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Proteomic analysis of the rat liver

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Abstract

Rat is a useful, widely used animal model for biological and toxicity studies. We analyzed total and cytosolic rat liver proteins by applying proteomics technologies. The proteins were separated by two-dimensional electrophoresis employing broad and narrow range immobilized pH gradient strips, followed by MALDI-MS analysis of the tryptic digests. Two hundred and seventy-three different gene products were identified, of which approximately 60% were enzymes with a broad spectrum of catalytic activities. Most of the identified proteins were detected in other rat protein samples as well, which were analyzed in our laboratory. Fifteen gene products were detected for the first time. These were represented by one spot each, whereas most of the frequently detected proteins were represented by multiple spots. In average, approximately five to 10 spots corresponded to one gene product. The database includes a large number of proteins known to be involved in toxicology-relevant pathways and may be useful in toxicity prediction studies.

Keywords: Proteomics; Two-dimensional database; Proteins, rat liver

1. Introduction

Proteomics is the high-throughput, large-scale, mainly automated analysis of protein mixtures. Proteomic analysis is a useful tool in investigating biological events, as it provides us with significant information about the particular proteome, i.e., which are the abundant gene products and how their levels and modifications change in response to the effect of various internal or external factors, such as diseases, toxic agents and environmental changes. Moreover, it facilitates protein–protein interaction and protein structure studies [1,2]. The sensitivity of the proteomics technologies has been largely improved the last few years and it now allows the detection and mapping of all species of a proteome, which are expressed in sufficient amounts to be detected in a two-dimensional (2-D) gel. Many laboratories, our own included, have undertaken the task to find and map the proteins of various proteomes. Thus, today many 2-D databases include several hundreds of different gene products [3–11]. The 2-D electrophoretic analysis has certain limitations, concerning the detection of hydrophobic proteins and proteins with extreme size and charge values [2,10–12]. Other emerging proteomics technologies not relying on 2-D gels appear to detect a higher number of gene products, but they are compromised by quantification weaknesses [13,14]. However, there is still a large discrepancy between possible and detected gene products in a proteome. To increase the likelihood of detection of low-abundance proteins in complex biological mixtures, a proteomic analysis should be

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directed to simpler protein fractions, each containing a lower number of components in comparison with the starting material. The separation of the protein mixture into organelle fractions prior to the 2-D electrophoretic analysis is usually the first step to increase the probability of detecting low-copy-number gene products [12].

One of the most frequent applications of proteomics is the investigation of toxic events, as it enables the efficient generation of toxicity-related protein patterns, which may be useful in predicting toxicity of drug candidates [15,16]. We have applied proteomics technologies to study changes in the levels of liver proteins of mice treated with acetaminophen [9], of rats treated with carbon tetrachloride, as well as changes of brain proteins of rats treated with the neurotoxin kainic acid, a cyclic analogue of glutamate [17,18]. In all cases, the differential protein expression studies revealed the presence of significant derangements in the levels of a series of protein classes, following administration of the toxic agents. To facilitate the performance of toxicity studies and the investigation of animal models of human diseases, we constructed two-dimensional databases for mouse liver total [9], cytosolic and microsomal proteins [10], as well as for rat brain total proteins [7]. In a previous study, we analyzed the rat liver mitochondrial proteins [19]. Here we performed a large-scale proteomic analysis of total and cytosolic rat liver proteins and identified 273 different gene products.

2. Experimental

2.1. Materials

Immobilized pH-gradient (IPG) strips were purchased from Amersham–Pharmacia Biotechnology (Uppsala, Sweden). Acrylamide was obtained from Serva (Heidelberg, Germany) and the other reagents for the polyacrylamide gel preparation were from Bio-Rad (Hercules, CA, USA). Ampholytes (Resolyte 3.5–10) were purchased from BDH Laboratory Supplies (Poole, UK). CHAPS and thiourea were from Sigma (St. Louis, MO, USA), urea, dithioerythritol and EDTA were obtained from Merck (Darmstadt, Germany). Adult, male Wistar rats were purchased from BRL (Füllingsdorf, Switzerland).

2.2. Sample preparation

Animals were sacrificed using CO_2. Livers from two control animals were flushed through the hepatic vein with a cold NaCl solution to eliminate excessive blood content. For preparation of the total protein extract, liver tissue (1.0 g) was suspended in 10 ml of sample buffer consisting of 20 m*M* Tris, 7 *M* urea, 2 *M* thiourea, 4% CHAPS, 10 m*M* 1,4-dithioerythritol, 1 m*M* EDTA and a mixture of protease inhibitors (1 m*M* PMSF and one tablet complete™ (Boehringer Mannheim) per 50 ml of suspension buffer) and phosphatase inhibitors (0.2 m*M* Na_2VO_3 and 1 m*M* NaF). The suspension was homogenized with the use of a Polytron homogenizer (Kinematica, Luzern, Switzerland) for approximately 1 min, sonicated for 30 s and centrifuged at 150 000 *g* for 45 min. The supernatant contained the total liver proteins solubilized in the IEF-compatible agents.

For the preparation of the cytosolic fraction, liver tissue (1.0 g) was suspended in 10 ml of 20 m*M* Hepes–OH, pH 7.5, containing 250 m*M* sucrose, 1 m*M* EDTA, 5 m*M* dithioerythritol and protease and phosphatase inhibitors as above. The suspension was homogenized with the use of a PTFE/potter homogenizer and centrifuged at 800 *g* for 10 min to remove nuclei and undissolved material. The supernatant was centrifuged at 10 000 *g* for 15 min to separate the mitochondrial proteins. The supernatant of this centrifugation step was centrifuged further at 100 000 *g* for 1 h to separate cytosolic and microsomal proteins. The cytosolic fraction was directly used for isoelectric focusing. The protein concentration was determined using the Coomassie blue method [20] and was approximately 15 mg/ml.

2.3. Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis was performed essentially as reported [21]. Samples of 1.5 mg protein were applied on immobilized pH 3–10 nonlinear and on pH 4–5, 4–7, 5–6 and 5.5–6.7 linear gradient strips, in sample cups at both ends. Each sample was analyzed in triplicate. Focusing started at 200 V and the voltage was gradually increased to 5000 V at 3 V/min and kept constant for a further 24 h (approximately 120 000 kVh totally). The second-dimensional separation was performed in

10% SDS–polyacrylamide gels. The gels (180×200×1.5 mm) were run at 40 mA per gel, in an ISO-DALT apparatus (Hoefer Scientific Instruments, San Francisco, CA), accommodating 10 gels. After protein fixation for 12 h in 40% methanol, containing 5% phosphoric acid, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 24 h. Molecular masses were determined by running standard protein markers (Gibco, Basel, Switzerland), covering the range 10–200 kDa. p*I* values were used as given by the supplier of the IPG strips. Excess of dye was washed out from the gels with H_2O and the gels were scanned in an Agfa DUOSCAN densitometer (resolution 200). Electronic images of the gels were recorded using Photoshop (Adobe) and PowerPoint (Microsoft) software. The images were stored as both tiff (about 5 Mbytes/file) and jpeg (about 50 kbytes/file) formats. Protein spots were outlined first automatically and then manually and quantified using the ImageMaster 2D Elite software (Amersham Biosciences). The percentage of the volume of the spots representing a certain protein was determined in comparison with the total proteins present in the 2-D gel.

2.4. Matrix-assisted laser desorption ionization mass spectroscopy (MALDI-MS)

MALDI-MS analysis was performed as described elsewhere [22] with certain modifications. Spots were automatically excised with a spot picker and placed into 96-well microtiter plates. Each spot was destained with 100 μl of 30% acetonitrile in 50 m*M* ammonium bicarbonate and dried in a speedvac evaporator. Each dried gel piece was rehydrated with 4 μl of 1 m*M* Tris–HCl, pH 9.0, containing 50 ng trypsin (Promega, Madison, WI, USA). After 16 h at room temperature, 7 μl of H_2O were added to each gel piece and the samples were shaken for 10 min. Four μl of 50% acetonitrile, containing 0.3% trifluoroacetic acid and the standard peptides des-Arg-bradykinin (Sigma, 904.4681 Da) and adrenocorticotropic hormone fragment 18–39 (Sigma, 2465.1989 Da), in water were added to each gel piece. The application of the samples was performed with a SymBiot I sample processor (PE Biosystems, Framingham, MA, USA). Of the peptide mixture, 1.5 μl were simultaneously applied with 1 μl of matrix, consisting of a saturated solution of α-cyano-4-hydroxycinnamic acid (Sigma) in 50% acetonitrile, containing 0.1% trifluoroacetic acid. Samples were analyzed in a time-of-flight mass spectrometer (Reflex 3, Bruker Analytics, Bremen, Germany). An accelerating voltage of 20 kV was used. Peptide matching and protein searches were performed automatically with the use of in-house developed software [23]. The peptide masses were compared to the theoretical peptide masses of all available proteins from all species. Monoisotopic masses were used and a mass tolerance of 0.0025% was allowed. The probability of a false-positive match with a given MS spectrum was determined for each analysis [23]. Four matching peptides was the minimal requirement for an identity assignment. Unmatched peptides or miscleavage sites were not considered. The automatically identified proteins were checked individually and only rat proteins or highly homologous counterparts from other species with p*I* and molecular mass values close to the theoretical were considered (a deviation of about 20% was allowed).

3. Results

3.1. Two-dimensional electrophoretic analysis

We prepared mitochondrial, microsomal and cytosomal protein fractions from rat liver tissue of male control animals. Each fraction and the total proteins were analyzed by 2-D electrophoresis and mass spectrometry. The proteomic analysis of the mitochondrial fraction has been described elsewhere [19]. Here we report the proteomic analysis of total and cytosolic proteins. The samples were analyzed on broad and narrow pH range IPG strips and the spots were visualized following stain with colloidal Coomassie blue. Fig. 1 shows a representative analysis of total liver proteins separated on a broad pH range 3–10 and Fig. 2 on a narrow pH range 4–7 2-D gel. Fig. 3 shows the separation of the cytosolic proteins on a broad pH range 3–10 gel. Figs. 4–6 show the separation of the cytosolic proteins on narrow pH range 4–5, 5–6 and 5.5–6.7 IPG strips, respectively. On each gel, 1.5 mg of total protein amount was applied. The use of the narrow pH range strips resulted in the detection of the heterogeneity pattern of certain proteins. For example, contrapsin-like protease inhibitor (P05545) was represented by

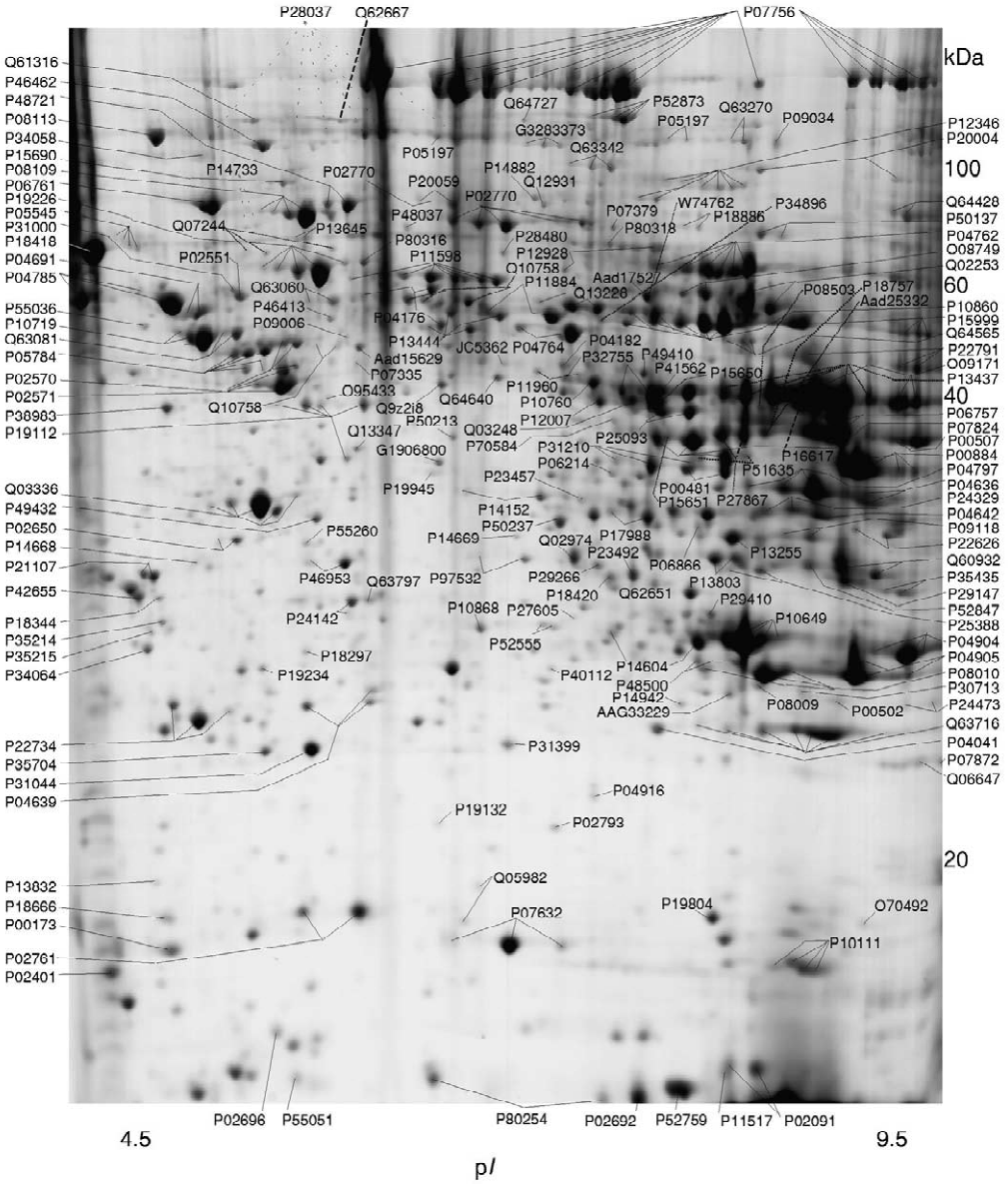

Fig. 1. Two-dimensional electrophoretic analysis of rat liver total proteins. The proteins were separated on a pH 3–10 nonlinear IPG strip, followed by a 10% SDS–polyacrylamide gel, as stated in Section 2. The gel was stained with Coomassie blue. The spots were analyzed by MALDI-MS. The proteins identified are designated with the accession numbers of the corresponding database. The identities assigned are listed in Table 1.

about 20 spots, detected in the narrow pH range 4–5 gel (Fig. 4). The heterogeneity was only partially evident in the 2-D gels prepared with the broad range IPG strips.

