Amino Acids (2011) 41:955–968
DOI 10.1007/s00726-010-0795-9

ORIGINAL ARTICLE

A first partial *Aplysia californica* proteome

Yanwei Sun · Francisco J. Monje · Daniela D. Pollak · Gert Lubec

Received: 20 October 2010 / Accepted: 20 October 2010 / Published online: 11 November 2010

Abstract *Aplysia* proteins have not been studied systematically and it was therefore the aim of the study to carry out protein profiling in ganglia from *Aplysia californica* (AC). AC ganglia were extirpated, proteins extracted and run on 2DE with subsequent in-gel digestion, followed by identification of proteins by nano-LC–ESI–MS/MS on an ion trap. Proteins were identified based upon a public *Aplysia* EST database. Out of 408 picked spots, 276 spots were identified corresponding to 172 ESTs and 118 individual proteins. The range of sequence coverage was between 14 and 80% and the average amount of peptides used for the identification of proteins was 9 (from 3 to 24). Mean score for protein identification was 516. Comparison of protein levels between cerebral, pleural, pedal and abdominal ganglia revealed a series of significant differences including: signaling, metabolism, cytoskeleton and structural, redox, chaperone, replication/transcription and electron/proton transport proteins. The generation of a protein map complements transcriptional studies carried out in AC ganglia. The findings provide the basis for investigation into post-translational modifications, splice variants and assist in the generation of antibodies against AC proteins. Moreover, differences in protein expression between ganglia may be valuable for the design of future studies in neurobiology of AC.

Keywords 2-DE · *Aplysia californica* · Nano-LC–ESI–MS/MS · Protein map · Protein profiling · Ganglia

Introduction

Aplysia californica (AC) is a well-accepted and established model animal to answer a series of questions in neuroscience, in particular learning and memory. Although this animal has been used for decades, it is intriguing that proteomic studies are limited and no systematic approach to analyze the proteome of the AC nervous system has been reported so far. It is well known that protein synthesis per se and specific and local protein synthesis is required for memory formation (Bergold et al. 1990; Ezzeddine and Glanzman 2003; Guan and Clark 2006; Kim et al. 2006; Martin et al. 1997a; Schacher et al. 1988; Skehel et al. 1995).

At the nucleic acid level, a cDNA library prepared from isolated AC sensory neurons (Moccia et al. 2003) and a neuronal transcriptome analysis were published so far (Moroz et al. 2006). At the protein level molecular weight distribution of proteins synthesized in single AC neurons (Wilson 1971), protein patterns in the R15 neuron (Strumwasser and Wilson 1976), newly synthesized proteins during synaptogenesis in AC neurons (Ambron et al. 1985) as well as the proposed use of MALDI-MS (Kruse and Sweedler 2003) were reported. In these publications no protein profiling was carried out.

Y. Sun and F. J. Monje have equally contributed to the work.

Electronic supplementary material The online version of this article (doi:10.1007/s00726-010-0795-9) contains supplementary material, which is available to authorized users.

Y. Sun · G. Lubec (✉)
Department of Pediatrics, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria
e-mail: gert.lubec@meduniwien.ac.at

F. J. Monje · D. D. Pollak
Department of Physiology, Center for Physiology and Pharmacology, Medical University of Vienna, Schwarzspanierstrasse 17, 1090 Vienna, Austria

A series of protein classes has been characterized in AC including brain receptor homologues (Angers et al. 1998; Barbas et al. 2005, 2002; Hansen et al. 2004; Hislop et al. 2004; Whim and Kaczmarek 1998), transporters and channels (Collado et al. 2009; Furukawa et al. 2006; Jezzini and Moroz 2004; Jezzini et al. 2006), transcription factors (Casadio et al. 1999; Chen et al. 2003; Dash and Moore 1996; Han et al. 2005; Si et al. 2003; Swanson et al. 1986). In addition, cytoskeleton elements (Han et al. 2004; Jang et al. 2005; Zappulla et al. 2005), a chaperone (Kuhl et al. 1992), neural elements (Cibelli et al. 1996; Martin et al. 1995; Reissner et al. 2008), a large series of kinases and phosphatases (Bergold et al. 1992; Bougie et al. 2009; Carroll et al. 2004; Dyer et al. 1996; Farah et al. 2009; Greenberg et al. 1987; Houeland et al. 2007; Kennedy et al. 1992; Lee et al. 2006; Liu et al. 2004; Martin et al. 1997b; Purcell et al. 2003; Sharma et al. 2003; Sung et al. 2004; Yanow et al. 1998) and a handfull of enzymes from different metabolic cascades (Fan et al. 1999; Fioravante et al. 2008; Hernandez et al. 2009; Juvvadi et al. 1997; Park et al. 2005; Wickham et al. 1999; Yang et al. 2005a) were reported.

It was the aim of the current study to describe a partial proteome from AC ganglia in a gel-based proteomic approach using two-dimensional gel electrophoresis with LC–MS/MS identification of protein spots that may be helpful in neuroscientific studies at the protein level. In addition, differences in protein levels between four specific AC ganglia were examined.

Materials and methods

Aplysia californica were obtained from the Rosenstiel School of Marine and Atmospheric Science, National Resource for Aplysia, University of Miami/RSMAS, 4600 Rickenbacker Causeway, Miami, Florida 33149, USA. Samples were sent on dry ice and the freezing chain was never interrupted.

Dissection of Aplysia neural ganglia

Aplysia californica handling and ganglia dissection procedure was performed following the methodologies and solutions described by Zhao et al. (2009) with minor modifications. Briefly, adult AC with body weights of about 120 g were used (Fig. 1a). Before the dissection of the ganglia animals were anesthetized by a direct injection of $MgCl_2$ (0.35 M) into the internal body cavity. The volume of isotonic $MgCl_2$ injected was equivalent to approximately 1/3 to 1/2 of the body weight of the animal. When general muscle contractions were no more noticeable and animals were completely anesthetized, dissection was performed on a standard wax dissecting pan (Fig. 1b). A longitudinal head-to-tail opening cut of the skin (arrows in Fig. 1b) was then carried out at the ventral region of the animals using small surgical fine scissors. Skin flaps were gently pulled apart and pinned down and the visceral mass was moved apart using fine forceps thus making the pairs of major AC ganglia visible to the naked eye (Fig. 1c).

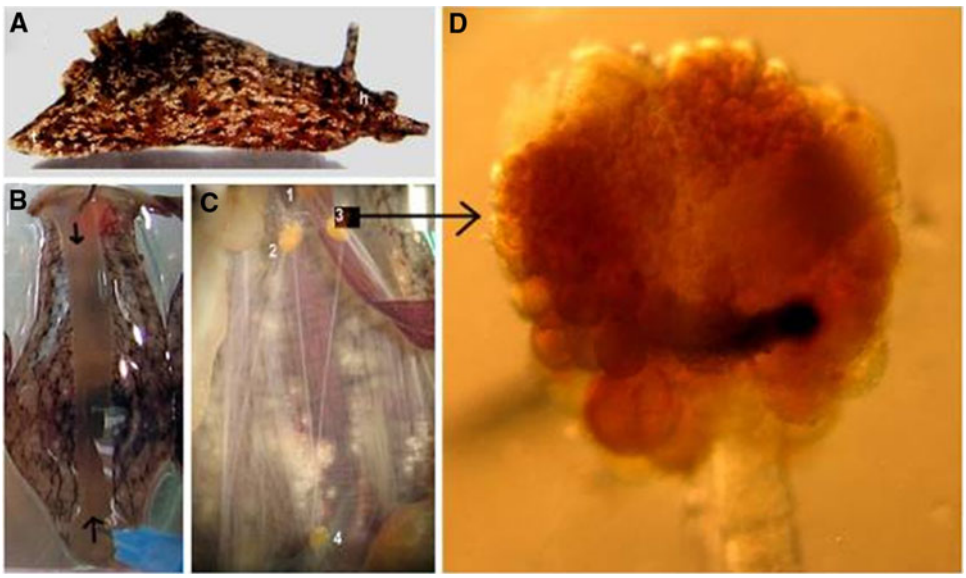

Fig. 1 Illustrative images of Aplysia, its nervous system and constitutive ganglia. **a** A representative adult *Aplysia californica* of ~120 g (*h* head; *t* tail). **b** An anesthetized animal lies pinned down on the dissecting pan. The semi transparent ventral section is clearly visible. **c** The skin was cut open (following the *arrows* in **b**), skin flaps were separated and visceral mass displaced; in this two-pictures assembly of the same animal some of the Aplysia ganglia are visible [*1* cerebral; *2* pedal; *3* part of the pleural is visible; *4* abdominal; buccal ganglia are not visible in the picture (Zhao et al. 2009)]. **d** High magnification digital photograph of a representative pleural ganglion hold by a black metal insect-pin. All images were taking with a Power Shot A510 digital camera of 3.2 Mega Pixels

