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Towards a high resolution separation of human cerebrospinal fluid

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Abstract

Human cerebrospinal fluid is an ultrafiltrate of plasma that is largely produced by the choroid plexus. It consists of a mixture of anorganic salts, various sugars, lipids and proteins from the surrounding brain tissues. The predominant proteins in cerebrospinal fluid are isoforms of serum albumin, transferrin and immunoglobulins, representing more than 70% of the total protein amount. A rough overview of the protein compounds of human cerebrospinal fluid including their respective concentrations is given by Blennow et al. [Eur. Neurol. 33 (1993) 129]. In contrast, the aim of this work is to display the detailed protein composition of CSF by two-dimensional gel electrophoresis and to identify both high and low concentrated proteins using different mass spectrometry techniques. This extensive overview of proteins in human cerebrospinal fluid will be highly relevant for clinical research. Furthermore, the comparison of 2D gels will help to analyze the standard protein variability in CSF of healthy persons and detect specific protein variations of patients with various neurological diseases (e.g., Alzheimer's disease, Huntington's chorea). Sample preparation for two-dimensional gel electrophoresis must include concentration and desalting steps such as precipitation and ultrafiltration due to the high amount of salts, sugars and lipids and the low total amount of protein of 0.3–0.7 μg/μl present in human CSF. Up to now we were able to identify more than 480 spots from suchlike generated 2D gels using MALDI- and ESI-mass spectrometry. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: High-resolution separation; Cerebrospinal fluid; Proteins

1. Introduction

The central nervous system (CNS) is surrounded by cerebrospinal fluid (CSF), which resembles an ultrafiltrate of plasma. The CSF provides buoyancy and protection to the CNS. Besides active concentration mechanisms, several factors contribute to the constitution of the CSF. The total volume of CSF in humans is approximately 140 ml, with a mean CSF production rate of 0.3–0.4 ml/min [1,2]. The CSF is distributed between the subarachnoid space surrounding the brain and the spinal cord and the interstitial space encircling the cells in the central nervous system. Metabolites from various tissues within the brain are secreted into the interstitial space and up in the cerebrospinal fluid. Therefore, the cerebrospinal fluid reflects the metabolic state of the brain under healthy conditions as well as in several diseases.

Our interest is to give a detailed overview of proteins present in CSF. For this purpose, we used a

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cerebrospinal fluid pool consisting of samples from different patients without any symptoms of neurological diseases. The age of the patients varied between 60 and 70 years. For protein identification we used different approaches such as peptide mass fingerprints using MALDI-MS [3,4] or fragmentation of peptide ions using MALDI-PSD [5,6]. Peptide mass fingerprints are a very fast and easy way to identify proteins with about 70–80% success rate using samples of human origin. However, the method normally reaches its limitations when there are more than two proteins in the digestion mixture. Though the MALDI-PSD technique is more time consuming than the MALDI-MS fingerprint, it allows the identification of more than one protein in a mixture.

Low-molecular-mass proteins are particularly difficult to identify because in most cases only few proteolytic fragments are generated by tryptic digestion and a database search with only three to five peptide masses does not allow an unequivocal protein identification. In this case we change our strategy to a nano-HPLC [7] separation of the digestion mixture followed by MS analysis of single fractions thereof. By this means we avoid the common suppression of weak peptide signals in digestion mixtures and are able to separate single peptides for high sensitive MALDI-PSD analysis leading to the identification even of low-molecular-mass proteins. Though this is a very time consuming method, it is also very helpful for the identification of posttranslational modifications in ''pearl necklet'' spot groups.

2. Materials and methods

2.1. Withdrawal and sample preparation of human cerebrospinal fluid

Human cerebrospinal fluid is usually taken from the bottom part of the lumbar spinal column while the patient is in a sitting position. The CSF is collected in 10 ml vials containing a proteinase inhibitor mixture (Complete Mini, Roche Diagnostics, Mannheim, Germany) to suppress any proteolytic activity, although mature liquor itself contains proteinase inhibitors (Table 1). The total protein amount in CSF is subsequently determined by amino acid analysis using the derivatizer 140D system (Applied Biosystems, Foster City, CA, USA).

To remove all disturbing components of mature CSF the fluid is concentrated by ultrafiltration using UltrafreeMC (Millipore, Bedford, USA) with a cut-off mass of 5000 Da. The flow-through is collected, concentrated by evaporation, and used for further μHPLC analysis to detect small peptides and proteins in the range up to 5000 Da. The CSF volume corresponding to 100 or 300 μg protein, respectively, is reduced to 30 μl and washed twice with reswelling buffer (see Section 2.2.1) used for the following isoelectric focusing.

2.2. Two-dimensional polyacrylamide gel electrophoresis

2.2.1. Isoelectric focusing using IPG strips (first dimension)

Isoelectric focusing for two-dimensional gel electrophoresis is performed using the Multiphor II system (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. For analytical gels 100 μg of total protein, for micro preparative gels 300 μg are solubilized in 60 μl (modified) reswelling buffer of the following composition according to the pH range (modified after Ref. [8]). pH 3–10: 7 *M* urea, 2 *M* thiourea, 2% CHAPS, 15 m*M* DTT, 2% carrier ampholytes pH 3–10; pH 4–7: 7 *M* urea, 2 *M* thiourea 2% CHAPS, 100 m*M* DTT, 2% carrier ampholytes pH 4–7; pH 6–11: 8 *M* urea, 2% CHAPS, 200 m*M* DTT, 2% carrier ampholytes pH 6–11.

Sample preparation is performed as described above. The IPG strips are reswollen overnight in a custom-built reswelling cassette using 350 μl of reswelling buffer already containing the sample. For high-resolution isoelectric focusing IPG strips of the pH ranges 4–7 and 6–11 with 18 cm length are used. To obtain an overview gel the broad range pH 3–10 is used.

Prefocusing to remove ionic components of CSF is performed as follows: 2 h 200 V, 1 h 400 V, 1 h 600 V, 1 h 1000 V, 2 h 2000 V. Focusing is done at 3.5 kV to 60 000 Vh. All focusing steps are conducted under oil. After focusing, the strips are removed, washed with water, and stored at −80 °C.

