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Journal of Chromatography B, 792 (2003) 5–21
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Catalogue of soluble proteins in human vitreous humor by one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electrospray ionization mass spectrometry including seven angiogenesis-regulating factors

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Received 8 November 2002; received in revised form 14 January 2003; accepted 21 January 2003

Abstract

A catalogue of proteins in the human vitreous humor may contribute to elucidating the pathogenesis of various diseases in ophthalmology. To improve the recovery of proteins in vitreous, we applied one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis (1D-PAGE). Proteins were extracted from unstained gel strips and digested in gel with trypsin and the peptides were analyzed by capillary-column reversed-phase high-performance liquid chromatography coupled with electrospray ionization-ion trap-mass spectrometry. From a patient with diabetic retinopathy, 84 different proteins were identified. Most of the proteins which we identified in vitreous previously using 2D-PAGE were also identified in the present study. In total, we identified 121 different proteins including five proteins seen at the genomic level only. Four angiogenic factors, insulin-like growth factor, vascular endothelial growth factor, fibroblast growth factor, and placental endothelial cell growth factor, and three anti-angiogenic factors, pigment epithelium-derived factor, endostatin, and thrombospondin, were found, and this may contribute to elucidating the pathological changes in the concentration and the modified structures of these proteins, in diseases of the retina, especially, diabetic retinopathy.

Keywords: Proteins; Angiogenesis-regulating factors

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 such diseases, the production of angiogenic and anti-angiogenic factors by retinal cells may change and, consequently, the concentration and modified structures of these factors may change. A variety of factors to regulate angiogenesis were expected to be observed in human VH. However, we could identify only two factors in 51 different proteins by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) coupled with electrospray ionization-ion trap-mass spectrometry (ESI-IT-MS) [1]. It has been

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1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00133-8

reported that proteins are lost during 2D-PAGE and extraction from stained gels [2]. Poor solubility of some proteins and the charge heterogeneity is often refractory to 2D-PAGE, the current paradigm technology for studying protein expression profiles. To identify more proteins, we improved the recovery of peptides by using 1D-sodium dodecyl sulfate (SDS)-containing PAGE, blind cutting of gels, and extraction from unstained gels. Here, we report the identified proteins, including seven vascular factors.

2. Materials and methods

2.1. Sample preparation, 1D-SDS–PAGE, and in-gel digestion

The vitreous humor (VH) was obtained from a patient with diabetic retinopathy. The VH was dialyzed with distilled water to remove salt using Biodialyzer™ (membrane: B010K; Cypress, Tokyo, Japan), which can remove molecules smaller than 1000 u (molecular mass). About 500 μl VH were dialyzed overnight at 4 °C with two changes of 3 l 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 (nonylphenoxy polyethoxy ethanol, Sigma, St. Louis, MO, USA), 30 m*M* dithiothreitol (DTT, Sigma), 50 m*M* Tris–HCl (pH 8.3). Electrophoresis was carried out at a constant current of 40 mA per gel until the tracking dye reached the cathode. After fixation with 10% acetic acid–50% methanol for 30 min, all of the unstained gel was cut into 50 slices, 1.5 mm in width, and the slices were washed by agitation for 30 min in 300 μl of 50% methanol and then dried under a vacuum. Disulfide bonds were reduced with 50 m*M* DTT in 50 m*M* ammonium bicarbonate (pH 8.3) by incubation for 1 h at 56 °C and alkylated with 100 m*M* iodoacetoamide in the same buffer for 45 min in the dark at room temperature. Excess reagents were removed and the gel was 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 50 μl of digestion buffer [50 m*M* ammonium bicarbonate (pH 8.3)] containing 250 ng TPCK modified trypsin (Promega, Madison, WI, USA) at 4 °C for 45 min. After the trypsin solution was discarded and 100 μl of the digestion buffer was added in the tube, the tube kept at 37 °C for 18 h. The peptide solution was recovered and the gel pieces were further extracted with 100 μl of 5% formic acid and 5% formic acid–50% acetonitrile. The combined solution was concentrated, resolved with 0.1% formic acid and stored frozen until use. Reagents not specified were purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Mass spectrometric identification

ESI-IT-MS–MS (ESI-IT-tandem mass spectrometry) experiments were performed with a LCQ^{DECA} (ThermoQuest, San Jose, CA, USA) equipped with a monitor C_{18} 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 μl/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). Solvents were purchased from Nacalai Tesque.