Ganglia were next dissected out (Zhao et al. 2009) and transferred to 35 mm Petri dishes containing 4 ml of L-15 media with 10 mg/ml of Dispase (Worthington, Catalogue Nr. LS02104, Lakewood, NJ). Petri dishes with cerebral, pleural, pedal and abdominal ganglia were transferred to an incubator set at 35°C and digestion proceeded for 1 h. Subsequently, ganglia were washed out five times with L-15/ASW (Artificial Sea Water; 50%/50%) and were then transferred to a Petri dish containing L-15 (Zhao et al. 2009). The L-15 (Leibovitz) medium (L5520, Sigma–Aldrich Corp. St. Louis, MO, USA) was systematically supplemented with NaCl 6.25 g; KCl 172 mg; $CaCl_2$ 744 mg; $MgSO_4$ $7H_20$ 3.12 g; $MgCl_2$ $6H_2O$ 2.85 g; Dextrose 3.12 g and $NaHCO_3$ 96 mg (per 500 ml of L-15). Ganglia were desheathed (removal of the external connective tissue layer) using fine forceps and stereo-binocular microscopy. Samples were stored in cryotubes at −80°C until used for further analysis.

Sample preparation

Cerebral, pleural, pedal and abdominal ganglia were homogenized and suspended in 1.2 ml of sample buffer (20 mM Tris, 7 M urea, 2 M thiourea, 4% (w/v) 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 on ice for approximately 30 s and centrifuged at 15,000×*g* for 120 min at 12°C. Desalting was carried out with an Ultrafree-4 centrifugal filter unit with a cut-off molecular weight of 10 kDa (Millipore, Bedford, MA) 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 content of the supernatant was determined by the Bradford assay.

Two-dimensional gel electrophoresis

Five gels per group of ganglia were produced. Samples of 700 µg of protein were subjected to immobilized pH 3-10 nonlinear gradient strips. Focusing started at 200 V and the voltage was gradually increased to 8,000 at 4 V/min and kept constant for a further 3 h (approximately 150,000 Vh totally). Prior to the second-dimensional run, strips were equilibrated twice for 15 min with gentle shaking in 10 ml of SDS equilibration buffer (50 mM pH 8.8 Tris–HCl, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, trace of bromophenol blue). DTT (1%) (w/v) was added at the first incubation for 15 min and 4% iodoacetamide (w/v) instead of DTT at the second incubation step for 15 min. 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) for 8 h and excess of dye was washed out from the gels with distilled water. Molecular masses were determined by running precision protein standard markers (Bio-Rad Laboratories, Hercules, CA), covering the range of 10–250 kDa. Isoelectric point values were determined as given by the supplier of the immobilized pH gradient strips.

In-gel digestion

Protein spots were manually excised and placed into 0.5 ml low-bind Eppendorf tubes. In-gel digestion and sample preparation for mass spectrometric analysis was performed as described (Chen et al. 2006).

Gel plugs were washed several times with 10 mM ammonium bicarbonate and with 50% acetonitrile in 10 mM ammonium bicarbonate. After addition of 100% acetonitrile to shrink the gel, gel plugs were dried in a Speedvac Concentrator 5301 (Eppendorf). The dried gel pieces were reswollen in digestion buffer (5 mM β-octylglucoside (OG) in 10 mM ammonium bicarbonate) containing 40 ng/µl trypsin (sequencing grade; Promega) and incubated for 12 h at 37°C. Chymotrypsin digestion was performed by 25 mM ammonium bicarbonate containing 25 ng/µl chymotrypsin (sequencing grade; Roche Diagnostics) and incubation for 12 h at 30°C. Peptide extraction was performed with 20 µl of 1% formic acid in 5 mM OG for 30 min, and subsequently 20 µl 0.1% formic acid for 30 min and 20 µl 0.1% formic acid in 20% acetonitrile for 30 min. Extracted fractions were pooled for analysis using nano-LC-ESI-(CID/ETD)-MS/MS.

Analysis of peptides by nano-LC–ESI–(CID/ETD)–MS/MS

A high capacity ion trap (HCT) was used to analyze 40 µl of extracted peptides. The HPLC used was an Ultimate 3000 system (Dionex Corporation, Sunnyvale, CA) equipped with a PepMap100 C-18 trap column (300 µm × 5 mm) and PepMap100 C-18 analytic column (75 µm × 150 mm). The column was run with a flow rate of 300 nl/min using a two-buffer system (a) 0.1% formic acid in water and (b) 0.08% formic acid in acetonitrile according to the following procedure: 4 to 30% B from 0 to 105 min, 80% B from 105 to 110 min, and 4% B from 110 to 125 min. An HCT ultra ETDII PTM discover system (Bruker Daltonics, Bremen, Germany) was used to record peptide spectra over the mass range of *m/z* 350–1,500, and MS/MS spectra in information dependent data acquisition over the mass range of *m/z* 100–2,800. Repeatedly, MS spectra were recorded followed by four data-dependent CID MS/MS spectra generated

from four highest intensity precursor ions. On neutral loss peaks of precursor mass −32.66 and −48.99, ETD MS/MS spectra of the corresponding precursor was recorded. An active exclusion of 0.4 min after two spectra was used to detect low abundant peptides. The voltage between ion spray tip and spray shield was set to about 1,500 V. Drying nitrogen gas was heated to 150°C and the flow rate was 10 l/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 4.0 (Bruker Daltonics). Then data were searched against the AC EST database (CU_ALL_APLYSIA_ASSEM_SP-060605; 1,503,600 protein entries) using MASCOT 2.2.06.

Enzyme was selected "as used" with three maximum missing cleavage sites, NO species limited for two times searching, a mass tolerance of 0.2 Da for peptide tolerance, 0.2 Da for MS/MS tolerance, fixed modification of carbamidomethyl (C) and variable modification of methionine (M) oxidation and phosphorylation (Tyr, Thr, and Ser). Positive protein identifications were based on significant MOWSE scores (peptides ion score cut-off = 30). Peptides matched were then submitted to the NCBI to BLAST against the UniProtKB protein database (Release 57.5 of 07 July 2009).

After protein identification, an error-tolerant search was done to detect unspecific cleavage and unassigned modifications. Protein identification and modification information returned from MASCOT were manually inspected and filtered to validate protein identification and modification.

Quantification of protein levels

Protein spots from each gel from the individual groups of ganglia (5 per group; total $n = 20$) were outlined (first automatically and then manually) and quantified using the Proteomweaver software (Definiens, Munich, Germany). The percentage of the volume of the spots representing a certain protein was determined in comparison with the total proteins present in the two-dimensional gel electrophoresis (2-DE) gel (Langen et al. 1999).

Statistical analysis

Statistical analysis to reveal between-group differences was performed by ANOVA. Bonferroni–Holm correction was applied for correction of multiple testing.

In all proteomic studies, a probability level of $P < 0.05$ was considered as statistically significant. All calculations were performed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

A representative gel of an AC abdominal ganglion with spot numbers is shown in Fig. 2.

The spot numbers are decoded in Table 1 that lists the proteins identified in terms of accession numbers, Blast scores/E values, GenBank accession numbers, total scores/matched peptides, and sequence coverage in percentages.

Figure 3 demonstrates the distribution of peptide scores versus peptide length (a); the distribution of delta values versus peptide length (b); and distribution of number of peptides/protein numbers (c). On average 9 peptides were generated from each protein in the range between 3 and 24.

The sequence coverage of identified proteins ranged from 14 to 80%; on average, sequence coverage was 44% (Fig. 3d).

Trypsin and chymotrypsin digestion produced 1,325 unique peptides matching 276 spots and 118 different proteins. Identification of the 276 proteins corresponded to 172 ESTs.

Figure 4a reveals assignment of identified proteins to functional categories. Subcellular distribution of identified proteins is shown in Fig. 4b.

Total number of spots was 1,121 and 408 spots were picked because they were well-separated in the gel.