Table 1
Summary of all identified proteins in human liquor

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
1	α_1-Antitrypsin	22 828	6.11	gi|28637
2	α_1-Antitrypsin	22 828	6.11	gi|28637
3	α_1-Antitrypsin	22 828	6.11	gi|28637
4	α_1-Antitrypsin	22 828	6.11	gi|28637
5	α_1-Antitrypsin precursor	46 707	5.51	gi|177836
6	α_1-Antitrypsin precursor	46 707	5.51	gi|177836
7	α_1-Antitrypsin	22 828	6.11	gi|28637
8	α_1-Antitrypsin	22 828	6.11	gi|28637
9	α_1-Antitrypsin	22 828	6.11	gi|28637
14	Serum albumin	69 366	6.05	gi|28592
15	Serum albumin	69 366	6.05	gi|28592
16	HCMVUL 71 herpesvirus protein	45 727	5.06	gi|136966
17	Serum albumin	69 366	6.05	gi|28592
18	Serum albumin	69 366	6.05	gi|28592
20	Serum albumin	69 366	6.05	gi|28592
21	Serum albumin	69 366	6.05	gi|28592
22	Serum albumin	69 366	6.05	gi|28592
23	Serum albumin	69 366	6.05	gi|28592
24	Serum albumin	69 366	6.05	gi|28592
25	Serum albumin	69 366	6.05	gi|28592
26	Serum albumin	69 366	6.05	gi|28592
27	Serum albumin	69 366	6.05	gi|28592
28	Serum albumin	69 366	6.05	gi|28592
29	Serum albumin	69 366	6.05	gi|28592
30	Serum albumin	69 366	6.05	gi|28592
31	Serum albumin precursor	69 226	5.92	gi|6013427
32	Serum albumin precursor	69 226	5.92	gi|6013427
33	Serum albumin precursor	69 226	5.92	gi|6013427
34	Serum albumin precursor	69 226	5.92	gi|6013427
35	Serum albumin precursor	69 226	5.92	gi|6013427
36	Serum albumin precursor	69 226	5.92	gi|6013427
37	Serum albumin	69 366	6.05	gi|28592
38	Serum albumin	69 366	6.05	gi|28592
39	Serum albumin	69 366	6.05	gi|28592
40	Serum albumin	69 366	6.05	gi|28592
42	Serum albumin	69 366	6.05	gi|28592
44	Serum albumin	69 366	6.05	gi|28592
45	Serum albumin	69 366	6.05	gi|28592
48	Serum albumin	69 366	6.05	gi|28592
49	Serum albumin	69 366	6.05	gi|28592
50	Serum albumin	69 366	6.05	gi|28592
51	Serum albumin	69 366	6.05	gi|28592
52	Serum albumin	69 366	6.05	gi|28592
53	Serum albumin	69 366	6.05	gi|28592
54	Serum albumin	69 366	6.05	gi|28592
55	Serum albumin	69 366	6.05	gi|28592
56	Serum albumin	69 366	6.05	gi|28592
57	Serum albumin	69 366	6.05	gi|28592
58	Serum albumin	69 366	6.05	gi|28592
59	Serum albumin	69 366	6.05	gi|28592

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
60	Serum albumin	69 366	6.05	gi|28592
61	Serum albumin	69 366	6.05	gi|28592
62	Serum albumin	69 366	6.05	gi|28592
63	Serum albumin	69 366	6.05	gi|28592
65	Lysozyme	15 872	9.30	gi|1127120
66	α_1-Antitrypsin	22 828	6.11	gi|28637
67	Serum transferrin	36 360	6.53	gi|7245523
68	α_1-Antitrypsin	22 828	6.11	gi|28637
69	α_1-Antitrypsin	22 828	6.11	gi|28637
	Ig κ chain	15 174	5.50	gi|386812
70	α_1-Antitrypsin	22 828	6.11	gi|28637
	Ig λ chain	10 187	4.60	gi|422897
71	Serum transferrin	36 360	6.80	gi|3318925
72	Ig heavy Chain	12 713	4.82	gi|2073436
74	Ig κ chain	15 174	5.50	gi|386812
75	Ig κ chain	15 174	5.50	gi|386812
76	Ig κ chain	15 174	5.50	gi|386812
77	Ig κ chain	15 174	5.50	gi|386812
78	Serum albumin	69 366	6.05	gi|28592
79	Serum albumin	69 366	6.05	gi|28592
80	Serum albumin	69 366	6.05	gi|28592
81	Ig κ chain	15 174	5.50	gi|386812
82	Serum albumin	69 366	6.05	gi|28592
83	Serum albumin	69 366	6.05	gi|28592
84	Serum albumin	69 366	6.05	gi|28592
85	Serum albumin	69 366	6.05	gi|28592
86	Serum albumin	69 366	6.05	gi|28592
87	Serum albumin	69 366	6.05	gi|28592
88	Serum albumin	69 366	6.05	gi|28592
89	Serum albumin	69 366	6.05	gi|28592
90	Serum albumin	69 366	6.05	gi|28592
91	Serum albumin	69 366	6.05	gi|28592
92	Serum albumin	69 366	6.05	gi|28592
93	Serum albumin	69 366	6.05	gi|28592
95	Transferrin precursor	77 050	6.81	gi|4557871
96	Zinc finger protein ZFP 30	23 290	9.39	gi|1613854
97	Serum transferrin N-terminal lobe	36 360	6.80	gi|3318925
98	Serum transferrin N-terminal lobe	36 360	6.80	gi|3318925
99	Transferrin	53 760	6.00	gi|553788
100	α_1-Antitrypsin precursor	46 707	5.51	gi|177836
102	Complement component 3 precursor	187 165	6.02	gi|4557385
103	Prostaglandin D2 synthase	21 028	7.65	gi|4506251
109	Serum albumin	69 366	6.05	gi|28592
110	Serum albumin	69 366	6.05	gi|28592
111	Serum albumin	69 366	6.05	gi|28592
112	Serum albumin	69 366	6.05	gi|28592
113	IgM heavy chain	9875	8.81	gi|3851273
114	Apolipoprotein E3	16 719	6.00	gi|230129
115	Serum albumin	69 366	6.05	gi|28592
116	Serum albumin	69 366	6.05	gi|28592
117	Serum albumin	69 366	6.05	gi|28592

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
118	Serum albumin	69 366	6.05	gi|28592
119	Serum albumin	69 366	6.05	gi|28592
120	Serum albumin	69 366	6.05	gi|28592
122	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
123	Serum albumin	69 366	6.05	gi|28592
124	Serum albumin	69 366	6.05	gi|28592
125	Serum albumin	69 366	6.05	gi|28592
126	Serum albumin	69 366	6.05	gi|28592
127	Serum albumin	69 366	6.05	gi|28592
128	Serum albumin	69 366	6.05	gi|28592
129	Serum albumin	69 366	6.05	gi|28592
130	Serum albumin	69 366	6.05	gi|28592
131	Serum albumin	69 366	6.05	gi|28592
132	Serum albumin	69 366	6.05	gi|28592
133	Serum albumin	69 366	6.05	gi|28592
134	Serum albumin	69 366	6.05	gi|28592
135	Serum albumin	69 366	6.05	gi|28592
136	Serum albumin	69 366	6.05	gi|28592
137	Serum albumin	69 366	6.05	gi|28592
138	Serum albumin	69 366	6.05	gi|28592
139	Serum albumin	69 366	6.05	gi|28592
140	Serum albumin	69 366	6.05	gi|28592
	Importin β chain	97 170	4.68	gi|2135476
141	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
142	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
143	Transthyretin	12 843	5.33	gi|339685
144	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12843	5.33	gi|339685
145	α_1-Antitrypsin	22 828	6.11	gi|28637
146	α_1-Antitrypsin	22 828	6.11	gi|28637
147	α_1-Antitrypsin	22 828	6.11	gi|28637
148	α_1-Antitrypsin	22 828	6.11	gi|28637
	Transferrin	77 050	6.81	gi|4557871
	Transthyretin	12 843	5.33	gi|339685
	Serum albumin	69 366	6.05	gi|28592
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
149	α_1-Antitrypsin	22 828	6.11	gi|28637
	Transthyretin	12 843	5.33	gi|339685
	Serum albumin	69 366	5.33	gi|28592
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
150	Serum albumin	69 366	6.05	gi|28592
151	Transthyretin	12 843	5.33	gi|339685
	Retinol binding protein	13 760	5.55	gi|494652
	Vimentin	53 651	5.06	gi|2119204
152	Glutathione *S*-transferase	27 053	6.05	gi|809436
	Transthyretin	15 887	5.33	gi|4507725
	Complement C4A precursor	192 336	6.80	gi|116602

