	974.58		
A40	0	362-369	RMVAIRTE	975.54	12.24	975.54 (1+), 488.27 (2+), 325.84 (3+)	in-source fragments match sequence	ND	ND		
A41	0	370-379	LTKLGASVEE	1046.57	14.75	1046.58 (1+), 523.79 (2+)	in-source fragments match sequence	25%	1046.62		
A43	0	383-390	YCIITPPE	992.48	20.92	992.48 (1+)	in-source fragments match sequence	ND	ND		
A47	0	403-416	HRMAMAFSLAACAE	1565.70	ND	ND		50, 70%	1565.79		
A48	0	417-433	VPVTIRDPGCTRKTFPD	1959.01	15.94	980.01 (2+), 653.67 (3+), 490.50 (4+)	in-source fragments match sequence	10, 25, 50, 70%	1959.10		
A50	0	437-444	VLSTFVKN (-)	907.53	16.32	907.53 (1+), 454.26 (2+)	in-source fragments match sequence	ND	ND		C-terminal; coelutes with A5

Table 4 (continued). Observed Glu-C (pH8) fragments of 2m-EPSPS. Cys residues were reduced and carboxyamidomethylated.

Fragment #	# of missed cleavages	Start - End	Sequence	Theor. mono. m/z (MH ⁺)	Retention Time, min	Observed m/z LC/MS *	Observed in-source peptide fragments	% ACN (elution from C18-zt; MALDI)	Observed m/z MALDI-MS	Modification	Comment
A1-2 + Met	1	(-1) - 13	(-) MAGAEEIVLQPIKE	1527.81	ND	ND		ND	ND		N-terminal
A1-2	1	1- 13	(-) AGAEEIVLQPIKE	1396.77	ND	ND		10, 25%	1396.82		N-terminal
A4-5	1	39- 50	GTTVVDNLLNSE	1261.63	23.07	1261.64 (1+), 631.31 (2+)	in-source fragments match sequence	ND	ND		
A6-7	1	51- 67	DVHYMLGALRTLGLSVE	1873.98	29.30	937.50 (2+)	in-source fragments match sequence	10, 25, 50, 70%	1874.07		
A8-9	1	68- 87	ADKAAKRAVVVGCGGKFPVE	2059.11	ND	ND		10, 25, 70%	2059.18		
A13-14	1	124-135	GVPRLMRERPIGD	1382.73	ND	ND		10, 25%	1382.81		
A21-22	1	200-211	IIDKLISIPYVE	1402.82	26.27	1402.84 (1+), 701.91 (2+)	in-source fragments match sequence	50, 70%	1402.86		
A25-26	1	226-231	HSDSWD	746.27	7.26, 7.67	746.27 (1+)	some in-source fragments match	ND	ND		
A30-31	1	274-288	CGGTSLSQGDVKFAE	1569.72	18.18	1569.70 (1+), 785.36 (2+)	some in-source fragments match	10, 25, 50, 70%	1569.79		
A34-35	1	302-331	TSVTVTGPPREPFGRLKLAIDVNMNMKMPD	3335.74	17.51	1668.36 (2+), 1112.58 (3+), 834.69 (4+), 667.95 (5+)	in-source fragments match sequence	10, 25, 50, 70%	3335.85		
A36-37	1	332-351	VAMTLAVVALFADGPTAIRD	2031.09	33.09	1016.05 (2+), 677.69 (3+)	in-source fragments match sequence	25, 50, 70%	2031.18		
A39-40	1	360-369	TERMVAIRTE	1205.63	ND	ND		10, 70%	1205.53		
A48-49	1	417-436	VPVTIRDPGCTRKTFPDYFD	2384.17	20.33	1192.61 (2+), 795.39 (3+), 596.79 (4+)	in-source fragments match sequence	25, 50, 70%	2384.26		
A49-50	1	434-444	YFDVLSTFVKV (-)	1332.68	ND	ND		50, 70%	1332.75		C-terminal
A8-10	2	68- 88	ADKAAKRAVVVGCGGKFPVED	2174.14	15.07	1087.56 (2+), 725.37 (3+)	some in-source fragments match	10, 25, 70%	2174.24		
A9-11	2	70- 92	KAARKRAVVVGCGGKFPVEDAKEE	2445.29	13.48	1223.18 (2+), 815.76 (3+), 612.08 (4+)	some in-source fragments match	ND	ND		
A17-19	2	149-197	CFLGTDCCPVVRVNGIGLPGGKVKLSGSISSQYLSALLMAAP LALGDVE	4985.59	ND	ND		50, 70%	4986.72		Asn161 could be deamidated
A25-27	2	226-250	HSDSWDRFYIKGGQKYKSPKNAYVE	3003.48	ND	ND		10, 25, 50, 70%	3003.59		
A31-33	2	284-301	VKFAEVLMMGAKVTWTE	2069.05	ND	ND		70%	2069.14		
S23-24	1	380-416	GPDYCIITPPEKLNVTADTYDDHRMAMAFSLAACAE	4156.91	ND	ND		70%	4156.96		
A48-50	2	417-444	VPVTIRDPGCTRKTFPDYFDVLSTFVKV (-)	3272.68	ND	ND		10, 25, 50, 70%	3272.80		C-terminal
A4-7	3	39- 67	GTTVVDNLLNSEDVHYMLGALRTLGLSVE	3116.59	ND	ND		50, 70%	3116.70		
A8-11	3	68- 92	ADKAAKRAVVVGCGGKFPVEDAKEE	2631.36	14.02	1316.17 (2+), 877.79 (3+), 658.59 (4+)	in-source fragments match sequence	10, 25, 50, 70%	2631.46		
A39-42	3	360-382	TERMVAIRTELTKLGASVEEGPD	2502.29	ND	ND		10, 25%	2502.42		
A44-47	3	391-416	KLNVTADTYDDHRMAMAFSLAACAE	2914.35	24.02	1457.74 (2+), 972.13 (3+), 729.35 (4+)	in-source fragments match sequence	25, 50, 70%	2914.46		

ND = not detected

i.c. = "incorrect" cleavage

Table 5. Amino acid sequence obtained for N-terminal T1 peptide of 2m-EPSPS from tryptic digest (a.a. 1-12; m/z 634.41; retention time 20.95 min).

