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RESEARCH ARTICLE

Proteomics analysis of cerebral cortex in Wistar rats

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Abstract To analyze the protein expression pattern of the cerebral cortex in Wistar rats using the proteomics approach, proteins were separated by two-dimensional gel electrophoresis, stained with Coomassie brilliant blue and digested with trypsin. Then, we analyzed the peptide section using a matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) and identified the protein by indexing special database (SwissProt) according to the finger printing of the peptide quality. Eighty-four protein spots were identified, including metabolic enzymes, skeleton proteins, heat shock proteins, antioxidant proteins, signaling proteins, proteasome related proteins, neuron and glial specific proteins and serum associated proteins. The result of this study enriches the database of the proteome in the cerebral cortex of rats and lays a foundation for further research of neurological disorders in rat models.

Keywords brain proteomics, two-dimensional gel electrophoresis (2-DE), matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS)

1 Introduction

Proteomics, the large-scale analysis of proteins, emerged along with the coming of post-genomic era (Pandey and Mann, 2000). It has developed dramatically in recent years and has been applied extensively in the fields of biology and clinical medicine. Two dimensional electrophoresis (2-DE) combined with biological mass-spectrum, the basic approach of proteome research, has attracted increasing attention in biomedical field (Walter and Weir, 1999).

Rat models are usually used in the research of human neurological disorders. The proteome expression map of rat serum, the whole brain and cerebellum as well as differential display proteomics for comparison of protein levels of rats in different sexes and age have been successfully constructed (Fountoulakis et al., 1999; Taoka et al., 2000; Tsugita et al., 2000). Here we analyzed the protein expression profile in the cerebral cortex of rats using a proteomics approach and established a database of proteome in rat cerebral cortex. This study may lay a foundation for further investigation of pathogenic mechanisms of neurological disorders in rat models.

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2 Materials and methods

2.1 Materials

2.1.1 Equipment

The equipments used in the study included: Universal 16R low-temperature high-speed centrifuge (Hettich, Germany), Beckman L7-80 low-temperature ultracentrifuge (Beckman, Germany), UV-2501PC UV spectrophotometer (Simadzu, Japan), IPGphor electrophoresis unit (Pharmacia Biotech, Sweden), PROTEIN- II ® XI Cell (BIORAD), Image scanner (Pharmacia Biotech, Sweden), ImageMaster 2D. v 3.01 analysis software (Pharmacia Biotech, Sweden), matrix-assisted laser desorption time of flight mass spectrometry (MAL-DI-TOF-MS, Bruker, Germany).

2.1.2 Reagents and materials

IPG gel (pH 3–10; NL, 18 cm), IPG buffer and low molecular mass protein marker were purchased from Amersham Pharmacia Biotech. acrylamide (ACM), methylene bisacrylamide, dithiothreitol (DTT), ammonium persulfate (APS), glycine (GLY) and phenylmethyl sulfonylfluoride (PMSF) were supplied by Sigma. Urea, 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), sodium dodecyl sulphate (SDS) and tetram ethylethylenediamine (TEMED) were provided by AMRESCO. Ethylenediamine tetraacetic acid (EDTA) was obtained

from Bebeo. Coomassie brilliant blue R-250 and Tris-base were purchased from USB. IAA was purchased from Fluka. Thiourea and other chemical agents were all of analytical purity produced in China.

2.2 Methods

2.2.1 Experimental animals

Adult healthy male Wistar rats ($n = 3$, weighing 250–300 g, rating II) were purchased from the Experimental Animal Center of the Chinese Academy of Medical Sciences. After acclimation in the cages for one week, the rats were sacrificed by decapitation. The brain tissue was immediately removed. The cerebral cortex was separated on ice and quickly frozen in liquid nitrogen and then stored at −80°C.

2.2.2 Protein sample preparation

Pooled cerebral cortex from each rat was triturated together with liquid nitrogen and then homogenized in lyses solution (3.5 μL per mg of tissue) consisting of 40 mM Tris, 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM DTT and 1 mM PMSF. The homogenates were centrifuged at 12000 g for 10 min, then at 150000 g for 1 h and 60000 g for 45 min. Following centrifugation, the supernatants were frozen at −80°C until further analysis. Protein concentration was measured according to Bollag and Edelstein (1999).