Table 1. Continued

No.	Protein	Mass[a] (Da)	pI[b]	Accession NCBInr
153	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	15 887	5.33	gi|4507725
	GC-rich sequence DNA-binding factor	91 002	9.56	gi|121059
	Apolipoprotein J	48 803.8	6.27	gi|178855
154	Serum albumin	69 366	6.05	gi|28592
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Transthyretin	15 887	5.33	gi|4507725
	Cartilage glycoprotein-39	42 613	8.69	gi|4557018
	Ceruloplasmin	122 205	5.44	gi|4557485
	KIAA0191	171 112	7.71	gi|1228035
155	Serum albumin	69 366	6.05	gi|28592
156	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
157	Transthyretin	12 843	5.33	gi|339685
	Retinol binding protein	13 760	5.55	gi|494652
	Serum albumin	69 366	6.05	gi|28592
158	Serum albumin	69 366	6.05	gi|28592
	β2-Microglobulin	12 799	5.77	gi|34616
	Transthyretin	12 843	5.33	gi|339685
159	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
160	Transferrin precursor	77 050	6.81	gi|4557871
	Serum albumin	69 366	6.05	gi|28592
	Cystatin C	15 799	9.00	gi|4503107
	β2-Microglobulin	12 799	5.77	gi|34616
	β-Trace (N-terminal)	2103	4.37	gi|261745
	IgG 3 heavy chain	12 822	7.55	gi|70031
161	Transthyretin	12 843	5.33	gi|339685
162	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
163	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
164	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
165	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
166	Serum albumin	69 366	6.05	gi|28592
	Transthyretin	12 843	5.33	gi|339685
167	Serum albumin	69 366	6.05	gi|28592
168	Serum albumin	69 366	6.05	gi|28592
169	Serum albumin	69 366	6.05	gi|28592
170	Serum albumin	69 366	6.05	gi|28592
171	Serum albumin	69 366	6.05	gi|28592
172	β-Trace (N-terminal)	2103	4.37	gi|261745
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592
173	β-Trace (N-terminal)	2103	4.37	gi|261745
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592
174	β-Trace (N-terminal)	2103	4.37	gi|261745
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
175	Serum albumin	69 366	6.05	gi|28592
176	Pro-Apolipoprotein-A-I	30 778	5.45	gi|178775
	Serum albumin	69 366	6.05	gi|28592
177	Pro-Apolipoprotein-A-I	30 778	5.45	gi|178775
	Serum albumin	69 366	6.05	gi|28592
178	Serum albumin	69 366	6.05	gi|28592
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
179	Serum albumin	69 366	6.05	gi|28592
180	Serum albumin	69 366	6.05	gi|28592
181	Serum albumin	69 366	6.05	gi|28592
182	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	β-Trace (N-terminal)	2103	4.37	gi|261745
	Ig λ light chain	24 655	7.54	gi|87890
	Serum albumin	69 366	6.05	gi|28592
183	Apolipoprotein J	52 495	6.27	gi|178855
184	Serum albumin	69 366	6.05	gi|28592
185	Serum albumin	69 366	6.05	gi|28592
186	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592
187	Serum albumin	69 366	6.05	gi|28592
188	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592
	β-Trace (N-terminal)	2103	4.37	gi|261745
189	Serum albumin	69 366	6.05	gi|28592
190	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592
	Keratin 10	59 528	5.01	gi|4557697
191	Serum albumin	69 366	6.05	gi|28592
192	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592
	KIAA0191	171 112	7.71	gi|1228035
193	Keratin 10	59 528	5.17	gi|71528
	KIAA0191	171 112	7.71	gi|1228035
	Serum albumin	69 366	6.05	gi|28592
194	Serum albumin	69 366	6.05	gi|28592
195	Keratin 9	159 419	5.14	gi|4557705
	Serum albumin	69 366	6.05	gi|28592
196	Keratin 9	159 419	5.14	gi|4557705
	Serum albumin	69 366	6.05	gi|28592
197	Keratin 10	59 528	5.17	gi|71528
198	Prostaglandin D2 synthase	59 528	5.17	gi|71528
	Serum albumin	69 366	6.05	gi|28592
	β-Trace (N-terminal)	2103	4.37	gi|261745
	Ig κ chain C region	10 997	5.60	gi|106529
199	Keratin 10	59 528	5.17	gi|71528
	Keratin 1	65 494	8.16	gi|5174491
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Keratin 9	159 419	5.14	gi|4557705
200	Apolipoprotein E	36 154	5.65	gi|4557325
	Keratin 9	159 419	5.14	gi|4557705

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
201	Tolloid-like protein	114 709	5.69	gi|6912722
	Keratin 9	159 419	5.65	gi|4557325
	Serum albumin	69 366	6.05	gi|28592
202	Superoxide dismutase 1	15 935	5.70	gi|15936
	Transthyretin	15 887	5.52	gi|4507725
203	Keratin 9	159 419	5.65	gi|4557325
	Keratin 10	59 528	5.17	gi|71528
	Serum albumin	69 366	6.05	gi|28592
	Apolipoprotein E	36 154	5.65	gi|5174491
204	Angiotensinogen precursor	53 154	5.87	gi|4557287
	Keratin 9	159 419	5.65	gi|4557325
	Apolipoprotein J	52 495	6.27	gi|178855
	α_1-Antichymotrypsin	47 651	5.33	gi|11437394
	KIAA0191	171 113	7.71	gi|1228035
205	Apolipoprotein J	52 495	6.27	gi|178855
	KIAA0191	171 113	5.33	gi|1228035
	DKFZp434C013.1	35 061	8.67	gi|7512538
206	Tolloid-like protein	114 709	5.69	gi|6912722
207	Keratin 10	59 528	5.17	gi|71528
	Apolipoprotein J	52 495	6.27	gi|178855
	Phosphoribosylpyrophosphate synthetase-associated protein 2	40 926	7.08	gi|4506133
208	Pigment epithelium-derived factor	36815	5.84	gi|284355
209	Keratin 9	15 9419	5.65	gi|4557325
	Ribosomal protein L35	14 551	11.04	gi|6005860
	DKFZp761E1824.1	63 713	6.08	gi|11360234
	Hypothetical protein DJ1186C01.1	190 926	8.87	gi|7512506
210	Serum albumin	69 366	6.05	gi|28592
211	–	–	–	–
212	Tolloid-like protein	114 709	5.69	gi|6912722
213	KIAA0191	171 113	7.71	gi|1228035
	Serum albumin	69 366	6.05	gi|28592
	Tolloid-like protein	114 709	5.69	gi|6912722
214	KIAA0191	171 113	7.71	gi|1228035
215	Tolloid-like protein	114 709	5.69	gi|6912722
	Serum albumin	69 366	6.05	gi|28592
	KIAA0191	171 113	7.71	gi|1228035
216	Transferrin	77 050	6.81	gi|4557871
217	–	–	–	–
218	Cystatin C	15 829	9.00	gi|181387
219	Cystatin C	15 829	9.00	gi|181387
	Ig heavy chain	8834	6.77	gi|1675254
220	Hemopexin	51 676	6.55	gi|11321561
221	–	–	–	–
222	Elastase IIB	28 936	7.98	gi|7705648
223	–	–	–	–
224	Haptoglobin	38 452	6.26	gi|1212947
225	Apolipoprotein A-IV precursor	45 399	5.28	gi|11440019
226	Apolipoprotein A-IV precursor	45 399	5.28	gi|11440019
227	–	–	–	–

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
228	β_{1B}-Glycoprotein	51 676	6.55	gi|11321561
229	–	–	–	–
230	Apolipoprotein A-IV precursor	45 399	5.28	gi|11440019
	Haptoglobulin-1 precursor	45 205	6.13	gi|4826762
231	Apolipoprotein A-IV Precursor	45 399	5.28	gi|11440019
	Haptoglobulin-1 precursor	45 205	6.13	gi|4826762
232	Apolipoprotein A-IV precursor	45 399	5.28	gi|11440019
	Haptoglobulin-1 precursor	45 205	6.13	gi|4826762
233	Apolipoprotein A-IV precursor	45 399	5.28	gi|11440019
234	Apolipoprotein A-IV precursor	45 399	5.28	gi|11440019
235	Ig κ chain	11 663	9.01	gi|407599
236	Serum albumin	69 366	6.05	gi|28590
237	Serum albumin	69 366	6.05	gi|28590
238	Serum albumin	69 366	6.05	gi|28590
239	–	–	–	–
240	Serum albumin	69 366	6.05	gi|28592
241	–	–	–	–
242	Serum albumin	69 366	6.05	gi|28590
243	Serum albumin	69 366	6.05	gi|28590
244	–	–	–	–
245	Serum albumin	69 366	6.05	gi|28590
246	–	–	–	–
247	Serum albumin	69 366	6.05	gi|28590
248	Transferrin	77 050	6.81	gi|4557871
	Cytochrome P450 monooxygenase	49 534	5.98	gi|10257439
249	Serum albumin	69 366	6.05	gi|28590
	Gelsolin	85 698	5.90	gi|4504165
250	–	–	–	–
251	Serum albumin	69 366	6.05	gi|28590
252	Serum albumin	69 366	6.05	gi|28590
253	–	–	–	–
254	Serum albumin	69 366	6.05	gi|28590
255	Serum albumin	69 366	6.05	gi|28590
256	Serum albumin	69 366	6.05	gi|28590
257	Serum albumin	69 366	6.05	gi|28590
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	β-Trace (N-terminal)	2103	4.37	gi|261745
258	Serum albumin	69 366	6.05	gi|28590
259	Keratin 9	62 129	5.19	gi|1082558
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	β-Trace (N-terminal)	2103	4.37	gi|261745
	Serum albumin	69 366	6.05	gi|28590
260	Serum albumin	69 366	6.05	gi|28590
261	Keratin 10	59 528	5.17	gi|71528
	Transferrin	77 050	6.81	gi|4557871
	Serum albumin	69 366	6.05	gi|28592
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	β-Trace (N-terminal)	2103	4.37	gi|261745
262	Serum albumin	69 366	6.05	gi|28590
263	Serum albumin	69 366	6.05	gi|28590
264	Actin β-Kette	42 009	5.24	gi|4501883
	Transferrin	77 050	6.81	gi|4557871
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	KIAA0191	171 112	7.71	gi|1228035

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
265	Transferrin	77 050	6.81	gi|4557871
266	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592
	β-Trace (N-terminal)	2103	4.37	gi|261745
267	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
268	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
269	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28590
270	Serum albumin	69 366	6.05	gi|28590
271	Keratin 10	59 528	5.17	gi|71528
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Serum albumin	69 366	6.05	gi|28592
	Caldesmon	93 250	5.63	gi|2498204
272	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
273	Serum albumin	69 366	6.05	gi|28590
273	β-Trace (N-terminal)	2103	4.37	gi|261745
	Complement factor I	65 720	7.72	gi|116133
274	Serum albumin	69 366	6.05	gi|28590
	β-Trace (N-terminal)	2103	4.37	gi|261745
275	Keratin 10	59 528	5.17	gi|71528
	Serum albumin	69 366	6.05	gi|28590
	Fibrinogen γ-A chain	49 497	5.37	gi|71828
276	Keratin 9	62 129	5.19	gi|1082558
	Serum albumin	69 366	6.05	gi|28590
277	Keratin 9	62 129	5.19	gi|1082558
	Serum albumin	69 366	6.05	gi|28590
278	Keratin 9	62 129	5.19	gi|1082558
	Serum albumin	69 366	6.05	gi|28590
279	Keratin 9	62 129	5.19	gi|1082558
	Serum albumin	69 366	6.05	gi|28590
280	Serum albumin	69 366	6.05	gi|28590
281	Serum albumin	69 366	6.05	gi|28590
	Fibrinogen γ-B chain	51 510	5.37	gi|71828
282	Serum albumin	69 366	6.05	gi|28590
283	Angiotensinogen	53 154	5.87	gi|4557287
	Serum albumin	69 366	6.05	gi|28590
284	Serum albumin	69 366	6.05	gi|28590
285	Serum albumin	69 366	6.05	gi|28590
286	SH3 Protein	78 960	5.94	gi|7705266
287	Serum albumin	69 366	6.05	gi|28590
288	DKFZp564P2062.1	46 420	5.84	gi|3766197
	Serum albumin	69 366	6.05	gi|28590
289	–	–	–	–
290	Keratin 2a	65 494	8.07	gi|4557703
	Serum albumin	69 366	6.05	gi|28590
291	Serum albumin	69 366	6.05	gi|28590
292	Serum albumin	69 366	6.05	gi|28590
293	Keratin 10	59 528	5.17	gi|71528
	Unnamed protein product	26 436	5.35	gi|10435546
	Serum albumin	69 366	6.05	gi|28590
294	Serum albumin	69 366	6.05	gi|28590
295	Serum albumin	69 366	6.05	gi|28590

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
296	Keratin 10	59 528	5.17	gi|4557697
	Serum albumin	69 366	6.05	gi|28590
	Transferrin	30 870	8.41	gi|339469
297	Keratin 10	59 528	5.17	gi|4557697
	Serum albumin	69 366	6.05	gi|28590
	Transferrin	30 870	8.41	gi|339469
298	Transferrin	30 870	8.41	gi|339469
	Keratin 10	59 528	5.17	gi|4557697
	Serum albumin	69 366	6.05	gi|28590
299	Serum albumin	69 366	6.05	gi|28590
300	Serum albumin	69 366	6.05	gi|28590
301	Serum albumin	69 366	6.05	gi|28590
302	Keratin 10	59 528	5.17	gi|4557697
	Serum albumin	69 366	6.05	gi|28590
	Transferrin	30 870	8.41	gi|339469
	KIAA0191	171 112	7.71	gi|1228035
303	Keratin 10	59 528	5.17	gi|4557697
	Serum albumin	69 366	6.05	gi|28590
	Transferrin	30 870	7.71	gi|339469
304	Keratin 10	59 528	5.17	gi|4557697
	Serum albumin	69 366	6.05	gi|28590
	Transferrin	30 870	7.71	gi|339469
305	Ig κ chain	11 663	9.01	gi|407599
306	Serum albumin precursor	69 366	5.92	gi|4502027
307	Transferrin	30 870	8.41	gi|339469
308	Serum albumin	69 366	6.05	gi|28590
309	Serum albumin	69 366	6.05	gi|28590
310	Serum albumin	69 366	6.05	gi|28590
311	Serum albumin	69 366	6.05	gi|28590
312	Serum albumin	69 366	6.05	gi|28590
	KIAA0052	118 243	6.19	gi|1177022
313	Serum albumin	69 366	6.05	gi|28590
314	–	–	–	–
315	Serum albumin	69 366	6.05	gi|28590
316	Serum albumin	69 366	6.05	gi|28590
317	α_1-Antitrypsin	22 828	6.11	gi|28637
318	–	–	–	–
319	–	–	–	–
320	Vitamin D binding protein	52 964	5.40	gi|139641
321	Vitamin D binding protein	52 964	5.40	gi|139641
322	–	–		–
323	Angiotensinogen	53 154	5.87	gi|4557287
	KIAA0191	171 112	7.71	gi|1228035
324	–	–	–	–
325	Transferrin	53 760	6.00	gi|553788
326	Transferrin	53 760	6.00	gi|553788
327	–	–	–	–
328	Serum albumin	69 366	6.05	gi|28590
329	Serum albumin	69 366	6.05	gi|28590
330	Serum albumin	69 366	6.05	gi|28590
331	Serum albumin	69 366	6.05	gi|28590
332	Serum albumin	69 366	6.05	gi|28590

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
333	Complement component 3	187 164	6.02	gi|4557385
	IgG 2 chain C region	35 885	7.66	gi|121043
	Serum albumin	69 366	6.05	gi|28590
	Transferrin	53 760	6.00	gi|553788
334	Serum albumin	69 366	6.05	gi|28590
335	Serum albumin	69 366	6.05	gi|28590
336	Serum albumin	69 366	6.05	gi|28590
337	Serum albumin	69 366	6.05	gi|28590
338	Serum albumin	69 366	6.05	gi|28590
339	Serum albumin	69 366	6.05	gi|28590
340	Serum albumin	69 366	6.05	gi|28590
341	Serum albumin	69 366	6.05	gi|28590
342	Serum albumin	69 366	6.05	gi|28590
343	Serum albumin	69 366	6.05	gi|28590
344	Serum albumin	69 366	6.05	gi|28590
345	Serum albumin	69 366	6.05	gi|28590
346	Serum albumin	69 366	6.05	gi|28590
347	Ig heavy chain V region	18 318	7.74	gi|478651
348	Ig heavy chain V region	18 318	7.74	gi|478651
349	β_2-Glycoprotein I	38 312	8.34	gi|4557327
	Keratin 10	59 528	5.01	gi|4557697
	IgG Fc fragment	28 325	7.59	gi|33069
	Transferrin	30 870	8.41	gi|339469
	Serum albumin	69 366	6.05	gi|28590
	Tolloid-like protein	114 709	5.69	gi|9247108
350	β_2-Glycoprotein I	38 312	8.34	gi|4557327
	Keratin 10	59 528	5.01	gi|4557697
	IgG Fc fragment	28 325	7.59	gi|33069
	Transferrin	30 870	8.41	gi|339469
	Serum albumin	69 366	6.05	gi|28590
	Tolloid-like protein	114 709	5.69	gi|9247108
351	Serum albumin	69 366	6.05	gi|28590
	Igα_1	73 377	5.14	gi|229585
352	Keratin 10	59 528	5.01	gi|4557697
	Serum albumin	69 366	6.05	gi|28590
	Transferrin	30 870	8.41	gi|339469
353	Keratin 9	62 129	5.14	gi|4557705
	Transferrin	30 870	8.41	gi|339469
354	Ig heavy chain V region	18 318	7.74	gi|478651
	Transferrin	30 870	8.41	gi|339469
355	Keratin 9	62 129	5.14	gi|4557705
	Transferrin	30 870	8.41	gi|339469
356	Keratin 9	62 129	5.14	gi|4557705
	Transferrin	30 870	8.41	gi|339469
357	Transferrin	30 870	8.41	gi|339469
358	Hemopexin	51 676	6.55	gi|11321561
	Transferrin	30 870	8.41	gi|339469
359	Hemopexin	51 676	6.55	gi|11321561
	Transferrin	30 870	8.41	gi|339469
360	Hemopexin	51 676	6.55	gi|11321561
361	Hemopexin	51 676	6.55	gi|11321561

Table 1. Continued

No.	Protein	Mass[a] (Da)	p*I*[b]	Accession NCBInr
362	Serum albumin	69 366	6.05	gi|28590
	Igα$_1$	73 377	5.14	gi|229585
363	Serum albumin	69 366	6.05	gi|28590
	Igα$_1$	73 377	5.15	gi|229585
364	Igα$_1$	73 377	5.14	gi|229v585
365	Serum albumin	69 366	6.05	gi|28590
	Igα$_1$	73 377	5.14	gi|229585
366	Serum albumin	69 366	6.05	gi|28590
	Igα$_1$	73 377	5.14	gi|229585
367	Serum albumin	69 366	6.05	gi|28590
	Igα$_1$	73 377	5.14	gi|229585
368	Hemopexin	51 676	6.55	gi|11321561
	Igα$_1$	73 377	5.14	gi|229585
369	Hemopexin	51 676	6.55	gi|11321561
	Igα$_1$	73 377	5.14	gi|229585
370	Igα$_1$	73 377	5.14	gi|229585
371	Igα$_1$	73 377	5.14	gi|229585
372	Serum albumin	69 366	6.05	gi|28590
	Igα$_1$	73 377	5.14	gi|229585
373	Igα$_1$	73 377	5.14	gi|229585
374	Igα$_1$	73 377	5.14	gi|229585
375	Igα$_1$	73 377	5.14	gi|229585
376	Igα$_1$	73 377	5.14	gi|229585
377	Serum albumin	69 366	6.05	gi|28590
378	Serum albumin	69 366	6.05	gi|28590
379	Serum albumin	69 366	6.05	gi|28590
380	Serum albumin	69 366	6.05	gi|28590
381	Serum albumin	69 366	6.05	gi|28590
382	Serum albumin	69 366	6.05	gi|28590
383	Serum albumin	69 366	6.05	gi|28590
384	Serum albumin	69 366	6.05	gi|28590
385	Serum albumin	69 366	6.05	gi|28590
386	KIAA1460	150 737	6.51	gi|7959181
	Serum albumin	69 366	6.05	gi|28590
387	Serum albumin	69 366	6.05	gi|28590
388	α$_{1B}$-Glycoprotein	51 941	5.65	gi|112892
	Serum albumin	69 366	6.05	gi|28590

[a] Theoretical mass.
[b] Theoretical p*I*. PMF, peptide mass fingerprint.

2.2.2. SDS–polyacrylamide gel electrophoresis (second dimension)

The defrosted IPG strips are successively incubated in equilibration buffer (30% glycerin, 6 *M* urea, 2% SDS, 0,05 *M* Tris base pH 8.8) first containing 10 mg/ml DTT and then 42.5 mg/ml IAA for 20 min, respectively. All gels are of a total acrylamide concentration of 11% with 2.6% cross-linker. The separation is carried out vertically in an ISO-DALT chamber (Hoefer, Germany) according to the manufacturer's instructions. Protein transfer from the first to the second dimension is achieved by running a constant current of 50 mA for 30 min. Electrophoresis is carried out at 10 °C by a constant current of 300 mA for 5 h. Analytical gels are silver stained, micropreparative gels are stained with Coomassie G 250 [9,10].

2.3. In-gel digestion for MALDI-MS and μHPLC

Proteins spots are cut out of the 2D gel and the gel pieces are washed for 10 min in digestion buffer (10

m*M* NH_4OAc) and digestion buffer–acetonitrile (1:1), respectively. These steps are repeated three times. A final washing step with acetonitrile leads to a shrinking of the gel piece. It is reswollen with 2 μl protease solution (trypsin at 0.05 μg/μl in digestion buffer). Digestion is performed overnight at 37 °C.