3. Results and discussion

3.1. 1D-PAGE of VH derived from a patient with diabetic retinopathy

The mass spectrometric analysis and database search of 50 gel slices of 1.5 mm width allowed us to characterize 84 different proteins. These are listed in Table 1. Some proteins were found from two or more fractions, probably due to fragmentation in vivo or during preparation. Fig. 1 shows silver-stained 1D-PAGE patterns of VH proteins derived from a patient with diabetic retinopathy and the positions of four angiogenic and three anti-an-

Table 1
Proteins identified from gel slices, fraction numbers, and peptides identified by ESI-IT-MS–MS and database analyses, mol.mass: molecular mass shown in data base, in which carbohydrate was not included

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
1	Zinc finger protein	164704	1047.4	229–237	FEVQVTVPK
			1395.3	124–135	NEDSLVFVQTDK
			1673.2	215–228	TEHPFTVEEFVLPK
	Matrixmetalloproteinase-1	53 988	926.1	444–450	QYKFDPK
			1385.7	376–388	HIDAALSEENTGK
			1553.1	152–165	VSEGQADIMISFVR
	α_2-Microglobulin*	163 259	1117.3	854–863	QTVSWAVTPK
			1019.2	812–820	ATVLNYLPK
			1085.5	522–531	GHFSISIPVK
			1449.7	665–676	DMYSFLEDMGLK
	Ig(Heavy chain)*	51 442	1187.4	122–133	GPSVFPLAPSSK
			1678.8	298–311	FNWYVDGVEVHNAK
	Tyrosine-protein kinase JAK2	191 065	1101.2	716–723	WYQFTSLR
			1230.3	783–793	TIVAVEVQDQK
			2002.6	853–869	AFVYLSNLLYPVPLVHR
	Kinesin-like protein KIF1A	130 645	1044.4	631–639	FGSLDTYLK
			1340.6	697–709	LSDPGISITVLPK
			1831.2	566–581	EVGDYGQLHETEVLLK
2	Thrombospondin-1	129 394	1571.2	180–192	TDSTDFFIEPLER
			1749.2	127–141	LVVPGSSVEWQEDFR
			1776.7	530–543	DCVGDVTENQICNK
	Tyrosine protein kinase receptor EHK-3	112 078	1073.3	893–901	MIRNPNSLK
			1235.6	802–811	WTAPEAIQYR
			1716.2	942–957	DNFTAAGYNSLESVAR
	Albumin*	69 348	960.7	427–434	FQNALLVR
			1095.6	35–44	FKDLGEENFK
			1640.3	438–452	KVPQVSTPTLVESR
3	Interphotoreceptor retinoid-binding protein	135 344	870.9	316–323	ALAILTLR
			1014.4	937–946	VPTVLQTAGK
			1346.8	736–747	TEVLPGQLGYLR
			1386.7	123–134	HEVLEGNVGYLR
	Tastin	83 740	785.3	124–130	SPLQVLK
			840.2	371–377	HLNGDER
			870.1	82–89	LVGISQPR
			1311.7	243–254	QIEASVVAIRPK
	Albumin*	69 227	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	Ceruloplasmin*	122 187	1192.4	548–558	DIFTGLIGPMK
			1241.5	610–619	EDEDFQESNK
			1432.7	721–732	QSEDSTFYLGER
			2705.2	577–598	EFYLFPTVFDENESLLLEDNIR
4	Interphotoreceptor retinoid-binding protein	135 344	871.3	316–323	ALAILTLR
			1014.2	937–946	VPTVLQTAGK
			1038.3	1062–1069	LLVEHIWK
			1169.5	1228–1236	EMLQHNQLR
			1346.4	736–747	TEVLPGQLGYLR
			1447.9	1071–1082	IMHTDAMIIDMR