2.2.3 Two-dimensional gel electrophoresis

The first dimensional immobilized pH gradient isoelectric focusing (IEF) electrophoresis was carried out according to the manufacturer's protocol. Using immobilized IPG gel (pH 3–10; NL, 18 cm), samples were loaded during rehydration and the sample for loading was 100 μg (for silver nitrate) or 1.5 mg (for Coomassie brilliant blue). The parameters were as follows: rehydration at 30 V for 12 h and electrophoresis at 200 – 500 V for 1 h, 500 – 1000 V for 1 h, 1000 – 5000 V for 1 h, and 5000 – 8000 V for 8 h. After IEF, the IPG gels were equilibrated for 15 min in a solution containing 65 mmol/L DTT, 6 mol/L urea, 30% w/v glycerol, 2% w/v SDS and 50 mmol/L Tris-HCl (pH 8.8). A second equilibration step was also carried out for 15 min in the same solution except for DTT, which was replaced by 259 mmol/L iodoacetamide. The spare equilibration solution on the strip was sucked by the filter-paper and then the second-dimensional electrophoresis was carried out in the PROTEAN II ® XI cell with 13% SDS-PAGE gel at a constant current of 20 mA/gel for the initial 40 min and 30 mA/gel for 4 h. The immobile gels were stained with silver nitrate or Coomassie brilliant blue R-250. The digitized pictures were taken by an image scanner and analyzed with Image Master 2 D Elite soft ware.

2.2.4 In-gel tryptic digestion

In-gel tryptic digestion of protein spots were performed according to the method described by Fernandez et al. (1998) with some modifications. The protein spots were cut from the gels, respectively, and cut into small pieces manually. The gel pieces were destained with 50 μL 100 mM NH_4HCO_3: acetonitrile (1:1). The gel pieces were dried in a vacuum centrifuge and rehydrated in 10 μL 50 mM NH_3HCO_3/10 mM $CaCl_2$, containing 4 μL trypsin, for 30 min at 4°C. After the excess solution was removed, the gel was added with 10 μL of 50 mM NH_3HCO_3 / 10 mM $CaCl_2$ and digested at 37°C for 12 h. The supernatant was collected and the peptides were extracted with 5 μL 5% trifluoroacetic acid (TFA), 5 μL 5% TFA/CAN (1:1) and finally with 5 μL CAN, respectively. The combined supernatants were concentrated to 2 μL in a vacuum concentrator and stored frozen until use.

2.2.5 Matrix-assisted laser desorption/ ionization time of flight mass spectrometry

The peptide mixture was reconstituted in 20 μL 0.1% TFA and purified with Zip Tip TM C18 from Millipore to remove salts according to the manufacturer's instructions. Mass spectra were obtained using a matrix-assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF-MS), equipped with a 337 nm nitrogen laser. The instrument was operated in the positive ion reflection mode at 20 kV accelerating voltage and 23 kV reflecting voltage.

Protein identification was performed using the MASCOT software (http://www.matrixscience.com) to compare the monoisotopic m/z values of the peptide mass finger to known proteins in the Swiss-Prot database

3 Results

3.1 2-DE maps of rat cerebral cortex proteome

The proteins in rat cerebral cortex were separated by 2-DE and stained with silver nitrate or Coomassie brilliant blue. About 1000 or 800 protein spots could be detected using the 2-D ImageMaster software in silver or Coomassie stained map (Figs. 1, 2).

3.2 Results of the protein spots

Protein identification was performed using MASCOT software (http://www.matrixscience.com) to compare the monoisotopic m/z values of the peptide mass fingerprint

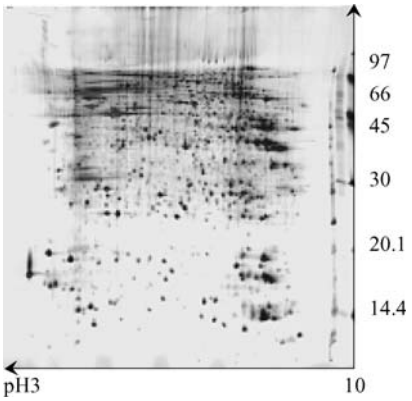


Fig. 1 Silver-stained 2-DE map of rat cerebral cortex proteins (pH 3–10; NL, 18 cm, IPG gel). Range of molecular weight markers represent, from top to bottom, 97, 66, 45, 30, 20.1 and 14.4 (kDa).

to known proteins in the Swiss-Prot database. One hundred and eight protein spots were identified, including 84 proteins. The rate of peptide matching was 11%–67%, which is consistent with other reports (Bienvenut et al., 2002). The typical PMF is shown in Fig. 3 and the detailed information of identified protein is shown in Table 1.