The spots representing total (Figs. 1 and 2) and cytosolic (Figs. 3–6) proteins were analyzed by mass spectrometry (see Section 3.2). In total, 273 different gene products were identified from all gels. Of these, 65 gene products were only detected in the gels carrying total, 52 in the gels carrying cytosolic, and the remaining proteins were found in both samples. Moreover, 45 proteins out of the 62 found in the gels

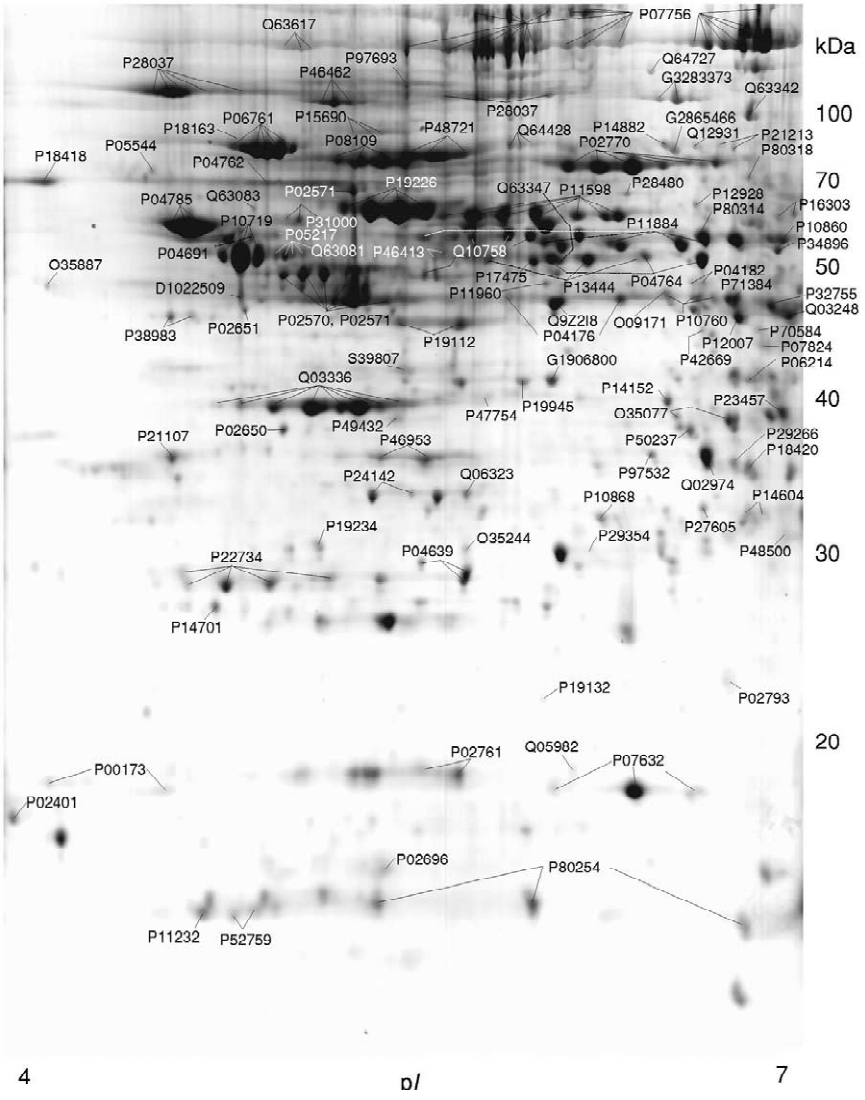

Fig. 2. Two-dimensional electrophoretic analysis of total proteins. The proteins were separated on a pH 4–7 IPG strip, followed by a 10% SDS–polyacrylamide gel. The gel was stained with Coomassie blue. Analysis was performed as stated in the legend to Fig. 1.

carrying total protein samples were detected in the broad pH range 3–10 gel (Fig. 1), 11 in the narrow pH range (Fig. 2) and nine in both types of gels. The 52 proteins only detected in the gels carrying the cytosolic fraction, except for six which were found in the broad pH range 3–10 gel (Fig. 3), were found in one of the narrow pH range gels only (Figs. 4–6). Thus, the narrow pH range strips helped to detect 46 proteins not found in the broad range gels. About 20 of them were detected in the pH 5.5–6.7 gel (Fig. 6). The protein distribution was solely based on the protein identification by mass spectrometry and may not be complete, as the analysis of many spots excised from the various gels may have not resulted in a successful identity assignment on account of technical limitations, such as spot loss during automatic excision, peptide loss mainly from weak spots, spot overlapping and small protein size. Nevertheless, it provides an indication that subcellular fractionation and use of narrow pH range strips can

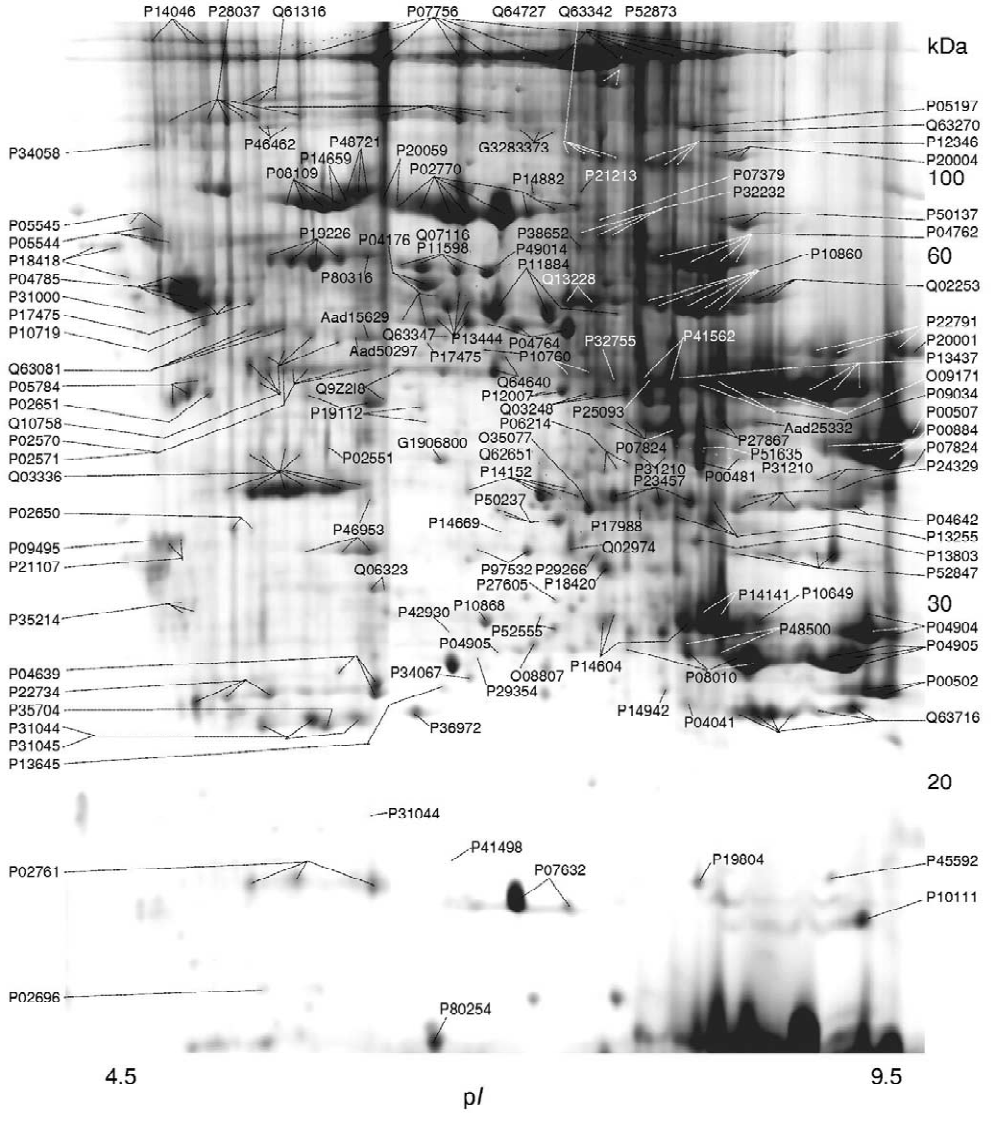

Fig. 3. Two-dimensional electrophoretic analysis of rat liver cytosolic proteins. The proteins were separated on a pH 3–10 nonlinear IPG strip, followed by a 10% SDS–polyacrylamide gel. The gel was stained with Coomassie blue. Analysis was performed as stated in the legend to Fig. 1.

result in the detection of additional gene products not found when the total protein sample is analyzed in broad pH range gels.

3.2. Identity assignment

The proteins were identified by MALDI-MS on the basis of peptide mass matching [24], following in-gel digestion with trypsin. About 5000 spots were excised from 13 2-D gels, five carrying total and eight carrying cytosolic proteins. The total proteins were separated in three broad pH range 3–10 nonlinear and in two pH 4–7 gels. The cytosolic proteins were separated in three broad pH range 3–10 nonlinear, in two pH 4–7 and in one of each pH 4–5, 5–6 and 5.5–6.7 gels. The spots from each gel were selected randomly with the goal to detect as many new gene products as possible. Each excised

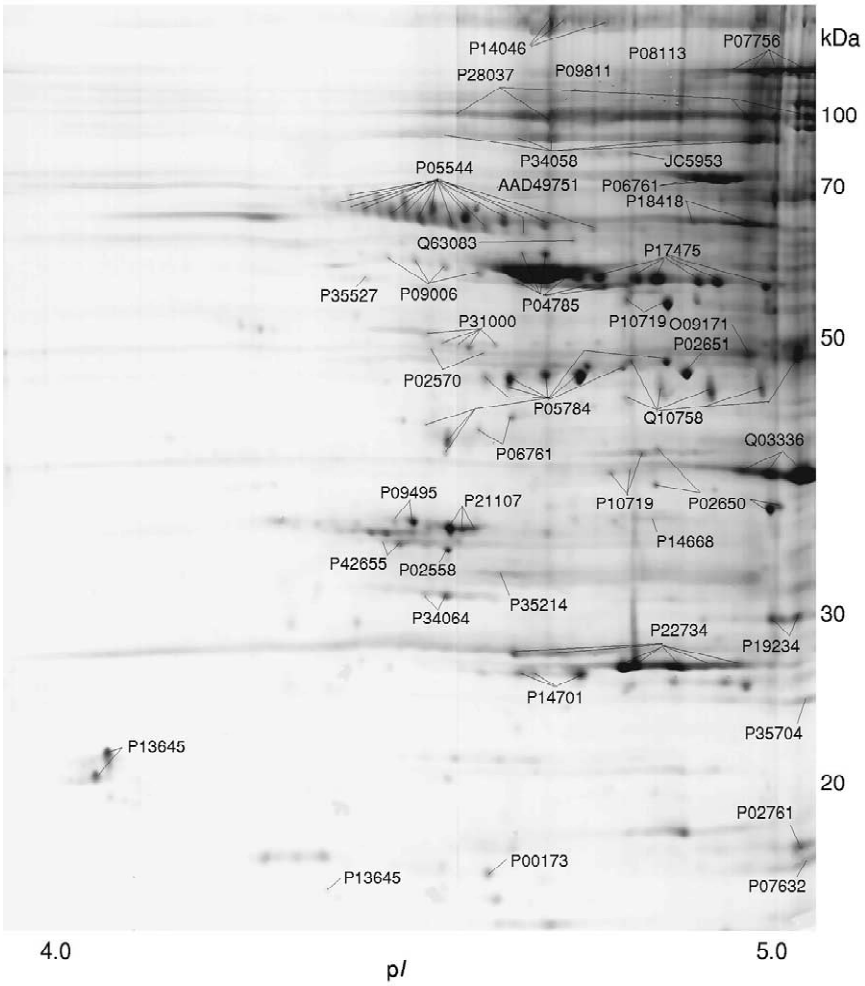

Fig. 4. Two-dimensional electrophoretic analysis of cytosolic proteins, separated on a pH 4–5 IPG strip, followed by a 10% SDS–polyacrylamide gel. Stain was with Coomassie blue.

spot was analyzed individually. The peptide masses were matched with the theoretical peptide masses of all known proteins from all species. The analysis resulted in the identification of about 3000 proteins, which were the products of 273 different genes (Table 1). In Table 1, the theoretical MW and p*I* values of the proteins identified are listed, together with data from the mass spectrometry analysis, i.e., the numbers of matching peptides and the probability of assignment of a random identity.