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
	Endotherial cell multimerin	138 053	1412.8	1016–1028	KPTVNLTTVLIGR
			1520.6	910–922	SIHLSINFFSLNK
			1784.1	989–1005	SLPGSLANVVKSQKQVK
5	HSP 71 kDa	70 880	1160.2	558–567	LQGKINDEDK
			1254.6	302–311	FEELNADLFR
			1304.5	540–550	NSLESYAFNMK
			2516.2	470–493	GVPQIEVTFDIDANGILNVSAVDK
	LA ribonucleoprotein	46 819	965.3	216–223	LEEDAEMK
			1317.6	317–327	IIEDQQESLNK
			1813.2	281–297	EALGKAKDANNGNLQLR
	Albumin*	69 367	927.6	162–168	YLYEIAR
			947.9	222–229	LKCASLQR
			960.5	427–434	FQNALLVR
			1925.3	589–603	ETCFAEEPTMRIRER
	Ig(Heavy chain)*	52 728	1857.6	477–493	GGLGHPLPELADELRRK
			1882.4	391–406	EPTSPPERPCPEPDEK
			2232.9	160–179	LLFAGSRSQLVQLPVADCMK
6	YL1 protein	40 576	989.3	101–110	VNTPAGSSQK
			1316.5	175–185	EAKITEELNLR
			1630.3	116–130	ALLPLELQDDGSDSR
	α-Catenin	100 062	1134.3	738–748	NTSDVISAAKK
			1379.7	684–695	IAEQVASFQEEK
			2160.3	634–651	TPEELDDSDFETEDFDVR
	Albumin*	69 367	960.4	427–434	FQNALLVR
			1450.7	106–117	ETYGEMADCCAK
			1640.4	438–452	KVPQVSTPTLVESR
			1925.3	589–603	ETCFAEEPTMRIRER
	Ig(Heavy chain)*	51 409	1186.4	122–133	GPSVFPLAPSSK
			1678.8	298–311	FNWYVDGVEVHNAK
7	Neutrophil gelatinase-associated lipocalin	20 530	820.2	162–168	ELTSELK
			1442.7	63–74	SYNVTSVLFRKK
			1786.3	129–143	VVSTNYNQHAMVFFK
	Platelet glycoprotein IV	53 035	1112.4	399–407	IQVLKNLKR
			1368.9	387–398	LQVNLLVKPSEK
			1958.5	369–385	TYLDIEPITGFTLQFAK
	Insulin-like growth factor 1a	15 159	728.9	110–115	HTDMPK
			984.2	116–123	TQKEVHLK
			1669.2	54–68	GFYFNKPTGYGSSSR
	Albumin*	69 227	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	Transferrin*	76 981	1249.3	454–464	SASDLTWDNLK
			1276.6	300–310	EFQLFSSPHGK
8	Insulin-like growth factor 1a	15 159	729.2	110–115	HTDMPK
			984.2	116–123	TQKEVHLK
			1669.2	54–68	GFYFNKPTGYGSSSR
	Albumin*	69 227	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
	ATP-dependent DNA helicaseII	69 825	790.2	181–187	ASRARTK
			1208.6	35–45	DSLIFLVDASK
			1342.7	115–123	RILELDQFK
9	G protein pathway suppressor I	53 354	635.8	308–312	DIIFK
			969.3	289–297	NVISSSSFK
			1244.7	298–307	LFLELEPQVR
	Acyl CoA dehydrogenase	46 570	1245.4	195–205	EGDYYVLNGSK
			1913.2	244–259	CSDTRGIVFEDVKVPK
			2009.4	71–88	FAQEQIAPLVSTMDENSK
	Carnitine palmitoyltransferase II	73 759	983.2	232–239	DELFTDDK
			1064.5	152–161	ATNMTVSAIR
			1636.2	168–182	AGLLEPEVFHLNPAK
	Albumin*	69 227	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	Transferrin*	76 981	1249.3	454–464	SASDLTWDNLK
			1276.6	300–310	EFQLFSSPHGK
10	Protein kinase C	78 429	886.2	91–98	GPQTDDPR
			1747.2	253–268	NDFMGAMSFGVSELLK
			3321.7	167–197	APTADEIHVTVGEARNLIPMDPNGLSDPYVK
	Transcription factor TMF	123 153	821.3	369–376	TVESAEGK
			1480.8	338–351	SVSEINSDDELSGK
			2977.2	774–799	QIENLQATLGSQTSSWEKLEKNLSDR
	Albumin*	69 227	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	Transferrin*	77 031	1249.3	454–464	SASDLTWDNLK
			1276.6	300–310	EFQLFSSPHGK
			1284.3	531–541	EGYYGYTGAFK
11	Granzyme M	27 428	793.2	95–101	AAIQHPR
			1136.6	218–228	VLAGVLSFSSR
			1202.4	165–174	ELDLQVLDTR
			1664.1	121–135	VKPSRTIRPLALPSK
	Albumin*	69 227	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	Transferrin*	77 031	979.3	216–225	DGAGDVAFVK
			1249.3	454–464	SASDLTWDNLK
			1276.6	300–310	EFQLFSSPHGK
			1284.3	531–541	EGYYGYTGAFK
12	Medium chain acyl-CoA dehydrogenase	46 570	1220.5	264–275	ENVLIGDGAGFK
			1377.8	62–73	EEIIPVAAEYDK
			1913.1	244–259	CSDTRGIVFEDVKVPK
	Finger protein 9	45 049	1145.5	186–194	IHTEEKPYK
			1168.4	145–154	AFNWSSTLNK
			1347.5	285–296	AFNLSSTLTKHK