A series of high abundance proteins (defined by Coomassie blue visualization) was identified, 118 different proteins represented by 276 protein spots were detected and were assigned to protein pathways: signaling, carbohydrate and intermediary metabolism, amino acid and protein metabolism, cytoskeleton and structural, redox, chaperone, replication and transcription, electron/proton transport ATP synthesis, neural, miscellaneous proteins and proteins with unknown function.

Subcellular localization assigned proteins to cytoplasm, extracellular space, cell membrane, nucleus, mitochondrion, endoplasmic reticulum, unknown and miscellaneous.

Mean score for protein identification was 516.

The peptide list sorted into tryptic and chymotryptic peptides, based upon Mascot searches is given in supplementary Table 1.

A protein list based upon the BLASTp results against UniProtKB database is provided in supplementary Table 2.

The results of the differences in protein expression between the four individual ganglia are shown in the supplementary Tables 3 and 4.

Differences in levels of several pathways and cascades including signaling, neurotransmission, metabolic, structural and miscellaneous proteins, were observed.

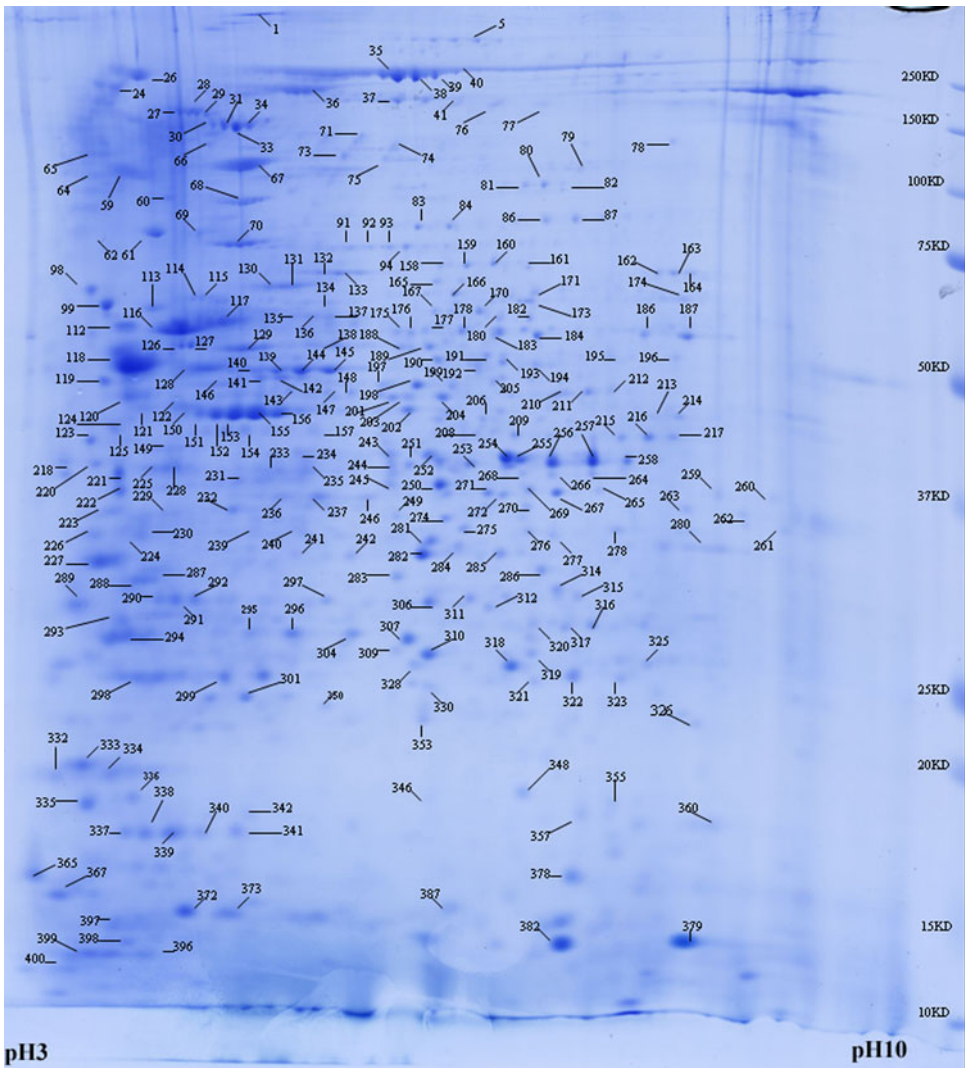

Fig. 2 Gel with spot numbers. All spots labeled were identified using trypsin and chymotrypsin digestion

Sequences were submitted to the PRIDE (PRoteomics IDEntifications) database and are available at http://www.ebi.ac.uk/pride/.

Discussion

Although the identification rate of AC ganglia proteins is not very high, only 276 proteins were identified out of 408 picked spots, we consider publishing the outcome a first necessary step to provide evidence for AC proteins identified by MS/MS sequencing using a gel-based proteomic method that was successfully used in the past (Kang et al. 2009; Zheng et al. 2009).

Trypsin and chymotrypsin in-gel digestions carried out on each spot warranted fair identification and herein the first systematic proteomic approach to generate a partial AC ganglia proteome is shown. Out of 276 spots 118 individual proteins were observed showing different expression forms probably due to post-translational modifications or splice variants that were not further studied in this current work. These data may be important for AC studies at the protein level and indeed, the existence of a series of proteins based upon information from nucleic acid sequences was confirmed and we introduce two unknown proteins and one protein with unknown function.

In the group of cytoskeleton and structural elements, a series of proteins resemble proteins observed in mammalian neural tissue (Fountoulakis et al. 2002, 1999; Krapfenbauer et al. 2003; Langen et al. 1999; Myung and Lubec 2006; Yang et al. 2004, 2005b), including the 70 kDa neurofilament protein, neural cell adhesion molecule 1, WD repeat-containing protein 1, lamins, tubulins, actins/actin-binding and -depolymerizing proteins and filamins. Myosin-10 and paramyosin were observed as high abundance proteins that are not regularly observed in brain or neural tissue proteomes as revealed by extensive literature searches. The collagen composition consisted of collagen alpha-4(VI),

Table 1 Proteins identified from Aplysia ganglia

Protein name	Accession number	Blast score/ E-value	Spot number	GenBank gi	Total score/match pept./seq. cov.%
Cytoskeleton and structural					
WD repeat-containing protein 1	Q6DIF4	238/2e-62	159, 160	gil121358745	549/10/54%
Basement membrane-specific heparan sulfate proteoglycan core protein	P98160	123/1e-27	26, 27, 28, 29, 31	gil121376175	576/12/41%
	P98160	123/1e-27	75	gil121361546	312/7/47%
	Q05793	62.8/2e-09	59, 335	gil121364101	256/5/19%
Basement membrane proteoglycan	Q06561	153/1e-36	230, 294	gil121322046	533/11/51%
Non-neuronal cytoplasmic intermediate filament protein B	P16275	384/3e-106	33, 34, 35, 36, 38, 83, 128, 131, 231	gil203637484	1,105/21/70%
Non-neuronal cytoplasmic intermediate filament protein	P22488	424/4.00E-117	37	gil121314058	469/8/35%
	P22488	236/6e-62	80, 306	gil121312821	578/11/55%
	P22488	333/8e-91	129	gil203603755	1,324/24/73%
	P22488	333/8e-91	130, 133	gil171860958	797/14/55%
	P22488	333/8e-91	132	gil171867799	780/12/53%
	P22488	392/1e-108	149, 226	gil171876541	781/14/58%
	P22488	392/1e-108	150	gil203633785	847/15/64%
70 kDa neurofilament protein	Q01241	193/1e-48	135, 136	gil121320515	509/7/29%
Neural cell adhesion molecule 1	P31836	60.8/5e-09	61, 66	gil121369397	604/8/45%
Lamin-A/C	P48678	175/3e-43	93	gil171873312	405/7/33%
Lamin-B2	P21619	98.2/1e-20	92	gil121377803	404/6/49%
Lamin-C	Q03427	84.7/3e-16	91	gil121460004	416/7/48%
Paramyosin	O96064	286/9e-77	307	gil171860674	1,065/16/45%
	O96064	286/9e-77	1	gil171875892	665/10/43%
Myosin-10	Q9JLT0	228/2e-59	67	gil121355847	330/5/28%
Muscle myosin heavy chain	P24733	264/2e-70	148	gil121378537	649/8/57%
	P24733	329/8e-90	68, 69, 70, 71, 73, 74, 82, 115	gil203591012	1,019/14/61%
Tubulin alpha-1 chain	Q8T6A5	449/1.00E-124	118, 193	gil171865999	1,093/20/79%
	Q8T6A5	471/1e-132	138	gil171866672	756/12/49%
	Q8T6A5	236/1e-61	139, 346	gil121364156	559/10/46%
	Q8T6A5	414/1e-115	192	gil121312913	498/10/56%
	Q8T6A5	430/4e-120	235	gil171862896	307/5/23%
	Q8T6A5	430/4e-120	236	gil203633445	368/7/37%
Tubulin alpha-3 chain	P05214	397/2e-110	336	gil121310797	224/4/26%
Tubulin alpha-2/alpha-4 chain	P41383	473/5e-133	233	gil171876774	623/11/41%
Tubulin beta-4 chain	P30883	485/1.00E-135	117	gil171869578	964/17/59%
	P30883	447/3e-125	237, 243	gil203632665	761/16/62%
	P30883	237/3e-62	334	gil121310913	207/4/29%
Tubulin beta chain	P41386	241/3e-63	114	gil203629384	536/6/29%
	P41386	472/7e-133	116, 201, 203	gil119565698	1,032/20/79%
	P11833	396/6e-110	137, 229	gil171877777	435/8/32%
Filamin-A	P21333	350/5e-96	81, 86	gil203633543	664/15/59%
	P21333	350/5e-96	87	gil203678435	177/4/18%
Fascin	Q16658	116/1e-25	134	gil121449120	384/6/31%
Severin	Q24800	236/1e-61	140	gil203668659	374/6/32%
	Q24800	181/3e-45	199	gil121375426	551/11/50%