4 Discussion

The etiopathogeneses of central nervous system diseases are complicated and the pathologic mechanism is related to many factors. There are various kinds of phenotypes for one clinical disease. Using proteomics technologies we can get more insight into the disease phenotype. It may also be used to develop new medicines or to find molecular markers for early diagnosis and the monitoring of disease progression. Moreover, it is helpful for new drug design by discovery of molecular targets. Studies of the subcellular fraction and membrane protein proteomics have been reported. It is expected that proteomics research will play a significant role in the diagnosis and monitoring of central nervous system disease, as well as the development of new drugs.

We investigated the proteome profiling of cerebral cortex in Wistar rats using 2-DE and MALDI-TOF-MS technology and identified 108 spots, including 84 proteins. Several spots turned out to be one protein. For example: three spots were identified as ubiquitin C extremity hydrolytic enzyme L1. Two spots were identified as myelin basic protein S. Phosphotriose isomerase was expressed by three spots. Homologous protein of heat shock 71 kD had five spots. We presumed this may be because that the isoelectric point and molecular weight of proteins changed through different modifications such as methylation and phosphorylation, or that proteins have different monomers. The identified proteins, including cytoskeletal protein and mid-metabolic enzymes (Lubec et al., 2003) enrich the database of rat brain proteome and may be helpful for the comparative proteomics research between various cerebropathies and normal brain tissue.

4.1 Cytoskeletal proteins

This group of proteins not only acts as cytoskeleton, but also plays an important role in the process of signal transduction. They included tubulin, myelin basic protein S and endonexin α actin.

4.2 Mid-metabolic enzymes

This group of proteins was mainly high-density protein, including glycolysis, TCA cycle related enzyme, such as 3GADP, AMP kinase isozyme, cytochrome C oxidase and malate dehydrogenase. They could be used to determine the metabolic state of brain cells or brain tissue in different pathologic and physiologic conditions in the research of cerebrosis. Therefore, this group of proteins was important in the study of encephalopathy.

4.3 Antioxidant proteins

Antioxidant proteins play an important role in nervous system diseases and physiological conditions as well. Reactive oxygen species are the products of normal metabolism of histiocytes, including oxygen free radical, hydrogen peroxide, hydroxyl radicals and nitrogen monoxidum. To protect cells from the influence of toxic effects of active oxygen and maintain the normal oxidoreduction potentiality of cells, an anti-oxygen mechanism is developed including enzyme mechanism (superoxide dismutase, hydrogen peroxidase, tathion peroxidase and glutathione S-transferase) and non-enzyme mechanism (vitamin E, coenzyme Q, carotenoid and antiscorbic acid). In this research, we successfully identified superoxide dismutase I, glutathione S-transferase P, glutathione S-transferase Yb-3 and antioxidant proteins 2.

4.4 Heat-shock protein and molecular chaperones

This group of protein has multi-functions, such as the protection of the newly synthesized protein in the ribosome, correction of the wrong folding of protein in pathologic and physiologic conditions, intervention of the right folding and assembly of protein and the transport of mitochondrial protein, regulation of the transduction pathway and transcription/replication and anti-oxidation. Stress-70 protein, mitochondrial, heat shock cognate 71 kDa protein and pre-mitochondrial matrix protein P1 were identified in this research.