An identity could be assigned to about 60% of the analyzed spots. The identification was based on four to 21 matching peptides. Proteins of low molecular mass, which deliver few peptides [25], were usually identified with four matches. The average molecular mass of the proteins identified with four matching peptides was 25 kDa and those identified with five matches 32 kDa. In general, the number of matching peptides is related to the molecular mass of the protein analyzed and usually increases with the protein size, as larger proteins carry a higher number of lysine and arginine residues, i.e., more trypsin cleavage sites than their shorter counterparts. This gives rise to a larger number of proteolytic products and consequently the identification relies on a higher number of matching peptides. When the identification was based on seven or more matches, the probability of a wrongly assigned identity was

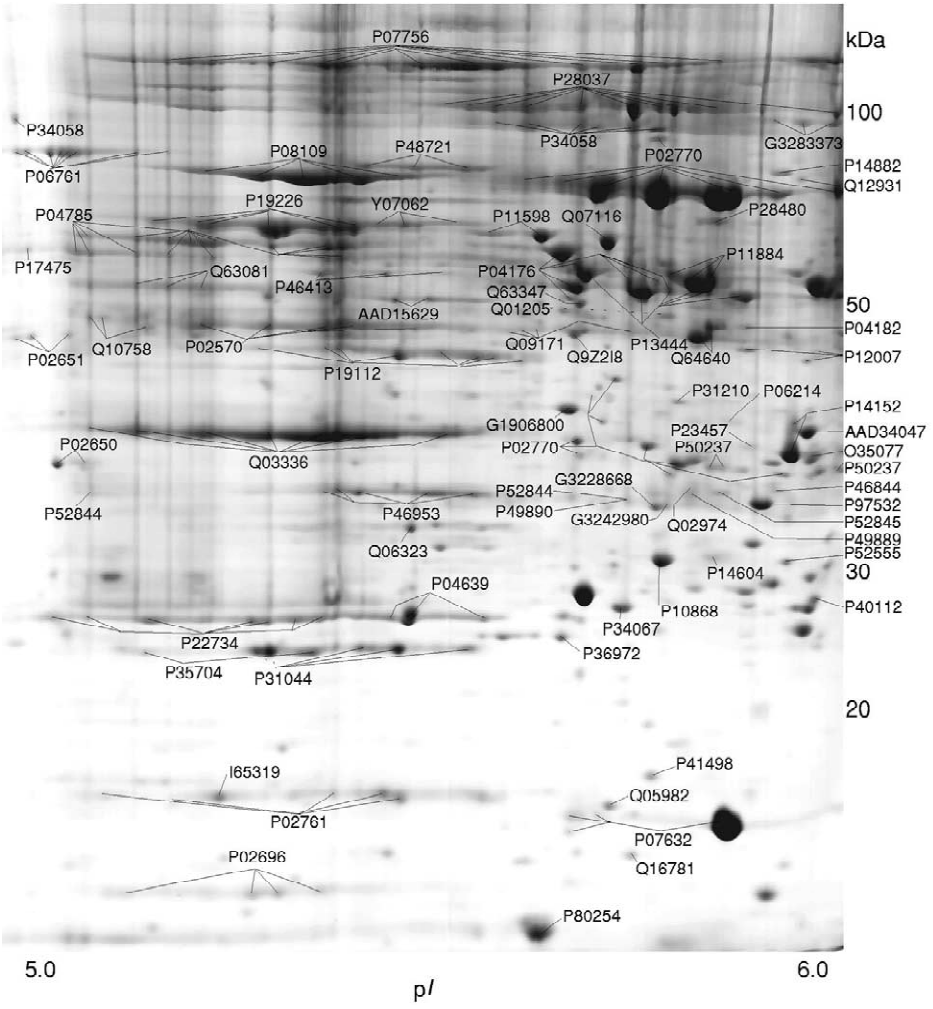

Fig. 5. Two-dimensional electrophoretic analysis of cytosolic proteins, separated on a pH 5–6 IPG strip, followed by a 10% SDS–polyacrylamide gel. The gel was stained with Coomassie blue.

practically zero (Table 1). For about 50% of the unidentified spots, good MS data were collected, but no identity could be assigned. This may be due to poorly defined genes, to insufficient precision in mass determination, or to spot overlapping, which did not allow an unambiguous identity assignment. For about 40% of the not identified spots, the MS data were insufficient for a protein identification. A low number of peptides were found mainly from spots of low intensity. For the remaining 10% of the spots, no MS data could be acquired. The major reasons for the latter were no signal acquisition for one of the standard peptides, very weak spots, which did not deliver a sufficient amount of peptides or peptide losses. No search on residual peptides was performed to detect additional proteins in the mixture.

The theoretical p*I* values of the proteins identified varied between 4 and 9. Twelve proteins had p*I* values between 9 and 10 and two higher than 10 (Fig. 7A). No proteins with p*I*s below 4 were detected (the lower p*I* detection limit was about 3.5). The proteins with theoretical p*I* values higher than 10 were probably represented by multiple spots. The spots representing protein forms with lower p*I* values were most likely those detected within the nominal

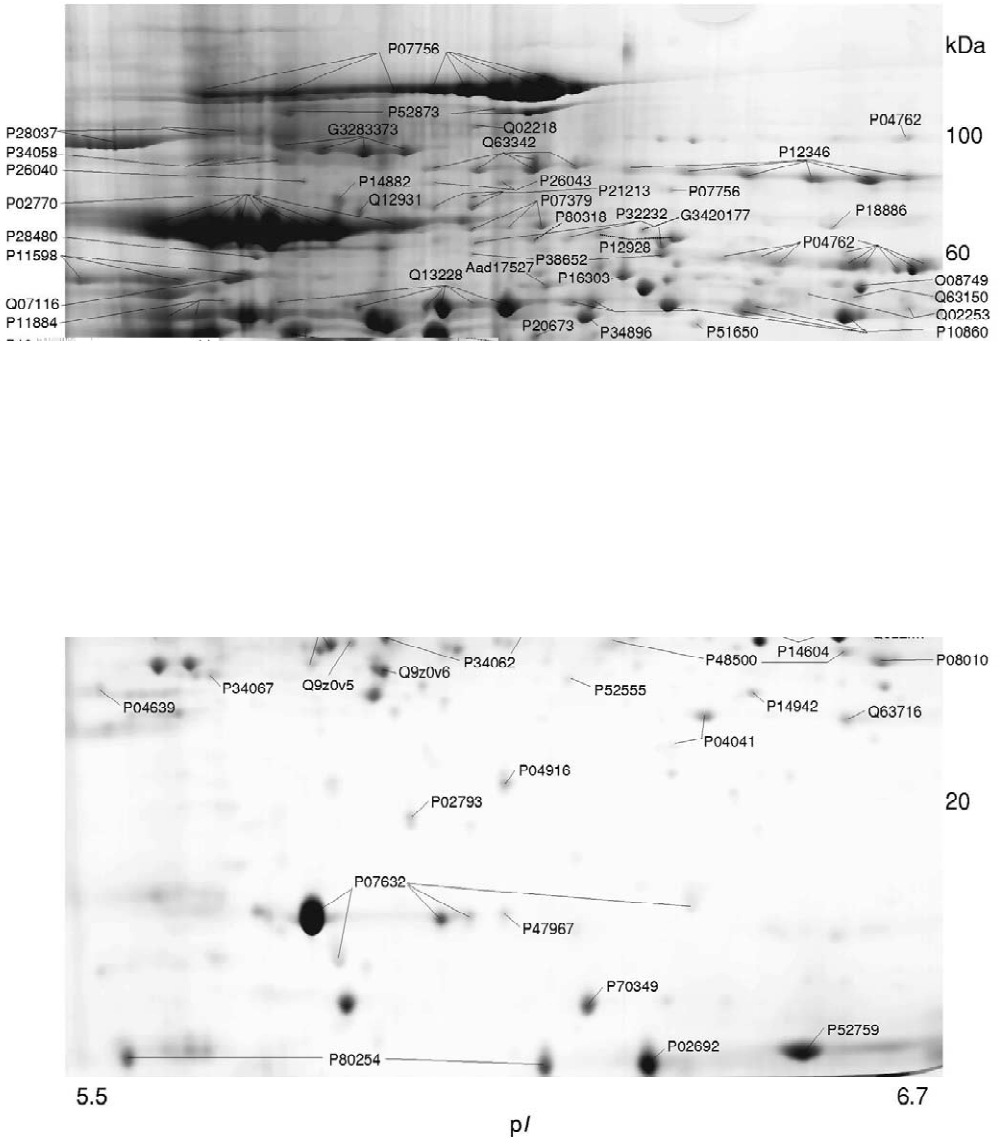

Fig. 6. Two-dimensional electrophoretic analysis of cytosolic proteins, separated on a pH 5.5–6.7 IPG strip, followed by a 10% SDS–polyacrylamide gel.

pH range of the strips. The molecular masses of about 70% of the identified proteins varied between 20 and 60 kDa. No protein smaller than 10 kDa was identified. Nine proteins were larger than 100 kDa (Fig. 7B). In general, low- and high-molecular mass proteins are underrepresented in Table 1. For the small proteins, this is most likely due to limitations of the gels (the lower mass limit of the gels was about 8 kDa) and of the staining methods (small proteins not do efficiently adsorb colored substances) and for the large proteins, to limitations of the IPG strips (large proteins enter the strips less efficiently).

3.3. Protein abundance and function

The relative abundance of the proteins was determined using the ImageMaster 2D Elite software. The sum of the relative volumes of the spots

Table 1
Rat liver proteins

Number	Protein	Full name	Level	Location	Frequency	Spots	GRAVY	TM	pI	MW	Matches	Probability	Figure
AAD15629	NRDBP:NTR_AAD15629	Guanine aminohydrolase (EC 3.5.4.3)	0.059						5.70	51 553	10	3.45E-12	1, 3
AAD17527	NRDBP:NTR_AAD17527	Leucine aminopeptidase	0.290						7.69	56 412	8	4.76E-06	1, 6
AAD25332	NRDBP:NTR_AAD25332	Glycolate oxidase (EC 1.1.3.15)	0.222						7.74	41 260	6	8.70E-05	1, 3
AAD34047	NTREMBL:NTR_AAD34047	AAD34047 CGI-52 protein							8.34	40 920	6	1.00E-05	5, 6
AAD49751	NRDBP:NTR_AAD49751	Ubiquilin							5.03	63 143	8	1.62E-07	4
AAD50297	NRDBP:NTR_AAD50297	Guanine deaminase							5.34	51 493	7	1.34E-05	3
AAG33229	SWTR_RAT:AAG33229	GTPase	0.045						7.53	24 578	5	1.80E-05	1
D1022509	PATCHX:D1022509	p47–*Rattus norvegicus*							4.90	40 655	7	1.26E-06	2
G1906800	PATCHX:G1906800	N^G,N^G-Dimethylarginine dimethylaminohydrolase–*Rattus norvegicus*	0.045						6.07	31 805	8	6.65E-08	1, 2, 3, 5
G2145013	PATCHX:G2145013	Homogentisate 1,2-dioxygenase–*Mus musculus* (house mouse)							7.20	50 755	7	1.00E-06	6
G2443753	PATCHX:G2443753	Pyridoxal kinase–*Rattus norvegicus* (Norway rat)							6.79	35 113	8	2.18E-09	6
G2865466	PATCHX:G2865466	Heat shock protein 75–*Homo sapiens*							6.49	74 199	9	8.88E-10	2
G3228668	PATCHX:G3228668	Nitrilase 1–*Mus musculus* (house mouse)			6	6			7.85	36 423	8	2.61E-06	5
G3237304	PATCHX:G3237304	Pyridoxine 5′-phosphate oxidase–*Rattus norvegicus* (Norway rat)							8.46	30 507	6	1.00E-03	6
G3242980	PATCHX:G3242980	Nitrilase homolog 1–*Mus musculus* (house mouse)			6	6			7.85	36 435	8	2.61E-06	5
G3283373	PATCHX:G3283373	Sarcosine dehydrogenase–*Rattus norvegicus*	0.070						6.60	102 573	17	4.25E-22	1, 2, 3, 5, 6
G3420177	PATCHX:G3420177	WDR protein–*Mus musculus* (house mouse)							6.58	67 049	6	1.00E-04	6
I65319	PIR2:I65319	α-2u-Globulin–rat							5.42	20 992	7	6.79E-07	5
JC5362	PIR2:JC5362	Adenosine kinase (EC 2.7.1.20)–rat	0.020		1	1			6.14	40 415	6	1.71E-04	1
JC5953	PIR3:JC5953	Inter-α-inhibitor H4P heavy chain–Rat							6.49	103 884	9	1.89E-06	4
O08749	SW:DLDH_MOUSE	Dihydrolipoamide dehydrogenase, mitochondrial precursor (EC 1.8.1.4)	0.384	MM	8	8			7.83	54 748	6	4.60E-06	1, 6
O09171	SW:BHMT_RAT	Betaine-homocysteine *S*-methyltransferase (EC 2.1.1.5)	4.300	C	1	1	−0.35	0	7.89	45 403	10	2.57E-14	1, 2, 3, 4, 5
O15144	SW:AR34_HUMAN	ARP2/3 complex 34-kDa subunit (Actin-related protein 2/3 complex subunit 2)			2	4			7.39	34 425	6	1.00E-08	6
O35077	SW:GPDA_RAT	Glycerol-3-phosphate dehydrogenase (NAD+), cytoplasmic (EC 1.1.1.8)		C	22	32	−0.42	0	6.76	37 869	8	7.87E-08	2, 3, 5, 6
O35244	NSW:AOX2_RAT	Antioxidant protein 2 (EC 1.11.1.7)		C					5.78	24 728	9	4.70E-15	2
O35887	NSW:CALU_MOUSE	Calumenin precursor		ERL					4.34	37 154	5	2.60E-06	2
O35952	SW:GLO2_RAT	Hydroxyacylglutathione hydrolase (EC 3.1.2.6) (Round spermatid protein RSP29)			2	3	−0.37	0	6.95	29 162	4	1.00E-05	6
O70492	SW:SNX3_MOUSE	Sorting nexin 3 (sdp3 protein).	0.014		2	2			9.19	18 802	4	1.00E-04	1
O95433	TRE_HUM:O95433	Hypothetical 38.3-kDa protein	0.006						5.33	38 421	6	2.37E-04	1
P00173	SW:CYB5_RAT	Cytochrome b5	0.134	MC	1	1	−0.59	1	4.74	15 214	5	1.77E-08	1, 2, 4
P00481	SW:OTC_RAT	Ornithine carbamoyltransferase precursor (EC 2.1.3.3) (ornithine transcarbamylase)	0.400	MM	27	82	−0.26	0	9.91	39 917	9	7.48E-12	1, 3, 6
P00502	SW:GTA1_RAT	Glutathione *S*-transferase YA (EC 2.5.1.18) (ligandin) (chain 1)	0.026	C	1	2	−0.28	0	9.44	25 577	6	1.00E-06	1, 3
P00507	SW:AATM_RAT	Aspartate aminotransferase (EC 2.6.1.1) (glutamate oxaloacetate transaminase-2)	1.025	MM	14	36	−0.23	0	9.62	47 683	10	2.00E-10	1, 3
P00884	SW:ALFB_RAT	Fructose-bisphosphate aldolase B (EC 4.1.2.13) (liver-type aldolase)	2.863		21	103	−0.27	0	8.43	39 918	9	2.54E-09	1, 3
P02091	SW:HBB1_RAT	Hemoglobin β chain, major-form	0.280		14	100	−0.06	0	8.19	15 952	8	1.55E-11	1
P02401	SW:RLA2_RAT	60S acidic ribosomal protein P2	0.159		3	4	−0.26	0	4.24	11 684	4	2.12E-05	1, 2
P02551	SW:TBA1_RAT	Tubulin α-1 chain	0.105		1	1			4.81	50 787	7	2.66E-07	1, 3