Sequence: AGAEEIVLQPIK

Fragment ion masses: monoisotopic

Peptide mass $[M+H]^{2+}$ (monoisotopic): 634.37

	A	G	A	E	E	I	V	L	Q	P	I	K
a(+1)	44.05	101.07	172.11	301.15	430.19	543.28	642.35	755.43	883.49	980.54	1093.63	
		101.07	172.11	301.15	430.20	543.27	642.33					
b(+1)	72.04	129.07	200.10	329.15	458.19	571.27	670.34	783.43	911.48	1008.54	1121.62	
		129.07	200.10	329.14	458.19	571.27	670.33	783.40	911.52		1121.54	
y(+1)		1196.69	1139.67	1068.63	939.59	810.55	697.46	598.39	485.31	357.25	260.20	147.11
		1196.72		1068.64	939.57	810.54	697.46	598.39	485.31	357.25	260.20	147.11

Upper rows = theoretical ions.

Lower rows (in **bold**) = observed fragment ions.

Table 6. Amino acid sequence obtained for N-terminal "i.c.1" peptide (see Table 3) of 2m-EPSPS from endoproteinase Asp-N digest (a.a. 1-12; m/z 634.37; retention time 20.99 min)

Sequence: AGAEEIVLQPIK

Fragment ion masses: monoisotopic

Peptide mass $[M+H]^{2+}$ (monoisotopic): 634.37

	A	G	A	E	E	I	V	L	Q	P	I	K
a(+1)	44.05	101.07	172.11	301.15	430.19	543.28	642.35	755.43	883.49	980.54	1093.63	
		101.07	172.11	301.15	430.19	543.26	642.34					
b(+1)	72.04	129.07	200.10	329.15	458.19	571.27	670.34	783.43	911.48	1008.54	1121.62	
		129.07	200.10	329.15	458.19	571.27	670.34	783.42				
y(+1)		1196.69	1139.67	1068.63	939.59	810.55	697.46	598.39	485.31	357.25	260.20	147.11
				1068.57	939.56	810.53	697.46	598.39	485.30	357.25	260.20	147.11

Upper rows = theoretical ions.

Lower rows (in **bold**) = observed fragment ions.

Table 7. Amino acid sequence obtained for C-terminal D27 peptide of 2m-EPSPS from endoproteinase Asp-N digest (a.a. 436-444; m/z 1022.59; retention time 21.55 min)

Sequence: DVLSTFVK^N

Fragment ion masses: monoisotopic

Peptide mass [M+H]²⁺(monoisotopic): 1022.5522

	D	V	L	S	T	F	V	K	N
a(+1)	88.04	187.11	300.19	387.22	488.27	635.34	734.41	862.50	
		187.13	300.18			635.31	734.39		
b(+1)	116.03	215.10	328.19	415.22	516.27	663.34	762.40	890.50	
		215.11	328.19	415.20	516.25	663.32	762.40	890.46	
y(+1)		907.53	808.46	695.37	608.34	507.29	360.22	261.16	133.06
		907.49	808.46	695.35	608.33	507.31	360.23	261.16	

Upper rows = theoretical ions.

Lower rows (in **bold**) = observed fragment ions.

Table 8. Amino acid sequence obtained for C-terminal R20 peptide of 2m-EPSPS from Arg-C digest (a.a. 429-444; m/z 961.54; retention time 29.22 min).

Sequence: KTFPDYFDVLSTFVKN

Fragment ion masses: monoisotopic

Peptide mass $[M+H]^{2+}$ (monoisotopic): 960.99

	K	T	F	P	D	Y	F	D	V	L	S	T	F	V	K	N
a(+1)	101.11	202.16	349.22	446.28	561.30	724.37	871.44	986.46	1085.53	1198.61	1285.65	1386.69	1533.76	1632.83	1760.93	
	101.11		349.21						1085.56	1198.50						
b(+1)	129.10	230.15	377.22	474.27	589.30	752.36	899.43	1014.46	1113.53	1226.61	1313.64	1414.69	1561.76	1660.83	1788.92	
	129.10	230.15	377.19		589.30	752.34		1014.46	1113.49	1226.61		1414.67				
y(+1)		1792.88	1691.83	1544.76	1447.71	1332.68	1169.62	1022.55	907.53	808.46	695.37	608.34	507.29	360.22	261.16	133.06
				1544.78		1332.75	1169.61	1022.56		808.43	695.37	608.31		360.19	261.16	

Upper rows = theoretical ions.

Lower rows (in **bold**) = observed fragment ions.

Table 9. Amino acid sequence obtained for internal R5 peptide of 2m-EPSPS from Arg-C digest (a.a. 106-127; m/z 1053.13; retention time 23.93 min) containing P to S mutation.

Sequence: **S**LTAAVTAAGGNATYVLDGVPR

Fragment ion masses: monoisotopic

Peptide mass $[M+H]^{2+}$ (monoisotopic): 1052.56

	<i>S</i>	<i>L</i>	<i>T</i>	<i>A</i>	<i>A</i>	<i>V</i>	<i>T</i>	<i>A</i>	<i>A</i>	<i>G</i>	<i>G</i>	<i>N</i>	<i>A</i>	<i>T</i>	<i>Y</i>	<i>V</i>	<i>L</i>	<i>D</i>	<i>G</i>	<i>V</i>	<i>P</i>	<i>R</i>
a(+1)	60.04	173.13	274.18	345.21	416.25	515.32	616.37	687.40	758.44	815.46	872.48	986.53	1057.56	1158.61	1321.68	1420.74	1533.83	1648.85	1705.88	1804.94	1902.00	
		173.11			416.24	515.29																
b(+1)	88.04	201.12	302.17	373.21	444.25	543.31	644.36	715.40	786.44	843.46	900.48	1014.52	1085.56	1186.61	1349.67	1448.74	1561.82	1676.85	1733.87	1832.94	1929.99	
		201.13	302.17	373.20	444.24	543.29	644.36	715.39	786.41	843.37	900.47			1187.57								
y(+1)		2017.07	1903.99	1802.94	1731.90	1660.87	1561.80	1460.75	1389.71	1318.68	1261.65	1204.63	1090.59	1019.55	918.50	755.44	656.37	543.29	428.26	371.24	272.17	175.12
					1660.81	1561.78	1460.65	1389.75	1318.68	1261.70	1204.59	1090.51	1019.55	918.48	755.43	656.36	543.29	428.26	371.20	272.17	175.12	

Upper rows = theoretical ions.