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
	Matrixmetalloproteinase-12	53 983	1001.4	380–388	DAAVFNPR
			1078.4	257–266	GIQSLYGDPK
			1259.5	166–177	GAHGDFHAFDGK
	Albumin*	69 227	927.7	162–168	YLYEIAR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	α1-Antichymotrypsin*	47 632	1095.3	351–360	NLAVSQVVHK
			1775.2	201–214	WEMPFDPQDTHQSR
			2260.2	222–239	WVMVPMMSLHHLTIPYFR
	α1-Antitrypsin*	46 718	1111.3	315–324	LSITGTYDLK
			1333.8	150–160	LVDKFLEDVKK
			1641.7	50–63	ITPNLAEFAFSLYR
			2574.1	126–149	TLNQPDSQLQLTTGNGLFLSEGLK
			3402.3	35–63	TDTSHHDQDHPTFNKITPNLAEFAFSLYR
13	Collagen α2(V)	144 702	1140.3	606–617	GQPGTMGLPGPK
			1311.6	570–583	GLTGNPGVQGPEGK
			1716.9	501–518	GPRGDPGTLGPPGPVGER
	ATP-dependent DNA helicase II	69 825	790.2	181–187	ASRARTK
			1162.4	115–123	RILELDQFK
			1208.6	35–45	DSLIFLVDASK
	Albumin*	69 348	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1075.1	206–214	LDELRDEGK
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	α1-Antichymotrypsin*	47 632	1095.3	351–360	NLAVSQVVHK
			1775.2	201–214	WEMPFDPQDTHQSR
	α1-Antitrypsin*	45 718	1111.3	315–324	LSITGTYDLK
			1641.7	50–63	ITPNLAEFAFSLYR
			2574.1	126–149	TLNQPDSQLQLTTGNGLFLSEGLK
			3402.3	35–63	TDTSHHDQDHPTFNKITPNLAEFAFSLYR
14	ATP-dependent DNA helicase II	69 442	947.3	275–283	FAVAVPQSK
			1103.5	249–258	IGVEAFILLK
			1897.2	301–318	SYSYGGSSVVFGSDELNK
	Albumin*	69 348	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1075.1	206–214	LDELRDEGK
			1468.5	361–372	RHPDYSVVLLLR
	α1-Antichymotrypsin*	47 632	1095.3	351–360	NLAVSQVVHK
			1775.2	201–214	WEMPFDPQDTHQSR
	α1-Antitrypsin*	46 718	1111.3	315–324	LSITGTYDLK
			1333.8	150–160	LVDKFLEDVKK
			1641.7	50–63	ITPNLAEFAFSLYR
			2574.1	126–149	TLNQPDSQLQLTTGNGLFLSEGLK
			3402.3	35–63	TDTSHHDQDHPTFNKITPNLAEFAFSLYR
	Apo (a)*	515 061	1043.3	38–47	GTYSTTVTGR
			1300.9	5–15	EVVLLLLLFLK
15	Albumin*	69 348	927.7	162–168	YLYEIAR