P02558	SW:TPMA_RABIT	α-Tropomyosin 5b							4.51	32 717	6	1.00E-06	4
P02570	SW:ACTB_RAT	Actin, cytoplasmic 1 (β-actin)	1.125	C	4	1			5.24	42 051	8	1.91E-08	1, 2, 3, 4, 5
P02571	SW:ACTG_RAT	Actin, cytoplasmic 2 (γ-actin)		C	6	2			5.26	42 107	8	1.91E-08	1, 2, 3, 4, 5
P02650	SW:APE_RAT	Apolipoprotein E precursor (APO-E)	0.088	E	24	41	−0.71	1	5.06	35 788	8	7.74E-10	1, 2, 3, 4, 5
P02651	SW:APA4_RAT	Apolipoprotein A-IV precursor (apo-aiv)		E	15	24	−0.67	1	4.98	44 428	9	8.12E-12	2, 3, 4, 5
P02692	SW:FABL_RAT	Fatty acid-binding protein, liver (l-fabp) (z-protein) (squalene- and sterol-carrier protein)	0.350	C	1	1	−0.43	0	8.39	14 320	4	7.73E-05	1, 6
P02696	SW:RET1_RAT	Retinol-binding protein I, cellular (crbp)	0.035	C	6	12	−0.62	0	4.97	15 863	7	1.38E-09	1, 2, 3, 5
P02761	SW:MUP_RAT	Major urinary protein precursor (mup) (15.5-kDa fatty acid binding protein)	0.371	C	23	53	−0.25	1	6.15	21 008	10	1.80E-10	1, 2, 3, 4, 5
P02770	SW:ALBU_RAT	Serum albumin precursor	0.712	E	49	625	−0.39	1	6.44	70 669	14	1.11E-19	1, 2, 3, 5, 6
P02793	SW:FRIL_RAT	Ferritin light chain	0.017		20	33	−0.50	0	6.41	20 718	5	9.06E-08	1, 2, 6
P04041	SW:GSHC_RAT	Glutathione peroxidase (EC 1.11.1.9) (gshpx-1) (cellular glutathione peroxidase)	0.100	C	24	35	−0.3	0	7.80	22 472	5	4.11E-06	1, 3, 6
P04176	SW:PH4H_RAT	Phenylalanine-4-hydroxylase (EC 1.14.16.1) (pah) (phe-4-monooxygenase)	0.272		30	188	−0.37	0	6.03	52 302	7	5.51E-08	1, 2, 3, 5
P04182	SW:OAT_RAT	Ornithine aminotransferase (EC 2.6.1.13) (ornithine-oxo-acid aminotransferase)	0.064	MM	16	26	−0.11	0	6.98	48 701	9	8.73E-13	1, 2, 5, 6
P04636	SW:MDHM_RAT	Malate dehydrogenase, mitochondrial precursor (EC 1.1.1.37)	1.153	MM	11	21	0.12	0	8.81	36 088	9	1.73E-14	1, 3
P04639	SW:APA1_RAT	Apolipoprotein A-I precursor (apo-ai)	0.159	E	27	53	−0.72	1	5.55	30 126	7	5.83E-08	1, 2, 3, 5, 6
P04642	SW:LDHM_RAT	L-Lactate dehydrogenase m chain (EC 1.1.1.27)	0.540	C	14	20			8.34	36 712	7	4.71E-08	1, 3
P04691	SW:TBB1_RAT	Tubulin β chain (t β-15)	0.274		20	5	−0.41	0	4.63	50 387	7	3.22E-07	1, 2
P04762	SW:CATA_RAT	Catalase (EC 1.11.1.6)	2.519	P	46	376	−0.64	0	7.51	60 061	16	9.06E-28	1, 2, 3, 6
P04764	SW:ENOA_RAT	α-Enolase (EC 4.2.1.11) (2-phospho-*d*-glycerate hydrolyase)	0.588	C	32	136	−0.21	1	6.54	47 297	11	2.35E-17	1, 2, 3, 6
P04785	SW:PDI_RAT	Protein disulfide isomerase precursor (PDI) (EC 5.3.4.1) (EC 1.14.11.2) (EC 3.8.1.4)	0.812	ERL	30	99	−0.38	1	4.66	57 314	14	1.09E-23	1, 2, 3, 4, 5
P04797	SW:G3P_RAT	Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) (gapdh)	1.691	C	20	41	−0.08	0	8.34	35 967	5	2.52E-06	1
P04904	SW:GTC1_RAT	Glutathione *S*-transferase YC-1 (EC 2.5.1.18) (chain 2) (gst yc1) (gst class-α)	1.328	C	11	17	−0.33	0	9.47	25 229	4	2.68E-05	1, 3
P04905	SW:GTM1_RAT	Glutathione *S*-transferase YB1 (EC 2.5.1.18) (chain 3) (class-mu)	2.027	C	38	129	−0.53	0	8.35	25 937	10	8.40E-16	1, 3, 6
P04916	SW:RETB_RAT	Plasma retinol-binding protein precursor (prbp) (rbp)	0.012		10	11	−0.51	0	5.83	23 547	4	1.67E-05	1, 6
P05197	SW:EF2_RAT	Elongation factor 2 (EF-2)	0.085	C	16	55	−0.21	1	6.81	96 192	8	3.71E-07	1, 3
P05217	SW:TBB2_HUMAN	Tubulin β-2 chain			4	6			4.63	50 255	7	2.24E-06	2
P05544	SW:CPI3_RAT	Contrapsin-like protease inhibitor 3 precursor (cpi-23) (serine protease inhibitor 1)			18	63	−0.20	2	5.46	46 419	13	9.81E-23	2, 3, 4
P05545	SW:CPI1_RAT	Contrapsin-like protease inhibitor precursor (kallikrein-binding protein)	0.060		15	34	−0.16	2	5.21	46 760	9	1.05E-10	1, 3
P05784	SW:K1CR_MOUSE	Keratin, type i cytoskeletal 18 (cytokeratin 18) (cytokeratin endo b) (keratin d)	0.484		10	15			5.08	47 344	7	6.70E-06	1, 3, 4
P06214	SW:HEM2_RAT	δ-Aminolevulinic acid dehydratase (EC 4.2.1.24) (aladh)	0.030		20	54	0.04	0	6.77	36 464	7	4.54E-08	1, 2, 3, 5, 6
P06757	SW:ADHA_RAT	Alcohol dehydrogenase A chain (EC 1.1.1.1)	3.020	C	8	16	0.21	2	8.08	40 400	6	3.50E-05	1
P06761	SW:GR78_RAT	78-kDa glucose regulated protein (immunoglobulin heavy chain binding protein)	0.620	ERL	36	205			4.90	72 473	18	7.08E-32	1, 2, 4, 5
P06866	SW:HPT_RAT	Haptoglobin precursor	0.035	E	11	24	−0.26	1	6.51	39 037	7	9.06E-06	1, 6
P07335	SW:KCRB_RAT	Creatine kinase, b chain (EC 2.7.3.2) (b-ck)	0.010	C	4	8	−0.47	0	5.36	42 970	6	7.92E-06	1

Table 1. Continued

Number	Protein	Full name	Level	Location	Frequency	Spots	GRAVY	TM	pI	MW	Matches	Probability	Figure
P07379	SW:PPCC_RAT	Phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (phosphoenolpyruvate carboxylase)	0.026	C	17	41	−0.31	0	6.46	70 112	8	2.14E-07	1, 3, 6
P07632	SW:SODC_RAT	Superoxide dismutase [Cu–Zn] (EC 1.15.1.1)	0.484	C	23	59	−0.42	0	6.34	15 941	5	8.07E-09	1, 2, 3, 4, 5, 6
P07756	SW:CPSM_RAT	Carbamoyl-phosphate synthase [ammonia] mitochondrial precursor (EC 6.3.4.16)	8.865	M	45	651	−0.12	1	6.75	165 672	20	6.37E-30	1, 2, 3, 4, 5, 6
P07824	SW:ARGI_RAT	Arginase 1 (EC 3.5.3.1) (liver-type arginase)	1.540	C	32	124	−0.20	0	7.28	35 122	9	4.OE-09	1, 2, 3, 6
P07872	SW:CAOP_RAT	Acyl-coenzyme A oxidase, peroxysomal (EC 1.3.3.6) (palmitoyl-CoA oxidase)	0.020	P	14	17			8.71	75 030	7	4.60E-08	1
P08009	SW:GTM3_RAT	Glutathione *S*-transferase YB3 (EC 2.5.1.18) (chain 4) (class-mu)	0.035		6	7	−0.41	0	7.37	25 704	5	1.28E-05	1, 2, 3
P08010	SW:GTM2_RAT	Glutathione *S*-transferase YB2 (EC 2.5.1.18) (chain 4) (class-mu)	1.145	C	39	131	−0.51	0	7.43	25 725	9	4.66E-13	1, 3, 6
P08109	SW:HS7C_RAT	Heat shock cognate 71-kDa protein	0.834		46	228			5.26	71 055	16	6.49E-28	1, 5
P08113	SW:ENPL_MOUSE	Endoplasmin precursor (endoplasmic reticulum protein 99) (erp99)	0.333	ERL	18	71			4.57	92 703	8	5.70E-10	1, 4
P08503	SW:ACDM_RAT	Acyl-CoA dehydrogenase, medium-chain specific precursor (EC 1.3.99.3)	0.100	MM	10	27	−0.29	0	8.52	46 924	7	3.60E-08	1
P09006	SW:CPI6_RAT	Contrapsin-like protease inhibitor 6 precursor (cpi-26) (serine protease inhibitor 3)	0.010		8	20	−0.11	2	5.21	46 793	7	4.17E-05	1, 4
P09034	SW:ASSY_RAT	Argininosuccinate synthase (EC 6.3.4.5) (citrulline-aspartate ligase)	0.054		19	40	−0.36	0	7.83	46 752	6	7.18E-05	1, 3
P09118	SW:URIC_RAT	Uricase (EC 1.7.3.3) (urate oxidase)	0.230	P	13	72	−0.46	0	8.24	35 008	11	2.60E-18	1
P09495	SW:TPM4_RAT	Tropomyosin 4, embryonic fibroblast isoform (tm-4)			5	6	−1.06	0	4.48	28 549	5	6.83E-05	3, 4
P09811	SW:PHS1_RAT	Glycogen phosphorylase, liver form (EC 2.4.1.1)			6	72	−0.33	0	7.20	97 877	11	5.41E-09	4
P10111	SW:CYPH_RAT	Peptidyl-prolyl *cis-trans* isomerase a (EC 5.2.1.8) (ppiase) (rotamase) (cyclophilin a)	0.535	C	13	22	−0.34	0	8.19	17 959	5	6.64E-05	1, 3
P10649	GTM1_MOUSE	Glutathione *S*-transferase gt8.7 (EC 2.5.1.18) (gst 1-1) (class-mu)	3.162	C					8.12	25 936	8	1.00E-09	1, 3
P10719	SW:ATPB_RAT	ATP synthase β chain, mitochondrial precursor (EC 3.6.1.34)	0.919	M	30	137	0.03	0	5.09	56 318	13	7.00E-22	1, 2, 3, 4
P10760	SW:SAHH_RAT	Adenosylhomocysteinase (EC 3.3.1.1) (*S*-adenosyl-l-homocysteine hydrolase)	0.235	C	34	85	−0.05	0	6.51	47 889	9	5.68E-10	1, 2, 3, 6
P10860	SW:DHE3_RAT	Glutamate dehydrogenase precursor (EC 1.4.1.3) (gdh)	2.418	MM	1	1	−0.32	0	8.04	61 731	11	3.04E-16	1, 2, 3, 6
P10868	SW:GAMT_RAT	Guanidinoacetate *N*-methyltransferase (EC 2.1.1.2)	0.065		25	34	−0.20	0	6.04	26 544	5	3.09E-05	1, 2, 3, 5, 6
P11232	SW:THIO_RAT	Thioredoxin			1	1	−0.01	0	4.63	11 876	4	7.13E-05	2
P11517	SW:HBB2_RAT	Hemoglobin β chain, minor-form	0.060		9	38	−0.02	0	9.25	15 955	6	9.62E-07	1
P11598	SW:ER60_RAT	Probable protein disulfide isomerase ER-60 precursor (EC 5.3.4.1) (erp60)	0.890	ERL	29	262			6.14	57 043	16	3.45E-29	1, 2, 3, 5, 6
P11884	SW:DHAM_RAT	Aldehyde dehydrogenase, mitochondrial precursor (EC 1.2.1.3) (class 2)	0.567	MM	38	202	−0.14	0	7.02	56 965	12	2.09E-17	1, 2, 3, 5, 6
P11960	SW:ODBA_RAT	2-Oxoisovalerate dehydrogenase α subunit precursor (EC 1.2.4.4)	0.064	MM	23	59	−0.59	0	7.86	50 418	10	7.31E-12	1, 2, 6
P12007	SW:IVD_RAT	Isovaleryl-CoA dehydrogenase precursor (EC 1.3.99.10)	0.230	MM	19	60	−0.11	0	7.87	46 861	7	3.72E-08	1, 2, 3, 5, 6
P12346	SW:TRFE_RAT	Serotransferrin precursor (siderophilin) (β-1-metal binding globulin)	0.221		32	125	−0.25	1	7.12	78 538	16	3.00E-28	1, 3
P12928	SW:KPYR_RAT	Pyruvate kinase, isozymes r/l (EC 2.7.1.40) (l-pk)	0.136		14	26	−0.004	1	6.90	62 503	9	3.39E-10	1, 2, 6
P13255	SW:GLMT_RAT	Glycine *N*-methyltransferase (EC 2.1.1.20) (folate-binding protein)	0.380	C	24	48	−0.26	0	7.45	32 796	6	8.54E-07	1, 3, 6
P13437	SW:THIM_RAT	3-Ketoacyl-CoA thiolase mitochondrial (EC 2.3.1.16) (acetyl-CoA acyltransferase)	4.311	M	33	170	−0.04	0	7.92	42 243	7	1.57E-07	1, 3, 6
P13444	SW:METL_RAT	*S*-Adenosylmethionine synthetase α and β forms (EC 2.5.1.6)	0.570		35	143	−0.18	0	5.83	44 240	12	1.07E-20	1, 2, 3, 5, 6
P13645	SW:K1CJ_HUMAN	Keratin, type i cytoskeletal 10 (cytokeratin 10) (k10) (ck 10)	0.045		20	39			4.99	59 710	6	4.17E-06	1, 3, 4
P13803	SW:ETFA_RAT	Electron transfer flavoprotein α-subunit precursor (α-etf)	0.440	MM	15	28	0.12	0	8.60	35 239	7	2.62E-10	1, 3, 6
P13832	SW:MLRA_RAT	Myosin regulatory light chain 2-a, smooth muscle isoform (myosin rlc-a)			6	10	−0.81	0	4.49	19 808	4	6.63E-05	1
P14046	SW:A1I3_RAR	α-1-Inhibitor III precursor			7	17	−0.22	1	5.96	165 038	14	1.95E-11	3, 4
P14141	SW:CAH3_RAT	Carbonic anhydrase iii (EC 4.2.1.1) (carbonate dehydratase iii)		C	28	104	−0.55	0	6.99	29 536	7	1.93E-09	3
P14152	SW:MDHC_MOUSE	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37)	0.097	C	12	24			6.54	36 494	5	1.97E-07	1, 2, 3, 5, 6
P14604	SW:ECHM_RAT	Enoyl-CoA hydratase, mitochondrial (EC 4.2.1.17) (short chain enoyl-CoA hydratase)	0.429	MM	22	78	−0.10	0	8.12	31 895	7	3.30E-08	1, 2, 3, 5, 6
P14659	SW:HS72_RAT	Heat shock-related 70-kDa protein 2 (heat shock protein 70.2)			16	31	−0.48	0	5.34	69 770	6	3.16E-06	3
P14668	SW:ANX5_RAT	Annexin V (lipocortin v) (endonexin ii) (placental antiCoAgulant protein i)	0.013		11	23	−0.33	0	4.75	35 648	5	1.23E-05	1, 4

