

Supplementary material

Methods for 2D electrophoresis and mass spectrometry

Materials - Immobilised pH-gradient strips and buffers were purchased from Amersham Biosciences, part of GE Healthcare (Milwaukee, WI, USA). Reagents for polyacrylamide gel preparation were purchased from Bio-Rad Laboratories (Hercules, CA, USA). CHAPS was obtained from Roche Diagnostics (Mannheim, Germany), urea from AppliChem (Darmstadt, Germany), thiourea from Fluka (Buchs, Switzerland), 1,4-dithioerythritol and EDTA from Merck (Darmstadt, Germany) and tributylphosphine from Pierce Biotechnology (Rockford, IL, USA).

Sample preparation – 23-month-old rat hippocampus tissue was powderised in liquid nitrogen and suspended in 2 ml sample buffer (20 mM Tris, 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM 1,4-dithioerythritol, 1 mM EDTA, 1 mM PMSF, 1 tablet Complete™ from Roche Diagnostics, and 0.2% v/v phosphatase inhibitor cocktail from Calbiochem). The suspension was sonicated for approximately 30 s and centrifuged at 15000 g for 60 min at 12 °C. Desalting was done with an Ultrafree-4 centrifugal filter unit with a cut-off molecular weight of 10 kDa (Millipore, Bedford, MA, USA) at 3,000 g at 12 °C until the eluted volume was about 4 mL and the remaining volume reached 100-200 µL. The protein concentration of the supernatant was determined by the Bradford assay.

Two-dimensional gel electrophoresis - Samples of 750 µg protein were applied onto immobilised pH 3–10 non-linear gradient strips. Focusing started at 200 V, and the voltage was gradually increased to 8000 V at 4 V/min and kept constant for a further 3 h (approximately 150,000 Vh in total). The second-dimensional separation was performed on 10–16% gradient SDS-PAGE. After protein fixation for 12 h in 50% methanol and 10% acetic acid, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 8 h, and the excess of dye was removed with distilled water. Molecular masses were determined by running standard protein markers (Bio-Rad Laboratories, Hercules, CA, USA), covering the range of 10–250

kDa. pI values were used as provided by the supplier of the immobilised pH gradient strips.

In-gel digestion - Spots of interest were excised and washed with 10 mM ammonium bicarbonate and 50% acetonitrile in 10 mM ammonium bicarbonate. After washing, gel plugs were shrunk by the addition of acetonitrile and dried in a SpeedVac (Eppendorf, Hamburg, Germany). The dried gel pieces were reswollen with 40 ng/ μ l trypsin (sequencing grade; Promega, Madison, WI) in digestion buffer (5 mM octyl β -D-glucopyranoside in 10 mM ammonium bicarbonate) and incubated for 30 min at 30 °C. Chymotrypsin digestion was performed by the addition of 25 mM ammonium bicarbonate containing 25 ng/ μ l chymotrypsin (sequencing grade; Roche diagnostic) and incubation for 1 h at 30 °C. Extraction was performed firstly with 15 μ l of 1% trifluoroacetic acid in 5 mM octyl β -D-glucopyranoside, secondly using 15 μ l 0.1% trifluoroacetic acid, 4% acetonitrile, and thirdly using 15 μ l 0.1% trifluoroacetic acid, 4% acetonitrile. Both extracted peptides were pooled.

Analysis of peptides by nano-LC-ESI-CID/ETD-MS/MS and data processing - 40 μ L extracted peptides were analysed on nano-LC-ESI-CID/ETD-MS/MS. The HPLC used was a bio-compatible Ultimate 3000 system (Dionex Corporation, Sunnyvale, CA, USA) equipped with a PepMap100 C-18 trap column (300 μ m x 5 mm) and PepMap100 C-18 analytical column (75 μ m x 150 mm). The gradient was (A = 0.1% formic acid in water, B = 0.08% formic acid in acetonitrile) 4% to 30% B from 0 min to 105 min, 80% B from 105 min to 110 min, 4% B from 110 min to 135 min. The flow rate was 300 nl/min from 0 min to 12 min, 75 nl/min from 12 min to 105 min, 300 nl/min from 105 min to 135 min. A HCTultra PTM discover system (Bruker Daltonics, Bremen, Germany) was used to record peptide spectra over the mass range of m/z 350-1500, and MS/MS spectra in information dependent data acquisition over the mass range of m/z 100-2800. Repeatedly, MS spectra were recorded followed by three data dependent CID MS/MS spectra and three ETD MS/MS spectra generated from three highest intensity precursor ions. An active exclusion of 0.4 min after 2 spectra was used to detect low-abundance peptides. The voltage between ion spray tip and spray shield was set to 1100 V. Drying nitrogen gas was heated to 170 °C and its flow rate was 10L/min. The collision energy was set automatically according to the

mass and charge state of the peptides chosen for fragmentation. Multiple charged peptides were chosen for MS/MS experiments due to their good fragmentation characteristics. MS/MS spectra were interpreted and peak lists were generated by DataAnalysis 3.4 (Bruker Daltonics, Bremen, Germany). Searches were done by using the MASCOT 2.2.04 (Matrix Science, London, UK) against Swiss-Prot 56.0 database for protein identification. Search parameters were set as follows: enzyme selected as trypsin or chymotrypsin with two maximum missing cleavage sites, species limited to rat, a mass tolerance of 500 ppm for peptide tolerance, 0.5 Da for MS/MS tolerance, fixed modification of carbamidomethyl (Cys) and variable modification of methionine oxidation and phosphorylation (Tyr, Thr, and Ser). Positive protein identifications were based on a significant MOWSE score. After protein was identified, error-tolerant search was done to detect unspecific cleavage and unassigned modifications. Protein identification and modification returned from MASCOT were manually inspected and filtered to create a confirmed protein identification and modification list.