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
			960.9	427–434	FQNALLVR
			1075.1	206–214	LDELRDEGK
	α1-Antichymotrypsin*	47 632	1095.3	351–360	NLAVSQVVHK
			1216.8	364–374	ITLLSALVETR
			1422.8	240–251	DEELSCTVVELK
	α1-Antitrypsin*	46 718	1009.1	180–187	QINDYVEK
			1111.3	315–324	LSITGTYDLK
			1333.8	150–160	LVDKFLEDVKK
			2574.1	126–149	TLNQPDSQLQLTTGNGLFLSEGLK
	Ig γ-3*	41 268	1287.4	275–285	EPQVYTLPPSR
16	Platelet endotherial cell growth factor	49 963	1056.1	147–157	GLGHTGGTLDK
			1143.6	254–265	TLVGVGASLGLR
			1414.5	266–279	VAAALTAMDKPLGR
			1493.1	236–249	FGGAAVFPNQEQAR
	Collagen α1(XI)	181 125	899.3	930–938	GQIGPIGEK
			1096.2	1362–1373	RGPPGAAGAEGR
			1358.8	900–913	GDVGLPGKPGSMDK
	Albumin*	69 227	927.7	162–168	YLYEIAR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	Vitamin D-binding protein*	52 932	1255.7	208–218	HLSLLTTLSNR
			1530.9	38–50	EDFTSLSLVLYSR
			1695.9	51–65	KFPSGTFEQVSQLVK
	Ig γ-3*	41 268	1287.4	275–285	EPQVYTLPPSR
	α1-Acid glycoprotein*	23 740	995.2	74–81	TEDTIFLR
			1710.3	139–153	NWGLSVYADKPETTK
17	Pigment epithelium derived factor	46 311	1056.3	307–316	TVQAVLTVPK
			1251.6	400–411	DTDTGALLFIGK
			1384.7	334–345	LQSLFDSPDFSK
			1560.4	54–68	LAAAVSNFGYDLYR
			1895.6	198–214	EIPDEISILLLGVAHFK
			1957.3	107–123	ALYYDLISSPDIHGTYK
	Glioma pathogenesis-related protein	26 554	924.4	136–142	ILEMDFR
			1158.2	144–152	GYINDDWFK
			2023.5	111–127	FPVTYSFLDANLQEHIK
	Zn-α2-glycoprotein*	34 718	1126.4	91–99	EDIFMETLK
			1128.2	246–255	AGEVQEPELR
			1409.8	25–36	YSLTYIYTGLSK
	Albumin*	69 367	927.6	162–168	YLYEIAR
			947.9	222–229	LKCASLQR
			960.5	427–434	FQNALLVR
			1925.3	589–603	ETCFAEEPTMRIRER
	Apo A-IV*	43 384	1708.3	245–259	QRLAPLAEDVRGNLR
			2045.8	74–90	DSEKLKEEIGKELEELR
			2705.6	285–306	RRVEPYGENFNKALVQQMEQLR
	α1-Acid glycoprotein*	23 740	995.2	74–81	TEDTIFLR
			1446.8	127–138	TYMLAFDVVDEK
			1710.3	139–153	NWGLSVYADKPETTK
	Cathepsin D*	44 534	1046.2	185–194	QPGITFIAAK
			1110.4	257–266	GSLSYLNVTR
			1960.5	206–222	ISVNNVLPVFDNLMQQK