Lower rows (in **bold**) = observed fragment ions.

Table 10. Amino acid sequence obtained for internal T12 peptide of 2m-EPSPS from tryptic digest (a.a. 106-127; m/z 1053.11; retention time 23.93 min) containing P to S mutation.

Sequence: **SL**TAAVTAAGGNATYVLDGVPR
Fragment ion masses: monoisotopic
Peptide mass [M+H]²⁺(monoisotopic): 1052.56

	S	L	T	A	A	V	T	A	A	G	G	N	A	T	Y	V	L	D	G	V	P	R
a(+1)	60.04	173.13	274.18	345.21	416.25	515.32	616.37	687.40	758.44	815.46	872.48	986.53	1057.56	1158.61	1321.68	1420.74	1533.83	1648.85	1705.88	1804.94	1902.00	
		173.11			416.24	515.29																
b(+1)	88.04	201.12	302.17	373.21	444.25	543.31	644.36	715.40	786.44	843.46	900.48	1014.52	1085.56	1186.61	1349.67	1448.74	1561.82	1676.85	1733.87	1832.94	1929.99	
		201.13	302.17	373.21	444.24	543.28	644.34		786.41								1561.77					
y(+1)		2017.07	1903.99	1802.94	1731.90	1660.87	1561.80	1460.75	1389.71	1318.68	1261.65	1204.63	1090.59	1019.55	918.50	755.44	656.37	543.29	428.26	371.24	272.17	175.12
					1731.71	1660.79	1561.77	1460.78	1389.75	1318.64	1261.65		1090.57	1019.55	918.54	755.45	656.36	543.28	428.26		272.17	175.12

Upper rows = theoretical ions.
Lower rows (in **bold**) = observed fragment ions.

Table 11. Amino acid sequence obtained for internal T11 peptide of 2m-EPSPS from tryptic digest (a.a. 91-105; m/z 724.99; retention time 25.56 min) containing T to I mutation.

Sequence: EEVQLFLGNAG**I**AMR

Fragment ion masses: monoisotopic

Peptide mass $[M+H]^{2+}$ (monoisotopic): 824.43

	E	E	V	Q	L	F	L	G	N	A	G	I	A	M	R
a(+1)	102.06	231.10	330.17	458.23	571.31	718.38	831.46	888.48	1002.53	1073.56	1130.58	1243.67	1314.71	1445.75	
	102.06	231.10			571.29										
b(+1)	130.05	259.09	358.16	486.22	599.30	746.37	859.46	916.48	1030.52	1101.56	1158.58	1271.66	1342.70	1473.74	
		259.09	358.16	486.23	599.30	746.38	859.49								
y(+1)		1518.81	1389.77	1290.70	1162.64	1049.56	902.49	789.40	732.38	618.34	547.30	490.28	377.20	306.16	175.12
				1290.63	1162.62	1049.55	902.49	789.40	732.37	618.33	547.29	490.28	377.19	306.16	175.12

Upper rows = theoretical ions.

Lower rows (in **bold**) = observed fragment ions.