4.5 Neuron and glial specific proteins

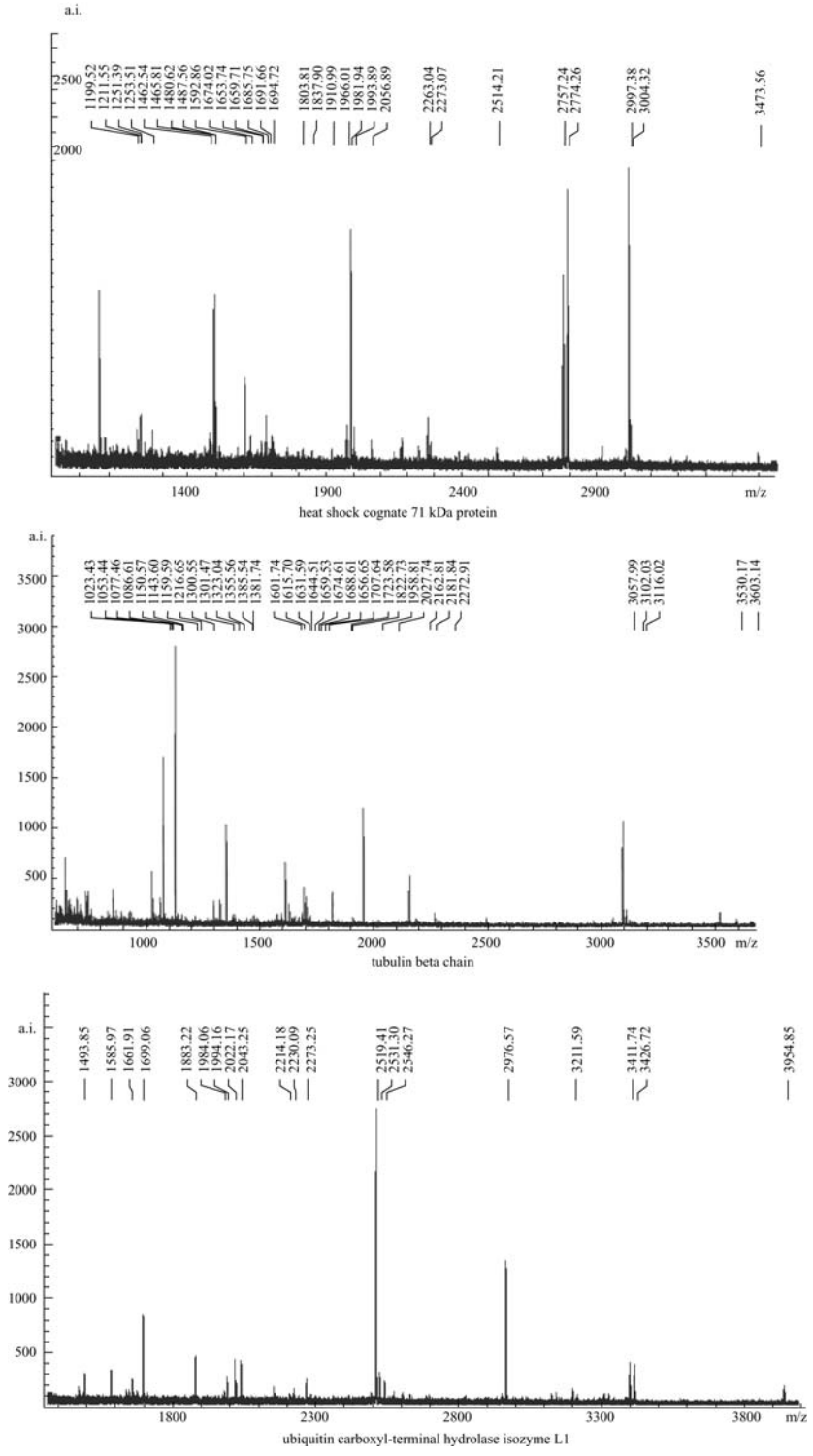
Neuron specific proteins in the brain tissue consist of synaptosome protein and neuron cytoskeletal protein,

Fig. 2 Coomassie blue-stained 2-DE image of rat cerebral cortex proteins. The identified proteins designated by their accession numbers in Swiss-prot (pH 3–10; NL, 18 cm, IPG gel).

such as voltage dependent anion-selective channel protein 1 and α-enolase. Colloid related proteins are the mark of astrocytic cells, such as glial fibrillary acidic protein and carbonate dehydratase II.

4.6 Ubiquitin and proteasomes related proteins

The Ubiquitin pathway is also known as the ubiquitin system or ubiquitin proteasomes pathway. There are four enzyme families: ubiquitin activating enzyme, ubiquitin conjugated enzyme, ubiquitin-protein joining enzyme and de-ubiquitin enzyme. All of them exist in a variety of isomeric forms. The Ubiquitin system is a non-lysosomed protein degradation pathway (DeSalle and Pagano, 2001) and an important cell regulation system. It may be regulated at protein degradation level but not at transcriptional level (Chait and Kent, 1992). The underlying mechanism for the protein degradation may be revealed using a proteomics approach. In this study, we identified ubiquitin carboxyl-terminal hydrolase L1, 26S