P14669	SW:ANX3_RAT	Annexin III (lipocortin iii) (placental anticoagulant protein iii) (35-α calcimedin)	0.012		21	29	−0.47	0	6.42	36 527	11	3.40E-19	1, 3
P14701	SW:TCTP_MOUSE	Translationally controlled tumor protein (tctp) (21-kDa polypeptide)		C	7	7			4.60	19 563	4	2.04E-04	2, 4
P14733	SW:LAM1_MOUSE	Lamin B1	0.015	N	1	1			4.95	66 940	7	4.23E-05	1
P14882	SW:PCCA_RAT	Propionyl-CoA carboxylase α chain precursor (EC 6.4.1.3)	0.037	MM	25	52	−0.22	0	6.76	78 289	16	2.16E-25	1, 2, 3, 5, 6
P14942	SW:GTA3_RAT	Glutathione *S*-transferase 8 (EC 2.5.1.18) (gst 8-8) (chain 8) (gst class-α)	0.021	C	2	2	−0.17	0	7.47	25 550	4	1.42E-06	1, 3, 6
P15650	SW:ACDL_RAT	Acyl-CoA dehydrogenase, long-chain specific precursor (EC 1.3.99.13)	0.114	MM	14	26	−0.22	1	7.73	48 241	7	5.12E-07	1, 6
P15651	SW:ACDS_RAT	Acyl-CoA dehydrogenase, short-chain (EC 1.3.99.2) (butyryl-CoA dehydrogenase)	0.070	MM	19	52	−0.15	0	8.34	45 022	6	5.30E-06	1, 6
P15690	SW:NUAM_BOVIN	NADH-ubiquinone oxidoreductase 75-kDa subunit (EC 1.6.5.3) (EC 1.6.99.3)	0.060	MIM	9	17			6.06	80 417	6	6.26E-10	1, 2
P15999	SW:ATPA_RAT	ATP synthase α chain, mitochondrial precursor (EC 3.6.1.34) (fragment)	1.224	MIM	15	190	−0.14	0	10.01	58 904	10	6.34E-14	1
P16303	SW:ES10_RAT	Liver carboxylesterase 10 precursor (EC 3.1.1.1) (carboxyesterase es-10)		ERL	9	16	−0.10	0	6.80	62 389	8	2.42E-06	2, 6
P16617	SW:PGK2_RAT	Phosphoglycerate kinase (EC 2.7.2.3), testis specific	0.180		9	9	−0.09	0	7.63	44 794	5	1.09E-05	1
P17475	SW:A1AT_RAT	α-1-Antiproteinase precursor (α-1-antitrypsin) (α-1-proteinase inhibitor)		E	20	24	−0.16	2	6.00	46 277	9	8.54E-12	2, 3, 4, 5
P17988	SW:SUAR_RAT	Aryl sulfotransferase (EC 2.8.2.1) (phenol sulfotransferase) (pst-1) (sulfokinase)	0.270	C	20	36	−0.40	0	6.85	34 169	6	1.21E-04	1, 3, 6
P18163	SW:LCFB_RAT	Long-chain-fatty-acid-CoA ligase, liver isozyme (EC 6.2.1.3)		OMM, P	8	17	−0.08	2	6.95	79 154	4	1.92E-05	2
P18297	SW:SPRE_RAT	Sepiapterin reductase (EC 1.1.1.153) (spr)	0.017	C	7	7	0.05	0	5.46	28 509	9	1.70E-13	1
P18344	SW:TPMZ_RAT	Tropomyosin α chain, brain-3 (tmbr-3)	0.027						4.55	28 383	5	1.00E-04	1
P18418	SW:CRTC_RAT	Calreticulin precursor (crp55) (calregulin) (calcium-binding protein 3)	0.800	ERL	13	28	−1.10	1	4.17	48 136	5	1.31E-04	1, 2, 3, 4
P18420	SW:PRC2_RAT	Proteasome component C2 (EC 3.4.99.46) (macropain subunit C2)	0.069	C, N	15	18			6.60	29 783	7	6.09E-07	1, 2, 3, 6
P18666	SW:MLRB_RAT	Myosin regulatory light chain 2-B, smooth muscle isoform	0.049		5	7	−0.05	0	4.60	19 751	6	1.20E-07	1
P18757	SW:CGL_RAT	Cystathionine γ-lyase (EC 4.4.1.1) (γ-cystathionase)		C	11	15	−0.05	0	7.89	44 261	6	1.70E-08	1
P18886	SW:CPT2_RAT	Mitochondrial carnitine palmitoyltransferase ii precursor (EC 2.3.1.21) (cpt ii)	0.034	MIM	20	27	−0.29	0	7.31	74 633	10	1.00E-13	1, 6
P19112	SW:F16P_RAT	Fructose-1,6-bisphosphatase (EC 3.1.3.11)	0.303		29	102	−0.13	1	5.56	39 909	8	6.74E-11	1, 2, 3, 5, 6
P19132	SW:FRIH_RAT	Ferritin heavy chain	0.003		2	3	−0.81	0	6.29	21 153	5	8.24E-08	1, 2
P19226	SW:P60_MOUSE	Mitochondrial matrix protein p1 precursor (heat shock protein 60) (GROEL protein)	1.028	MM	34	157			6.02	61 088	9	1.26E-10	1, 2, 3, 5
P19234	SW:NUHM_RAT	NADH-ubiquinone dehydrogenase 24-kDa subunit (EC 1.6.5.3) (EC 1.6.99.3)	0.029	MIM	9	13	−0.35	0	6.36	26 853	5	1.73E-04	1, 2, 4
P19804	SW:NDKB_RAT	Nucleoside diphosphate kinase b (EC 2.7.4.6) (ndk b) (ndp kinase b) (p18)	0.152	C, PM	17	20	−0.27	0	7.51	17 385	5	1.34E-06	1, 3
P19945	SW:RLA0_RAT	60S acidic ribosomal protein p0 (l10e)	0.011		9	14	0.05	0	6.18	34 364	7	1.92E-09	1, 2
P20001	SW:EF11_RAT	Elongation factor 1-α 1		ER	8	11			9.72	50 424	6	1.94E-08	3
P20004	SW:ACON_BOVIN	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (citrate hydrolyase)	0.100	M	11	10			7.91	86 045	6	2.25E-04	1, 3
P20059	SW:HEMO_RAT	Hemopexin precursor	0.205	E	20	64	−0.42	1	7.59	51 999	8	4.07E-10	1, 3
P20673	SW:ARLY_RAT	Argininosuccinate lyase (EC 4.3.2.1) (arginosuccinase) (asal)			8	21	−0.19	0	6.38	51 745	7	1.87E-05	6
P21107	SW:TPMI_MOUSE	Tropomyosin 5, cytoskeletal type	0.181		5	7	−1.07	0	4.57	29 230	6	6.62E-06	1, 2, 3, 4
P21213	SW:HUTH_RAT	Histidine ammonia-lyase (EC 4.3.1.3) (histidase)			17	53	−0.09	0	6.48	72 922	13	2.27E-19	2, 3, 6
P22626	SW:ROA2_HUMAN	Heterogeneous nuclear ribonucleoproteins a2/b1 (hnrnp a2 and hnrnp b1)	0.127	N	9	13			9.78	37 463	7	1.24E-10	1
P22734	SW:COMT_RAT	Catechol *O*-methyltransferase, membrane-bound form (EC 2.1.1.6) (mb-comt)	0.460	C, MB	39	142	0.04	1	5.41	29 806	8	1.78E-09	1, 2, 3, 4, 5
P22791	SW:HMCM_RAT	Hydroxymethylglutaryl-CoA synthase, mitochondrial precursor (EC 4.1.3.5)	0.450	M	30	105	−0.36	0	8.98	57 331	7	1.30E-07	1, 3
P23457	SW:DIDH_RAT	3-α-Hydroxysteroid dehydrogenase (EC 1.1.1.50)	0.025	C	34	72	−0.34	0	7.07	37 517	5	2.86E-06	1, 2, 3, 5, 6
P23492	SW:PNPH_MOUSE	Purine nucleoside phosphorylase (EC 2.4.2.1) (inosine phosphorylase)	0.150		4	5			6.12	32 541	4	8.20E-05	1, 6
P24142	SW:PHB_MOUSE	Prohibitin (B-cell receptor associated protein 32) (bap 32)	0.101	C	13	46			5.55	29 858	9	5.04E-13	1, 2
P24329	SW:THTR_RAT	Thiosulfate sulfurtransferase (EC 2.8.1.1) (rhodanese) (fragment)	0.440	MM	18	74	−0.46	0	7.89	33 383	9	1.26E-14	1, 3, 6
P24473	SW:GTK1_RAT	Glutathione *S*-transferase (EC 2.5.1.18) (glutathione *S*-transferase subunit 13)	0.040	MM	2	3	−0.12	0	9.82	25 459	8	4.24E-10	1