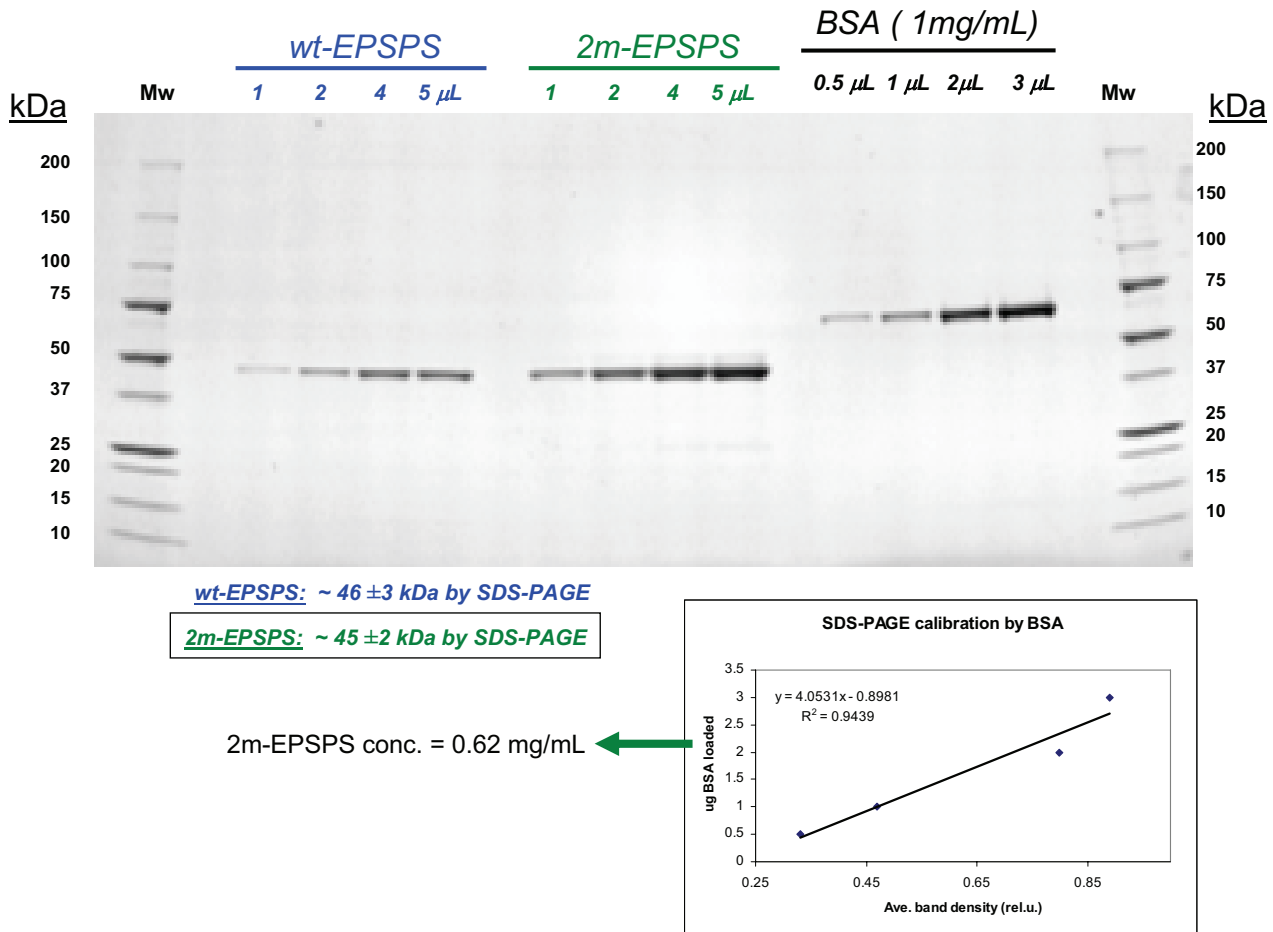
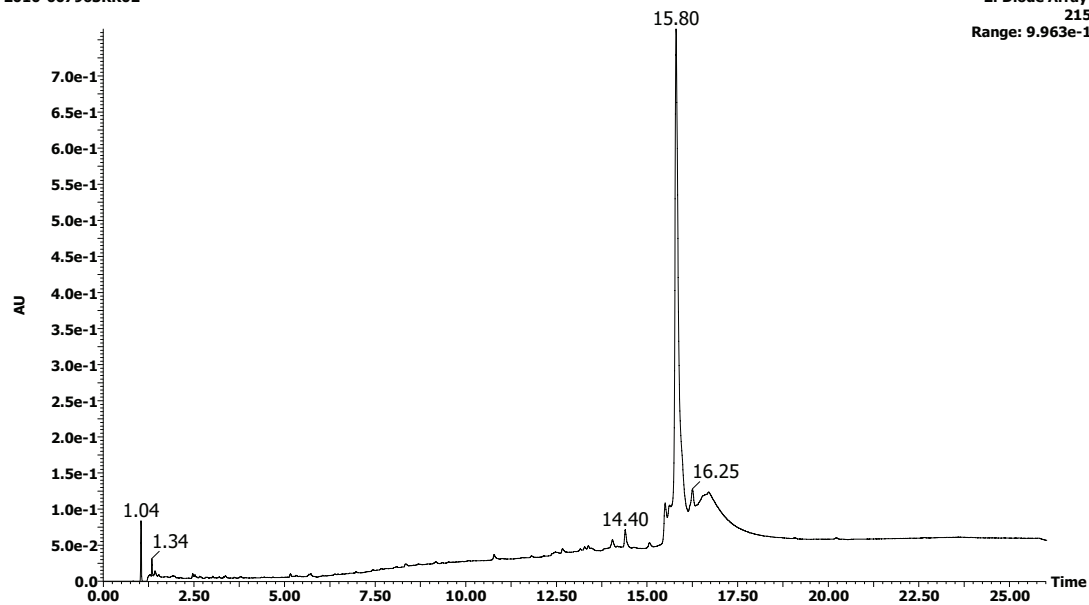


Figure 1. SDS-PAGE analysis of 2m-EPSPS protein sample. Lanes on the left of gel: wt-EPSPS reference material. Lanes in the center of gel: 2m-EPSPS sample. Lanes on the right of gel: BSA protein standard. Bottom panel: estimate of 2m-EPSPS protein quantity based on known amounts of loaded BSA standard. MW = molecular weight standard (Bio-Rad). BSA = bovine serum albumin standard (Sigma). wt-EPSPS = wild-type EPSPS reference ².

(A)

Krishna Kuppannan 06-May-2010 17:21:23
2010-007963KK02

2m EPSPS TIPS
2: Diode Array
215
Range: 9.963e-1



(B)

Krishna Kuppannan 06-May-2010 17:21:23
2010-007963KK02 922 (16.075) Sb (5,5.00); Cm (904:923)

2m EPSPS TIPS
1: TOF MS ES+
455

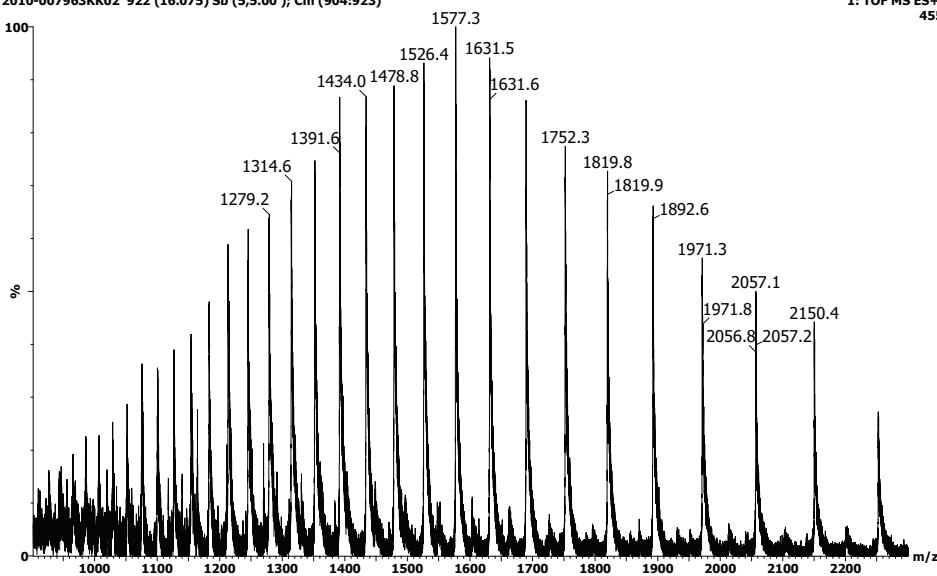


Figure 2 (continued on next page).