a.i.
1199.52
1211.55
1251.39
1253.51
1462.54
1465.81
1480.62
1487.56
1592.86
1674.02
1653.74
1659.71
1685.75
1691.66
1694.72
1803.81
1837.90
1910.99
1966.01
1981.94
1993.89
2056.89
2263.04
2273.07
2514.21
2757.24
2774.26
2997.38
3004.32
3473.56
2500
2000
1400
1900
2400
2900
m/z
heat shock cognate 71 kDa protein
a.i.
1023.43
1053.44
1077.46
1086.61
1150.57
1143.60
1159.59
1216.65
1190.55
1301.47
1323.04
1355.56
1385.54
1381.74
1601.74
1615.70
1644.51
1659.53
1674.61
1688.61
1656.65
1707.64
1723.58
1822.73
1958.81
2027.74
2162.81
2181.84
2272.91
3057.99
3102.03
3116.02
3530.17
3603.14
3500
3000
2500
2000
1500
1000
500
1000
1500
2000
2500
3000
3500
m/z
tubulin beta chain
a.i.
1493.85
1585.97
1661.91
1699.06
1883.22
1984.06
1994.16
2022.17
2043.25
2214.18
2230.09
2273.25
2519.41
2531.30
2546.27
2976.57
3211.59
3411.74
3426.72
3954.85
3000
2500
2000
1500
1000
500
1800
2300
2800
3300
3800
m/z
ubiquitin carboxyl-terminal hydrolase isozyme L1