Table 1 continued

Protein name	Accession number	Blast score/ E-value	Spot number	GenBank gi	Total score/match pept./seq. cov.%
Actin	Q26065	453/4e-127	147, 151, 152, 156	gil203610982	916/18/65%
	Q964E0	481/1e-135	146, 153, 304	gil171867107	1,071/22/79%
	Q5JAK2	111/4e-24	154	gil203608325	175/4/19%
	Q964E0	481/1e-135	155	gil171879120	1,169/23/67%
	P12716	379/8e-105	157	gil121343472	553/10/57%
	Q964E0	410/3e-114	227, 287	gil121312538	414/7/44%
	Q964E1	467/3e-131	290, 291, 292, 399	gil171871823	1,085/20/69%
	P53461	187/3e-47	387	gil121310645	197/3/22%
Actin-depolymerizing factor	Q68FP1	184/3e-46	197, 198	gil203628756	433/10/48%
Coactosin-like protein	Q2HJ57	120/8e-27	367	gil203673181	612/10/49%
Cofilin	Q4P6E9	88.2/3e-17	341, 342	gil121345512	377/8/64%
Transgelin-2	Q9WVA4	60.8/3e-09	360	gil121311093	172/3/26%
Flotillin-1	O61491	320/7e-87	194, 195, 196	gil203629812	566/14/59%
Collagen alpha-4(VI) chain	A2AX52	231/1e-11	24, 396	gil121475019	186/3/19%
	A2AX52	318/3e-17	124, 224	gil171860439	507/11/35%
	A2AX52	335/7e-19	225	gil203688464	518/8/36%
	A2AX52	121/3e-05	400	gil121415879	216/4/17%
Collagen alpha-2(IV) chain	P27393	377/4e-88	39, 40, 41	gil121326424	310/6/41%
Collagen alpha-1(XII) chain	P13944	346/7e-19	60, 62, 64, 65	gil121410720	453/7/39%
	Q91145	46.6/2e-04	122	gil203591416	534/17/57%
Collagen alpha-1(II) chain	P28481	1287/2e-15	84	gil203637462	190/3/14%
Chaperone					
Heat shock cognate 71 kDa protein	P19120	412/9e-115	76, 77, 78, 79	gil203671998	1,366/21/80%
	P19120	346/5e-95	284	gil121315709	519/8/40%
Small heat shock protein p36	Q7YZT0	103/1e-21	269, 272	gil203608765	569/11/52%
Protein disulfide isomerase	Q2HWU2	303/5e-82	220	gil203599579	722/15/60%
	P04785	303/7e-82	125	gil203629777	764/15/54%
T-complex protein 1 subunit alpha	Q9XT06	363/6e-100	165	gil171861678	395/8/39%
T-complex protein 1 subunit beta	Q5XIM9	324/2e-88	171	gil121343886	817/14/67%
Peptidyl-prolyl cis–trans isomerase 6	P52014	189/2e-47	332	gil121374848	695/10/45%
	P52014	182/1e-45	333	gil121362241	216/4/19%
DnaJ homolog subfamily B member 11	Q9UBS4	252/9e-67	208	gil121390198	198/3/19%
Putative universal stress protein SERP1273	Q5HNJ5	38.5/0.023	382	gil121398433	329/5/20%
Electron/proton transport and ATP synthesis					
Flavoprotein subunit of complex II B	Q801S2	380/4e-105	94	gil203652504	458/8/50%
Reductase-related protein	AAC13268	264/6.00E-69	113	gil203662567	690/12/43%
ATP synthase subunit alpha	P19483	332/2e-90	186, 187	gil171867387	447/9/41%
Obg-like ATPase 1	Q7ZU42	251/2e-66	212	gil121373900	368/6/39%
ATP synthase beta subunit	Q25117	426/7e-119	126, 127	gil203628134	1,000/20/67%
Cytochrome b-c1 complex subunit 2	P22695	134/5e-31	190	gil203613168	694/13/49%
Carbohydrate and intermediary metabolism					
Aconitate hydratase	P20004	310/3e-84	98, 99	gil121333467	523/9/60%
Enolase	Q27655	288/3e-77	141, 142	gil203608453	539/10/42%
	O02654	369/9e-102	144	gil203618084	619/12/52%
	Q27655	348/1e-95	145	gil203590432	677/14/58%
Transketolase-like protein 2	Q9D4D4	279/6e-75	162	gil121313130	507/11/80%
	Q9D4D4	313/5e-85	163, 164	gil171868378	459/10/48%