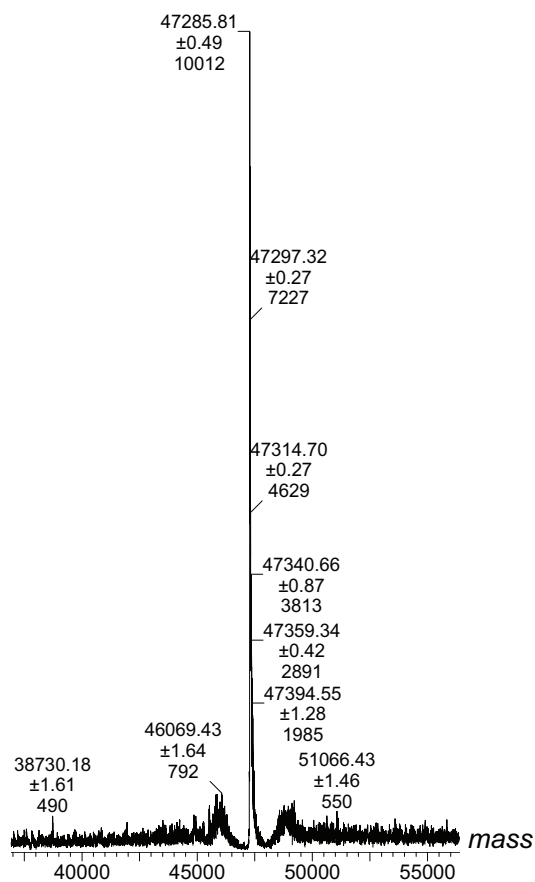
(C)

Figure 2 (continued). (A) 2m-EPSPS intact MW analysis: LC/MS chromatogram (UV at 215 nm). (B) 2m-EPSPS intact MW analysis: raw ESI mass spectrum (multiple charge envelope). (C) 2m-EPSPS intact MW analysis: Deconvoluted mass-spectrum corresponding to the principal sample component eluting at 15.80 min.

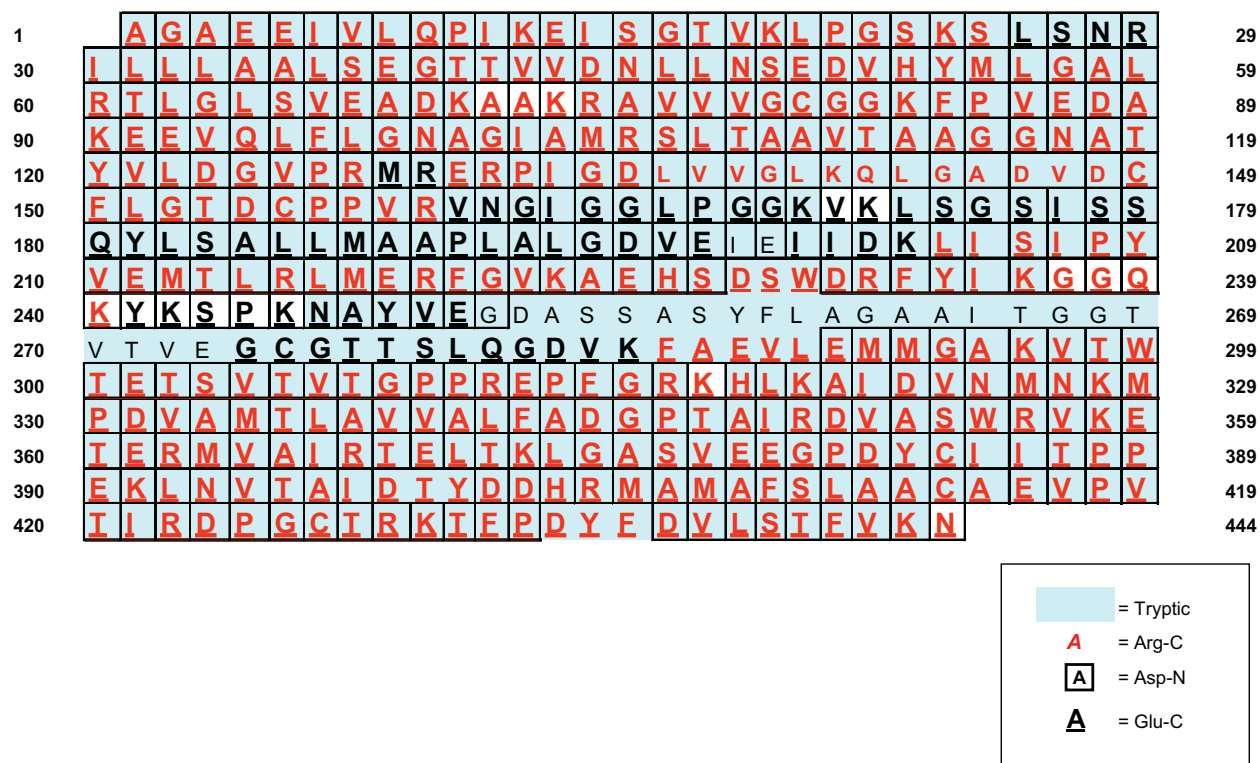


Figure 3. Theoretical amino acid sequence of 2m-EPSPS, with Tryptic, Arg-C, Asp-N, and Glu-C fragments detected by mass-spectrometry highlighted. Sequence coverage is complete (100%). The corresponding observed proteolytic fragments are listed in **Tables 1** through **4**.