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
	Glutathione *S*-transferase*	27 488	1125.3	32–41	TVDLVKGQHK
			1744.5	1–15	SCESSMVLGYWDIR
			1995.4	83–98	YIARKHNMCGETEEEK
			3312.5	198–225	IAAYLQSDQFCKMPINNKMAQWGNKPVC
	Serine proteinase inhibitor EPC-1	40 280	1026.1	260–268	LSYEGEVTK
			1517.5	167–178	TSLEDFYLDEER
			1895.4	139–155	EIPDEISILLLGVAHFK
18	Zn-α2-glycoprotein*	34 718	929.3	140–146	DYIFNK
			1126.4	91–99	EDIFMETLK
			1128.2	246–255	AGEVQEPELR
	Albumin*	69 367	927.6	162–168	YLYEIAR
			947.9	222–229	LKCASLQR
			960.5	427–434	FQNALLVR
	Apo A-IV*	45 317	1084.2	201–209	LTPYADEFK
			1236.4	113–123	LLPHANEVSQR
	α1-Acid glycoprotein*	23 740	995.2	74–81	TEDTIFLR
			1113.4	171–179	SDVVYTDWK
19	α-Actin 2	41 990	999.1	186–193	DLTDYLMK
			1131.5	199–208	GYSFVTTAER
			1502.9	87–97	IWHHSFYNELR
	Ig γ-3*	41 268	1287.4	275–285	EPQVYTLPPSR
	Zn-α2-glycoprotein*	34 718	929.2	140–146	DYIEFNK
			1409.8	25–36	YSLTYIYTGLSK
			1776.9	208–224	QDPPSVVVTSHQAPGEK
	Albumin*	69 367	927.6	162–168	YLYEIAR
			947.9	222–229	LKCASLQR
			960.5	427–434	FQNALLVR
			1925.3	589–603	ETCFAEEPTMRIRER
	Apo A-IV*	45 317	1084.2	201–209	LTPYADEFK
			1236.4	113–123	LLPHANEVSQR
	α1-Acid glycoprotein*	23 740	995.3	74–81	TEDTIFLR
			1113.4	171–179	SDVVYTDWK
			1446.8	127–138	TYMLAFDVNDEK
	Complement C3*	187 144	1093.3	1442–1450	NTLIIYLDK
			1402.9	892–904	SSLSVPYVIVPLK
			1472.8	914–926	AAVYHHFISDGVR
20	Blue-sensitive opsin	39 116	825.2	277–283	NHGLDLR
			1139.6	284–293	LVTIPSFFSK
			1452.8	3–13	KMSEEEFYLFK
			1461.8	229–242	AVAAQQQESATTQK
	Zn-α2-glycoprotein*	34 718	1126.5	91–99	EDIFMETLK
			1128.4	246–255	AGEVQEPELR
	α1-Acid glycoprotein*	23 493	1161.5	43–51	WFYIASAFR
			1754.2	109–123	YVGGQEHFAHLLILR
21	Apo LAL2*	20 530	953.4	100–108	LVPILQAAK
			1053.2	78–87	ALPEGVTTHK
			1530.2	114–127	VTAHLHESAPLIIK
22	Macrophage scavenger receptor type I & II	49 744	862.2	309–317	GAIGFPGSR
			1137.7	294–305	GFPGPIGPPGLK