Table 1 continued

Protein name	Accession number	Blast score/ E-value	Spot number	GenBank gi	Total score/match pept./seq. cov.%
Phosphoglucomutase	Q7KHA1	252/1e-66	167	gil171868050	223/5/35%
Aldehyde dehydrogenase	Q27640	213/5e-55	180, 183	gil121415687	968/18/74%
Dihydrolipoyl dehydrogenase	Q8CIZ7	334/2e-91	182, 184	gil203629011	450/9/52%
Phosphoglycerate kinase	O61471	328/1e-89	209	gil203658207	679/13/68%
Fructose-bisphosphate aldolase	Q9GP32	473/5e-133	234, 271	gil171865624	678/11/53%
	Q9GP32	270/6e-72	251, 255	gil171860680	353/5/36%
	Q9GP32	270/6e-72	252	gil171861157	232/5/34%
Pyruvate dehydrogenase E1 component subunit beta	P11966	276/6e-74	239	gil171860036	408/8/41%
Glyceraldehyde-3-phosphate dehydrogenase	P29497	69.3/1e-11	245	gil121312064	223/4/20%
	Q05025	342/8e-94	249, 253	gil121312173	501/9/48%
	Q5R2J2	359/1e-98	254, 257	gil203651247	541/13/60%
	P00356	375/1e-103	256, 258, 264, 266, 268, 323	gil171861973	606/13/74%
	Q9N2D5	176/8e-44	270	gil121312365	499/7/36%
	Q05025	351/2e-96	316	gil203613170	724/15/66%
Glyoxylate reductase/hydroxypyruvate reductase	Q91Z53	156/6e-38	246	gil121404017	393/8/59%
	Q9UBQ7	208/2e-53	250	gil171870064	719/14/60%
Malate dehydrogenase	P00346	261/2e-69	265, 267	gil18002163	562/11/49%
	Q3T145	234/3e-61	398	gil121314512	236/4/25%
Phosphonoacetaldehyde hydrolase	A0LMC1	164/6e-40	281	gil171876508	657/11/41%
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	Q0BBK5	288/2e-77	314, 321	gil171863921	723/12/43%
Isochorismatase domain-containing protein 1	Q96CN7	219/7e-57	353, 355	gil121387713	324/5/29%
Citrate synthase	P00889	225/1e-58	211	gil171859933	263/6/33%
Enolase-phosphatase E1	Q28C69	138/4e-32	218	gil171870078	187/4/14%
Caffeoyl-CoA O-methyltransferase	O04854	79.7/2e-14	310	gil121381477	281/5/26%
Inorganic pyrophosphatase	Q6FRB7	305/2e-82	228	gil121374295	289/7/30%
Amino acid and protein metabolism					
Calreticulin	P14211	341/2e-93	112	gil171878099	408/8/29%
	Q4VIT5	349/1e-95	223	gil121376125	148/4/14%
Calumenin-B	B5X4E0	176/1e-43	121	gil203604283	331/7/39%
Calcineurin subunit B type 1	P63100	259/1e-68	365	gil171862791	399/7/38%
Prolyl 4-hydroxylase subunit alpha-1	P13674	150/6e-36	170	gil171875075	639/9/37%
Putative aminopeptidase	Q27245	222/2e-57	173	gil203619558	476/10/47%
Alpha-aminoadipic semialdehyde dehydrogenase	Q2KJC9	346/7e-95	175	gil171872176	426/9/40%
S-adenosylhomocysteine hydrolase	P51893	438/1e-122	205	gil121363904	326/6/32%
Aspartate aminotransferase	Q5REB0	255/2e-67	213, 214	gil203590279	436/8/44%
Arginine kinase	P51544	354/3e-97	215, 216, 217	gil203599216	768/15/69%
60S acidic ribosomal protein P0	P19889	211/2e-54	232	gil121316179	303/4/21%
Elongation factor 1-alpha	Q92005	408/1e-113	259, 260	gil171861265	229/4/28%
Eukaryotic translation initiation factor 3 subunit H	Q6P9U8	268/3e-71	244	gil171868782	280/5/26%
Translocon-associated protein subunit beta	P23438	200/4e-51	330	gil121315985	220/3/21%
Endoplasmic reticulum protein ERp29	P81628	154/5e-37	317	gil203650892	318/7/31%
Heterogeneous nuclear ribonucleoprotein F	Q60HC3	150/6e-36	177, 178	gil171875550	388/9/32%

Table 1 continued

Protein name	Accession number	Blast score/ E-value	Spot number	GenBank gi	Total score/match pept./seq. cov.%
Heterogeneous nuclear ribonucleoprotein 87F	P48810	207/3e-53	261, 262, 263, 319, 357	gil121380249	616/12/60%
Proteasome subunit alpha type-2	P24495	382/1e-105	322	gil203598569	834/12/55%
Proteasome subunit alpha type-4	P25789	394/2e-109	311	gil171871599	870/19/71%
Proteasome subunit alpha type-7	Q3ZBG0	332/2.00E-89	315	gil121442473	458/10/39%
Proteasome subunit beta type-2	P49721	251/4e-66	326	gil121364267	345/5/25%
Ubiquitin-conjugating enzyme E2 N	P61088	270/6e-72	378, 379	gil121353541	270/5/28%
Dihydropyrimidinase/related protein	Q14117	269/8e-72	161	gil121361409	411/8/39%
Purine nucleoside phosphorylase	P55859	124/1e-28	309	gil121378293	549/5/38%
Replication and transcription					
Histone H4	Q6WV72	166/2e-41	158, 166	gil121377319	258/5/36%
TAR DNA-binding protein 43	Q5R5W2	171/5e-42	206, 210, 275	gil171861979	476/10/37%
DAZ-associated protein 1	Q98SJ2	146/5e-35	276, 277, 278	gil121408319	481/7/36%
Transcriptional activator protein Pur-beta	Q68A21	221/4e-57	240, 241	gil18002677	262/5/21%
Far upstream element-binding protein 1	Q96AE4	32.7/1.9	298	gil203644593	262/4/25%
Putative ATP-dependent RNA helicase R458	Q5UQD1	35.4/0.085	397	gil121331310	292/4/29%
Signaling					
Rab GDP-dissociation inhibitor alpha	P21856	125/1e-28	143, 202, 204	gil121341499	331/7/61%
Rho GDP-dissociation inhibitor 1	P52565	181/3e-45	297	gil171870070	412/9/37%
Voltage-dependent anion-selective channel protein 2	P82013	298/2e-80	242	gil121313789	291/7/45%
	P82013	353/6e-97	282, 285	gil171859816	587/11/49%
Guanine nucleotide-binding protein subunit beta-2-like 1	O42248	407/3e-113	280	gil121321423	587/11/49%
Neural element					
Synaptic vesicle membrane protein VAT-1 homolog-like	Q9HCJ6	266/6e-71	188	gil121438255	545/8/56%
	Q9HCJ6	323/8e-88	189, 191	gil121358164	533/11/50%
Synaptotagmin-1	P41823	329/6e-90	273	gil18002293	260/5/33%
14-3-3 protein epsilon	P62258	273/8e-73	288	gil121311271	465/6/29%
Acetylcholine-binding protein	P58154	139/1e-32	289	gil121313732	352/6/36%
Redox					
Thioredoxin domain-containing protein 4	Q3T0L2	250/9e-66	176	gil121375394	398/7/27%
Probable glutathione transferase	P81124	153/3e-37	283	gil121392222	483/9/62%
Probable glutathione S-transferase 7	P91253	142/1e-33	299, 301	gil203608041	1,053/23/78%
	P91253	126/1e-28	348	gil203594285	241/4/17%
Glutathione S-transferase	O18598	125/2e-28	350	gil121373806	295/7/31%
Glutathione S-transferase Mu 5	Q9Z1B2	209/7e-54	318	gil121399221	879/20/70%
	Q9Z1B2	207/6e-53	328	gil171860291	1063/21/59%
Glutathione-dependent PGD synthetase	O73888	110/9e-24	325	gil203598288	606/13/41%
Catalase	Q9PT92	334/2e-91	174	gil121341576	401/9/48%
Putative uncharacterized oxidoreductase	Q9UT59	64.7/5e-10	293	gil171869268	676/12/65%
Peroxiredoxin-4	Q13162	335/1e-91	312	gil171879655	380/7/36%
Superoxide dismutase	O73872	201/2e-51	372	gil18002128	238/5/43%
Peroxisomal antioxidant enzyme	P99029	177/5e-44	373	gil18001985	178/4/22%
Miscellaneous					
Methyl-accepting chemotaxis protein	P54576	32.3/2.2	119, 337, 339	gil121310487	602/11/54%
	P54576	32.3/2.2	120, 123, 338, 340,	gil121313356	752/14/58%

Table 1 continued

Protein name	Accession number	Blast score/ E-value	Spot number	GenBank gi	Total score/match pept./seq. cov.%
Neural/ectodermal development factor IMP-L2	Q09024	176/8e-44	274	gil121349061	341/5/27%
Ag	AAB17098	347/5.00E-94	295	gil121323050	393/6/42%
	AAB17098	347/5.00E-94	296	gil121368416	688/8/44%
Unknown					
Neighbor of COX4	O43402	164/3e-40	320	gil203657740	283/5/27%
Unknown	Unknown	Unknown	286	gil121422856	411/7/24%
Unknown	Unknown	Unknown	5, 30	gil121469388	147/3/18%

Protein name, accession number and blast score/E-value are from the best BLASTp result of ganglia EST translated peptides

Genbank gi is the matched EST identity in GenBank. Match Pept includes the unique peptides after trypsin and chymotrypsin digestion

Sequence coverage means the number of amino acids observed by the EST translated peptide amino acid length