Supplementary Table 1. Identification and characterisation of rat Profilin-2 (PROF2_RAT) by MS/MS spectrum.

Spot	PI/MW (KDa)	Enzyme	total score	Sequence Coverage (%)	MS/MS Peptide sequence (ion score)	PTMS
1	5.60/ 12.86	Trypsin	727	50	29 YVWAATAGGVFQSITPAEIDVIIGK 53 (108) 29 YVWAATAGGVFQSITPAEIDVIIGKDR 55 (50) 54 DREGFFTNGTLGGK 68 (58) 54 DREGFFTNGTLGGK 68 (47) 56 EGFFTNGTLGGK 68 (56) 56 EGFFTNGTLGGK 68 (48) 75 DSLYVSDCTMDIR 88 (105) 75 DSLYVSDCTM*DIR 88 (73) 89 TKSQ*GGEPTYNVAVGR 104 (45) 91 SQGGEPTYNVAVGR 104 (74) 91 SQ*GGEPTYNVAVGR 104 (63)	Deamidation: N61, Q92 Oxidation: M85
		Chymotrypsin	193	27.1	7 VDNLMCDGCCQEAAIVGY 24 (50) 7 VDNLMCDGCCQEAAIVGY 24 (47) 40 QSITPAEIDVIIGKDREGF 58 (52) 40 QSITPAEIDVIIGKDREGFF 59 (44)	Deamidation: Q17
2	6.14/ 12.92	Trypsin	1863	80	1 A*GWQSYVDNLMCDGCCQEAAIVGYCDAK 28 (62) 1 A*GWQSYVDNLM*CDGCCQEAAIVGYCDAK 28 (49) 29 YVWAATAGGVFQSITPAEIDVIIGK 53 (119) 29 YVWAATAGGVFQSITPAEIDVIIGKDR 55 (112) 29 YVWAATAGGVFQ*SITPAEIDVIIGKDR 55 (119) 54 DREGFFTNGTLGGK 68 (84) 54 DREGFFTNGTLGGK 68 (109) 54 DREGFFTNGTLGGK 69 (61) 56 EGFFTNGTLGGK 68 (102) 56 EGFFTNGTLGGK 68 (74) 56 EGFFTNGTLGGK 69 (75) 70 CSVIRDSLYVSDCTMDIR 88 (70) 70 CSVIR*DSLYVSDCTMDIR 88 (43) 75 DSLYVSDCTMDIR 88 (112) 75 DSLYVSDCTM*DIR 88 (71) 75 DSLYVSDCTMDIR* 88 (52) 89 TKSQGGEPTYNVAVGR 104 (79) 89 TKSQ*GGEPTYNVAVGR 104 (133) 89 TKSQ*GGEPTYN*VAVGR 104 (59) 91 SQGGEPTYNVAVGR 104 (85) 91 SQ*GGEPTYNVAVGR 104 (47) 91 SQGGEPTYN*VAVGR 104 (62) 91 SQGGE*PTYNVAVGR 104 (40) 108 VLVFVMGK 115 (44)	Acetylation: A1 Deamidation: Q40, N61, Q92, N99 Deimination: R74, R88 Methylation: E95 Oxidation: M11, M85
		Chymotrypsin	932	82.9	9 VDNLMCDGCCQEAAIVGY 24 (89) 9 VDNLM*CDGCCQEAAIVGY 24 (70) 9 VDN*LMCDGCCQEAAIVGY 24 (68) 9 VDNLMCDGCCQEAAIVGYCDAKY 29 (47) 30 VWAATAGGVF 39 (43) 32 AATAGGVFQSITPAEIDVIIGKDREGF 58 (50) 32 AATAGGVFQSITPAEIDVIIGKDREGFF 59 (48) 32 AATAGGVFQ*SITPAEIDVIIGKDREGFF 59 (63) 40 QSITPAEIDVIIGKDREGF 58 (45) 40 QSITPAEIDVIIGKDREGFF 59 (89) 66 GGKKCSVIRDSLY 78 (55)	Deamidation: N9, Q40 Oxidation: M11, M113

					79 VSDCTMDIRTKSQGGEPTY 98 (56) 99 NVAVGRAGRVL 109 (53) 110 VFVMGKEGVHGGGL 123 (44) 110 VFVM*GKEGVHGGGLNKKAY 128 (47) 112 VMGKEGVHGGGLNKKAY 128 (65)	
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