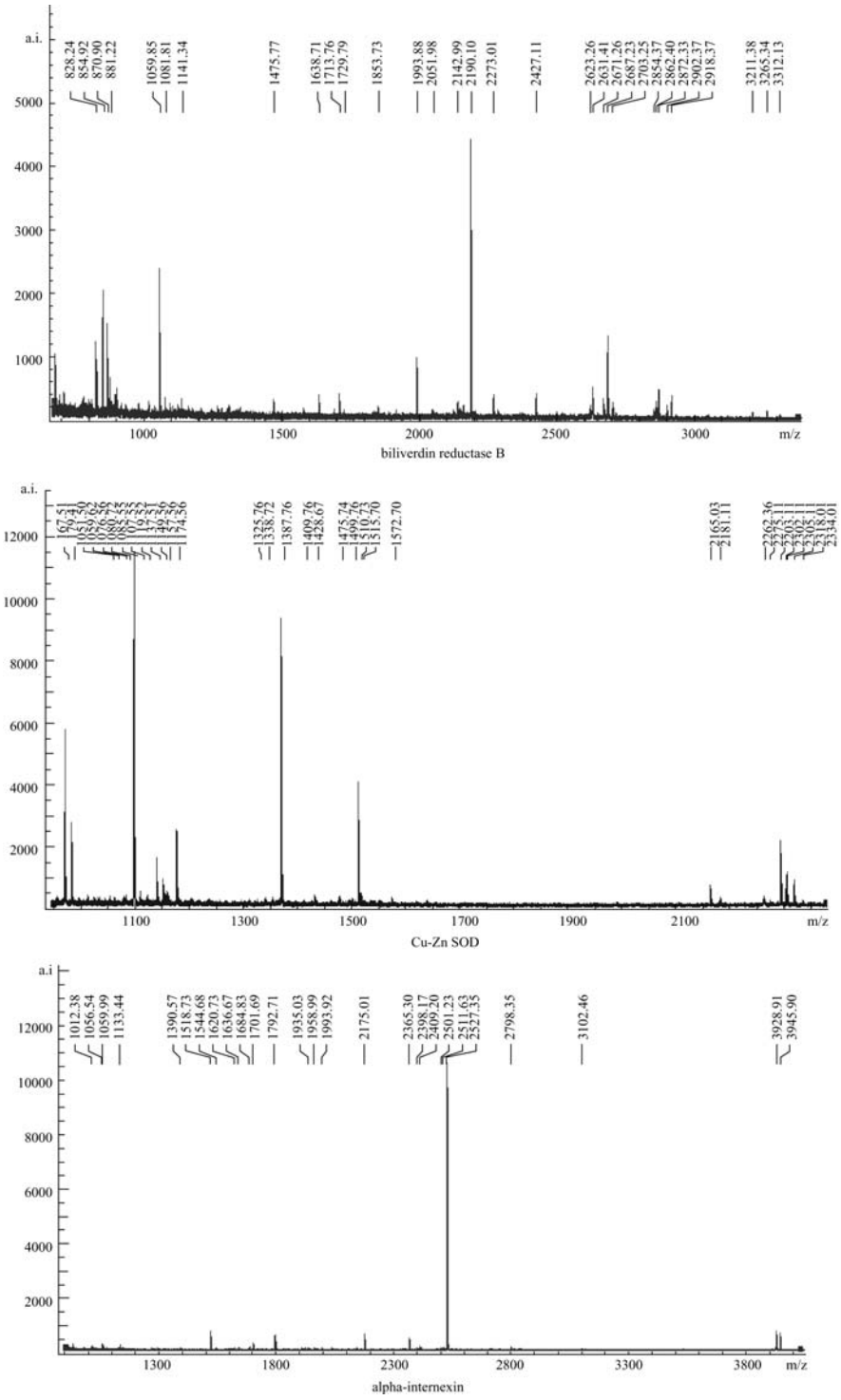


Fig. 3 The typical PMF

proteasome subunit alpha type 8 and ubiquitin protein conjugated enzyme in the cerebral cortex tissue.

4.7 Serum related proteins

This group of proteins include hemoglobin β chain and pre-seralbumin.

4.8 RNA binding protein and transcription, shearing and extension related proteins

Proteins such as heterogeneous nuclear ribonucleoproteins H and the subunit of regulate-associated protein AP-4β1 belong to this group.

Table 1 Rat cerebral cortex proteins identified by MALDI-TOF-MS

	accession no.	protein name	Mr.		cal. PI	peptides		sequence coverage/%	scor.
			theor.	cal.		matching	total		
1	P02688-03-00-00	myelin basic protein S	14000	14071	11.75	9	24	53	91
2	P02688-03-00-00	myelin basic protein S	14000	14184	11.75	8	38	51	94
3	P12075-00-00-00	cytochrome c oxidase polypeptide Vb, mitochondrial	13915	13906	7.68	7	25	35	62
4	P63018	heat shock cognate 71 kDa protein	70871	70827	5.37	14	30	32	108
5	P63018	heat shock cognate 71 kDa protein	70871	70827	5.37	11	28	23	84
6	P63018	heat shock cognate 71 kDa protein	70871	70827	5.37	10	19	21	99
7	P63018	heat shock cognate 71 kDa protein	70871	70827	5.37	12	38	29	82
8	P63018	heat shock cognate 71 kDa protein	70871	70827	5.37	11	22	22	84
9	P48721	stress-70 protein, mitochondrial	73843	73814	5.97	8	18	17	76
10	P50516	vacuolar ATP synthase catalytic subunit A	68268	68225	5.62	16	32	31	84
11	P23565	alpha-internexin	56115	56082	5.20	9	26	28	82
12	O88569	heterogeneous nuclear ribonucleoproteins A2/B1	35993	35971	8.67	15	31	60	156
13	Q9Z2L0	voltage-dependent anion-selective channel protein 1	30624	30606	8.63	10	34	43	95
14	Q9Z2L0	voltage-dependent anion-selective channel protein 1	30624	30606	8.63	8	21	29	80
15	O08550	trithorax homolog 2	31202	31183	9.39	7	20	24	67
16	P07632	superoxide dismutase [Cu-Zn]	15780	15771	5.89	9	32	47	96
17	P39069	adenylate kinase isoenzyme 1	21602	21588	7.71	5	8	24	72
18	P39069	adenylate kinase isoenzyme 1	21602	21588	7.71	10	31	32	98
19	P04906	glutathione S-transferase P	23307	23293	7.30	6	21	43	71
20	P04906	glutathione S-transferase P	23307	23521	7.4	5	14	35	65
21	P19227	60 kDa heat shock protein, mitochondrial	60955	61095	6.0	11	18	28	89
22	P16858	glyceraldehyde-3-phosphate dehydrogenase	35679	35656	8.45	6	13	31	68
23	Q9R0P9	ubiquitin carboxyl-terminal hydrolase isozyme L1	24838	24822	5.14	8	20	60	109
24	P54311	guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 1	37377	37353	5.60	12	32	44	94
25	P54311	guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 1	37377	37353	5.60	9	22	42	90
26	P02094	Hemoglobin beta-major chain	15860	15850	7.33	5	21	34	89
27	P70349	Histidine triad nucleotide-binding protein 1	13646	13637	6.39	9	30	66	69
28	P02600	myosin light chain 1, skeletal muscle isoform	20548	20535	4.99	10	34	67	94
29	P02600	myosin light chain 1, skeletal muscle isoform	20548	20535	4.99	8	29	52	96
30	P02770	serum albumin	68719	70669	6.4	13	25	25	90
31	P14141	carbonic anhydrase III	29300	29282	6.97	12	25	66	120
32	P21670	proteasome subunit alpha type 4	29498	29479	7.59	10	27	58	97
33	P25113	phosphoglycerate mutase 1	28514	28497	6.21	8	40	48	80
34	P25113	phosphoglycerate mutase 1	28514	28497	6.21	13	30	45	93
35	P25113	phosphoglycerate mutase 1	28514	28553	6.7	6	18	29	80
36	P25113	phosphoglycerate mutase 1	28514	28497	6.21	16	46	52	93
37	P20788	ubiquinol-cytochrome c reductase iron-sulfur subunit, mitochondrial	29445	29427	9.04	7	24	18	67
38	Q61553	fascin	54273	54240	6.21	18	41	49	159
39	P04691	tubulin beta chain	49963	49931	4.79	8	14	22	78
40	P04691	tubulin beta chain	49963	49931	4.79	13	35	27	74
41	P19945	60S acidic ribosomal protein P0	34215	34194	5.91	7	28	34	69
42	P47757	F-actin capping protein beta subunit	31214	31195	5.47	11	23	34	101
43	P47757	F-actin capping protein beta subunit	31214	30478	5.69	7	29	44	65
44	O08709	peroxiredoxin 6	24739	24837	5.8	6	18	14	69
45	P04764	alpha enolase	47069	47297	6.5	11	18	29	98
46	P04797	glyceraldehyde-3-phosphate dehydrogenase	35705	35967	8.3	6	18	24	60