0.1mL/min;30 min gradient;5-40%B;50C;214nm
2010-007963KK07

2m EPSPS tryptic digest

1: TOF MS ES+

TIC

3.68e4

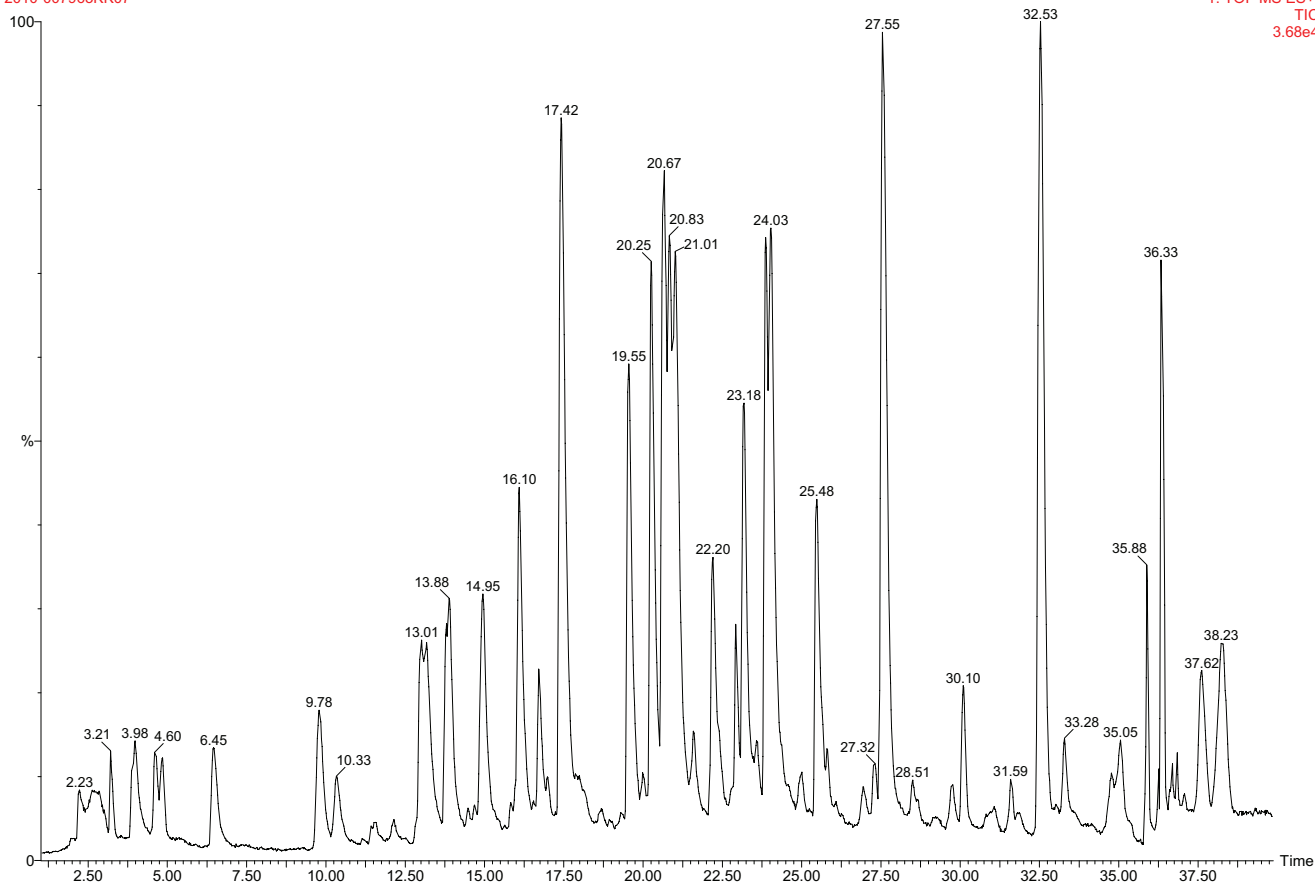


Figure 4. LC/MS chromatogram (TIC) of 2m-EPSPS Tryptic digest. The corresponding observed proteolytic fragments are listed in **Table 1**.