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
			1172.2	258–266	DWEHSQTLR
			1356.9	219–230	VYNVSAEIMAMK
	Apo J*	52 476	948.2	82–89	EDALNETR
			1075.2	159–167	IDSLLENDR
			1076.3	69–79	RPHFFFPK
			1394.5	183–194	ASSIIDELFQDR
23	Albumin*	69 367	927.6	162–168	YLYEIAR
			947.9	222–229	LKCASLQR
			960.5	427–434	FQNALLVR
			1925.3	589–603	ETCFAEEPTMRIRER
	Apo J*	52 476	1076.4	159–167	RPHFFFPK
			1289.4	326–336	ELDESLQVAER
	Apo E*	36 136	969.2	199–207	LGPLVEQGR
			1034.2	270–278	LQAEAFQAR
			1115.3	261–269	LEEQAQQIR
24	Transthyretin*	13 742	1367.6	22–34	GSPAINVAVHVFR
			1523.6	35–48	KAADDTWEPFASGK
	α1-Microglobulin*	38 981	962.3	141–149	HHGPTITAK
			1022.1	159–166	ETLLQDFR
			2385.9	63–85	MTVSTLVLGEGATEAEISMTSTR
25	Albumin*	69 227	927.7	162–168	YLYEIAR
			960.9	427–434	FQNALLVR
			1468.5	361–372	RHPDYSVVLLLR
			2045.4	397–413	VFDEFKPLVEEPQNLIK
	Apo J*	52 476	1072.4	45–54	EIQNAVNGVK
			1075.5	159–167	IDSLLENDR
			1289.4	326–336	ELDESLQVAER
26	Golgi-associated particle 102 K chain	102 469	973.1	672–680	QLAELAISK
			1051.4	760–768	TYLPSQVSR
			1580.2	656–669	IAYQLAVEAESEQK
	α1-Microglobulin*	38 981	708.1	107–111	FLYHK
			1022.1	159–166	ETLLQDFR
			2129.4	206–226	AVLPQEEEGSGGGQLVTEVTK
	Transthyretin*	13 742	1367.6	22–34	GSPAINVAVHVFR
			1522.7	55–68	KAADDTWEPFASGK
			2451.4	81–103	ALGISPHEHAEVVFTANDSGPR
27	Ig(κ)*	22 968	1193.8	116–126	TPAWTFGQGTK
			1839.2	1–16	MDMRVPAQLLGLLLLR
			2381.3	47–67	ASQSISSYLNWYQQKPGKAPK
	α1-Microglobulin*	38 981	707.9	107–111	FLYHK
			1022.1	159–166	ETLLQDFR
			2006.5	167–185	VVAQGVGIPEDSIFTMADR
28	G protein-coupled receptor 10	40 805	1252.4	160–169	YVVLVHPLRR
			1441.7	210–22	LCEEFWGSQER
			1466.8	357–370	IAPHGQNMTVSVVI
	Carbonic anhydrase I*	28 852	715.2	151–157	VGEANPK
			971.2	161–169	VLDALQAIK
			986.3	82–90	GGPFSDSYR