Table 1. Continued

Number	Protein	Full name	Level	Location	Frequency	Spots	GRAVY	TM	pI	MW	Matches	Probability	Figure
P25093	SW:FAAA_RAT	Fumarylacetoacetase (EC 3.7.1.2) (fumarylacetoacetate hydrolase)	1.012		25	59	−0.18	0	7.16	46 231	10	7.43E-14	2, 3, 5
P25113	SW:PMGB_RAT	Phosphoglycerate mutase, brain (EC 5.4.2.1) (EC 5.4.2.4) (EC 3.1.3.13)			10	12	−0.50	0	6.65	28 553	4	1.33E-05	6
P25388	SW:GBLP_RAT	Guanine nucleotide-binding protein β subunit-like protein 12.3 (p205)	0.100		7	20			7.64	35 510	7	9.93E-08	1
P26040	SW:EZRI_MOUSE	Ezrin (p81) (cytovillin) (villin-2)		C	1	1			6.00	69 285	9	5.83E-07	6
P26043	SW:RADI_MOUSE	Radixin		AJ	3	3			5.98	68 253	5	2.65E-06	6
P27605	SW:HPRT_RAT	Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8)	0.005	C	17	27	−0.1	0	6.51	24 689	5	1.32E-05	1, 2, 3, 6
P27867	SW:DHSO_RAT	Sorbitol dehydrogenase (EC 1.1.1.14) (l-iditol 2-dehydrogenase)	0.900		20	39	0.03	1	7.22	43 377	7	8.94E-05	1, 3
P28037	SW:FTDH_RAT	10-Formyltetrahydrofolate dehydrogenase (EC 1.5.1.6) (fbp-ci)	0.800	C	31	194	−0.15	0	6.04	99 976	13	3.53E-17	1, 2, 3, 4, 5, 6
P28480	SW:TCPA_RAT	T-Complex protein 1, α subunit (tcp-1-α)	0.035	C	19	22	−0.02	0	6.13	60 834	9	1.70E-08	1, 2, 5, 6
P29147	SW:BDH_RAT	D-β-hydroxybutyrate dehydrogenase precursor (EC 1.1.1.30)	0.218	MM	10	31	−0.23	0	8.99	38 721	8	6.68E-10	1
P29266	SW:D3HI_RAT	3-Hydroxyisobutyrate dehydrogenase precursor (EC 1.1.1.31)	0.058	M	11	14	0.03	0	8.33	36 741	5	2.23E-07	1, 2, 3, 6
P29354	SW:GRB2_HUMAN	Growth factor receptor-bound protein 2 (grb2 adaptor protein)			7	7			6.27	25 304	5	5.74E-06	2, 3
P29410	SW:KAD2_RAT	Adenylate kinase isoenzyme 2 (EC 2.7.4.3) (ATP-AMP transphosphorylase)	0.056	M	11	14	−0.33	0	6.78	26 516	6	1.90E-04	1, 6
P30713	SW:GTT2_RAT	Glutathione S-transferase yrs-yrs (EC 2.5.1.18) (glutathione S-transferase 12)	0.020	C, N	3	5	−0.01	0	7.98	27 461	6	2.29E-04	1
P31000	SW:VIME_RAT	Vimentin	0.031		6	12	−0.85	0	4.89	53 626	11	1.04E-15	1, 2, 3, 4
P31044	SW:PBP_RAT	Phosphatidylethanolamine-binding protein (23-kDa morphine-binding protein)	0.265	C, MB	15	21			5.63	20 902	5	8.90E-06	1, 3, 5
P31210	SW:3O5B_RAT	3-Oxo-5-β-steroid 4-dehydrogenase (EC 1.3.99.6)	0.382	C	41	157			6.61	37 639	10	1.82E-14	1, 3, 5, 6
P31399	SW:ATPQ_RAT	ATP synthase D chain, mitochondrial (EC 3.6.1.34)	0.061		9	16	−0.72	0	6.56	18 677	5	9.10E-07	1
P32232	SW:CBS_RAT	Cystathionine β-synthase (EC 4.2.1.22) (serine sulfhydrase)		C	10	20	−0.22	3	6.45	62 025	7	2.57E-06	3, 6
P32755	SW:HPPD_RAT	4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27) (4hppd) (fragment)	0.630		27	49	−0.5	0	6.76	43 591	7	1.61E-08	1, 3, 6
P34058	SW:HS9B_RAT	Heat shock protein hsp 90-β (hsp 84)	0.020	C	20	72	−0.68	0	4.90	83 475	8	4.36E-08	1, 3, 4, 5, 6
P34062	SW:PRCI_RAT	Proteasome iota chain (EC 3.4.99.46) (multicatalytic endopeptidase complex i chain)		C, N	17	19			6.72	27 837	4	1.71E-04	6
P34064	SW:PRCZ_RAT	Proteasome ζ chain (EC 3.4.99.46) (multicatalytic endopeptidase complex z chain)	0.071	C, N	10	10			4.63	26 545	5	3.40E-06	1, 4
P34067	SW:PRCB_RAT	Proteasome β chain precursor (EC 3.4.99.46) (macropain β chain)		C, N	2	2			6.93	29 349	5	1.00E-04	3, 5, 6
P34896	SW:GLYC_HUMAN	Serine hydroxymethyltransferase, cytosolic (EC 2.1.2.1)	0.023	C	2	2	0.10	0	7.67	53 619	7	3.85E-05	1, 2, 6
P35214	SW:143G_RAT	14-3-3 Protein γ (protein kinase C inhibitor protein-1) (kcip-1)	0.036	C	9	10			4.63	28 324	6	1.35E-06	1, 3, 4
P35215	SW:143Z_RAT	14-3-3 Protein ζ/δ (protein kinase C inhibitor protein-1)	0.026	C	8	11			4.55	27 924	5	5.20E-08	1
P35435	SW:ATPG_RAT	ATP Synthase γ chain, mitochondrial (EC 3.6.1.34)	0.136	M	2	3	−0.22	0	9.61	30 228	6	6.34E-06	1
P35527	SW:K1CI_HUMAN	Keratin, type i cytoskeletal 9 (cytokeratin 9) (k9)			1	1			5.00	62 177	7	8.43E-05	4
P35704	SW:TDX1_RAT	Thioredoxin peroxidase 1 (thioredoxin-dependent peroxide reductase 1)	0.083	C	11	20			5.35	21 941	5	1.00E-04	1, 3, 4, 5
P36972	SW:APT_RAT	Adenine phosphoribosyltransferase (EC 2.4.2.7) (APRT).		C	4	5	0.11	0	6.52	19 761	5	1.00E-04	3, 5
P38652	SW:PGMU_RAT	Phosphoglucomutase (EC 5.4.2.2) (glucose phosphomutase) (pgm)		C	6	16	−0.13	1	6.73	61 518	11	1.37E-14	3, 6
P38983	SW:RSP4_RAT	40S ribosomal protein sa (p40) (34/67-kDa laminin receptor)	0.112	C	16	43	−0.30	0	4.63	32 917	8	6.22E-14	1, 2
P40112	SW:PRCT_RAT	Proteasome theta chain (EC 3.4.99.46) (macropain theta chain)	0.010	C, N	5	5			6.51	23 234	4	2.55E-05	1, 5
P41498	SW:PPAC_RAT	Low-molecular-mass phosphotyrosine protein phosphatase ACP1/ACP2 (EC 3.1.3.48)		C	12	13	−0.48	0	6.48	18 696	7	1.00E-12	3, 5
P41562	SW:IDHC_RAT	Isocitrate dehydrogenase (NADP) (EC 1.1.1.42) (oxalosuccinate decarboxylase)	0.200	C	34	76	−0.04	0	6.99	47 046	6	8.98E-06	1, 3, 6
P42655	SW:143E_RAT	14-3-3 Protein epsilon (mitochondrial import stimulation factor l subunit)	0.098	C	10	12			4.46	29 326	6	1.76E-07	1, 4
P42669	SW:PUR_MOUSE	Transcriptional activator protein pur-α		N	1	1			6.41	34 976	4	2.24E-04	2, 6
P42930	SW:HS27_RAT	Heat shock 27-kDa protein (HSP 27).			9	19	−0.48	0	6.54	22 935	7	1.00E-10	3

P45592	SW:COF1_RAT	Cofilin, non-muscle isoform		C, N	2	2	−0.37	0	8.16	18 748	4	1.00E-04	3
P46413	SW:GSHB_RAT	Glutathione synthetase (EC 6.3.2.3) (glutathione synthase)	0.031		9	14	−0.19	0	5.48	52 597	6	1.85E-05	1, 2, 5
P46462	SW:TERA_RAT	Transitional endoplasmic reticulum ATPase (ter atpase)	0.232		29	81	−0.35	0	4.99	89 976	12	9.52E-15	1, 2, 3
P46844	SW:BIEA_RAT	Biliverdin reductase a precursor (EC 1.3.1.24) (biliverdin-ix α-reductase)		C	5	5	−0.25	0	6.06	33 715	4	6.11E-05	5
P46953	SW:3HAO_RA	3-Hydroxyanthranilate 3,4-dioxygenase (EC 1.13.11.6)	0.175	C	36	88			5.71	32 846	12	7.12E-19	1, 2, 3, 5
P47754	SW:CAZ2_MOUSE	F-Actin capping protein α-2 subunit (capz)			3	3			5.72	33 117	6	5.65E-11	2
P47967	SW:LEG5_RAT	Galectin-5 (rl-18)			2	2	−0.17	0	6.66	16 283	5	8.60E-05	6
P48037	SW:ANX6_RAT	Annexin vi (lipocortin vi) (calphobindin-ii) (calcium-binding protein cata 65/67)	0.026		8	11	−0.43	0	5.31	75 974	10	1.28E-11	1
P48500	SW:TPIS_RAT	Triosephosphate isomerase (ec 5.3.1.1) (tim)	0.134		25	62	−0.12	0	6.84	27 285	7	1.26E-09	1, 2, 3, 6
P48721	SW:GR75_RAT	Mitochondrial stress-70 protein precursor (75-kDa glucose regulated protein) (grp 75)	0.339	M	32	85	−0.48	2	6.22	74 098	13	1.48E-19	1, 2, 3, 5
P49014	SW:PRS4_RAT	26S protease regulatory subunit 4 (p26s4)		C, N	4	6			6.14	49 324	8	5.36E-09	3
P49410	SW:EFTU_BOVIN	Elongation factor tu, mitochondrial precursor	0.074	M	17	26			7.19	49 709	12	1.30E-14	1
P49432	SW:ODPB_RAT	Pyruvate dehydrogenase E1 component, β subunit precursor (EC 1.2.4.1)	0.079	MM	9	14	0.11	0	6.27	39 336	6	1.50E-07	1, 2
P49889	SW:SUO3_RAT	Estrogen sulfotransferase, isoform 3 (EC 2.8.2.4) (sulfotransferase, estrogen-pref.)		C	2	3	−0.55	0	5.61	35 734	6	3.07E-06	5
P49890	SW:SUO6_RAT	Estrogen sulfotransferase, isoform 6 (EC 2.8.2.4) (sulfotransferase, estrogen-pref.)		C	7	22	−0.48	0	5.78	35 621	5	9.71E-05	5
P50137	SW:TKT_RAT	Transketolase (EC 2.2.1.1)	0.148		12	27	−0.13	0	7.45	68 341	5	1.09E-06	1, 3
P50213	SW:IDHA_HUMAN	Isocitrate dehydrogenase (NAD), mitochondrial subunit α precursor (EC 1.1.1.41)	0.041	M	1	1			6.91	40 022	6	1.04E-05	1
P50237	SW:SUAC_RAT	*N*-Hydroxyarylamine sulfotransferase (EC 2.8.2.-) (hast-i)	0.131	C	35	83	−0.65	0	6.53	35 854	9	1.80E-12	1, 2, 3, 5, 6
P50399	SW:GDIB_RAT	Rab gdp dissociation inhibitor β (rab gdi β) (gdi-2)		C, MB	4	6			5.69	51 165	8	2.18E-08	6
P51635	SW:ALDX_RAT	Alcohol dehydrogenase (NADP(+)) (EC 1.1.1.2) (aldehyde reductase)	..09		17	30	−0.30	0	7.33	36 579	6	4.64E-06	1, 3
P51650	SW:SSDH_RAT	Succinate semialdehyde dehydrogenase (EC 1.2.1.24)			1	1	−0.02	0	6.76	52 668	6	8.83E-06	6
P52555	SW:ER29_RAT	Endoplasmic reticulum protein erp29 precursor (erp31)	0.041	ERL	16	33	−0.27	1	6.59	28 613	8	3.04E-11	1, 3, 5, 6
P52759	SW:UK14_RAT	14.5-kDa translational inhibitor protein (perchrolic acid soluble protein)	0.642	C, N	8	18	0.20	0	8.39	14 220	4	1.42E-05	1, 2, 6
P52844	SW:SUO1_RAT	Estrogen sulfotransferase, isoform 1 (EC 2.8.2.4)		C	4	4	−0.55	0	6.00	35 827	5	1.00E-04	5
P52845	SW:SUO2_RAT	Estrogen sulfotransferase, isoform 2 (EC 2.8.2.4)		C	2	2	−0.53	0	5.61	35 683	6	3.07E-06	5
P52847	SW:SUDY_RAT	Dopa/tyrosine sulfotransferase (EC 2.8.1.-)	0.170	C	14	31	−0.46	0	8.21	35 040	6	3.04E-06	1, 3
P52873	SW:PYC_RAT	Pyruvate carboxylase precursor (EC 6.4.1.1) (pyruvic carboxylase)	0.358	MM	31	136	−0.17	0	6.70	130 348	21	4.31E-32	1, 3, 6
P55036	SW:PSD4_HUMAN	26S proteasome regulatory subunit s5a (multiubiquitin chain binding protein)	0.034		3	3			4.52	40 939	6	1.39E-05	1
P55051	SW:FABB_RAT	Fatty acid-binding protein, brain (b-fabp) (brain lipid-binding protein)	0.027	C	2	2	−0.30	0	5.37	15 008	4	6.85E-06	1
P55260	SW:ANX4_RAT	Annexin iv (lipocortin iv) (36-kDa zymogen granule membrane associated protein)	0.019		5	8	−0.44	0	5.16	36 063	7	1.03E-07	1
P70349	SW:IPK1_MOUSE	Hint protein (protein kinase c inhibitor 1) (pkci-1)		C, N	2	2			6.88	13 751	5	9.45E-06	6
P70473	SW:2APE_RAT	2-Arylpropionyl-CoA epimerase (EC 5.-.-.-)		P, M	9	18			6.52	40 025	5	1.74E-06	6
P70584	SW:ACDB_RAT	Acyl-CoA dehydrogenase, short/branched chain specific precursor (EC 1.3.99.-)	0.026	MM	11	20	−0.13	0	8.06	48 249	8	3.50E-11	1, 2
P80254	SW:DOPD_RAT	D-Dopachrome tautomerase	0.150	C	8	15	0.03	0	6.52	13 107	5	6.72E-08	1, 2, 3, 5, 6