0.1mL/min;30 min gradient;5-40%B;50C;214nm
2010-007963KK13

2m EPSPS ArgC digest
1: TOF MS ES+
TIC
4.55e4

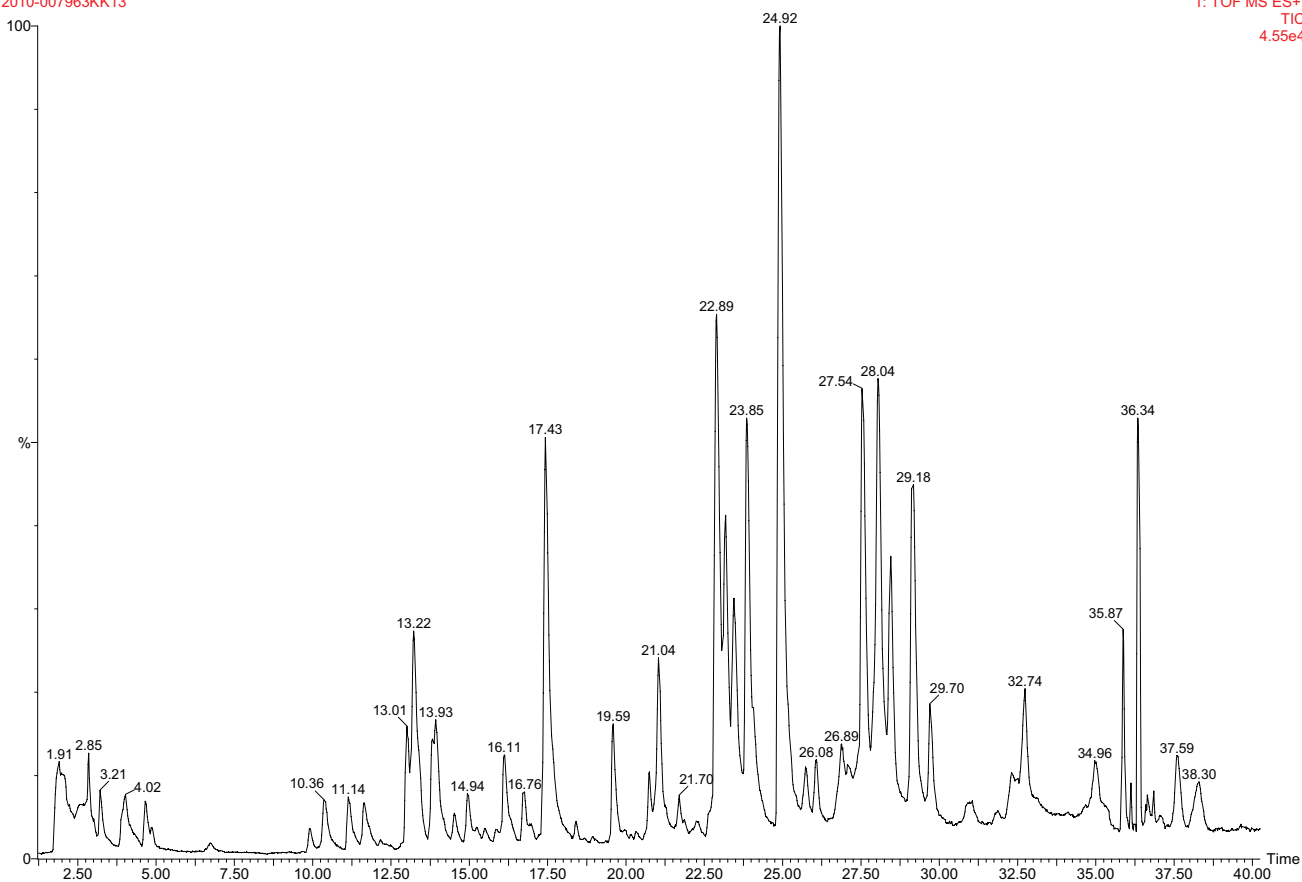


Figure 5. LC/MS chromatogram (TIC) of 2m-EPSPS Arg-C digest. The corresponding observed proteolytic fragments are listed in **Table 2**.

0.1mL/min;30 min gradient;5-40%B;50C;214nm
2010-007963KK09

2m EPSPS AspN digest
1: TOF MS ES+
TIC
3.72e4

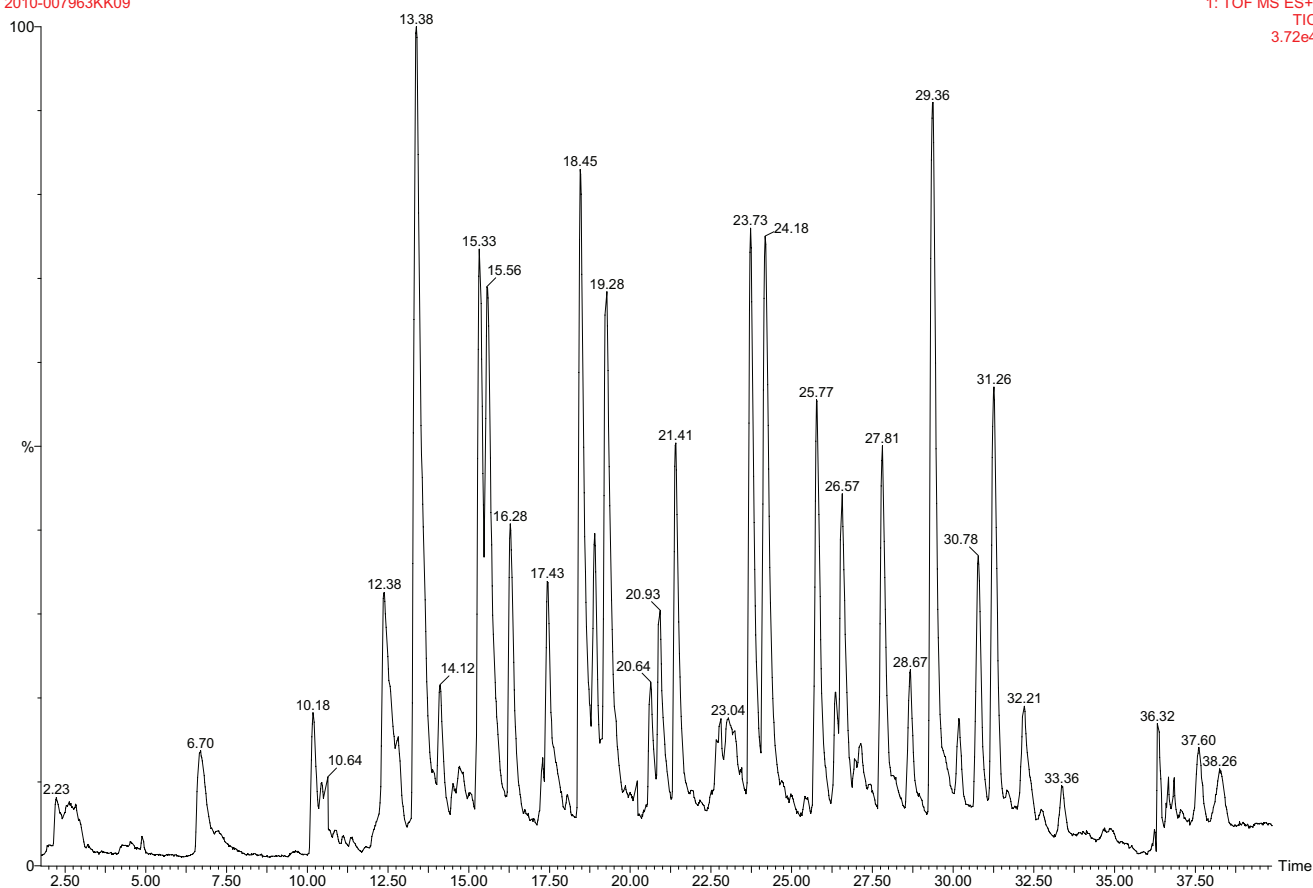


Figure 6. LC/MS chromatogram (TIC) of 2m-EPSPS Asp-N digest. The corresponding observed proteolytic fragments are listed in **Table 3**.

