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/µl 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/µl chymotrypsin (sequencing grade; Roche diagnostic) and incubation for 1 h at 30 °C. Extraction was performed firstly with 15 µl of 1% trifluoroacetic acid in 5 mM octyl β-D-glucopyranoside, secondly using 15 µl 0.1% trifluoroacetic acid, 4% acetonitrile, and thirdly using 15 µl 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 µL 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 µm x 5 mm) and PepMap100 C-18 analytical column (75 µm 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 DREGFFTNGLTLGGK 68 (58) 54 DREGFFTN*GLTLGGK 68 (47) 56 EGFFTNGLTLGGK 68 (56) 56 EGFFTN*GLTLGGK 68 (48) 75 DSLYVDSDCTMDIR 88 (105) 75 DSLYVDSDCTM*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 VDNLMCDGCCQ*EAAIVGY 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 DREGFFTNGLTLGGK 68 (84) 54 DREGFFTN*GLTLGGK 68 (109) 54 DREGFFTNGLTLGGKK 69 (61) 56 EGFFTNGLTLGGK 68 (102) 56 EGFFTN*GLTLGGK 68 (74) 56 EGFFTNGLTLGGKK 69 (75) 70 CSVIRDSLYVDSDCTMDIR 88 (70) 70 CSVIR*DSLYVDSDCTMDIR 88 (43) 75 DSLYVDSDCTMDIR 88 (112) 75 DSLYVDSDCTM*DIR 88 (71) 75 DSLYVDSDCTMDIR* 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 Deimidation: 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 VDSDCTMDIRTKSQGGEPTY 98 (56) 99 NVAVGRAGRVL 109 (53) 110 VFVMGKEGVHGGGL 123 (44) 110 VFVM*GKEGVHGGGLNKKAY 128 (47) 112 VMGKEGVHGGGLNKKAY 128 (65)	