Table 1. Continued

Number	Protein	Full name	Level	Location	Frequency	Spots	GRAVY	TM	*p*I	MW	Matches	Probability	Figure
P80314	SW:TCPB_MOUSE	T-Complex protein 1, β subunit (tcp-1-β) (cct-β)		C	3	6			6.38	57 753	6	2.23E-05	2
P80316	SW:TCPG_MOUSE	T-Complex protein 1, ϵ subunit (tcp-1-ϵ (cct-ϵ)	0.071	C	8	8			5.91	60 042	7	1.37E-05	1, 3
P80318	SW:TCPG_MOUSE	T-Complex protein 1, γ subunit (tcp-1-γ) (cct-γ) (matricin)	0.030	C					6.68	61 161	10	1.60E-09	1, 2, 6
P97532	SW:THTM_RAT	3-Mercaptopyruvate sulfurtransferase (EC 2.8.1.2) (mst)	0.055	C, M	35	66	−0.30	1	6.29	33 073	10	2.67E-17	1, 2, 3, 5, 6
P97693	TRE_ROD:P97693	P105 coactivator			7	5			6.74	100 011	6	2.23E-04	2
Q01205	SW:ODO2_RAT	Dihydrolipoamide succinyltransferase of 2-oxoglutar. dehydrog. complex (EC 2.3.1.61)		M	8	9	−0.13	0	8.08	47 667	5	2.79E-05	5
Q02218	SW:ODO1_HUMAN	2-Oxoglutarate dehydrogenase E1 component precursor (EC 1.2.4.2)		MM	2	3			7.06	11 4600	6	4.38E-09	6
Q02253	SW:MMSA_RAT	Methylmalonate-semialdehyde dehydrogenase precursor (acylating) (EC 1.2.1.27)	1.308	M	34	151	−0.05	0	8.24	58 226	12	4.73E-17	1, 3
Q02974	SW:KHK_RAT	Ketohexokinase (EC 2.7.1.3) (hepatic fructokinase)	0.153		13	22	−0.17	1	6.64	33 298	7	5.68E-09	1, 2, 3, 5, 6
Q03248	SW:BUP_RAT	β-Ureidopropionase (EC 3.5.1.6) (*N*-carbamoyl-β-alanine amidohydrolase)	0.200	C	31	68	−0.34	0	6.92	44 584	8	6.84E-11	1, 2, 3, 6
Q03336	SW:SM30_RAT	Senescence marker protein-30 (smp-30) (regucalcin) (rc)	0.967	C	36	151	−0.31	0	5.32	33 937	10	3.38E-16	1, 2, 3, 4, 5
Q05982	SW:NDKA_RAT	Nucleoside diphosphate kinase a (EC 2.7.4.6) (ndk a)	0.017	C, N	11	15	−0.24	0	6.29	17 295	4	1.10E-04	1, 2, 5
Q06323	SW:IGUP_HUMAN	Interferon γ up-regulated I-5111 protein precursor (igup i-5111)							5.90	28 876	8	1.99E-09	2, 3, 5
Q06647	SW:ATPO_RAT	ATP synthase oligomycin sensitivity conferral protein mitochondrial (EC 3.6.1.34)	0.038	MM	3	6	−0.02	0	10.84	23 439	7	1.93E-09	1
Q07116	SW:SUOX_RAT	Sulfite oxidase precursor (EC 1.8.3.1)		M	20	31	−0.41	0	6.19	54 605	13	1.09E-22	3, 5, 6
Q07244	SW:ROK_HUMAN	Heterogeneous nuclear ribonucleoprotein k (hnrnp k) (dc-stretch binding protein)	0.046	C, N	4	7			5.28	51 229	6	5.61E-07	1
Q10758	SW:K2C8_RAT	Keratin, type ii cytoskeletal 8 (cytokeratin 8) (cytokeratin endo a)	0.230		32	138	−0.62	0	5.83	53 854	12	5.80E-18	1, 2, 3, 4, 5
Q12931	SW:TRA1_HUMAN	Tumor necrosis factor type 1 receptor associated protein (trap-1) (fragment)	0.029	M	4	23			8.34	75 694	10	3.92E-12	1, 5, 6
Q13228	SW:SBP1_HUMAN	Selenium-binding protein 1	0.581	C	3	3			6.57	52 906	8	7.42E-07	1, 3, 6
Q13347	SW:IF34_HUMAN	Eukaryotic translation initiation factor 3 δ (tgf-b receptor interacting protein 1)	0.033		7	11			5.45	36 877	5	8.31E-07	1
Q16781	SW:UBCC_HUMAN	Ubiquitin-conjugating enzyme E2-17-kDa (EC 6.3.2.19) (ubiquitin carrier protein)			2	2			6.55	17 183	6	1.30E-04	5
Q60932	SW:POR1_MOUSE	Voltage-dependent anion-selective channel protein 1 (vdac1)	1.560	OMM	11	38			9.04	30 719	7	1.36E-11	1
Q61316	SW:HS74_MOUSE	Heat shock 70-related protein apg-2	0.010	C	7	9			5.00	94 871	8	8.54E-06	1, 3
Q62651	SW:ECH1_RAT	Probable peroxisomal enoyl-CoA hydratase (EC 4.2.1.17)		M, P	18	29	−0.09	1	7.98	36 520	9	9.60E-10	1, 3, 6
Q62667	SW:MVP_RAT	Major vault protein (mvp)	0.065	C	7	15	−0.33	2	5.78	98 956	6	1.24E-05	1
Q63060	SW:GLPK_RAT	Glycerol kinase (EC 2.7.1.30) (ATP:glycerol 3-phosphotransferase)	0.100	C, M	9	13	−0.03	0	5.48	58 238	7	2.49E-04	1
Q63081	SW:ERP5_RAT	Probable protein disulfide isomerase p5 precursor (EC 5.3.4.1)	0.173	ERL	16	45			4.79	47 589	8	7.94E-12	1, 2, 3, 5
Q63083	SW:NUBN_RAT	Nucleobindin precursor (nucb1) (bone 63-kDa calcium-binding protein)		C, MB	7	8			4.90	53 473	9	7.52E-11	2, 4
Q63150	SW:DPYS_RAT	Dihydropyrimidinase (EC 3.5.2.2) (dhpase) (hydantoinase) (dhp)			9	9	−0.25	0	7.19	57 309	6	3.84E-09	6
Q63270	SW:IRE1_RAT	Iron-responsive element binding protein 1 (ire-bp 1) (EC 4.2.1.3)	0.100	C	16	28	−0.15	0	7.14	98 749	10	1.07E-11	1, 3
Q63342	SW:M2GD_RAT	Dimethylglycine dehydrogenase precursor (EC 1.5.99.2) (me2glydh)	0.130	M	26	109			7.38	96 272	14	1.42E-20	1, 2, 3, 6
Q63347	SW:PRS7_RAT	26S protease regulatory subunit 7 (mss1 protein)		C, N	14	16	−0.41	0	5.55	48 943	11	1.46E-16	2, 3, 5
Q63617	TRE_ROD:Q63617	150-kDa oxygen regulated protein		ERL					4.95	111 448	9	7.51E-09	2
Q63716	SW:TDX2_RAT	Thioredoxin peroxidase 2 (thioredoxin-dependent peroxide reductase 2)	1.175	C	41	124			8.19	22 323	9	3.20E-16	1, 3, 6
Q63797	SW:PSE1_RAT	Proteasome activator complex subunit 1 (Proteasome activator 28-α subunit)	0.038		18	29	−0.64	0	5.90	28 730	8	1.30E-09	1
Q64428	SW:ECHA_RAT	Mitochondrial trifunctonal enzyme α subunit (EC 4.2.1.17)/(EC 1.1.1.35)	0.148	MM	1	1	−0.05	1	9.59	83 201	10	5.00E-14	1, 2
Q64565	SW:AGT2_RAT	Alanine-glyoxylate aminotransferase 2 (EC 2.6.1.44) (b-Ala-pyruvate aminotransferase)	0.250	M	5	5	−0.15	0	8.12	57 869	6	9.23E-06	1

Table 1. Continued

Number	Protein	Full name	Level	Location	Frequency	Spots	GRAVY	TM	p*I*	MW	Matches	Probability	Figure
Q64640	SW:ADK_RAT	Adenosine kinase (EC 2.7.1.20) (ak) (adenosine 5′-phosphotransferase)	0.063		27	68	−0.31	0	6.71	37 630	7	7.27E-08	1, 3, 5, 6
Q64727	SW:VINC_MOUSE	Vinculin	0.021	AP	6	8			5.76	117 171	5	8.64E-06	1, 3
Q9Z0T0	TRE_ROD:Q9Z0T0	Thiopurine *S*-methyltransferase (EC 2.1.1.67)		C					6.79	27 959	8	7.39E-08	6
Q9Z0V5	TRE_ROD:Q9Z0V5	PRX IV			11	14			6.63	31 216	7	1.32E-05	5, 6
Q9Z0V6	TRE_ROD:Q9Z0V6	PRX III			4	7			7.55	28 588	6	2.02E-05	6
Q9Z2I8	TRE_ROD:Q9Z2I8	GTP-specific succinyl-CoA synthetase β subunit (fragment)	0.072						6.13	44 115	8	2.19E-06	1, 3, 5
Q9Z2M7	TRE_ROD:Q9Z2M7	Phosphomannomutase 2 (EC 5.4.2.8) (PMM 2)		C					8.12	31 895	6	4.59E-08	6
S39807	PIR2:S39807	3-Methyl-2-oxobutanoate dehydrogenase (lipoamide) (EC 1.2.4.4)–mouse			3	3			6.68	43 651	6	2.72E-06	2
W74762	NRDBP:GSP_W74762	Human secreted protein encoded by gene 32 clone HRDEW41	0.099						7.92	56 650	8	3.42E-06	1
Y07062	NRDBP:GSP_Y07062	Renal cancer associated antigen precursor sequence							5.50	60 194	8	9.51E-05	5

Total and proteins from the cytosolic fraction of rat liver were extracted, separated by 2-D electrophoresis and identified by MALDI-MS, following in-gel digestion with trypsin, as described in Section 2. The search in protein databases was performed with in house developed software. The software usually assigned masses to 22 peptides of a mass spectrum. At least four matching peptides were required for an identity assignment. The number of matching peptides is listed in Table 1 (''Matches''). The mass spectra usually included a number of peptides higher than 22, which were not considered for protein search. The spots representing the identified proteins are indicated in Figs. 1–6 and are designated with their accession numbers of the SWISS-PROT or of the other databases. The theoretical MW and p*I* values, as well as the probability of assignment of a random identity are given. The probability was determined as described in [23]. In the column ''Protein'', the abbreviated name of the protein and the database used for protein search are indicated. In the column ''Level'', the approximate percentage of the volume of the spots representing the particular protein, in comparison with the total proteins present in the gel of Fig. 1 is given. In the column ''Location'', the annotated subcellular location for the particular protein in the SWISS-PROT database is indicated. In the column ''Frequency'', the number of times the particular protein has been detected in the 50 rat liver samples analyzed in our laboratory is indicated. In the column ''Spots'', the number of spots, which represent the particular protein and which were identified by MS in the 50 rat liver samples analyzed in our laboratory, is given. In the column ''GRAVY'', the grand average hydrophobicity values according to Kyte-Doolittle [26] and in the column ''TM'', the number of predicted transmembrane regions according to Klein et al. [27] are given. In the column ''Figure'', the figure number is given in which the spot representing the corresponding protein can be seen. The data are sorted according to ascending accession numbers. AJ, adhesion junction; AP, adhesion plaques; C, cytoplasmic; E, extracellular; ER, endoplasmic reticulum; ERL, endoplasmic reticulum lumen; M, mitochondria; MIM, mitochondrial inner membrane; MB, membrane bound; MC, microsomal; MM, mitochondrial matrix; N, nuclear; OMM, outer mitochondrial matrix; P, peroxisomal.

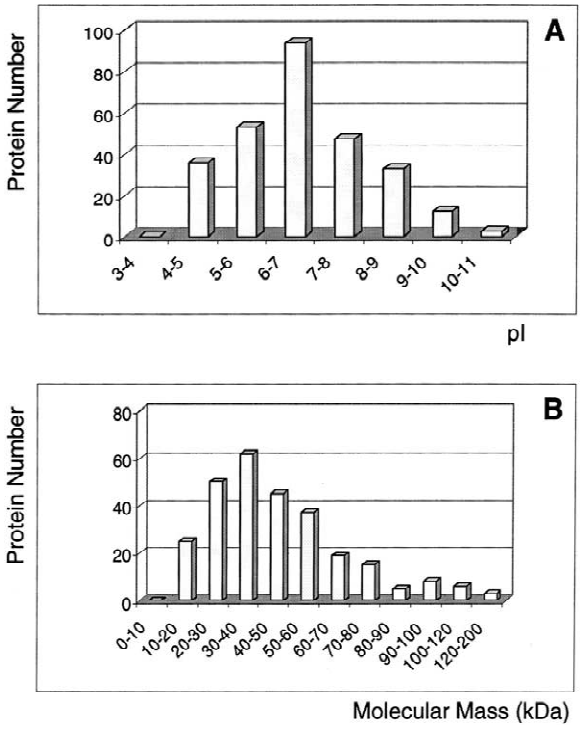

Fig. 7. Distribution of the rat liver proteins in relation to their theoretical p*I* (A) and molecular mass (B) values. The bars indicate the number of proteins found in the p*I* and the molecular mass intervals indicated.

representing the various protein forms of the same gene product was compared to total proteins present in the broad pH range gel of Fig. 1. The most abundant components were several house-keeping enzymes, represented by strong spots mainly at the basic region of the gel, such as carbamoyl-phosphate synthase, 3-ketoacyl-CoA thiolase, betaine-homocysteine *S*-methyltransferase, fructose-biphosphate aldolase, as well as heat shock proteins (Table 1). The less abundant species include enzyme subunits and proteins with various functions, such as structural and hypothetical proteins. The major components are localized in mitochondria and the cytosol. The minor components are mainly localized in the cytoplasma and nucleus.

About 60% of the liver proteins of Table 1 are enzymes or enzyme subunits (approximately 165) with various catalytic activities. Other major classes of the identified proteins include approximately 20 structural species, such as tubulin, about 14 heat shock proteins (glucose-regulated proteins, T-complex protein chains, etc.), ribosomal proteins, transport proteins, channels, elongation factors, urinary proteins and others (Fig. 8). Several calcium-binding proteins were also detected, such as calreticulin, probable protein disulfide isomerase, annexins, myosin regulatory chains, tubulin chains and necleobindin.