(*Continued*)

	accession no.	protein name	Mr.		cal. PI	peptides		sequence coverage/%	scor.
			theor.	cal.		matching	total		
47	P04797	Glyceraldehyde-3-phosphate dehydrogenase	35705	35682	8.44	8	23	31	65
48	P04797	glyceraldehyde-3-phosphate dehydrogenase	35705	35682	8.44	11	33	36	64
49	P04797	glyceraldehyde-3-phosphate dehydrogenase	35705	35682	8.44	8	25	31	68
50	P08009	glutathione S-transferase Yb-3	25549	25704	7.4	5	18	28	68
51	P10111	peptidyl-prolyl cis-trans isomerase A	17743	17959	8.2	7	20	37	79
52	P13668	stathmin	17157	17146	5.9	5	18	27	63
53	P14152	malate dehydrogenase, cytoplasmic	36346	36494	6.5	8	16	26	91
54	P18669	phosphoglycerate mutase 1	28672	28768	7.2	6	18	30	83
55	P19804	nucleoside diphosphate kinase B	17283	17385	7.5	8	18	55	92
56	P21707	synaptotagmin-1	47453	47764	8.4	7	18	19	76
57	P21796	voltage-dependent anion - selective channel protein 1	30641	37463	9.8	14	20	47	79
58	P24142	prohibitin	29820	29858	5.5	9	18	42	87
59	P25388	guanine nucleotide-binding protein beta subunit 2-like 1	35076	35510	7.6	9	18	44	93
60	P31044	phosphatidylethanolamine- binding protein	20670	20902	5.6	5	19	48	86
61	P31399	ATP synthase D chain, mitochondrial	18632	18677	6.6	5	18	31	70
62	P31943	heterogeneous nuclear ribonucleoprotein H	49098	49483	6.3	10	20	34	112
63	P34022	Ran-specific GTPase-activating protein	23596	23738	5.0	6	20	36	110
64	P34064	proteasome subunit alpha type 5	26391	26545	4.6	6	20	28	72
65	P34067	proteasome subunit beta type 4	29197	25891	6.8	5	18	28	71
66	P35704	peroxiredoxin 2	21784	21941	5.3	5	18	35	86
67	P37996	ADP-ribosylation factor-like protein 3	20456	20614	7.3	6	18	45	110
68	P45592	cofilin, non-muscle isoform	18401	18748	8.2	5	18	36	88
69	P48500	triosephosphate isomerase	26790	27285	6.8	7	15	37	70
70	P48500	triosephosphate isomerase	26790	26773	6.51	9	32	53	83
71	P48500	triosephosphate isomerase	26790	26773	6.51	10	29	52	110
72	Q00981	ubiquitin carboxyl-terminal hydrolase isozyme L1	24782	24766	5.12	8	21	41	80
73	Q00981	ubiquitin carboxyl-terminal hydrolase isozyme L1	24782	24766	5.12	10	30	43	82
74	P61089	ubiquitin-conjugating enzyme E2	17138	17183	6.5	6	20	43	78
75	Q61206	platelet-activating factor acetylhydrolase IB beta subunit	25492	25647	6.2	5	18	20	77
76	P37377	alpha-synuclein form 1 and 3	14515	14506	4.6	4	18	41	120
77	Q63228	glia maturation factor beta	16605	16765	5.2	6	19	37	100
78	Q63754	beta-synuclein	14504	14495	4.3	4	18	40	116
79	P60711	actin, cytoplasmic 1	41736	41710	5.29	8	21	26	65
80	P70566	neuronal tropomodulin	39492	39468	5.34	4	5	20	65
81	P70566	neuronal tropomodulin	39492	39468	5.34	14	40	41	93
82	P49432	pyruvate dehydrogenase E1 component beta subunit, mitochondrial [Precursor]	38848	38823	5.94	14	31	45	67
83	P49432	pyruvate dehydrogenase E1 omponent beta subunit, mitochondrial [Precursor]	38848	38823	5.94	14	39	40	65
84	P54311	guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 1	37377	37353	5.60	9	31	30	79
85	P11232	thioredoxin	11542	11876	4.6	6	18	41	87
86	Q9Z1Z2	serine-threonine kinase receptor-associated protein	38513	38489	4.99	10	20	39	114
87	P60901	proteasome subunit alpha type 6	27399	27382	6.34	11	27	33	82
88	P41498-01-00-00	low molecular weight phosphotyrosine protein phosphatase	18020	17795	5.93	5	9	52	65
89	P41498-01-00-00	low molecular weight phosphotyrosine protein phosphatase	18020	17795	5.93	8	13	61	62
90	P19637	tissue-type plasminogen activator [Precursor]	62903	62862	8.55	6	13	20	60