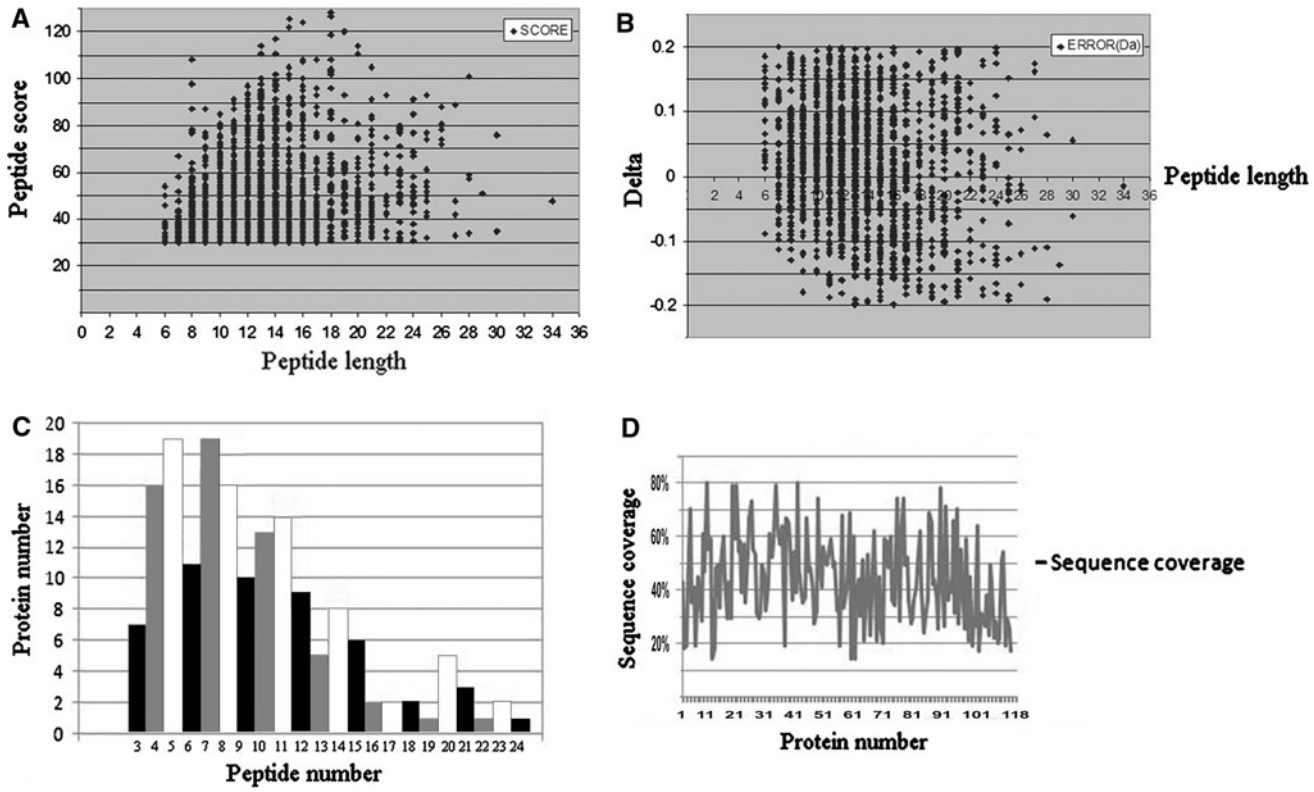

Fig. 3 **a** All peptides showed ion scores higher than 30. **b** All Delta values of peptides are strictly limited between −0.2 and +0.2 Da. **c** The number of peptides matching is ranging from 3 to 24. On average, from each protein spot 9 matching peptides were obtained. **d** Sequence coverage is 14–80%, on average 44%

collagen alpha-2-(IV), collagen alpha-1(XII) and collagen alpha-1-(II) chains, that is clearly different from mammalian neural tissues and may be specific for AC ganglia. As to chaperones, small heat shock protein p36, DnaJ homolog subfamily B member 11, and putative universal stress protein SERP1273, have not been reported before in excitable tissues to the best of our knowledge.

In the panel of metabolism-related proteins isochorismatase domain-containing protein-1 and caffeoyl-CoA O-methyltransferase were observed, proteins that were not described so far in mammalian neural tissues, although isochorismatase was observed in the mouse Harderian gland (Yang et al. 2006). Translocon-associated protein subunit beta protein was reported in chicken brain and is

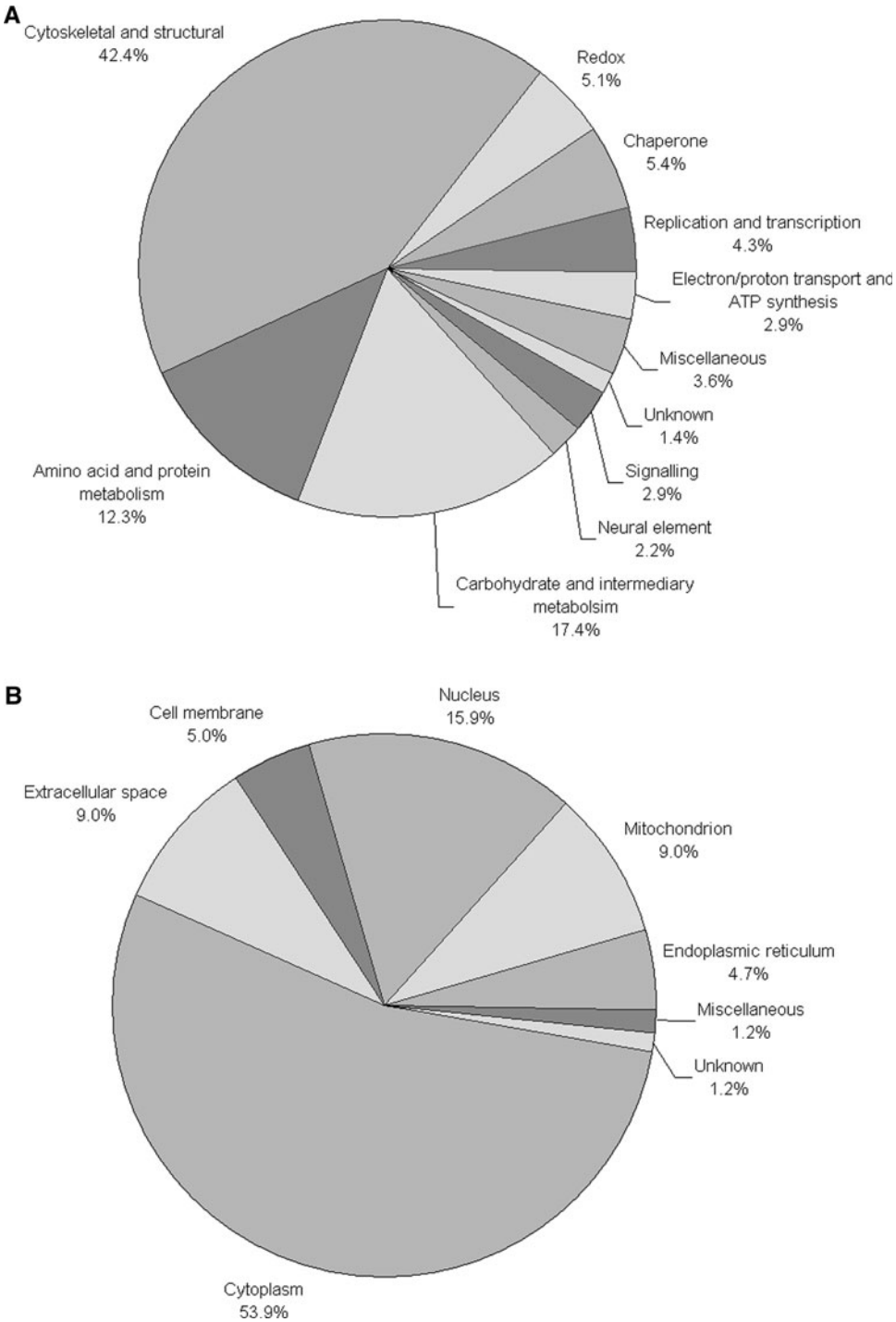

Fig. 4 **a** Proteins identified from aplysia ganglia presented by functional categories. **b** Proteins identified from aplysia ganglia presented by subcellular localization

herein demonstrated as a high abundance protein (Zarkadis et al. 1997).

In the category of replication and transcriptional proteins, DAZ-associated protein is a poorly characterized RNA binding protein and Putative ATP-dependent RNA helicase R458 has not been reported in AC or neural tissues before (Kress et al. 2004).

Acetylcholine-binding protein is a poorly described protein modulating neural transmission (Brejc et al. 2001; Smit et al. 2001) and is herein demonstrated as a high abundance protein of AC.

In the proteins of the redox system, putative uncharacterized oxidoreductase that was so far reported at the mRNA level in schizosaccharomyces pombe only was observed in AC ganglia as a single spot (Wood et al. 2002).

Neural/ectodermal development factor IMP-L2 is implicated in neural development as an essential factor and was reported so far only in drosophila (Garbe et al. 1993).

In the current work it is observed in AC ganglia as a single spot—high abundance protein.