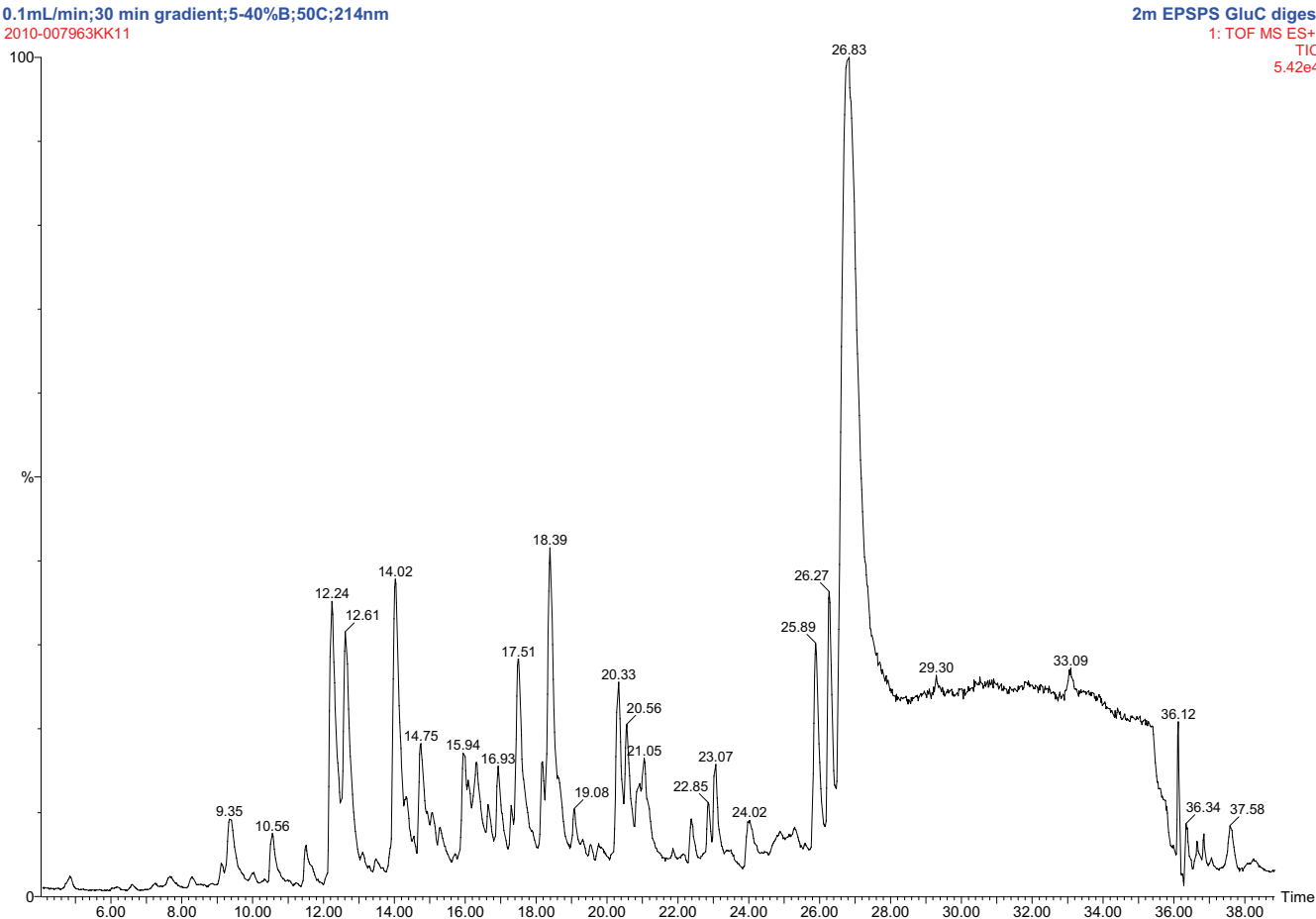


Figure 7. LC/MS chromatogram (TIC) of 2m-EPSPS Glu-C (pH8) digest. The corresponding observed proteolytic fragments are listed in **Table 4**.

Study Director: Karnoup, Anton (AS) (U369292)

Study: 2010-007963

Date Result Required: 6/30/2010
Client Project: Other R&D Support - DOW AGROSCIENCES
Charge Number: 83941000; Strain and Process Improvements; 010/16/YF
Description: Detailed characterization of 2mEPSPS with TIPS mut
Client:
Schafer, Barry (BW) (U097380)

Sample Data:
Sample-174582: 2010-007963-001
Required Date: 6/30/2010
Description:
Operator: Karnoup, Anton (AS) (U369292)

Aliquot Data:
Aliquot-146226: 2010-007963-001-001
Description: 2mEPSPS -TIPS mutation
Operator: Karnoup, Anton (AS) (U369292)

Test Data:
Test-
Operator:
Description:
Method or SOP Ref:
Method or SOP Ref:
Aliquot-146227: 2010-007963-001-002
Description: 2mEPSPS -TIPS mutation
Operator: Kuppannan, Krishna (K) (U386368)

Test Data:
Test-
Operator:
Description:
Method or SOP Ref:
Method or SOP Ref: