

Journal of Chromatography B, 776 (2002) 89-100

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Catalogue of soluble proteins in the human vitreous humor: comparison between diabetic retinopathy and macular hole

Toyofumi Nakanishi^a, Reiko Koyama^b, Tsunehiko Ikeda^b, Akira Shimizu^{a,*}

^aDepartment of Clinical Pathology, Osaka Medical College, 2–7 Daigakucho, Takatsuki, Osaka 569-8686, Japan ^bDepartment of Ophthalmology, Osaka Medical College, 2–7 Daigakucho, Takatsuki, Osaka 569-8686, Japan

Abstract

Two-dimensional gel electrophoresis and mass spectrometry were used to make a catalogue of soluble proteins in the human vitreous humor (VH). Fifty-one different proteins were identified on silver-stained two-dimensional (2D) gel patterns with VH proteins obtained from diabetic retinopathy and macular hole. Thirty of these have not been listed in the reported 2D profiles of plasma. Immunoglobulin (Ig), α 1-antitrypsin, α 2-HS glycoprotein, and complement C₄ fragment showed stronger spots in VH with diabetic retinopathy patient samples than those with macular hole. Pigment epithelium-derived factor, a potent inhibitor of angiogenesis in the cornea and vitreous, was clearly detected in VH with diabetes. It is impressive that the inhibitor increases in the vitreous with proliferative angiogenesis. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Human vitreous humor; Diabetic retinopathy; Macular hole; Proteins

1. Introduction

The analysis of soluble protein profiles in the vitreous humor (VH) may elucidate the pathogenesis of various retinopathies, especially those accompanied by blood vessel growth into the vitreous. In diseases such as retinopathy of prematurity and proliferative diabetic retinopathy, the main causes of visual loss are the development of new blood vessels at the junction of vascularized tissue and the avascular retina, and vascular growth into the vitreous, resulting in hemorrhage and retinal detachment [1–3]. In such diseases, the production of angiogenic and antiangiogenic factors by retinal cells may

change, and consequently, the concentration of these factors in the VH may also change [4-6]. Since Raymond and Jacobson [7] indicated an inhibitory factor of angiogenesis presented in an extract of bovine VH, a number of researchers have analyzed VH proteins from the eyes of animals [8,9]. However, there have been no reports of experiments in humans, probably because of the difficulty of sample collection and the limitations of detection by conventional protein analysis techniques. Modern mass spectrometry assisted by complete genome information will play an essential role in the study of proteins because of its sensitivity, resolution, and ability to analyze complex mixtures of molecules quickly. The combination of protein separation by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) with mass spectrometric analysis of proteins digested enzymatically in-gel, followed by protein database searching is a very efficient tool for

^{*}Corresponding author. Tel.: +81-726-84-6448; fax: +81-726-84-6548.

E-mail address: shimizu@poh.osaka-med.ac.jp (A. Shimizu).

protein identification in complex biological systems [10,11].

In this study, we began assembling a catalogue of proteins expressed in human VH using 2D-PAGE and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI-TOF-MS), and electrospray ionization (ESI) ion-trap tandem mass spectrometry (MS–MS). We compared the expressed proteins in VH between diabetic retinopathy and macular hole. Diabetic retinopathy accompanies abnormally high vascular growth, and macular hole does not.

2. Materials and methods

2.1. Sample preparation, 2D-PAGE, image analysis and in-gel digestion

The vitreous humors (VHs) were obtained from three patients with diabetic retinopathies and two patients with macular hole. The VHs were dialyzed with distilled water to remove salt using Biodialyzer[™] (membrane: B010K; Cypress, Tokyo), which can remove molecules smaller than 1000 Da. About 500 µl VH were dialyzed overnight at 4 °C with two changes of 31 distilled water. The solution was freeze-dried under a vacuum. One hundred µg of protein, which was determined by the Lowry method, were solubilized in the rehydration buffer (8 M urea, 2.0% NP-40, 30 mM dithiothreitol (DTT), 2.0% immobilized pH gradient (IPG) buffer 3-10 NL) and the samples were stored at 4 °C for 12 h. For first-dimension gel electrophoresis, isoelectric focusing (IEF) was performed using a Pharmacia Multiphor II apparatus according to the manufacturer's instructions as modified as by Arnott et al. [10]. IPG gel strips (18 cm, non-linear pH 3-10, Amersham-Pharmacia, Sweden) were rehydrated overnight in a cassette with standard rehydration buffer (described above), and aligned on the IEF tray. Samples were loaded adjacent to the anode, and the voltage was linearly increased from 300 to 3500 V over 4 h, followed by 4 h at 3500 V. A total volt hour product of 15 kVh was used.

After IEF, the IPG gel strip was equilibrated with 5 ml of 50 m*M* Tris–HCl, pH 8.8, 6 *M* urea, 2% sodium dodecyl sulfate (SDS), 30% glycerol and 100 m*M* DTT for 20 min.

Second-dimensional electrophoresis was carried out at a constant current of 10 mA per gel for 30 min, and 20 mA per gel until the tracking dye reached the cathode.

After removing the carrier ampholytes and fixation, the gel was visualized by 1% Coomassie brilliant blue (CBB) staining for 30 min or nonglutaraldehyde silver staining with 0.02% sodium thiosulfate for 20 min at 4 °C. The images of the stained gels were studied with ImegaMaster 2D Elite software (Amersham-Pharmacia). The CBB and silver-stained gels were digitized and the gels were matched using a constellation-matching algorithm whereby each spot was located according to its spatial relation to a defined number of nearest-neighbor spots. The matched spots were relatively quantified by adding the intensities of all pixels against that of spot number 61 containing apolipoprotein A-I, which was selected because spots found in 2D-PAGE for both diabetic retinopathy and macular hole were of similar intensity. All visible spots were numbered arbitrarily.

Protein spots were excised from the gel and placed in 1.5-ml microtubes (AMR, Tokyo, Japan). The gel slices were washed by shaking for 30 min in 25 µl of 50% methanol and then dried under a vacuum. Disulfide bonds were reduced with 50 mM DTT in 50 mM ammonium bicarbonate (pH 8.5) by incubation for 1 h at 56 °C and alkylated with 100 mM iodoacetoamide in the same buffer for 45 min in the dark at room temperature. Excess reagents were removed and the gel slices were washed twice. After the buffer was discarded, the gel pieces were dehydrated with 100% acetonitrile and then dried by vacuum centrifugation. The gel pieces were then re-constituted in 10 µl of digestion buffer containing 250 ng TPCK modified trypsin (Promega, Madison, WI) at 37 °C for 18 h. The peptide solution was recovered and the gel pieces were extracted with 30 µl of digestion buffer and 100% acetonitrile. The combined solution was concentrated, resolved with 0.1% trifluoroacetic acid (TFA) and stored frozen until use.

VH proteins were also initially isolated using ionexchange (PL-SAX; Cypress) column chromatography. The buffer system was a linear gradient of buffer A mixed with buffer B from 1% B to 99% B in 40 min. Buffer A was 0.01 *M* Tris-hydrochloride, pH adjusted to 7.4. Buffer B was 0.01 *M* Trishydrochloride, 0.4 M NaCl, pH adjusted to 7.4. The flow-rate was 1.0 ml/min. The freeze-dried proteins from each fraction were identified in the same way as described for proteins from 2D-PAGE spots.

2.2. Mass spectrometric identification

MALDI-TOF mass spectra were acquired on a Voyager DE-PRO MALDI-TOF mass spectrometer (PE Biosystems, Framingham, MS) equipped with a delayed extraction source and a 337-nm pulsed nitrogen laser. For the sample matrix, α -cyano-4-hydroxycinnamic acid was dissolved in a solution containing 50% acetonitrile and 0.1% TFA at a concentration of 10 g/l.

ESI-MS–MS experiments were performed with a LCQ^{DECA} (ThermoQuest, San Jose, CA, USA) equipped with a Monitor C₁₈ column (0.2×50 mm). The solvent system for on-line reversed-phase liquid chromatography was a linear gradient of solvent A mixed with solvent B from 5% B to 60% B in 40 min. Solvent A was 0.1% formic acid and solvent B was 0.1% formic acid in acetonitrile. The flow-rate was 1–2 μ l/min. A collision energy of 28–35 eV, depending on the charge state of the daughter ions, was applied; the gas pressure in the collision cell was regulated to 6.0×10^{-5} mbar. Protein identification was performed via a peptide mass, collision-induced dissociation (CID) mass spectra database using MS-fit and MS-tag (SwissProt).

3. Results and discussion

3.1. 2D-PAGE of VH derived from diabetic retinopathy patients and macular hole

Fig. 1a,b shows silver-stained 2D gel electrophoresis patterns of VH proteins derived from a patient with diabetic retinopathy (a) and from a patient with macular hole (b). We repeated the 2D-PAGE with the same materials, and with materials obtained from three patients with diabetic retinopathy and two with macular hole. The profiles were reproducible in the same materials and were similar among materials from the same disease. The mass spectrometric analysis and database search of 412 spots expressed in VH derived from a patient with diabetic retinopathy (Fig. 1a) allowed us to characterize 113 spots. However, a large number of the spots were isoforms of one another. The number of different protein species identified was 50 by the analysis shown in Fig. 1a. These are listed in Table 1. By 2D gel from another diabetic patient, additionally aquapolin-CHIP (peptide sequence. NDLADLLDIDYNHWIFW 5-21 was assigned by ESI-MS-MS, and the observed pI and MW coincided with those reported, pI, 7.17; MW, 9723.4) ATP synthase and vacuolar subunit E (IKVLQAQDDLVNAMKEAASK 79-98; pI, 6.00; MW, 26 128.4) were identified. The proteins identified by 2D-PAGE with VH were 52 in total. The molecular mass and pl observed for most proteins were close to the theoretical values based on a database, SwissProt. From spot no. 48 (pI, 4.2; MW, 34 600), transthyretin (TTR) peptides were detected clearly. However, the pI and molecular mass of the spot did not coincide with those of TTR. A molecule in spot no. 48 is probably a covalent polymer of a TTR fragment, or a molecule formed by a disulfide bridge between TTR and another unknown protein due to inappropriate reducing.

Most of these spots were also found in the profiles of patients with macular hole. Some spots found in VH samples corresponded to major plasma proteins, which were reported by 2D-PAGE [12]. These are albumin, α_1 -antitrypsin, α_2 -HS glycoprotein, transferrin, haptoglobin α_1 - and α_2 -chains, complement C₄ fragment, Gc globulin, apolipoprotein A-I, immunoglobulin (Ig) heavy and light chains, hemoglobin (Hb) α - and β -chains and transthyretin, and so on. The relative amount of the major proteins to the levels of albumin in VH was markedly different from those of plasma. For example, the ratios of transthyretin to albumin and of transferrin to albumin were ca. 1:10 and 1:3 in VH, although the ratios are ca. 1:100 and ca. 1:15 in plasma, respectively. Although Hb chains were detected in VH, spots of coagulation factors or orosomucoid were very faint or not visible. These data support the idea that plasma proteins revealed on the 2D-PAGE with VH were not contaminated by plasma during sampling, but systematically transferred through a specific blood-retina barrier in vivo.

We also isolated VH proteins from a patient with diabetic retinopathy using ion-exchange column chromatography, and identified five VH-specific proteins by ESI-MS-MS analysis with tryptic peptides.



Fig. 1. Silver-stained gel image of proteins in VH derived from a patient with diabetic retinopathy (a) and a patient with macular hole (b). The protein load was 100 μ g on the first-dimensional IEF gel. The positions of molecular mass markers are shown on the left and isoelectric points are shown at the top in the gels.

These were DNA binding protein (sequence assigned peptide: SSSPYSKSPVSK; 146–157), aquapolin-CHIP (NDLADLLDIDYNHWIFW; 5–21), glial fibrillary acidic protein (LALDIEIATYR; 356–367), thyroid receptor interaction protein (HPCDCLGQKHK; 45–55) and uracil-DNA glycosylase (QWMLANIADNK; 5–15). The proteins identified by 2D-PAGE and column chromatography with VH were 56 in total.

The stained spots shown in Fig. 1a,b were relatively quantified, and proteins, α_1 -antitrypsin, α_2 -HS glycoprotein, complement C₄ fragment, immunoglobulin (Ig) heavy and light chains, showed stronger spots in VH with diabetic retinopathy patient samples than those with macular hole. A few unidentified spots clearly showed stronger spots in VH with diabetic retinopathy patient samples than those with macular hole, and vice versa.

3.2. VH-specific proteins

By comparing the 2D-PAGE profiles obtained from VH protein with those of plasma reported in literature, many spots were detected in only VH, but



Fig. 1. (continued)

not in plasma. The specific proteins in VH were located at isoelectric points between 5.0 and 9.0, and at molecular weights between 20 and 65 kDa. This was a relatively smaller region with higher p*I*. The VH-specific proteins (29 proteins) identified are shown in Table 1. The five peptides detected by preliminary experiment using an ion-exchange column were also not reported in plasma [13]. In total,

35 VH-specific proteins were recognized in the present work.

Cystatin C (spot no. 336) spot was relatively strong. This protein is a mammalian cysteine protease inhibitor, synthesized in various amounts by many kinds of cells and appearing in most body fluids [14]. The concentration of human cystatin C is markedly higher in cerebrospinal fluid than in blood

Table 1								
Peptide sequences	of proteins	contained i	n VH as	determined	by	mass	spectrometr	y

Spot no.	Protein	Reported in plasma ^a	MW ^b	pI ^b	[M+H]+ ^c	Residue	Identified peptides, sequence from database	MW^d	pI ^d
6–9	Transferrin	+	76 981.3	6.70	1249.6	454– 464	SASDLTWDNLK	74.1-77.0	5.9–6.7
160-162					1276.6	300-310	EFQLFSSPHGK		
184-187	Fatty acid coenzyme A ligase5	-	72 196.2	6.45	1479.0	84-97	GLAVSDNGPCLGYR		
387,388					2186.1	379-398	VRVIVTGAAPMSTSVMTFFR		
					2564.0	167-188	GFTPSLKVIILMDPFDDDLKQR		
	SnoN2	-	71 795.8	6.57	2384.6	561-579	LEQIMKQKCTCDSNLEKDK		
					2707.1	594-615	LDHAEADROELODELROEREAR		
					3339.2	507-534	OOLOMEVKMLSSSKSMKELTEEOONLOK		
9	ETF-ubiquinone oxidoreductase	-	68 564.5	7.17	1211.9	255-263	QLYKKFDLR	75.0	6.7
	1				1429.6	345-357	HHPSIRPTLEGGK		
					1451.7	264-276	ANCEPOTYGIGLK		
11	SP100-B	_	78 145.0	7.81	1274.4	88-97	MFEDSODSCR	73.7-74.1	6.9-7.3
189 190	51100 2		10 1 1010	/.01	1479.0	70-81	KTEPELEGLEDR	/01/ / 111	017 710
10),170					1833.9	431-446	APMTSRSTSTWRIPSR		
					2096.9	88-104	MEEDSODSCENI VPVOR		
					2563.1	506-528	TESSOASDMMDTMDVENNSTI FK		
	Angiotensin-converting enzyme	+	79 334 5	7.41	1314.5	1_10	MI NI CEMI TR		
	Auglotensin-converting enzyme	1	17 554.5	7.41	2058.8	05 111	KEDVNOI ONTTIKRIIK		
					2006.0	148 463	IA FIDESVI VDOWDWD		
					2090.9	61 03	NVNTNITTETSKII LOKNMOJANHTI KVGTOAR		
10	Nuclear receptor subfamily 1	_	54 400 8	6 11	18123	442 455	TENHHHAEMI MSWP	61.2	64
19	Nuclear receptor subraining 1		54 409.8	0.11	1012.5	180 204	EMGMI AECI I TEIOCK	01.2	0.4
					22274 7	01 110	MDAETI VOGETEVAEMDVTK		
					2224.7	252 270			
					2203.0	271 205			
20	Drusteenhin (uteenhin accorded		55 660 9	6.00	2600.7	3/1-393	SIDELNIT QEE TALL TATVILSPDK	50.2	5.2
29	Dystrophin/utrophin-associated	-	33 009.8	0.00	1507 4	2/0-20/		30.2	3.5
	protein				1307.4	13-90			
	Dismont onithalium donived factor		16 206 2	6.00	2020.2 1251.6	150-105			
	Fightent epithenum-derived factor	-	40 380.5	0.00	1201.0	400-411			
					1564.7	534-545			
					1005.6	54-0/ 109 214			
					1057.2	196-214			
					1957.5	107-125			
25			10 1 10 1	5 40	2090.6	263-281	IAQLPLIGSMSIIFFLPLK	17.0	5.6
33	Guanine nucleotide binding protein	-	42 145.4	5.48	11/9.8	93-102	AMDILKIPIK	47.9	5.0
					1320.7	61-72	IIHGSGYSDEDK		
					1493.9	108-120	AHAQLVREVDVEK		
			12 20 1 0	<i></i>	2626.9	93-114	AMDILKIPYKYEHNKAHAQLVK		
	Apolipoprotein A-IV	+	43 384.8	5.22	1708.1	245-259	QRLAPLAEDVRGNLR		
					2045.7	74–90	DSEKLKEEIGKELEELR		
					2705.7	285-306	RRVEPYGENFNKALVQQMEQLR		
48	Transthyretin	+	15 859.2	5.10	1366.7	42–54	GSPAINVAVHVFR	34.6	4.2
					1522.7	55-68	KAADDTWEPFASGK		
					2451.2	81-103	ALGISPHEHAEVVFTANDSGPR		
					2489.3	69–96	TSESGELHGLTTEEEFVEGIYVEIDTK		
61	S100 calcium-binding protein	-	13 229.0	6.00	1456.6	26-38	LGHPDTLNQGEFK	27.0	5.2
					1808.0	11-25	NIETIINTFHQYSVK		
					2177.4	94-114	MHEGDEGPGHHHKPGLGEGTP		
	Glutathione S-transferase	+	26 689.0	5.37	1744.5	1-15	SCESSMVLGYWDIR		
					1995.4	83-98	YIARKHNMCGETEEEK		
					3312.5	198-225	IAAYLQSDQFCKMPINNKMAQWGNKPVC		

Table	1.	Continued

Spot no.	Protein	Reported in plasma ^a	MW ^b	pI ^b	[M+H]+ ^c	Residue	Identified peptides, sequence from database	MW ^d	pI ^d
	Apolipoprotein A-I	+	30 726.3	5.30	1235.7	13-23	DLATVYVDVLK		
					1386.7	251-262	VSFLSALEEYTK		
62	Phosphoglycerate mutase	-	28 766.4	8.99	1046.1	34-43	GTEEAKRGAK	26.7	7.8
					1488.3	22-33	FCGWFDAELSEK		
					1544.3	117-129	PSFDIPPPPMDEK		
					1835.8	47-61	DAKMEFDICYTSVLK		
					2240.5	158-176	DTIARALPFWNEEIVPQIK		
	Syntaxin 5	-	34 086.4	9.00	1012.0	129-137	LASMSNDFK		
					1152.9	188-197	DVAIDMMDSR		
					1708.5	33-46	AVRQRSEFTLMAKR		
					1943.3	138-153	SVLEVRTENLKQQRSR		
	Fibroblast growth factor-23	+	27 9541.1	9.17	1813.0	100-114	GNIFGSHYFDPENCR		
	0				2211.8	162-179	NEIPLIHFNTPIPRRHTR		
					2665.1	1-23	LGARLRWVCALCSVCSMSVLR		
63	GOS28/P28 protein	_	28 966.3	9.36	1298.6	40-50	LCTSYSHSSTR	27.0	8.1
	1				1640.9	217-230	FPAVNSLIORINLR		
					1795.4	71-85	MFETMAIEIEQLLAR		
					1925.8	217-232	FPAVNSLIORINLRKR		
85	Prostaglandin D2 synthase	+	21 085.8	8.33	1582.6	43-56	WFSAGLASNSSWLR	17.7	7.5
	0 ,				1745.3	93-108	TMLLOPAGSLGSYSYR		
					1911.4	169-185	AOGFTEDTIVFLPOTDK		
					1919.8	67-85	SVVAPATDGGLNLTSTFLR		
111	Apoptosis inhibitor hiap-2	_	69 922.4	6.27	947.9	442-448	REEEKEK	69.1	5.7
	1 1 1 1 1 1 1				960.8	234-241	DDAMSEHR		
					1728.7	50-65	MSTYSTFPAGVPVSER		
					2129.6	242-258	RHEPNCPELENSLETLR		
	Albumin	+	69 227 2	5 99	927.7	162-168	YLYEIAR		
	, nounni		07 22712	0.77	947.9	222-229	LKCASLOK		
					960.8	427-434	FONALLVR		
					1468 3	361-372	RHPDYSVVLLLR		
					1925.4	589-603	ETCEAEEPTMRIRER		
112	Albumin	+	69 367 4	5.92	927.6	162-168	YLYEIAR	69.4	5.0
				•	960.7	427-434	FONALLVR		
					1450.9	106-117	ETYGEMADCCAK		
					1467.9	361-372	RHPDYSVVLLR		
					1640.2	438-452	KVPOVSTPTLVESR		
					2045.2	397-413	VFDEFKPLVEEPONLIK		
114 115	α -Antitrynsin	+	46 689 0	5 10	1110.6	315-324	I SITGTYDI K	59 9-61 0	47-45
111,115	u] muu ypsii	'	10 007.0	5.10	1333.8	150-160		57.7 01.0	1.7 1.5
					1641.8	50-63	ITPNI AFFAFSI YR		
					2574.3	126-149	TI NOPDSOL OL TTGNGLEL SEGLK		
					2374.5	35 63			
126	Apolipoprotein A IV	+	15 335 1	5 23	1083.5	201 200	I TRVA DEEK	177	57
120	Apolipopiotelli A-1V		45 555.4	5.25	1/188 1	201-209		47.7	5.7
					2045.7	04 110	ALVQQMEQLKQK		
					2043.7	305 206	DOURDENENIKAI VOOMEOLD		
	CCI 190 motoin		41 260 2	5 27	2703.0	303-320			
	COI-160 protein	-	41 209.2	5.57	1640.4	1-10			
					1040.4	1-14			
					1911.3	286 202			
					1923.4	200-302	KUTNAEUKINLI IAEUK		

Table 1. Continued

Spot no.	Protein	Reported in plasma ^a	MW ^b	pI ^b	[M+H]+ ^c	Residue	Identified peptides, sequence from database	MW ^d	pI ^d
163	Protein kinase C	_	57 010.6	5.42	2560.1	434-454	KEIQPPYKPKACGRNAENFDR	59.4	4.8
					2663.1	162-184	LTDFNFLMVLGKGSFGKVMLSER		
					2721.6	5-28	NLVPMDPNGLSDPYVKLKLIPDPK		
					2721.6	76–99	NDFMGSLSFGISELOKASVDGWFK		
	Gc globin	+	52 950.2	5.40	2560.1	66-87	EVVSLTEACCAEGADPDCYDTR		
					3339.8	304-332	TAMDVECTYEMPAAOL PEL PDVEL PTNK		
186	Glycerol-3-phosphate_dehydro-	_	80 834 4	7 23	1210.1	580-588	FI NWDDYKK	76.0	69
100	denase		00 00 111	1.20	1283.8	114-124	LIHGGVRYLOK	7010	0.7
	genuse				1580.3	255 260	KTDPOTGKVHVSGAP		
					1601.2	507 608	KELVVEMGVKSP		
					1641.7	558 572			
105	To heavy shain		51 400 2	7.80	1196.6	122 122	CDEVEDI ADSSV	96.1	7.0
195	ig neavy chain	т	51 409.5	7.80	1100.0	208 211		80.1	7.9
222	6 1 1 1 1 1 1 1		10 626 7	0.60	10/7.8	298-311	FINW I VDGVEVHNAK		
222	Signal recognition particle protein		49 636.7	8.68	1101.3	37-46	ALIQSDVNIK	55.5	7.6
					1133.4	102-112	ILFLGLQGSGK		
	Ig λ heavy chain	+	52 728.2	8.74	1882.2	391-406	EPTSPPFRPCPEPDEK		
					1857.9	477-493	GGLGHPLPELADELRRK		
					2232.9	160-179	LLFAGSRSQLVQLPVADCMK		
					2248.8	160-179	LLFAGSRSQLVQLPVADCMK		
	Unknown protein (Acc. no. 15929862)	-	52 265.7	8.24	1882.2	314-330	GPLNSDRSDYFAAWGAR		
					2248.8	186-203	LHPVLHKEEKQHLERLNK		
					2284.5	91-108	QICGTHRQTKKMFCDMDK		
					2704.8	313-336	RGPLNSDRSDYFAAWGARVFSFGK		
231	ER81 protein	-	55 157.7	6.00	850.9	325-331	EGPTYQR	52.0	6.6
					1037.2	206-213	EGRPMYQR		
					1109.2	380-388	NRPAMNYDK		
240	α-Actinin	_	68 762.8	5.07	948.1	381-388	SIVNYKPK	70.3	4.6
					1712.1	207-220	LI ETIDOL YL EYAK		
					1925.8	1-16	LENRVPENTMHAMOOK		
					2808.4	155_179			
	EVVE finger protein EID1	_	60 078 7	5 63	031.8	103 200	ITDVI DOK		
	1 1 VE-miger protein En 1		07 070.7	5.05	1026.5	201 208	NVVEEI ND		
					1050.5	542 551	N I VEELNK OCEVEESISD		
					1265.1	221 222	QUEREFSISK TEMELANIZH EV		
					1455.4	521-552			
	~				1494.2	521-533	EVNQALKGHAWLK		
	Guanine nucleotide exchange factor	-	66 313.5	5.17	883.7	584-590	LRSHENK		
					1036.5	1–9	MDNLSDTLK		
					1902.4	242-258	EMIFEVLAPLAENDAIK		
	α_{1B} -Glycoprotein	+	51 890.3	5.00	2471.2	453-474	SWVPHTFESELSDPVELLVAES		
254	Lipoprotein Gln I	+	28 346.2	5.27	1012.1	210-218	AKPALEDLR	24.4	4.9
					1226.3	1 - 10	DEPPQSPWDR		
					1301.4	77-87	LNLEKETGELR		
					1380.4	164-174	THLAPYSDELR		
					1723.3	122-136	VEPLRAELQEGARQK		
					1815.1	24-40	DSGRDYVSQFQGSALGK		
269	Lipoprotein Gln I	+	28 346.2	5.27	1012.1	210-218	AKPALEDLR	24.5	5.3
					1226.3	1-10	DEPPQSPWDR		
					1302.3	77-87	LNLEKETGELR		
					1380.4	101-111	VQPYLDDFOKK		
					1815.1	24-40	DSGRDYVSOFOGSALGK		
271	Iо к light chain	+	22,968,0	8 64	1193.6	116-126	TPAWTEGOGTK	24.3	58
-/1	-5		22 700.0	0.01	1838.9	1_16	MDMRVPAOLIGLUUR	21.5	5.0
					2381.2	47_67	ASOSISSVI NWYOOKPOKADK		
					2301.2	+/-0/	ATRAUSICE INTERNET COLOGIC		

Table 1. Continued

272 274Da prosonal prosein - 274555 6.00 1170.3 172-181 0TSTSFLEK 25.3 5.9 273 Ig κ light chain + 229650 8.64 118.05 116-16 DDROTYCQEK 24.0 6.0 273 Ig κ light chain + 229650 8.64 118.0 116-126 TRWTFQQTK 24.0 6.0 1830 116-126 TRWTFQQTK 24.0 6.0 183.0 116-126 TRWTFQQTK 24.0 6.0 1830 116-126 TRWTFQQTK 24.0 6.0 183.0 116-126 TRUTKPQLTK 24.0 6.0 1812 47-67 ASQISSTUNVQQRUKTKAK 25.0 117.0 119-128 FGCVQFLMEK 26.6 5.9 1812 113-117 FACELENSGRWERK 26.6 5.9 118.2 113-117 FACELENSGRWERK 28.1 113-10 115.1 116.1 KTLALENCULEK 28.1 115.0 116.1 KTLALENCULEK 28.3 7.6 276 Indolethylamine, N-methylansferaze - 28.01.9 8.09 106.1 23.24<	Spot no.	Protein	Reported in plasma ^a	MW ^b	pI ^b	[M+H]+ ^c	Residue	Identified peptides, sequence from database	MW ^d	pI ^d
273 Ig × figle chain + 2960 1674 0F1400 0DRAGYNCOFK 2.0 60 1673 01670 106700 000000000000000000000000000000000000	272	27-kDa prosomal protein	-	27 456.5	6.00	1170.3	172-181	QTESTSFLEK	25.3	5.9
13 Is a fight chain 4 2960 164 105-17 YUTEPUDMICKS 240 6.0 18 Hyoshetical protein (Ac: no. 1472652) - 21 45.3 5.05 106.6 93.00 1-16 MUMRVPAQLICALLIR 20 6.0 19 Hyoshetical protein (Ac: no. 1472652) - 21 45.3 5.05 106.6 93.00 INTUPOSK - 7.0 5.00 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00 7.0 10.00						1338.4	154-164	CDPAGYYCGFK		
273 Ig κ light chain + 2980 864 103.6 10-50 TWATTGOORK 20 6.0 1838 1-6 MDNPRVQLCLLLLL<						1678.1	105 - 117	YGYEIPVDMLCKR		
Hamiltonian protein (Acc. no. 1472652) - 21 45.3 5.05 100.5 9-10 KULUNSR - - 5.05 (SUSUMVVQQRKGKAFK 27.6 Indekthylamine Armethyltransfense - 27.7 10-128 GEYQPE MEK 10-128 FECKULEK 26.6 5.9 27.6 Indekthylamine Armethyltransfense - 28.75 5.2 100.24 10-124 FECKULEK 26.6 5.9 27.6 Indekthylamine Armethyltransfense - 28.975 5.2 100.24 10-124 KURSEVNER 26.6 5.9 27.10 Indekthylamine Armethyltransfense - 29.75 7.4 103.3 205-21 CHEHORE 7.6 <td>273</td> <td>Ig к light chain</td> <td>+</td> <td>22 968.0</td> <td>8.64</td> <td>1193.6</td> <td>116-126</td> <td>TPAWTFGQGTK</td> <td>24.0</td> <td>6.0</td>	273	Ig к light chain	+	22 968.0	8.64	1193.6	116-126	TPAWTFGQGTK	24.0	6.0
is a state state is a state is a state state a state is a state is a stat						1838.9	1-16	MDMRVPAQLLGLLLLR		
Hypothetical protein (Acc. no. 1472552) - 21 445.3 5.05 100.10 KTNL/DSR 307 IIo-128 50.91 KTNL/DSR IIO-128 KIN-16708 59 276 Indolethylamine M-methyltransferace - 28 75.2 52.3 IIO2.4 210-218 FEXVLEK 26.6 59 181.21 II-107 FACELEDSORWERK 26.6 59 181.21 II-1317 FACELEDSORWERK 26.6 59 181.21 II-141 MEESQUCEEPMOR 211.3 I06-124 KTTALIAEYKQUSQLSTR 28.3 7.6 296 Liver-specific BHLH-ZIP transcription factor - 28.041.9 80.0 104.12 VITAI VISPOSRSPRAMIK 28.3 7.6 2032 Ig s light chain + 23.018.0 8.00 701.10 III-118 TVARSPSPERDARK 28.3 7.6 238.329 Hemoglobin δ + 16.027.7 7.6 110-127 TVARSPSPERDARK 15.0 7.5 238.49 Hemoglobin δ + 15.07.7						2831.2	47-67	ASQSISSYLNWYQQKPGKAPK		
276 Indeethylamine A-methyltransferate - 28 775 2 98 715 2 98 -01 CERVSORVKEK 26.5 5.9 1970 8 0.802.4 210-218 ERCNSLEWVERK 26.5 5.9 1970 9 113-127 PACUELCONSORVERATION 28.42 19.93 DULATYDENDSPREAMUK 5.9 1970 1 113-127 PACUELCONSORVERATION 28.12 19.93 DULATYDENDSPREAMUK 5.9 2010 1 100211 113-127 PACUELCONSORVERATION 28.3 7.6 2113 1 106-124 KTAILAEYKORSOLSOLST 28.3 7.6 22013 1 5.9 123.8 22-21 ORICHORNER 28.3 7.6 233.29 Faright chain - 20.018 8.0 10.61 23.74 EAQEPDAR 4.9 6.9-7 233.29 Reinglobin δ + 16.027 7.8 1002.7 15.00 1004.000K 1.9 6.9-7 234.9 Apartiphylitylitylitylitylitylitylitylitylitylit		Hypothetical protein (Acc. no. 14726525)	-	21 445.3	5.05	1045.6	93-101	KTNLIVDSR		
276 Indole Intrast I						1307.7	119-128	FGEYQFLMEK		
276 Modelshylamine N-methylamadenae - 28752. 52.3 108.4 210-218 ENSYALEK 26.6 5.9 1812.1 113-127 FACELEGONGRWERK 26.6 5.9 1812.1 113-127 FACELEGONGRWERK 26.6 5.9 1812.1 113-127 FACELEGONGRWERK 26.8 5.9 2814.2 105-310 CPLL <proscongrwerk< td=""> 28.3 7.6 2815.3 106-112 MEESQLQUEDPOR 28.3 7.6 2816.4 Inverspecific BHLH-ZIP transcription factor - 28.04.9 104.61 233-24 ELAGEDPOR 28.3 7.6 283.2 Jg. s light chain + 23.018.0 8.40 110-117 TMARSVFIDEDQLK 28.8 7.6 283.29 Hemoglobin δ + 16.027.3 7.84 126.7 18-30 DIVENASVFIDEDQLK 4.90 7.5 33.6 Quadrit Shuffinghold + 15.07.7 8.74 126.7 18-30 MVNDAVGGEALER 15.0 7.5</proscongrwerk<>						1475.8	39-51	EKLPSSEVVKFGR		
276 Indoleftylamine M-methylamanferase - 28 75.2 5.23 108.24 210-218 ENCAURIEK 26.6 5.9 Hypothetical protein (Acc. no. 4200222) - 29 501.7 5.47 105.28 205-212 102LEIGNSGRWEIK 36.9 37.6 296 Liver-specific BHLH-ZIP transcription factor - 28 041.9 8.09 106.12 237.41 ELAQPDPAR 28.3 7.6 302 Ig x light chain + 23 018.0 8.40 701.4 111-118 TMARTYPEDADLQ(GCR 28.3 7.6 302 Ig x light chain + 23 018.0 8.40 701.4 111-118 TMARTYPE TMADLQ(GCR 7.6 328,329 Hemoglobin \bar{bar} + 16 027.3 7.84 125.7 18.30 VINDAVGCRELIES 14.9 6.9-7 328,329 Hemoglobin \alpha + 16 027.3 7.84 125.7 18.30 VINDAVGCRELIES 15.0 7.5 336 Cystatin C + 15 092.5 8.33 125.7 12.8-19 Hemoglobin \alpha + 15 092.5 8.74 121.11 <t< td=""><td></td><td></td><td></td><td></td><td></td><td>1707.8</td><td>64–77</td><td>QVSRVQFSLQLFKK</td><td></td><td></td></t<>						1707.8	64–77	QVSRVQFSLQLFKK		
Hypothetical protein (Acc. no. 420022) - 29 5017 5.47 1052.8 205-212 QELEHQR - - 296 Liver-specific BHLH-ZIP transcription factor - 28 0419 8.09 104.1 233-241 ELAQCPDPAR 28.3 7.6 296 Liver-specific BHLH-ZIP transcription factor - 28 0419 8.09 104.61 233-241 ELAQCPDPAR 28.3 7.6 202 Ig v light chain - 28 0419 8.09 104.61 231-241 CAURDER -	276	Indolethylamine N-methyltransferase	-	28 775.2	5.23	1082.4	210-218	EFSCVALEK	26.6	5.9
384.2 19-39 0VL3TYSPOSPEABULK Pypohetical protein (Acc. no. 420022) - 29 501.7 5.47 1052.8 205.21 QELEHQR - 206 Liver-specific BHLH-ZIP transcription factor - 20 401.9 800 106-12 KTALARX QCQCUSTR 28.3 7.6 302 Liver-specific BHLH-ZIP transcription factor - 20 401.9 800 106-12 KHALARX QCQCUSTR 28.3 7.6 302 Ig k light chain - 20 401.9 800 106-12 11-111 TMACPOPTARMR 7.6 303 Ig k light chain + 20 01.7 7.6 DSTYSLSSTILTAS 7.6 DSTYSLSSTILTAS 7.6 7.6 TMADSYNFEPSEDEQLK 7.6 7.6 TMADSYNFEPSEDEQLK 7.6 7.6 TMADSYNFEPSEDEQLK 7.6 7.6 TMADSYNFEPSEDEQLK 7.6 7.6						1812.1	113-127	FACELEGNSGRWEEK		
Hypothetical protein (Acc: no. 420022) - 29 501.7 5.47 1052.8 205-212 JCELBQR 296 Liver-specific BHLH-ZIP transcription factor - 28.0 104.1 233.52 106-124 KTAILAEYKQICSQLSTR 28.3 7.6 296 Liver-specific BHLH-ZIP transcription factor - 28.0 104.1 233.52 105.118 CRUPTVREELAQFD9ARKR 28.3 7.6 302 Ig x light chain + 23 01.80 8.09 104.1 123.53 211.21V/VEELAQFD9ARKR 28.8 7.6 328.329 Hemoglobin δ + 16 027.3 7.84 1502.7 55.68 DSTYSISSTILT.SK 14.9 6.9-7 3360 Cystain C + 16 027.3 7.84 1526.7 18.90 VIVADAVGEPALER 15.0 7.5 336 Cystain C + 15 077.15 8.10 1081.4 6.3-71 ASNDMYHENVFLOVELCR 15.0 7.5 342 Hemoglobin α + 15 092.5 8.31 122.67 18.90						2384.2	19-39	DYLATYYSFDGSPSPEAEMLK		
296 Liver-specific BHLH-ZIP transcription factor - 82 104 233-241 ELAQEDPAR 28.3 7.6 296 Liver-specific BHLH-ZIP transcription factor - 82 104.1 233-241 ELAQEDPAR 28.3 7.6 302 Ig x light chain + 20.018.0 701.4 111-118 TVAAPSVP (MAQAKER 28.3 7.6 328.32 Henoglobin δ + 20.018.0 701.4 111-118 TVAAPSVP (MAQAKER 4.6 7.6 328.32 Henoglobin δ + 10.027.3 7.6 101.127 TVAAPSVPTEPPSDEQLK - 7.6 328.32 Henoglobin δ + 10.027.3 7.6 12.50.7 7.5 20.01MOGGALER 4.9 6.9-7 336 Qyatain C + 15.07.3 7.6 18.30 WINAGVNYELOVELGR 5.5 7.5 414.17 12.126 5.21.6 ADEPAVOYNELOVELGR 15.9 7.5 12.6 7.5 12.6 7.5 12.6 7.5 12.6 <td></td> <td>Hypothetical protein (Acc. no. 4200222)</td> <td>-</td> <td>29 501.7</td> <td>5.47</td> <td>1052.8</td> <td>205-212</td> <td>IQELEHQR</td> <td></td> <td></td>		Hypothetical protein (Acc. no. 4200222)	-	29 501.7	5.47	1052.8	205-212	IQELEHQR		
296 Liver-specific BHLH-ZIP transcription factor - 28 0419 8.09 1061 233-241 ELAOPDPAR 28.3 7.6 296 Liver-specific BHLH-ZIP transcription factor - 28 0419 8.09 10461 233-241 ELAOPDPAR 28.3 7.6 302 Ig κ light chain + 23 018.0 8.40 791.4 111-18 TVAIAUEVKQICSQUSTR - - 7.6 302 Ig κ light chain + 23 018.0 8.40 791.4 111-18 TVAIAUEVKQICSQUSTR -						1795.0	1-14	MRESQLQQEDPMDR		
296 Liver-specific BHLH-ZP transcription factor - 28 04.9 8.09 104.1 237-241 EUQPEPAR 28.3 7.6 302 Ig κ light chain + 23 018.0 8.40 711-118 CREIT/HDADLQIGK 7.6 302 Ig κ light chain + 23 018.0 8.40 75-66 DSTYSLSSTLTLSK 2.6 7.6 328,329 Hemoglobin δ + 16 027.3 7.84 1256.7 18-30 VIVDAVGCREALER 14.9 6.9-7 328,329 Hemoglobin δ + 16 027.3 7.84 1256.7 18-30 VIVDAVGCREALER 14.9 6.9-7 336 Cystain C + 15 07.1 301 1081.4 15.0 7.5 1226.9 52-61 ALDFAVGYNE 15.0 7.5 342 Hemoglobin α + 15 092.5 8.33 1252.7 17-31 VIVDAVGCROAELER 15.5 8.3 342 Hemoglobin β + 15 29.77 8.72 1530.1 18-32 VIAGKVGR						2211.3	106-124	KTTAIIAEYKQICSQLSTR		
302 Ig κ light chain + 23 08.0 840 791.4 111-118 TVARPSVF 26.8 7.6 328,329 Hemoglobin δ + 16 027.3 7.84 125.7 55-68 DSTVISISTILTSK 6.9-7 328,329 Hemoglobin δ + 16 027.3 7.84 125.7 18-30 VNVDAVGGEALER 14.9 6.9-7 336 Cystatin C + 15 07.13 7.01 1081.4 63-71 ASMOMYHSR 15.0 7.5 346 Cystatin C + 15 07.13 9.01 1081.4 63-71 ASMOMYHSR 15.0 7.5 374 Hemoglobin α + 15 092.5 8.33 125.7 128.19 PLAVSTVLTSK 15.0 7.5 374 Hemoglobin α + 15 092.5 8.74 121.10 18-50 PUPHOPLOSUGAR 15.5 8.3 374 Hemoglobin β + 15 998.5 6.74 112.11 18-30 PUPHOPLOSUGAR 15.5 8.3 375.1 122.13 124.19 INVYPEVEQGEALER 15.5 8.3	296	Liver-specific BHLH-ZIP transcription factor	-	28 041.9	8.09	1046.1	233-241	ELAQFDPAR	28.3	7.6
302 Ig κ light chain + 23 24 226-244 UTYVEKELAQEPDARMR 302 Ig κ light chain + 23 926-244 UTYVEKELAQEPDARMR 26.8 7.6 302 Ig κ light chain + 23 914 111-118 TAARSVE 26.8 7.6 328,29 Henoglobin δ + 16 027.3 7.84 1256.7 18-30 VNVDAVGGEALER 14.9 6.9-7 336 Cystatin C + 15 7.1 141.7 121-132 EFTPQMOAAYQK 5.6 5.7 18-30 VNDAVGGEALER 15.0 7.5 336 Cystatin C + 15<77.1						1235.8	212-221	GYRIQADKER		
302 Ig κ light chain + 23 018.0 8.40 79.4 11.118 TVAAPSVF1PPSDEQLK 26.8 7.6 328,329 Hemoglobin δ + 16 027.3 7.8 1946.0 110-127 TVAAPSVF1PPSDEQLK 4.9 6.9-7 328,329 Hemoglobin δ + 16 027.3 7.84 126.7 18-20 VNVDAVGELALER 14.9 6.9-7 336 Cystatin C + 15.771.3 9.01 108.14 63-71 ASNDMYH5R 15.0 7.5 336 Cystatin C + 15.791.3 9.01 108.14 63-71 ASNDMYH5R 15.0 7.5 342 Hemoglobin α + 15.975.7 8.72 125.9 7.131 <u>124.8VCYTUTSK</u> 15.5 8.3 342 Hemoglobin α + 15.257.7 8.72 1530.1 18-32 VGAHAGEYGAEALER 15.5 8.3 342 Hemoglobin β + 15.998.5 6.74 112.1 97-105 LIVVDENGRAQVK 15.5 8.3 343 Gene pp21 protein - 18.505.9 8.7						1765.5	103-118	GREITIVHDADLQIGK		
302 Ig κ light chain + 23 018.0 8.40 791.4 111-118 TVAAPSVF 26.8 7.6 328,329 Hemoglobin δ + 16 027.3 7.84 1256.7 18-30 VNVDAVGGEALER 14.9 6.9-7 328,329 Hemoglobin δ + 16 027.3 7.84 1256.7 18-30 VNVDAVGGEALER 14.9 6.9-7 336 Cystatin C + 15 771.3 9.01 1081.4 63-71 ASNDMYHSR 15.0 7.5 342 Hemoglobin α + 15 092.5 8.33 122.7 128-139 ELASVSTVLTSK 15.0 7.5 342 Hemoglobin α + 15 092.5 8.72 1530.1 183.4 42-57 TYPHFDLSHGSAQVK 15.5 8.3 342 Hemoglobin β + 15 995.5 6.74 1127.1 97-105 LHVDPENR 15.5 8.3 343 Gene pp21 protein - 18 505.9 8.06 1087.2 95-101 HNDEGOR/ALER						2383.9	226-244	VLYYVEKELAQFDPARRMR		
328,329 Hemoglobin δ + 16 027.3 7.84 126.7 110-127 TVALSPUTIFINGE I 338,329 Hemoglobin δ + 16 027.3 7.84 125.67 18-30 VNNDAVGCEALER 14.9 6.9-7 336 Cystatin C + 15 771.3 9.01 1081.4 63-71 ASINOM'ISR 15.0 7.5 336 Cystatin C + 15 771.3 9.01 1081.4 63-71 ASINOM'ISR 15.0 7.5 336 Cystatin C + 15 092.5 8.33 1252.7 17.31 WOYAGYNEIDVELOR 15.0 7.5 342 Hemoglobin α + 15 092.5 8.33 14-55 TYPHPTOLSHGSAQVK 15.5 8.3 342 Hemoglobin β + 15 995.5 6.74 1127.1 97-105 LHVDPENFR 15.5 8.3 343 Gene pp21 protein - 18 356.4 12.4 197.5 142.1 147.00 131.4 142.1 147.00 <t< td=""><td>302</td><td>Ig к light chain</td><td>+</td><td>23 018.0</td><td>8.40</td><td>791.4</td><td>111-118</td><td>TVAAPSVF</td><td>26.8</td><td>7.6</td></t<>	302	Ig к light chain	+	23 018.0	8.40	791.4	111-118	TVAAPSVF	26.8	7.6
328,329 Hemoglobin δ + 16 027.3 7.84 126.67 18.00 VNVDAVGGEALER 14.9 6.9-7 336 Cystain C + 15 771.3 9.01 1081.4 63-71 ASDWAYKS 15.0 7.5 336 Cystain C + 15 771.3 9.01 1081.4 63-71 ASDWAYKS 15.0 7.5 Hemoglobin α + 15 092.5 8.33 1252.7 128-139 FLASVSTVLEVELOR 15.0 7.5 342 Hemoglobin α + 15 297.7 8.72 1530.1 18-32 VGAHAGEYGAEALER 15.5 8.3 342 Hemoglobin β + 15 297.7 8.72 1530.1 18-32 VGAHAGEYGAEALER 15.5 8.3 342 Hemoglobin β + 15 99.5 67.4 127.1 9.10 HVDENFR 15.5 8.3 343 Hemoglobin β + 15 99.5 67.4 127.1 32-441 LLVVPVPOR 14.5 8.4						1502.7	55-68	DSTYSLSSTLTLSK		
328,329 Hemoglobin δ + 16 027.3 7.84 1256.7 18-30 VUDAVGCEALER 14.9 6.9-7 336 Cystatin C + 15 771.3 9.01 1081.4 63-71 ASNDMYHSR 15.0 7.5 336 Cystatin C + 15 771.3 9.01 1081.4 63-71 ASNDMYHSR 15.0 7.5 Hemoglobin α + 15 092.5 8.33 125.7 128-139 FLASWTVLTSK 15.0 7.5 342 Hemoglobin α + 15 092.5 8.33 125.7 17.31 VGVHAGEYGAEALER 15.5 8.3 342 Hemoglobin β + 15 998.5 6.74 112.1 97-105 LHVDEVGGEALER 15.5 8.3 342 Hemoglobin β + 15 998.5 6.74 112.1 97-105 LHVDEWRR 15.5 8.3 155 97.5 42-60 FFESFGDLSTPDAVMGNPK 15.5 8.3 1379.1 122-135 EFTPPVQAAYQK 15.0 16.1 15.0 16.1 17.5 18.50 11.51.26 11.52.50 11.						1946.0	110-127	TVAAPSVFIFPPSDEQLK		
336 Cystatin C + 15 771.3 9.01 1681.4 63 -11 ASNDMYHSR 15.0 7.5 336 Cystatin C + 15 771.3 9.01 1681.4 63 -11 ASNDMYHSR 15.0 7.5 342 Hemoglobin α + 15 092.5 8.7 122.7 128-139 FLASVSTVLTSK 15.0 7.5 342 Hemoglobin β + 15 25.7 8.72 1530.1 18-32 VGMHAGCYGAEALER 15.5 8.3 141.7 121.71 97-105 1479470 1450 TYPHFDLSHGSAQVK 15.5 8.3 342 Hemoglobin β + 15 998.5 6.74 1127.1 97-105 147047004004704 15.5 8.3 141.7 121.71 97-105 14704704704 15.0 1	328,329	Hemoglobin δ	+	16 027.3	7.84	1256.7	18-30	VNVDAVGGEALER	14.9	6.9–7.1
336 Cystain C + 15 77.13 9.01 1081.4 63-71 ASNDMYHSR 15.0 7.5 336 Cystain C + 15 77.13 9.01 1081.4 63-71 ASNDMYHSR 15.0 7.5 Hemoglobin α + 15 092.5 8.33 1252.7 128-139 FLASVSTVLTSK 70044GEYGAEALER 15.0 8.3 342 Hemoglobin α + 15 257.7 8.72 17-31 YGQHAGEYGAEALER 15.5 8.3 183.4 42-57 TYFPHFDLSHGSAQVK 15.5 8.3 342 Hemoglobin β + 15 998.5 6.74 1127.1 97-105 LHVDPENFR 183.4 42-57 TYFPHFDLSHGSAQVK 15.5 8.3 1998.5 6.74 1127.1 97-105 LHVDPENFR 15.0 8.3 199.4 Hemoglobin β + 15 998.5 6.74 1127.1 97-105 LHVDPENFR 15.0 8.3 199.4 Hemoglobin β + 15 90.5 8.06 1087.8 14-20 IPYDMKVR 15.0 8.4						1441.7	121-132	EFTPQMQAAYQK		
336 Cystatin C + 15 771.3 9.01 1081.4 63-71 ASNDMYHSR 15.0 7.5 Hemoglobin α + 15 092.5 8.3 1226.9 52-61 ALDFAVGYNK 120.7 128-139 FLASVSTVLTSK 1339 41-50 TYFPHFDLSHGSAQVK 15.0 7.5 342 Hemoglobin α + 15 998.5 6.74 128-139 FLASVSTVLTSK 15.5 8.3 344 Hemoglobin β + 15 998.5 6.74 1127.1 18-32 VGMAGEYGAEALER 15.5 8.3 342 Hemoglobin β + 15 998.5 6.74 1127.1 18-32 VGMAGEYGAEALER 15.5 8.3 345 Gene plobin β + 15 998.5 6.74 1127.1 97-105 LIVVPENR 15.0 15.0 8.14 343 Gene pp21 protein - - 18 505.9 8.06 1087.5 12-61 IMYASSKDAIKK 15.0 8.4 343 Gene pp21 protein + 15 970.3 6.74 1149.7 133-16 INSNEEMIQAADELEEMK						2044.9	41-59	FFESFGDLSSPDAVMGNPK		
4126.9 52-61 ALDFAVGYNK 1793.8 81-96 QUVAGVNYFLDVELGR 1793.8 81-96 QUVAGVNYFLDVELGR 128.9 125.7 128-139 FLASVSTVLTSK 1529.7 17-31 VGVHAGEVGAEALER 155 133.9 41-56 TYFPHFDLSHGSAQVK 342 Hemoglobin α + 15 257.7 8.72 1530.1 18-32 VGAHAGEVGAEALER 15.5 8.3 342 Hemoglobin β + 15 998.5 6.74 1127.1 97-105 LHVDPENFR 1275.1 32-41 LIVVPWTQR 1315.1 19-31 VNVDEVGGEALGR 15.5 8.3 1315.1 19-31 VNVDEVGGEALGR 1379.1 122-133 EFTPPVQAAYQK 1315.1 19-31 VNVDEVGGEALGR 15.0 8.4 1379.1 122-133 EFTPVQAAYQK 15.0 8.4 1379.1 122-135 IFYDMKVR 15.0 8.4 1379.1 122-133 EFTPVQAAYQK 15.0 8.4 1379.1 152-165 LGSSLIVAFEGCPV 15.0 8.4	336	Cystatin C	+	15 771.3	9.01	1081.4	63-71	ASNDMYHSR	15.0	7.5
Hemoglobin α + 15 092.5 8.33 1252.7 128-139 FLASUSTVLTSK 342 Hemoglobin α + 15 257.7 8.72 1501 18-52 VG4HAGEYGAEALER 15.5 8.3 342 Hemoglobin β + 15 257.7 8.72 1501 18-52 VG4HAGEYGAEALER 15.5 8.3 1834.4 42-57 TYFPHFDLSHGSAQVK 15.5 8.3 15.7 8.72 15.0 18-52 VG4HAGEYGAEALER 15.5 8.3 1834.4 42-57 TYFPHFDLSHGSAQVK 15.5 8.3 175.1 32-41 LIVVPWTQR 15.5 8.3 1998.5 6.74 1127.1 97-105 LHVDPENFR 15.5 8.3 205.5 42-60 FFESFGLSTDDAVMGNPK 15.5 8.3 205.5 42-60 FFESFGLSTDDAVMGNPK 14.8.5 152-165 LGGSLIVAFEGCPV 343 Gene pp21 protein - 18 364.1 12.48 1007.2 93-101 HILEEGIFK 15.0 8.4 4418.5 152-165 LGGSLIVAFEGCPV 15.0 8.4 205.3						1226.9	52-61	ALDFAVGYNK		
Hemoglobin α + 15 092.5 8.33 1252.7 128-139 FLASVSTVLTSK 342 Hemoglobin α + 15 257.7 8.72 1530.1 18-32 VGUHAGEYGAEALER 15.5 8.33 342 Hemoglobin β + 15 257.7 8.72 1530.1 18-32 VGUHAGEYGAEALER 15.5 8.33 Hemoglobin β + 15 998.5 6.74 1127.1 97-105 LHVDPENFR 15.5 8.34 Destrin - - 18 505.9 6.74 1127.1 97-105 LHVDPENFR 15.5 8.35 343 Gene pp21 protein - - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 15.0 8.4 343 Gene pp21 protein - 18 364.1 12.48 1097.2 93-101 HNLEGIFK 15.0 8.4 343 Gene pp21 protein - 18 364.1 12.48 1097.3 119-136 SKMIYASSKDAIKK 15.0 8.4 134.3 Gene pp21 protein - 18 364.1 12.48 2095.3 119-136						1793.8	81-96	QIVAGVNYFLDVELGR		
342 Hemoglobin α + 15 257. 8.72 1530.1 18-32 VGQHAGEYGAEALER 15.5 8.3 342 Hemoglobin β + 15 257. 8.72 1530.1 18-32 VGAHAGEYGAEALER 15.5 8.3 Hemoglobin β + 15 998.5 6.74 117.1 97-105 LHVDPENFR 15.5 8.3 Destrin - - 18 505.9 6.74 117.1 97-105 LHVDPENFR 15.5 8.3 343 Gene pp21 protein - - 18 505.9 8.06 1087.8 14-21 FPPPVQAAYQK 15.0 8.4 343 Gene pp21 protein - 18 364.1 12.48 1087.2 93-101 HNLEGGIFK 15.0 8.4 343 Gene pp21 protein + 15 970.3 6.74 149.7 133-144 VVAGVANALAHK 15.0 8.4 343 Gene pp21 protein + 15 970.3 6.74 149.7 133-144 VVAGVANALAHK 15.0 8.4 150 113-10 NLSNEEMIQAADELEEMK 15.0 15.0 <td></td> <td>Hemoglobin a</td> <td>+</td> <td>15 092.5</td> <td>8.33</td> <td>1252.7</td> <td>128-139</td> <td>FLASVSTVLTSK</td> <td></td> <td></td>		Hemoglobin a	+	15 092.5	8.33	1252.7	128-139	FLASVSTVLTSK		
342 Hemoglobin α + 15 257.7 8.72 1530.1 18-32 VGAHAGEYGAEALER 15.5 8.3 342 Hemoglobin β + 15 998.5 6.74 1127.1 97-105 LHVDPENFG Hemoglobin β + 15 998.5 6.74 1127.1 97-105 LHVDPENFR Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 343 Gene pp21 protein - 18 364.1 1248 1087.2 93-101 HN2ESGLASTPANMGNPK 4Hemoglobin β + 15 970.3 6.74 1149.7 1315.1 19-31 VNVDEVGGEALGR 15.0 8.4 1375.1 122-133 EFTPPVQAAYQK 2075.5 42-60 FFESFGDLSTPDAVMGNPK 15.0 8.0 1418.5 152-165 LGGSLIVAFEGCPV 1355.0 115-126 MYASSKDAIKK 15.0 8.4 343 Gene pp21 protein - 18 364.1 12.48 1087.2 93-101 HNLEGIFK 15.0 8.4 44-30 PSPS.3 119-136 NLSNEEMIQAADELEEMK 5.0 8.4 </td <td></td> <td></td> <td></td> <td></td> <td></td> <td>1529.7</td> <td>17-31</td> <td>VGVHAGEYGAEALER</td> <td></td> <td></td>						1529.7	17-31	VGVHAGEYGAEALER		
342 Hemoglobin α + 15 257.7 8.72 1530.1 18-32 VGAHAGEYGAEALER 15.5 8.3 Hemoglobin β + 15 98.5 6.74 1127.1 97-105 LHVDPENFR 15.5 8.3 Hemoglobin β + 15 98.5 6.74 1127.1 97-105 LHVDPENFR 15.5 8.3 Destrin - 18 505.9 8.06 1087.8 14-21 IFVDMKVR 15.5 8.4 343 Gene pp21 protein - 18 364.1 12.48 1087.2 93-010 HNLEEGIFK 15.0 8.4 Hemoglobin β + 15 970.3 6.74 1149.7 133-144 VVAGVAADLEEMK 8.4 418.5 15.9 8.06 1087.8 14-21 INVASKDAIKK 8.4 343 Gene pp21 protein 18 364.1 12.48 1087.2 93-010 HNLEEGIFK 15.0 8.4 15.0 β 19.73 119-136 NLSNEEMIQAADELEEMK 15.0 8.4 1418.5 127.7 31-40 LLVVYPWTQR 1314.7 18-30						1833.9	41-56	TYFPHFDLSHGSAQVK		
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	342	Hemoglobin a	+	15 257.7	8.72	1530.1	18-32	VGAHAGEYGAEALER	15.5	8.3
Hemoglobin β + 15 998.5 6.74 1127.1 97-105 LHVDPENFR 1275.1 32-41 LLVVYPWTQR 1315.1 19-31 VNVDEVGGEALGR 1315.1 19-31 VNVDEVGGEALGR 1379.1 122-133 EFTPPVQAAYQK 2075.5 42-60 FFESFGDLSTPDAVMGNPK Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 343 Gene pp21 protein - 18 364.1 12.4 1087.2 93-101 HNLEGGFV 1343 Hemoglobin β + 15 970.3 6.74 1149.7 133-144 VVAGVANALAHK 126 SKMIYASSKDAIKK 15.0 8.4 1274.7 31-40 LLVVYPWTQR 1314.7 18-30.9 VNVDEVGGEALGR 2058.9 41-59 FFESFGDLSTPDAVMGNPK 1274.7 31-40 LLVVYPWTQR 151-47 18-50 VNVDEVGGEALGR 2058.9 41-59 FFESFGDLSTPDAVMGNPK 255.9 255.9 150-70 115-126 Destrin - - 18 505.9 8.06 1087.8						1834.4	42-57	TYFPHFDLSHGSAQVK		
1275.1 32-41 LLVVYPWTQR 1315.1 19-31 VNVDEVGGEALGR 1379.1 122-133 EFTPPVQAAYQK 2075.5 42-60 FFESFGDLSTPDAVMGNPK 2075.5 42-60 FFESFGDLSTPDAVMGNPK 1350.0 115-126 MIYASSKDAIKK 1418.5 152-165 LGGSLIVAFEGCPV 1570.0 113-126 SKMIYASSKDAIKK 1418.5 152-165 LGSSLIVAFEGCPV 1570.0 113-126 SKMIYASSKDAIKK 1418.5 152-165 LGSSLIVAFEGCPV 1570.0 113-126 SKMIYASSKDAIKK 1418.5 152-165 LGSSLIVAFEGCPV 150 8.44 12.48 1087.2 93-101 Hemoglobin β + 15 970.3 6.74 1149.7 133-144 VVAGVAALAHK 1274.7 1314.7 18-30 VNVDEVGGEALGR 15.0 8.4 1314.7 18-30 VNVDEVGGEALGR 2058.9 41-59 FFESFGDLSTPDAVMGNPK Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 155.0		Hemoglobin B	+	15 998.5	6.74	1127.1	97-105	LHVDPENFR		
343 Gene pp21 protein - 18 364.1 12.48 1087.2 93-10 VNVDEVGGEALGR 343 Gene pp21 protein - 18 364.1 12.48 1087.2 93-10 HNLEEGIFK 15.0 8.4 Hemoglobin β + 15 970.3 6.74 1149.7 133-140 LLVVYPWTQR Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 343 Gene pp21 protein - 18 364.1 12.48 1087.2 93-101 HNLEEGIFK 15.0 8.4 18 364.1 12.48 1087.2 93-101 HNLEEGIFK 15.0 8.4 2095.3 119-136 NLSNEEMIQAADELEEMK 15.0 8.4 119-136 NLSNEEMIQAADELEEMK 15.0 8.4 1274.7 31-40 LLVVYPWTQR 140.4 140.4 140.4 Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 1255.0 118 505.9 8.06 1087.8 14-21 IFYDMKVR 140.4 140.4 140.4 140.4 140.4						1275.1	32-41	LLVVYPWTQR		
Destrin - 18 505.9 8.06 1087.8 142-10 IFTPPVQAAYQK 2075.5 42-60 FFESFGDLSTPDAVMGNPK 1355.0 115-126 MIYASSKDAIKK 1418.5 152-165 LGGSLIVAFEGCPV 1570.0 113-126 SKMIYASSKDAIKK 343 Gene pp21 protein 18 364.1 12.48 1087.2 93-101 HNLEEGIFK 15.0 8.4 148.5 119-136 NLSNEEMIQAADELEEMK 15.0 8.4 2095.3 119-136 NLSNEEMIQAADELEEMK 15.0 8.4 1149.7 133-144 VVAGVAALAHK 15.0 8.4 1147.1 18-30 VNVDEVGGEALGR 15.0 8.4 1147.1 18-30 VNVDEVGGEALGR 15.0 8.4 1147.1 18-30 VNVDEVGGEALGR 15.0 15.0 1147.1 18-30 14-21 IFYDMKVR 15.0 15.0 115.0 - 1855.9 8.06 1087.8 14-21 IFYDMKVR 1355.0 115-126 MIYASSKDAIKK 115-126 MIYASSKDAIKK 115.0 </td <td></td> <td></td> <td></td> <td></td> <td></td> <td>1315.1</td> <td>19-31</td> <td>VNVDEVGGEALGR</td> <td></td> <td></td>						1315.1	19-31	VNVDEVGGEALGR		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$						1379.1	122-133	EFTPPVQAAYQK		
Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 1355.0 115-126 MIYASSKDAIKK 1418.5 152-165 LGGSLIVAFEGCPV 1570.0 113-126 SKMIYASSKDAIKK 343 Gene pp21 protein 18 364.1 12.48 1087.2 93-101 <u>HNLEEGIFK</u> 15.0 8.4 Hemoglobin β + 15 970.3 6.74 1149.7 133-144 <u>VVAGVANALAHK</u> 1274.7 31-40 <u>LLVVYPWTQR</u> 1314.7 18-30 <u>VNVDEVGGEALGR</u> 2058.9 41-59 <u>FFESFGLSTPDAVMGNPK</u> 1570.0 113-126 MIYASSKDAIKK						2075.5	42-60	FFESFGDLSTPDAVMGNPK		
343 Gene pp21 protein 18 364.1 12.48 1087.2 93-101 HNLEEGIFK 15.0 8.4 Hemoglobin β + 15 970.3 6.74 1149.7 133-144 VVAGVANALAHK 15.0 8.4 Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR		Destrin	-	18 505.9	8.06	1087.8	14-21	IFYDMKVR		
343 Gene pp21 protein 18 364.1 12.48 1087.2 93-101 HNLEEGIFK 15.0 8.4 Hemoglobin β + 15 970.3 6.74 1149.7 133-144 VVAGVANALAHK 15.0 8.4 I2147.7 31-40 LLVVYPWTQR 1314.7 18-30 VNVDEVGGEALGR 15.0 8.4 Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 15.0 15.0 15.0 15.0 15.0 15.0 15.0 15.0 15.0 8.4						1355.0	115-126	MIYASSKDAIKK		
343 Gene pp21 protein Hemoglobin β + 15 970.3 6.74 1149.7 33-144 VAGVANALAHK 1274.7 31-40 LLVVYPWTQR 1314.7 18-30 VNVDEVGGEALGR 2058.9 41-59 FFESFGLSTPDAVMGNPK Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 135.0 115-126 MIYASSKDAIKK						1418.5	152-165	LGGSLIVAFEGCPV		
343 Gene pp21 protein 18 364.1 12.48 1087.2 93-101 HNLEEGIFK 15.0 8.4 Hemoglobin β + 15 970.3 6.74 1149.7 133-144 VVAGVANALAHK 1000000000000000000000000000000000000						1570.0	113-126	SKMIYASSKDAIKK		
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	343	Gene pp21 protein		18 364.1	12.48	1087.2	93-101	HNLEEGIFK	15.0	8.4
Hemoglobin β + 15 970.3 6.74 1149.7 133-144 VVAGVANALAHK 1274.7 31-40 LLVVYPWTQR 1314.7 18-30 VNVDEVGGEALGR 2058.9 41-59 FFESFGDLSTPDAVMGNPK Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 135.0 115-126 MIYASSKDAIKK						2095.3	119-136	NLSNEEMIQAADELEEMK		
1274.7 31-40 LLVVYPWTQR 1314.7 18-30 VNVDEVGGEALGR 2058.9 41-59 FFESFGDLSTPDAVMGNPK Destrin - 18 505.9 8.06 1087.8 14-21 IFYDMKVR 1355.0 115-126 MIYASSKDAIKK		Hemoglobin B	+	15 970.3	6.74	1149.7	133–144	VVAGVANALAHK		
1314.7 18–30 VNVDEVGGEALGR 2058.9 41–59 FFESFGDLSTPDAVMGNPK Destrin – 18 505.9 8.06 1087.8 14–21 IFYDMKVR 1355.0 115–126 MIYASSKDAIKK						1274.7	31-40	LLVVYPWTQR		
2058.9 41–59 FFESFGDLSTPDAVMGNPK Destrin – 18 505.9 8.06 1087.8 14–21 IFYDMKVR 1355.0 115–126 MIYASSKDAIKK						1314.7	18-30	VNVDEVGGEALGR		
Destrin – 18 505.9 8.06 1087.8 14–21 IFYDMKVR 1355.0 115–126 MIYASSKDAIKK						2058.9	41-59	FFESFGDLSTPDAVMGNPK		
1355.0 115–126 MIYASSKDAIKK		Destrin	-	18 505.9	8.06	1087.8	14-21	IFYDMKVR		
						1355.0	115-126	MIYASSKDAIKK		
1418.5 152–165 LGGSLIVAFEGCPV						1418.5	152-165	LGGSLIVAFEGCPV		
1570.0 113–126 SKMIYASSKDAIKK						1570.0	113-126	SKMIYASSKDAIKK		

Table 1. Continued

Spot no.	Protein	Reported in plasma ^a	MW ^b	pI ^b	[M+H]+ ^c	Residue	Identified peptides, sequence from database	MW ^d	pI ^d
	Cystatin C	+	15 771.3	9.01	1097.6	51-61	ALDFAVGEYNK		
					1664.8	35-51	LVGGPMDASVEEEGVR		
					1900.9	102-118	TQPNLDNPFHDQPHLK		
355	Hemoglobin a	+	15 257.7	8.72	1071.5	32-40	VGAHAGEYGAEALER	14.8	7.9
					1530.1	17-31	VGAHAGEYGAEALER		
					1834.4	41-56	TYFPHFDLSHGSAQVK		
358-360	Transthyretin	+	15 859.2	5.10	1366.7	22-34	GSPAINVAVHVFR	15.9-15.7	5.4-4.5
					2451.2	81-103	ALGISPFHEHAEVVFTANDSGPR		
					2455.1	49-70	TSESGELHGLTTEEEFVEGIYK		
					2645.4	124-147	RYTIAALLSPYSYSTTAVVTNPKE		
361	Putative HLA-associated protein	-	15 382.2	4.25	2270.4	32-61	LEGLTDEFEELEFLLSDNR	15.8	5.0
	Transthyretin	+	15 859.2	5.10	1366.8	42-54	GSPAINVAVHVFR		
					1522.7	55-68	KAADDYWEPFASGK		
					2148.2	36-55	VLDAVRGSPAINVAVHVFRK		

Underlined residues were confirmed by MS-MS product ion spectra.

^a +, Proteins reported to be in plasma (searched by Medline); -, proteins not reported to be in plasma (searched by Medline).

^b Theoretical values based on SwissProt.

^c Observed monoisotopic.

^d Observed values.

[14]. The retina is ontogenetically a part of the central nervous system and cystatin C might therefore be expected to also be contained in retinal neurons.

Prostaglandin D2 (PGD2) synthase (no. 85) modulates several functions in the central nervous system, such as sleep-wake behavior, body temperature, luteinizing hormone release, and odor responses. This enzyme, a 26-kDa glycoprotein, is a member of the lipocalin gene family, a group of secretory proteins and hydrophobic molecule transporters such as β -lactoglobin and retinol-binding protein [15]. PDG2 is the major prostaglandin formed in the eye [16].

3.3. Example of protein identification

Fig. 2 shows CID spectra of the tryptic digests of a spot (no. 29) in VH derived from a patient with diabetic retinopathy. The CID spectra matched the sequences of five peptides of a protein, pigment epithelium-derived factor (PEDF). These spectra covered 23% of the total PEDF sequence, which strongly support its identification. PEDF was found in 1995 in the conditioning medium of pigment epithelium cells, and the factor had a neurotrophic function for cerebellar granule cells and inhibited microglial growth [17]. Successively, Dawson et al. [18] reported that a factor isolated from a cultured medium of retinoblastoma cells, had a strong antiangiogenic activity and was revealed to be the same as PEDF. This factor was reported to be a potent inhibitor of vascular growth in the cornea and vitreous [18]. Spot no. 29 was clearly detected in the 2D gel profile of all cases with diabetic retinopathy, but the corresponding spot on the 2D gel profile of two cases with macular hole was faint. We roughly estimated that PEDF protein had a higher concentration in VH with diabetes than with macular hole, although this important factor needs to be quantified more correctly by immunoassay, or MS method using isotope coded affinity tag, etc. If the inhibitor increases constantly in proliferative diabetic retinopathy, i.e., an excessive neovascularization state, a fall in PEDF is not essential for diabetic retinopathy to proceed.

In conclusion, we detected 56 kinds of proteins in human VH by 2D-PAGE electrophoresis, ion-exchange column, and MS. Some were VH-specific proteins. PEDF, a potent antiangiogenic factor, was first characterized in human VH derived from diabetic retinopathy using this technique. The other factors, either angiogenic or antiangiogenic, must be



Fig. 2. ESI-MS-MS spectra of a peptide from the in-gel digest of a 2D gel spot (no. 29) from a diabetic retinopathy patient. Four of the resulting CID spectra are shown here, along with the database sequence of a peptide from PEDF. Peaks representing y and b series ions are marked.

identified, and quantitative analyses of these factors are required.

Acknowledgements

This work was supported by a 1997–2000 Grantin-Aid for Scientific Research (B) (09557220) from the Ministry of Education, Science and Culture of Japan.

References

- [1] D. McLeod, Trans. Ophthalmol. Soc. UK 103 (1983) 44.
- [2] C. Lahrmann, T. Bek, Acta Ophthalmol. Scand. 78 (2000) 169.
- [3] N. Ogata, J. Tombran-Tink, M. Nishikawa, T. Nishimura, Y. Mitsuma, T. Sakamoto, M. Matsumura, Am. J. Ophthalmol. 132 (2001) 378.
- [4] R. Cassey, W.W. Li, Am. J. Ophthalmol. 124 (1997) 521.
- [5] L.P. Aiello, R.L. Avery, P.G. Arrigg, New Engl. J. Med. 331 (1994) 1480.

- [6] V. Stellmach, S.E. Crawford, W. Zhou, N. Bouck, Proc. Natl. Acad. Sci. USA 98 (2001) 2593.
- [7] L. Raymond, B. Jacobson, Exp. Eye Res. 34 (1982) 267.
- [8] Y. Nishizawa, N. Komori, J. Usukura, K.W. Jackson, A. Tobin, H. Matsumoto, Exp. Eye Res. 69 (1999) 195.
- [9] K.-W. Yau, Invest. Ophthalmol. Vis. Sci 36 (1994) 9.
- [10] D. Arnott, K.L. O'Connell, K.L. King, J.T. Stults, Anal. Chem. 258 (1998) 1.
- [11] H. Matsumoto, N. Komori, Methods Enzymol. 316 (2000) 492.
- [12] J.-C. Sanchez, R.D. Appel, O. Golaz, C. Pasquali, F. Ravier, A. Bairoch, D.F. Hoschstrasser, Electrophoresis 16 (1995) 1131.
- [13] http://www.expasy.ch/ch2dothergifs/publi/plasma-acidic and basic.gif
- [14] J. Wasselius, K. Hakansson, K. Jobansson, M. Abrabamson, B. Ebinger, Invest. Ophthalmol. Vis. Sci. 42 (2001) 1901.
- [15] C.T. Beuckmann, W.C. Gordon, Y. Kanaoka, N. Eguchi, V.L. Marcheselli, D.Y. Gerashchenko, Y. Urade, O. Hayashi, N.G. Bazan, J. Neurosci. 16 (1996) 6119.
- [16] Y. Goh, Y. Urade, N. Fujimoto, O. Hayashi, Biochem. Biophys. Res. Commun. 921 (1987) 302.
- [17] T. Tanawaki, S.P. Becerra, G.J. Chader, J.P. Schwartz, J. Neurochem. 64 (1995) 2509.
- [18] D.W. Dawson, O.V. Volpert, P. Gillis, S.E. Crawford, H.-J. Xu, W. Benedict, N.P. Bouck, Science 285 (1999) 245.