Two proteins were assigned to unknown proteins with unknown function: three spots could be assigned to two ESTs from AC (Table 1) and these proteins are herewith shown to be expressed at the protein level. Based upon limited sequence information no domain analysis could be performed yet.

While this work initiated the generation of the AC proteome, studies are going on in our laboratory to use additional proteases for in-gel digestion (Lubec and Afjehi-Sadat 2007), to use different gel systems to analyze highly hydrophobic and highly insoluble proteins and to search for post-translational modifications (Kang et al. 2009), once highest sequence coverages are obtained and subsequently, de novo sequencing will be carried out.

AC proteins reveal remarkable identity to mammalian proteins and one may therefore expect cross-reactivity of antibodies against mammalian proteins with those from AC proteins that may be useful in comparison of protein cascades and pathways in neuroscience studies, even at the immunochemical level.

In the current study only high abundance proteins (i.e. in our case positive Coomassie blue staining was considered "high abundance") were undergoing analysis which is a limitation of the study but even identification of the high abundance proteins remains a challenge that we have to cope with, before proceeding to low abundance proteins. We are aware of the fact that other than gel-based studies may have been generating more peptides and derived protein data but the gel-based approach we are using is offering further advanced analysis on protein spots, including determination of enzyme activity (Afjehi-Sadat and Lubec 2007) or even conformational and functional studies from spots following electroelution (Chen et al. 2010).

As to quantitative differences between AC cerebral, pleural, pedal and abdominal ganglia, these may reflect functional differences. As an example, acetylcholine-binding protein probably representing cholinergic innervation, showed remarkably higher levels in cerebral than in other ganglia.

In conclusion, we provided a partial AS proteome that will be extended continuously and systematically and fosters neurochemical work with this well-established animal model of learning and memory. Moreover, data herein are considered a seedling of an AC protein MS/MS database that is planned to be established before long. The finding of differences in protein levels between four different ganglia may assist for designing future studies and interpretation of studies from the past.

Acknowledgment We thank Isabella Divisch for her help on quantification.

Conflict of interest The authors herewith declare that there is no financial/commercial conflict of interest.

References

Afjehi-Sadat L, Lubec G (2007) Identification of enzymes and activity from two-dimensional gel electrophoresis. Nat Protoc 2:2318–2324

Ambron RT, Den H, Schacher S (1985) Synaptogenesis by single identified neurons in vitro: contribution of rapidly transported and newly synthesized proteins. J Neurosci 5:2857–2865

Angers A, Storozhuk MV, Duchaine T et al (1998) Cloning and functional expression of an Aplysia 5-HT receptor negatively coupled to adenylate cyclase. J Neurosci 18:5586–5593

Barbas D, Zappulla JP, Angers S et al (2002) Functional characterization of a novel serotonin receptor (5-HTap2) expressed in the CNS of Aplysia californica. J Neurochem 80:335–345

Barbas D, Campbell A, Castellucci VF, DesGroseillers L (2005) Comparative localization of two serotonin receptors and sensorin in the central nervous system of Aplysia californica. J Comp Neurol 490:295–304

Bergold PJ, Sweatt JD, Winicov I et al (1990) Protein synthesis during acquisition of long-term facilitation is needed for the persistent loss of regulatory subunits of the Aplysia cAMP-dependent protein kinase. Proc Natl Acad Sci USA 87:3788–3791

Bergold PJ, Beushausen SA, Sacktor TC et al (1992) A regulatory subunit of the cAMP-dependent protein kinase down-regulated in aplysia sensory neurons during long-term sensitization. Neuron 8:387–397

Bougie JK, Lim T, Farah CA et al (2009) The atypical protein kinase C in Aplysia can form a protein kinase M by cleavage. J Neurochem 109:1129–1143

Brejc K, van Dijk WJ, Klaassen RV et al (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 411:269–276

Carroll M, Warren O, Fan X, Sossin WS (2004) 5-HT stimulates eEF2 dephosphorylation in a rapamycin-sensitive manner in Aplysia neurites. J Neurochem 90:1464–1476

Casadio A, Martin KC, Giustetto M et al (1999) A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. Cell 99:221–237

Chen A, Muzzio IA, Malleret G et al (2003) Inducible enhancement of memory storage and synaptic plasticity in transgenic mice expressing an inhibitor of ATF4 (CREB-2) and C/EBP proteins. Neuron 39:655–669

Chen WQ, Kang SU, Lubec G (2006) Protein profiling by the combination of two independent mass spectrometry techniques. Nat Protoc 1:1446–1452

Chen WQ, Salmazo A, Myllykoski M et al (2010) Purification of recombinant growth hormone by clear native gels for conformational analyses: preservation of conformation and receptor binding. Amino Acids 39:859–869

Cibelli G, Ghirardi M, Onofri F et al (1996) Synapsin-like molecules in Aplysia punctata and Helix pomatia: identification and distribution in the nervous system and during the formation of synaptic contacts in vitro. Eur J Neurosci 8:2530–2543

Collado MS, Khabour O, Fioravante D et al (2009) Post-translational regulation of an Aplysia glutamate transporter during long-term facilitation. J Neurochem 108:176–189

Dash PK, Moore AN (1996) Characterization and phosphorylation of CREB-like proteins in Aplysia central nervous system. Brain Res Mol Brain Res 39:43–51

Dyer JR, Sossin WS, Klein M (1996) Cloning and characterization of aplycalcin and Aplysia neurocalcin, two new members of the calmodulin superfamily of small calcium-binding proteins. J Neurochem 67:932–942

Ezzeddine Y, Glanzman DL (2003) Prolonged habituation of the gill-withdrawal reflex in Aplysia depends on protein synthesis, protein phosphatase activity, and postsynaptic glutamate receptors. J Neurosci 23:9585–9594

Fan X, Qian Y, Fricker LD et al (1999) Cloning and expression of Aplysia carboxypeptidase D, a candidate prohormone-processing enzyme. DNA Cell Biol 18:121–132

Farah CA, Weatherill D, Dunn TW, Sossin WS (2009) PKC differentially translocates during spaced and massed training in Aplysia. J Neurosci 29:10281–10286

Fioravante D, Liu RY, Byrne JH (2008) The ubiquitin-proteasome system is necessary for long-term synaptic depression in Aplysia. J Neurosci 28:10245–10256

Fountoulakis M, Schuller E, Hardmeier R et al (1999) Rat brain proteins: two-dimensional protein database and variations in the expression level. Electrophoresis 20:3572–3579

Fountoulakis M, Juranville JF, Dierssen M, Lubec G (2002) Proteomic analysis of the fetal brain. Proteomics 2:1547–1576

Furukawa Y, Miyawaki Y, Abe G (2006) Molecular cloning and functional characterization of the Aplysia FMRFamide-gated Na+ channel. Pflugers Arch 451:646–656

Garbe JC, Yang E, Fristrom JW (1993) IMP-L2: an essential secreted immunoglobulin family member implicated in neural and ectodermal development in Drosophila. Development 119:1237–1250

Greenberg SM, Castellucci VF, Bayley H, Schwartz JH (1987) A molecular mechanism for long-term sensitization in Aplysia. Nature 329:62–65

Guan X, Clark GA (2006) Essential role of somatic and synaptic protein synthesis and axonal transport in long-term synapse-specific facilitation at distal sensorimotor connections in Aplysia. Biol Bull 210:238–254

Han JH, Lim CS, Lee YS et al (2004) Role of Aplysia cell adhesion molecules during 5-HT-induced long-term functional and structural changes. Learn Mem 11:421–435

Han JH, Lee C, Cheang Y, Kaang BK (2005) Suppression of long-term facilitation by Rab3-effector protein interaction. Brain Res Mol Brain Res 139:13–22

Hansen SB, Talley TT, Radic Z, Taylor P (2004) Structural and ligand recognition characteristics of an acetylcholine-binding protein from Aplysia californica. J Biol Chem 279:24197–24202

Hernandez AI, Wolk J, Hu JY et al (2009) Poly-(ADP-ribose) polymerase-1 is necessary for long-term facilitation in Aplysia. J Neurosci 29:9553–9562

Hislop J, Dyer JR, Scott D et al (2004) Characterization of a novel molluskan tyrosine kinase receptor that inhibits neurite regeneration. J Neurobiol 60:127–136