2.4. MALDI-MS

2.4.1. Sample preparation for MALDI-MS

The next morning 10 μl fresh digestion buffer are added to the gel piece and incubated for 30 min in a sonification bath. The supernatant is collected and concentrated using ZipTip pipette tips (Millipore) according to the manufacturer's instructions. The samples are mixed on the MALDI target with a saturated matrix solution of 4-hydroxy-α-cinnamic acid in 0.1% TFA–acetonitrile (1:1, v/v).

2.4.2. MALDI-mass spectrometry

MALDI-MS is done using a Reflex III equipped with a SCOUT 384 ion source (Bruker Daltonik, Bremen, Germany). The acceleration voltage is set to 20 kV, the reflection voltage is set to 21.6 kV. PSD spectra are obtained with a parent ion selection of±30 Da. The reflection voltage is reduced in 14 steps from 21.6 to 0.65 kV. Data acquisition is done on a SUN Ultra using XACQ software, version 4.0. Postanalysis data processing is carried out using XMASS software, version 5.0.

2.5. Nano-HPLC

2.5.1. Sample preparation for nano-HPLC

Fifteen μl 5% formic acid buffer are added to the gel piece and incubated for 15 min in a sonification bath. The supernatant is used for the nano-HPLC autosampler and preconcentration is done with a 0.3×1 mm C_{18}-Pepmap column (LC-Packings, Amsterdam)

2.5.2. Peptide separation

Digestion mixtures are separated using a nano-HPLC equipped with a solvent delivery system containing an inert Ultimate pump (LC-Packings, Amsterdam) running at a column flow-rate of 150 nl/min. Nano-HPLC is performed on a 75 μm I.D.× 250 mm column (LC Packings) packed with reversed-phase material (C_{18}-PepMap, 300 Å pore size, 3 μm particle size). The gradient (solution A, 0.1% FA versus solution B, 0.1% FA–84% acetonitrile) starts at 0% solution B and rises to 50% solution B in 45 min.

Detection is carried out using a dual channel UV detector (LC-Packings) equipped with a 3 nl flow cell (LC Packings) at wavelengths of 215 nm (peptide bond) and 295 nm (tryptophan sidechains).

2.6. Peptide mass fingerprint database search

Monoisotopic peptide masses obtained from in-gel digestion are used for searching the NCBI nonredundant protein database with ProFound [11] (http://prowl.rockefeller.edu/cgi-bin/ProFound). The standard parameters are as follows: Species, mammals; molecular mass, 5–300 kDa; tryptic digest with a maximum of one missed cleavage site. Peptide masses are assumed to be monoisotopic masses and cysteines are assumed to be carbamidomethylated. The peptide mass tolerance is set to 0.1 Da after internal calibration with two trypsin autoproteolyse peptides at 842,5100 and 2211,1046 Da.

2.7. Interpretation of MALDI-PSD and ESI-MS–MS spectra

For the automatic database search of fragment-ion spectra the SEQUEST algorithm (version 27) [12–14] is used. For all searches the complete actual OWL and dbEST databases (ftp://ncbi.nlm.nih.gov/repository) are screened. The search parameters are set as follows: mass tolerance of 0.2 Da for the parent ion, 0.5 Da for fragment ions, tryptic digest, oxidation of methionine and carbamidomethylation of cysteine as differential modifications. The weighting of the different ion series is as follows: neutral loss ions of A- and B-series 1, b- and y-ions 1, and a-ions 0.5.

We commonly take into consideration various posttranslational modifications: N-terminal acetylation, deamidation of N-terminal glutamine to pyroglutamic acid, phosphorylation of serine, threonine and tyrosine, acrylamidation of cysteine, carbamidomethylation of cysteine, deamidation of internal glutamine and asparagine, oxidation of methionine [15].

3. Results and discussions

3.1. 2D-PAGE of human cerebrospinal fluid

The major challenge of 2D-PAGE of human CSF was the prior removal of all disturbing components without loss of protein. We tested both precipitation of all proteins with 80% acetone at −20 °C overnight and ultrafiltration using non-adhesive membranes with a cut-off mass of 5000 Da for sample concentration and salt removal. Though both techniques were successful, ultrafiltration showed better protein yields under the described conditions (Fig. 1). However, prefocusing prior to isoelectric focusing was essential in all cases. As described above, the pH ranges 3–10, 4–7 and 6–11 were used for the 2D-PAGE separations. Therefore the composition of the respective focussing buffer systems was individually optimized with regard to the content of urea, thiourea, non-ionic detergents and reducing agent. In Fig. 2 the influence of the urea–thiourea ratio is demonstrated for the pH ranges 3–10, 4–7 and 6–11. The addition of thiourea enhances the protein solubilisation in the pH ranges 3–10 and 4–7 but perturbs at pH 6–11. As for non-ionic detergents, CHAPS, Nonidet P40 and Triton X-100 in different concentrations were tested. Isolectric focusing in the presence of 2% CHAPS lead to the best protein resolution during 2D-PAGE for the pH range 4–7 (Fig. 3) as well as for pH 3–10 and pH 6–11 (data not shown). Further on, we compared the effect of tributyl phosphine (TBP) and DTT as reducing agents during isoelectric focusing. As shown in Fig. 4 DTT resulted in higher resolution in both narrow pH ranges. Fig. 5 demonstrates the impact of DTT concentration in the rehydration buffer for the pH ranges 4–7 and 6–11. However, cerebrospinal fluid requires very high concentration of DTT for proper protein separation especially in the upper pH range.

Even though higher protein application in 2D-PAGE promises easier protein identification afterwards, overloading of gels particularly in the first dimension must be prevented. Therefore we commonly use three times more material for micropreparative gels (300 μg of total protein) than for analytical gels (100 μg of total protein) which still resulted in high resolution of proteins (data not shown).

3.2. Identification of proteins from 2D gel spots

3.2.1. Identification of proteins using MALDI mass fingerprints

MALDI mass fingerprint is a highly sensitive method for the identification of proteins from polyacrylamide gels. Sample preparation, especially the

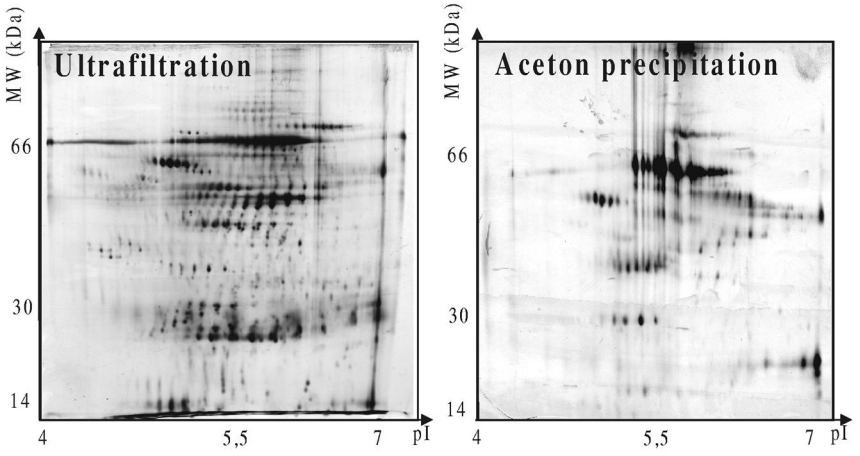

Fig. 1. Removal of disturbing components and concentration of human liquor. Left: preparation of 100 μg protein using ultrafiltration. Right: preparation of 100 μg protein using acetone precipitation. For each preparation human liquor derived from the same donor was used. Proteins are separated in the p*I* range 4–7, detection was done using silver staining. Protein recovery and protein resolution show better results using ultrafiltration.