(*Continued*)

	accession no.	protein name	Mr.		cal. PI	peptides		sequence coverage/%	scor.
			theor.	cal.		matching	total		
91	P19234	NADH-ubiquinone oxidore- ductase 24 kDa subunit, mitochondrial [Precursor]	26528	26511	6.00	5	17	29	65
92	P11598	protein disulfide-isomerase A3	56623	56588	5.88	13	29	31	105
93	P11598	protein disulfide-isomerase A3	56623	56588	5.88	15	39	36	109
94	Q9D892	inosine triphosphate pyrophosphatase	21897	21883	5.60	4	9	21	63
95	P62994-00-00-00	growth factor receptor-bound protein 2	25206	25190	5.89	10	29	60	100
96	P12007	isovaleryl-CoA dehydrogenase, mito- chondrial [Precursor]	46435	46406	8.03	11	25	38	79
97	O70467	protein arginine N –methyl- transferase 3	59419	59382	5.05	8	22	16	62
98	P07171	calbindin	29863	30072	4.5	5	18	20	87
99	P68255	14-3-3 protein tau	27778	15447	4.43	5	15	41	108
100	Q05982	nucleoside diphosphate kinase A	17193	17182	5.96	6	17	57	95
101	Q9Z339	glutathione transferase omega 1	27669	27651	6.25	11	33	45	79
102	Q8R5L7	fibroblast growth factor-14	27720	27702	10.06	5	13	34	60
103	Q9WV76	adapter-related protein complex 4 beta 1 subunit	82618	82566	5.87	6	9	11	72
104	Q9CWZ7	gamma-soluble NSF attachment protein	34732	34710	5.31	8	17	28	71
105	Q60641-00-00-00	bile acid receptor	56036	56000	6.42	8	22	28	69
106	P05218	tubulin beta-5 chain	49671	49639	4.78	8	15	25	89
107	P62198	26S protease regulatory subunit 8	45626	45597	7.11	10	18	30	93
108	P40112	proteasome subunit beta type 3	23234	25000	6.5	6	20	35	79

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