Houeland G, Nakhost A, Sossin WS, Castellucci VF (2007) PKC modulation of transmitter release by SNAP-25 at sensory-to-motor synapses in aplysia. J Neurophysiol 97:134–143

Jang DH, Han JH, Lee SH et al (2005) Cofilin expression induces cofilin-actin rod formation and disrupts synaptic structure and function in Aplysia synapses. Proc Natl Acad Sci USA 102:16072–16077

Jezzini SH, Moroz LL (2004) Identification and distribution of a two-pore domain potassium channel in the CNS of Aplysia californica. Brain Res Mol Brain Res 127:27–38

Jezzini SH, Reagin S, Kohn AB, Moroz LL (2006) Molecular characterization and expression of a two-pore domain potassium channel in the CNS of Aplysia californica. Brain Res 1094:47–56

Juvvadi S, Fan X, Nagle GT, Fricker LD (1997) Characterization of Aplysia carboxypeptidase E. FEBS Lett 408:195–200

Kang SU, Fuchs K, Sieghart W et al (2009) Gel-based mass spectrometric analysis of a strongly hydrophobic GABAA-receptor subunit containing four transmembrane domains. Nat Protoc 4:1093–1102

Kennedy TE, Kuhl D, Barzilai A et al (1992) Long-term sensitization training in Aplysia leads to an increase in calreticulin, a major presynaptic calcium-binding protein. Neuron 9:1013–1024

Kim H, Lee SH, Han JH et al (2006) A nucleolar protein ApLLP induces ApC/EBP expression required for long-term synaptic facilitation in aplysia neurons. Neuron 49:707–718

Krapfenbauer K, Fountoulakis M, Lubec G (2003) A rat brain protein expression map including cytosolic and enriched mitochondrial and microsomal fractions. Electrophoresis 24:1847–1870

Kress TL, Yoon YJ, Mowry KL (2004) Nuclear RNP complex assembly initiates cytoplasmic RNA localization. J Cell Biol 165:203–211

Kruse R, Sweedler JV (2003) Spatial profiling invertebrate ganglia using MALDI MS. J Am Soc Mass Spectrom 14:752–759

Kuhl D, Kennedy TE, Barzilai A, Kandel ER (1992) Long-term sensitization training in Aplysia leads to an increase in the expression of BiP, the major protein chaperon of the ER. J Cell Biol 119:1069–1076

Langen H, Berndt P, Roder D et al (1999) Two-dimensional map of human brain proteins. Electrophoresis 20:907–916

Lee JA, Lee SH, Lee C et al (2006) PKA-activated ApAF-ApC/EBP heterodimer is a key downstream effector of ApCREB and is necessary and sufficient for the consolidation of long-term facilitation. J Cell Biol 174:827–838

Liu J, Hu JY, Schacher S, Schwartz JH (2004) The two regulatory subunits of aplysia cAMP-dependent protein kinase mediate distinct functions in producing synaptic plasticity. J Neurosci 24:2465–2474

Lubec G, Afjehi-Sadat L (2007) Limitations and pitfalls in protein identification by mass spectrometry. Chem Rev 107:3568–3584

Martin KC, Hu Y, Armitage BA et al (1995) Evidence for synaptotagmin as an inhibitory clamp on synaptic vesicle release in Aplysia neurons. Proc Natl Acad Sci USA 92:11307–11311

Martin KC, Casadio A, Zhu H et al (1997a) Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. Cell 91:927–938

Martin KC, Michael D, Rose JC et al (1997b) MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in Aplysia. Neuron 18:899–912

Moccia R, Chen D, Lyles V et al (2003) An unbiased cDNA library prepared from isolated Aplysia sensory neuron processes is enriched for cytoskeletal and translational mRNAs. J Neurosci 23:9409–9417

Moroz LL, Edwards JR, Puthanveettil SV et al (2006) Neuronal transcriptome of aplysia: neuronal compartments and circuitry. Cell 127:1453–1467

Myung JK, Lubec G (2006) Use of solution-IEF-fractionation leads to separation of 2673 mouse brain proteins including 255 hydrophobic structures. J Proteome Res 5:1267–1275

Park H, Lee JA, Lee C et al (2005) An Aplysia type 4 phosphodiesterase homolog localizes at the presynaptic terminals of Aplysia neuron and regulates synaptic facilitation. J Neurosci 25:9037–9045

Purcell AL, Sharma SK, Bagnall MW et al (2003) Activation of a tyrosine kinase-MAPK cascade enhances the induction of long-term synaptic facilitation and long-term memory in Aplysia. Neuron 37:473–484

Reissner KJ, Boyle HD, Ye X, Carew TJ (2008) Aplysia synapse associated protein (APSAP): identification, characterization, and selective interactions with Shaker-type potassium channels. J Neurochem 105:1006–1018

Schacher S, Castellucci VF, Kandel ER (1988) cAMP evokes long-term facilitation in Aplysia sensory neurons that requires new protein synthesis. Science 240:1667–1669

Sharma SK, Sherff CM, Shobe J et al (2003) Differential role of mitogen-activated protein kinase in three distinct phases of memory for sensitization in Aplysia. J Neurosci 23:3899–3907

Si K, Giustetto M, Etkin A et al (2003) A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in aplysia. Cell 115:893–904

Skehel PA, Armitage BA, Bartsch D et al (1995) Proteins functioning in synaptic transmission at the sensory to motor synapse of Aplysia. Neuropharmacology 34:1379–1385

Smit AB, Syed NI, Schaap D et al (2001) A glia-derived acetylcholine-binding protein that modulates synaptic transmission. Nature 411:261–268

Strumwasser F, Wilson DL (1976) Patterns of proteins synthesized in the R15 neuron of Aplysia. Temporal studies and evidence for processing. J Gen Physiol 67:691–702

Sung YJ, Walters ET, Ambron RT (2004) A neuronal isoform of protein kinase G couples mitogen-activated protein kinase nuclear import to axotomy-induced long-term hyperexcitability in Aplysia sensory neurons. J Neurosci 24:7583–7595

Swanson ME, Elste AM, Greenberg SM et al (1986) Abundant expression of ras proteins in Aplysia neurons. J Cell Biol 103:485–492

Whim MD, Kaczmarek LK (1998) Expression of a foreign G-protein coupled receptor modulates the excitability of the peptidergic bag cell neurons of Aplysia. Neurosci Lett 258:143–146

Wickham L, Zappulla JP, DesGroseillers L (1999) Molecular cloning, sequence analysis and expression distribution of an aminopeptidase in Aplysia california. Comp Biochem Physiol B Biochem Mol Biol 124:429–437

Wilson DL (1971) Molecular weight distribution of proteins synthesized in single, identified neurons of Aplysia. J Gen Physiol 57:26–40

Wood V, Gwilliam R, Rajandream MA et al (2002) The genome sequence of Schizosaccharomyces pombe. Nature 415:871–880

Yang JW, Czech T, Lubec G (2004) Proteomic profiling of human hippocampus. Electrophoresis 25:1169–1174

Yang H, Johnson PM, Ko KC et al (2005a) Cloning, characterization and expression of escapin, a broadly antimicrobial FAD-containing L-amino acid oxidase from ink of the sea hare Aplysia californica. J Exp Biol 208:3609–3622

Yang JW, Rodrigo R, Felipo V, Lubec G (2005b) Proteome analysis of primary neurons and astrocytes from rat cerebellum. J Proteome Res 4:768–788

Yang JW, Afjehi-Sadat L, Gelpi E et al (2006) Proteome profiling in the rat Harderian gland. J Proteome Res 5:1751–1762

Yanow SK, Manseau F, Hislop J et al (1998) Biochemical pathways by which serotonin regulates translation in the nervous system of Aplysia. J Neurochem 70:572–583

Zappulla JP, Angers A, Barbas D et al (2005) A novel actin isoform is expressed in the ovotestis of Aplysia californica. Comp Biochem Physiol B Biochem Mol Biol 140:403–409

Zarkadis I, Vezyri E, Kouvelas E et al (1997) cDNA cloning of the translocon associated protein beta-subunit in the chick cerebellum. Gene 201:1–4

Zhao Y, Wang DO, Martin KC (2009) Preparation of Aplysia sensory-motor neuronal cell cultures. J Vis Exp 28:pii:1355. doi: 10.3791/1355

Zheng JF, Patil SS, Chen WQ et al (2009) Hippocampal protein levels related to spatial memory are different in the Barnes maze and in the multiple T-maze. J Proteome Res 8:4479–4486