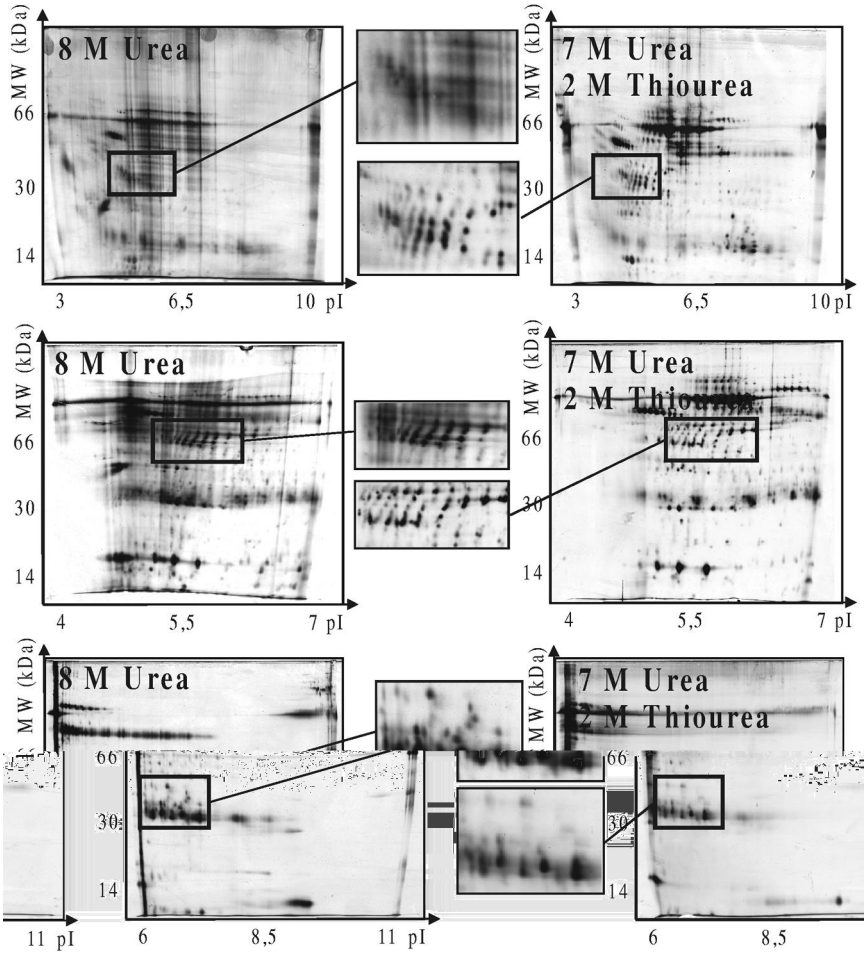

Fig. 2. Solubilization effect of thiourea. The addition of thiourea to the rehydration buffer enhances isoelectric focussing in p*I* ranges 3–10 and 4–7. In the p*I* range 6–11 the use of thiourea worsened the isoelectric focussing. The enhancement of solubilization is demonstrated by the decrease of stripes in the 2D gel. For each gel 100 μg of protein were used. Isoelectric focussing was done in 8 *M* urea, 2% CHAPS, 2% carrier ampholines and 15 m*M* DTT (left) or 7 *M* urea, 2 *M* thiourea, 2% CHAPS, 2% carrier ampholines and 15 m*M* DTT (right).

digestion and peptide extraction steps are critical for a successful result. MALDI mass spectrometry shows sensitivity in the upper attomole to the low femtomole range. For the identification of proteins we used colloidal Coomassie-stained protein spots from micropreparative 2D gels. From each visible spot a MALDI mass fingerprint spectrum could be recorded and with nearly 80% of the obtained mass spectra a successful database search was possible. An exemplary mass fingerprint derived from spot 114 (see Fig. 9) is shown in Fig. 6. The database search with ProFound lead clearly to the identification of apolipoprotein E3.

3.2.2. Identification of proteins using fragment ion spectra

It is a common problem of 2D gels that some spots still consist of more than one protein. The interpretation of MALDI peptide mass fingerprints of mixtures containing peptides of several proteins is quite difficult and often an unequivocal result cannot be achieved. To overcome this problem we used

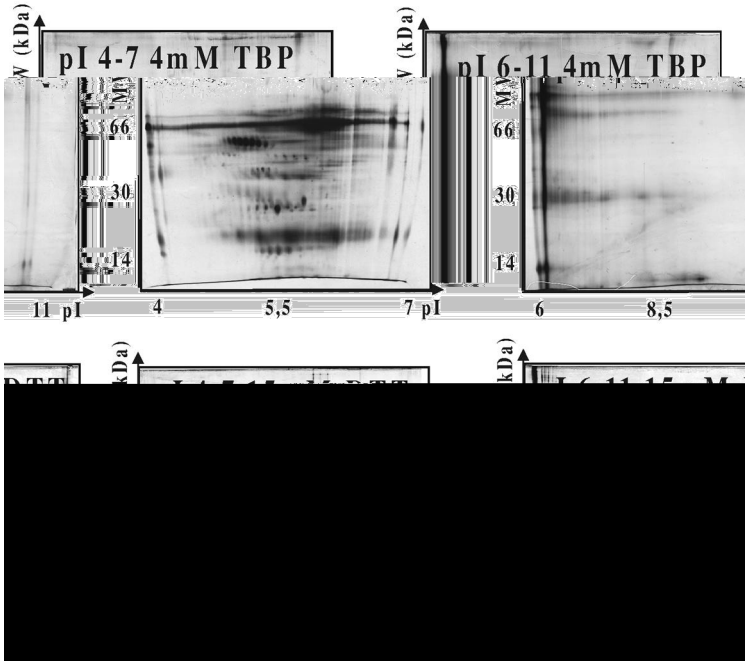

Fig. 4. Effect of the reducing agent in the rehydration buffer. For each gel 100 μg of protein were prepared by ultrafiltration and solubilized in rehydration buffer. Isolectric focussing is done in 7 *M* urea, 2 *M* thiourea, 2% CHAPS, 2% carrier ampholines for the p*I* range 4–7 and 8 *M* urea, 2% CHAPS, 2% carrier ampholines for the p*I* range 6–11. For the upper gels 4 m*M* TBP was used instead of 15 m*M* DTT. Isoelectric focussing show a much more better resolution when DTT is used.