3.4. Subcellular location

For about 63% of the proteins of Table 1, the annotation in the SWISS-PROT database includes their subcellular location. Seventy-two proteins are annotated as cytosolic and 72 as organelle-associated. Of the latter, 47 are annotated as mitochondrial, 11 as endoplasmic reticulum, four as peroxisomal and three as nuclear proteins (Fig. 9). Seven proteins are annotated as extracellular and only one protein is annotated as microsomal (cytochrome b5).

The species detected in the total protein sample (Figs. 1 and 2) are mainly annotated as cytosolic or mitochondrial (Table 1). Some cross-contamination of the subcellular fractions could not be avoided. Thus, the cytosolic fraction mainly consisted of cytosolic proteins but also some species of mitochondrial origin, such as carbamoyl-phosphate synthase, glutamate dehydrogenase and 3-ketoacyl CoA-thiolase, were detected. Similarly, in the mitochondrial fraction previously analyzed, we detected several cytosolic proteins. However, they were mainly minor components accounting for approximately 13% of the total spot volume [19]. The presence of cytosolic proteins in the mitochondrial fraction and

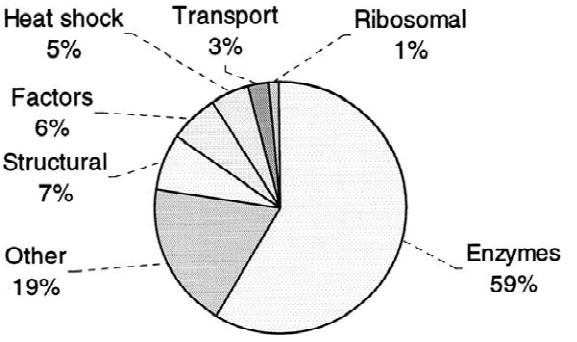

Fig. 8. Functions of the rat liver proteins. The proteins identified in this study were classified into functional groups. Proteins with no catalytic activities and proteins with hypothetical or unknown functions were taken together into the group ''Other''.

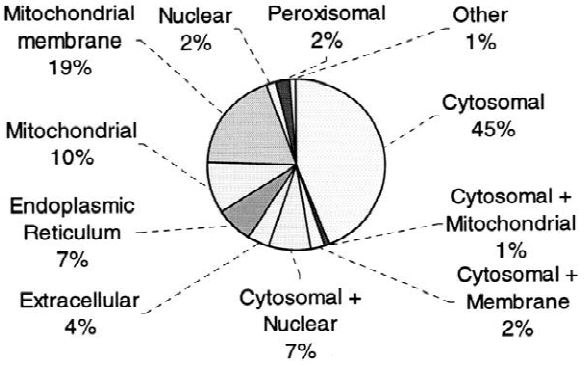

Fig. 9. Subcellular location of rat liver proteins as annotated in the SWISS-PROT database. For 37% of the proteins no annotation existed.

vice-versa may be attributed to the fact that the separation of the organelles was incomplete. Thus, the mitochondrial species found in the cytosolic fraction were mainly high-abundance components, for which the complete separation from the cytosolic proteins was not very efficient. About 50% of the proteins detected in the total protein sample (Figs. 1 and 2) were also detected in one or more of the gels carrying cytosolic proteins (Figs. 3–6). This is to be expected as the total proteins sample mainly included cytosolic and mitochondrial species.

3.5. Hydrophobicity

The grand average hydrophobicity (GRAVY) values for 170 proteins of Table 1, which are annotated in the SWISS-PROT database, were determined according to Kyte-Doolittle [26] and the number of the theoretical transmembrane (TM) regions was determined according to Klein et al. [27]. GRAVY values usually vary in the range ± 2. Positive scores indicate hydrophobic and negative scores hydrophilic proteins. 14 (8%) proteins had low positive (below 0.21) and the remaining negative values (down to -1.10). Fig. 10A shows the distribution of the GRAVY scores of the rat liver proteins. For comparison, in Fig. 10B, the distribution of the GRAVY scores of about 2900 rat proteins of the SWISS-PROT database is shown. About 590 (20%) rat proteins have positive GRAVY values (between 0 and 1.5), which indicates that hydrophobic proteins are underrepresented in our list. Twenty-five proteins of Table 1 include one theoretical transmembrane

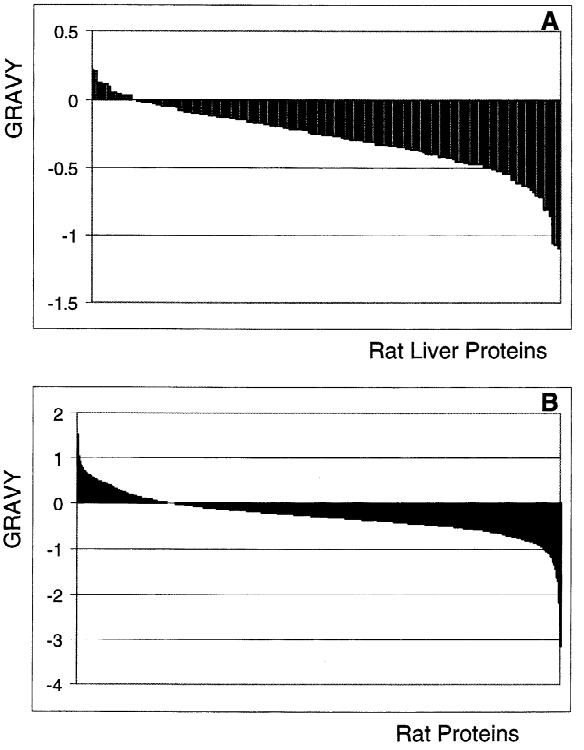

Fig. 10. Grand average hydrophobicity values (GRAVY) of 170 rat liver proteins (A), and of 2900 rat proteins of the SWISS-PROT database (B). The values were calculated according to Kyte-Doolittle [26]. Negative GRAVY scores mean hydrophilic and positive scores mean hydrophobic proteins.

region, eight proteins include two and one protein carries three predicted transmembrane domains. The three-transmembrane domain protein, cystathionine β-synthase, was found in 13 rat protein samples analyzed in our laboratory. The two-transmembrane domain proteins were in average detected in 25 samples, the one-transmembrane domain proteins in 40 and the proteins lacking a transmembrane domain were in average found in 30 samples. The GRAVY values of the species in the total protein sample (-0.26) were comparable with the values of the cytosolic species (-0.25). The GRAVY scores provide an image of the hydrophobicity of the whole protein. The proteins detected in 2-D gels are in general hydrophilic with negative GRAVY values [28]. The GRAVY scores do not seem to represent a reliable criterion to predict whether a protein will enter an IPG strip. More important may be the particular hydrophobic stretches, which could hinder the whole protein from entering the strip.

3.6. Heterogeneity

Table 1 includes a column with the number of spots identified for each protein in the ~50 rat liver samples analyzed by MS in our laboratory. Most proteins were represented by more than one spot. For 15 proteins, mainly enzyme subunits and structural proteins, only one spot was found. For 50 proteins up to five spots were detected. All other proteins were represented by a larger number of spots, seven high abundance proteins by more than 200. Six hundred and fifty-one spots were found representing carbamoyl-phosphate synthase, 625 representing serum albumin and 376 catalase. In average, we estimate that about five to 10 spots correspond to one gene product. In the mouse liver and the rat liver mitochondrial proteomes, we also detected a high heterogeneity, calculating approximately 10–20 spots per gene product [10,19]. The multiple spots can be partly due to artifacts of the 2-D electrophoresis, but in most cases they represent post-translationally modified protein forms. For most of the observed heterogeneities, we know neither the origin nor the biological significance. Efficient analysis of the post-translational modifications requires a significant improvement in sensitivity and throughput of the analytical techniques.

3.7. Frequency of detection

We observed a variation in the frequency with which the components of a proteome are detected by mass spectrometry. Certain abundant, hydrophilic and easily to solubilize proteins are present in most gels. They can be easily digested and deliver a sufficient number of peptides, so that an identity can be almost always assigned. We evaluated the frequency of detection of the proteins of Table 1 in the about 50 rat liver samples analyzed in our laboratory. Fifteen gene products, seven of which are low-abundance enzymes, were detected in only one of the gels, whereas the other proteins were found in two or more samples. Fifty-eight proteins were found in five or less samples, whereas about 35% of the proteins were detected in more than 20 samples. The most frequently detected in 40 or more samples, were heat shock cognate (P08109), serum albumin (P02770) and house-keeping enzymes, such as catalase (P04762), thioredoxin peroxidase (Q63716) and carbamoyl-phosphate synthase (P07756) (Table 1). The most frequently detected protein, heat shock cognate, has been most frequently detected in other proteomes as well, for example mouse liver [10] and human brain [29]. Therefore, it can be considered as a positive control in a protein identification process. The frequency of detection provides an indication of the importance of information deriving from the detection of a gene product in the proteome, which is currently analyzed. The detection of a species is probably of limited value if that protein has been already detected for example in 100 or more samples. The less frequently detected gene products are more interesting in proteomic studies, as they can be most likely involved in disease-related changes and their changed levels or modifications may carry more significant biological information than of their frequently detected counterparts.

4. Discussion

In a previous study, we prepared a 2-D database for mitochondrial rat liver proteins and detected 192 different gene products, a first step towards the establishment of a rat liver proteome [19]. Here, we performed a proteomic analysis of the total and cytosolic liver proteins, which resulted in the identification of 273 different gene products, 20% of which were detected only in the cytosolic fraction. Detection of additional species demands sophisticated protein enriching techniques, involving detailed organelle fractionation, efficient use of solubilizing agents and chromatographic separation [12,28]. The proteins identified were in the majority hydrophilic, usually represented by multiple spots, in average five to 10 per gene product. The most heterogeneous species were the abundant and frequently detected proteins. The average heterogeneity and the frequency of detection may be higher than the estimated or found because not all spots representing an abundant gene product are excised for a MS analysis and usually 60–70% of the MALDI-MS analyses result in an identity assignment.

Early detection of toxic effects of drug candidates increases the performance of the drug design process and the safety of pharmaceuticals. Genomics and proteomics are emerging, high-throughput technologies, which can easily generate toxicity patterns,

i.e., alterations in gene or protein levels, an information which can lead to drug toxicity prediction [15,16,30]. Changes in the mRNA [31–33] and the protein levels [9,15,16,34–37] on account of exposure to toxic agents have been reported. However, an unambiguous relationship between toxicity and gene or protein pattern derangement has not been established yet. Up to now, mainly model compounds, such as acetaminophen [9,34,38], thioacetamide [39] or carbon tetrachloride [40,41], are usually administered to animals and tissue samples are analyzed by employing the new approaches for the generation of toxicity databases, which will function as a guiding cue in predicting toxicity in similar cases.

The present results represent a contribution to the proteomic approach. The database could be useful in the quantification of differences in the levels of liver proteins of animals serving as models in toxicity studies. In our list, several proteins are included which have been described to be involved in the toxicity of acetaminophen, such as selenium-binding protein, N^{10}-formyl tetrahydrofolate dehydrogenase, glutathione *S*-transferases, glutathione peroxidase, proteasome proteins, superoxide dismutase, calreticulin and others [9,34,42–44]. Best documented is the involvement of selenium- or acetaminophen-binding protein, which is arylated and translocated into the nucleus, following administration of a toxic dose of acetaminophen [45]. Applying proteomics technologies, we found a decrease in the levels of the selenium-binding protein in the livers of mice treated with acetaminophen at 300 mg/kg. We also observed reduced levels for the antioxidant enzymes, glutathione *S*-transferases and glutathione peroxidase. It has been reported that oxidative stress is involved in the progression of acetaminophen-induced hepatotoxicity [46].

Deranged levels were also found for mitochondrial proteins included in our list, such as glutamate dehydrogenase, aldehyde dehydrogenase, the heat shock proteins mitochondrial matrix protein 1 and glucose-regulated protein 75 kDa, which suggests for mitochondria damage by the toxic agent. Mitochondria play a critical role in cell apoptosis and necrosis and are involved in the acetaminophen toxicity pathway and the pathways of other substances [37,47–49]. Moreover, we found reduced levels for the mitochondrial heat shock protein 75 kDa, also called tumor necrosis factor (TNF) receptor-associated protein, in animals treated with low and high doses of acetaminophen and with the non-toxic isomer [9]. This protein binds to the intracellular domain of the TNF receptor type 1 and has been proposed to play a role in the acetaminophen-induced toxicity [50,51]. TNFα has been found to be involved in both necrosis and apoptosis [52–54], however, its role in toxicity events is not clear [37].

In a recent study, we also employed genomics and proteomics technologies to investigate the effect of toxic doses of carbon tetrachloride on gene and protein levels in the liver [55]. The proteomic analysis revealed that the levels of three peroxisomal proteins, catalase, uricase and 3-ketoacyl-CoA thiolase A, were increased in the livers of the treated animals. Catalase and uricase are involved in stress defence, whilst the 17 down-regulated proteins are mainly enzymes participating in lipid and amino acid metabolism.

In summary, we constructed a 2-D database for total and cytosolic proteins from rat liver. The database is one of the largest databases for high eukaryotic proteomes, comprising 273 different gene products. They resulted from the MALDI-MS analysis of approximately 5000 spots, taken from 13 2-D gels. About 60% of the identified proteins are enzyme subunits. Fifteen gene products were detected for the first time. The most frequently detected proteins are heat shock proteins and house-keeping enzymes. In average, approximately five to 10 spots correspond to one gene product. The list includes many proteins which are known to be involved in toxicity and for which deranged levels have been reported in animals exposed to toxic agents. It may be useful in toxicity studies, in particular in prediction of toxic effects of drug candidates, on the basis of changes in the protein levels, resulting from the administration of compounds under investigation. Future efforts should be dedicated to optimize the subcellular fractionation in order to achieve a more detailed proteome image.

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