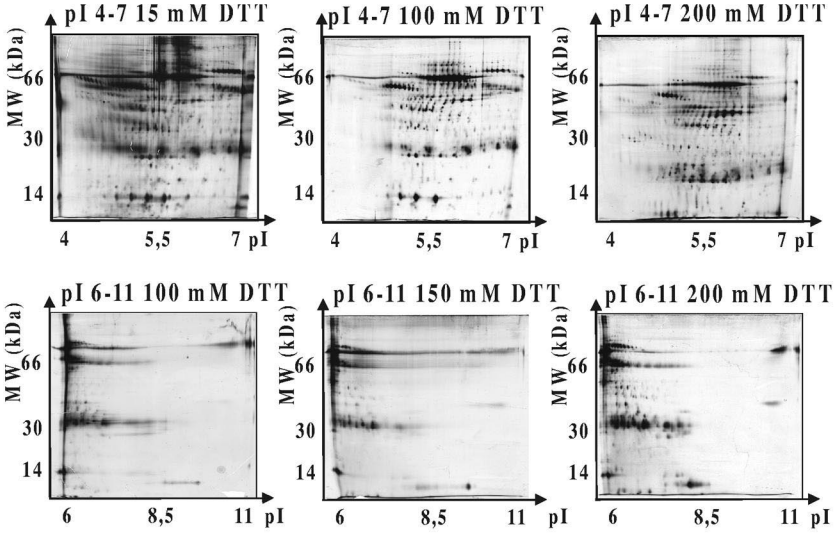

Fig. 5. Effect of DTT concentration during isoelectric focussing. Above: isoelectric focussing was done in 7 *M* urea, 2 *M* thiourea, 2 *M* CHAPS, 2% carrier ampholines. The DTT concentration was changes between 15 m*M* DTT (left) and 200 m*M* DTT (right). The best resolution was achieved using 100 m*M* DTT. Bottom: isoelectric focussing was done in 8 *M* urea, 2 *M* CHAPS, 2% carrier ampholines. The DTT concentration was changes between 100 m*M* DTT (left) and 200 m*M* DTT (right). The best resolution was achieved using 200 m*M* DTT.

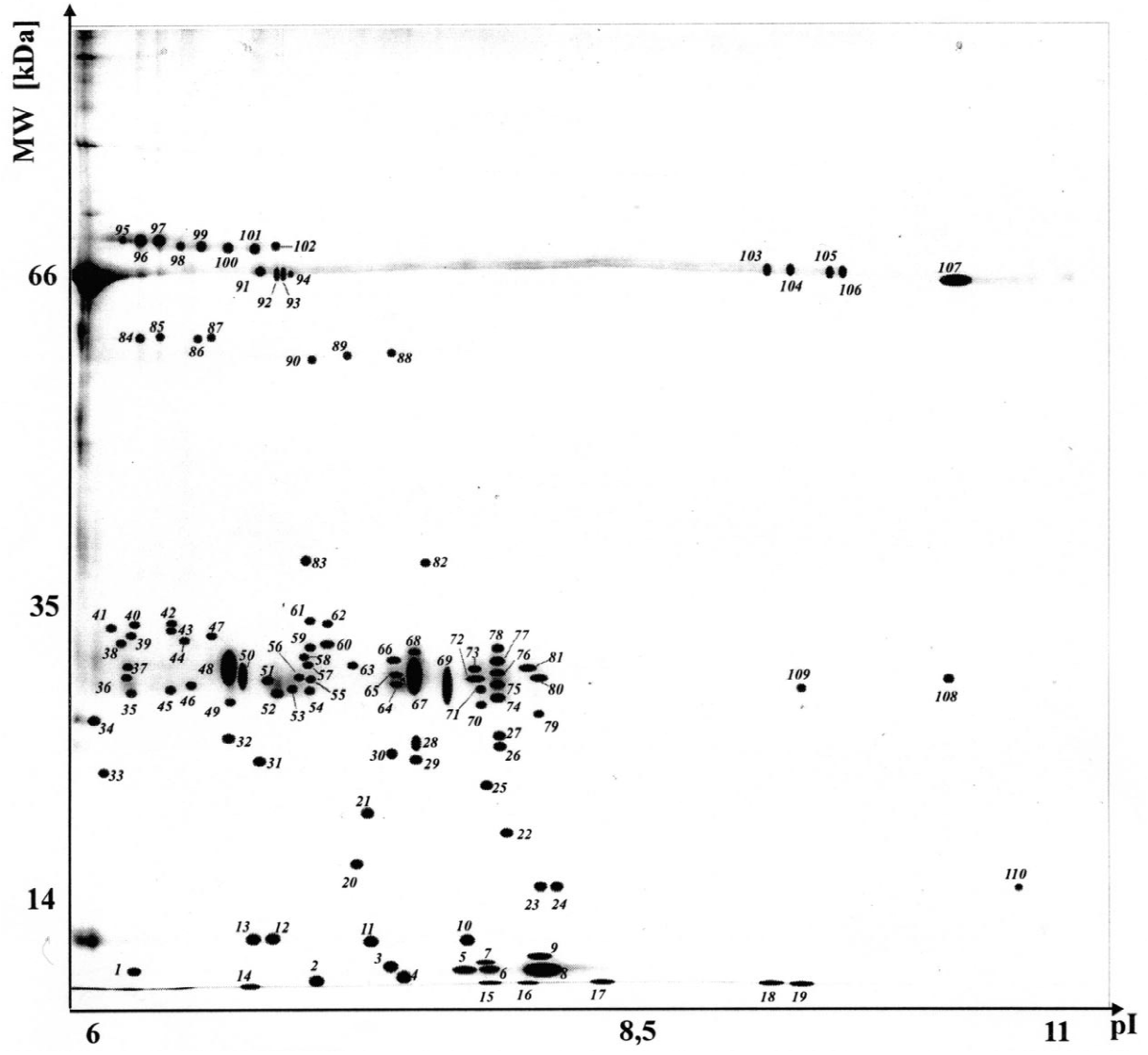

Fig. 10. 2D gel of human cerebrospinal fluid in the p*I* range 6–11. Altogether 110 protein spots are punched out and analyzed. A summary of all identified proteins is given in Table 2.

problem of characterization of post-translational modifications in ''pearl necklet'' spot groups.

As the pool of CSFs taken from various patients without any neurological symptoms represents the average protein composition of human cerebrospinal fluid it should be of great use for comparison studies of patients suffering from brain or CNS diseases.

5. Nomenclature

CHAPS	3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate
CSF	cerebrospinal fluid
DTT	dithiothreitol
ESI	electrospray ionization

Table 2
Summary of all identified proteins in human liquor (p*I* range 6–11)

No.	Protein	Mass	p*I*	Accession NCBInr
1	KIAA0191 protein	171 113	7.71	gi|1228035
2	Ig λ chain V–VI	11 966	4.64	gi|126575
3	Cystatin C	15 799	9.00	gi|4503107
4	Cystatin C	15 799	9.00	gi|4503107
5	Cystatin C	15 799	9.00	gi|4503107
6	Cystatin C	15 799	9.00	gi|4503107
7	Cystatin C	15 799	9.00	gi|4503107
8	Cystatin C	15 799	9.00	gi|4503107
9	Transthyretin	15 887	5.52	gi|4507725
10	Cystatin C	15 799	9.00	gi|4503107
11	Transthyretin	15 887	5.52	gi|4507725
12	Transthyretin	15 887	5.52	gi|4507725
13	Transthyretin	15 887	5.52	gi|4507725
14	β_2-Microglobulin	12 799	5.77	gi|34616
15	Ig κ chain C region	10 997	6.60	gi|106529
16	Cystatin C	15 799	9.00	gi|4503107
17	Hemoglobin α2	15 257	8.72	gi|4504345
	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
	Ig κ chain C region	10 997	6.60	gi|106529
18	Transthyretin	15 887	5.52	gi|4507725
19	Complement component 3 precursor	187 164	6.02	gi|4557385
20	–	–	–	–
21	–	–	–	–
22	Prostaglandin D2 synthase	21 029	7.65	gi|4506251
23	–	–	–	–
24	KIAA191 protein	171 113	7.71	gi|n