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
			1187.8	139–150	ADGLAVIGVLMK
			1614.2	115–128	YSAELHVAHWNSAK
	Ig(κ)*	14 226	1007.3	66–74	LLPHANEVSQR
			1304.4	87–99	FSGSGSGTDFTLK
	Fab fragment (L)*	24 376	759.9	61–67	ESGVPDR
			1123.3	52–60	LLIYWASTR
			1535.9	176–189	DSTYSMSSTLTLTK
			1593.2	162–175	QNGVLNSWTDQDSK
29	Apo A-I*	30 745	1231.5	240–250	QGLLPVLESFR
			1235.5	13–23	DLATVYVDVLK
			1386.5	251–262	VSFLSALEEYTK
	Ig(λ)*	22 780	1744.9	176–190	YAASSYLSKTPEQWK
	Albumin*	69 367	927.6	162–168	YLYEIAR
			947.9	222–229	LKCASLQR
			960.5	427–434	FQNALLVR
30	Apo A-I*	30 745	1159.6	202–212	LEALKENGGR
			1235.5	13–23	DLATVYVDVLK
			1386.5	251–262	VSFLSALEEYTK
	Ig(κ&λ)*	14 226	1007.3	66–74	LLIYGASNR
			1304.6	87–99	FSGSGSGTDFTLK
	Ig(Fab)*	24 376	1123.3	52–60	LLIYWASTR
31	Apo A-I*	30 745	832.2	213–219	LAEYHAK
			1160.2	202–212	LEALKEKGGAR
			1216.4	220–230	ATEHLSTLSEK
			1401.7	52–64	DYVSQFEGSALGK
	Ig(λ)*	10 350	1010.3	30–38	LLIYGTSSR
			1124.3	68–80	SGTSASLAISGLR
32	Glutathione peroxidase*	25 791	749.2	202–208	TTVSNVK
			1029.4	209–216	MDILSYMR
			1315.8	186–197	FLVGPDGIPIMR
	Apo A-I*	30 745	1159.6	202–212	LEALKENGGR
			1235.5	13–23	DLATVYVDVLK
			1386.5	251–262	VSFLSALEEYTK
	Ig(λ)*	10 350	1010.3	30–38	LLIYGTSSR
			1124.3	68–80	SGTSASLAISGLR
	Ig(Fab)*	23 191	761.2	75–81	GSWTGPR
			1027.3	49–60	ALGPGAPGGSSR
			2238.7	91–111	HNSVTHVFGSGTQLTVLSQPK
	Retinol binding protein*	22 849	1166.3	156–166	DPNGLPPEAQK
			1162.4	38–47	FSGTWYAMAK
			1661.2	3–17	WVWALFLLAALGSGR
33	Hyaluronidase*	36 435	949.3	179–186	ALMEDTLR
			1048.1	95–103	AESKQELDK
			1114.4	169–178	AYTGFEQAAR
	Glutathione peroxidase*	25 487	819.4	148–153	FYTFLK
			951.1	169–175	LFWEPMK
			1315.9	186–197	FLVGPDGIPIMR

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
	Hemoglobin β*	15 964	1127.4	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1315.4	19–31	VNVDEVGGEALGR
			1671.1	68–83	VLGAFSDGLAHLDNLK
34	Phosphotransferase	47 507	903.2	224–232	DAVAASIQK
			938.3	329–336	HHSQTSLK
			1332.6	337–347	VENLEQDNGWK
35	pHL E1F1	15 078	614.9	122–126	DRPAR
			714.2	101–106	QLSLPR
			1035.1	127–134	HPQEQPLW
			1603.1	107–120	FPSVSLQEASSFFR
	β2-Microglobulin*	13 696	753.3	33–39	HPAENGK
			766.3	27–32	IQVYSR
			1123.3	102–111	VNHVTLSQPK
			1149.4	69–78	VEHSDLSFSK
	Lysozyme C*	16 519	911.3	141–148	QYVQGCGV
			982.4	60–68	ATNYNAGDR
			1013.2	52–59	WESGYNTR
			1401.7	69–80	STDYGIFQINSR
36	Endostatin	20 676	925.1	71–79	AAVPIVNLK
			1107.5	58–66	LQDLYSIVR
			1212.4	122–132	SVWHGSDPNGR
	Glial fibrillary acidic protein	49 862	857.3	398–405	SVSEGHLK
			1033.2	331–339	LEEEGQSLK
			1278.5	357–367	LALDIEIATYR
			1931.2	377–390	ITIPVQTFSNLQIR
	Lysozyme C*	16 519	788.9	126–131	AWVAWR
			1401.5	69–80	STDYGIFQINSR
37	Hemoglobin β*	15 964	1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
38	Hemoglobin β*	15 964	1275.8	32–41	LLVVYPWTQR
			1315.4	19–31	VNVDEVGGEALGR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
39	Vascular endothelial growth factor	23 175	902.3	176–182	QLELNER
			2134.4	23–42	WSQAAPMAEGGGQNHHEVVK
	Serine-threonine protein kinase	53 642	1188.5	141–151	LGEGSYATVYK
			1492.2	168–180	LQEEEGTPFTAIR
			1625.2	190–203	HANIVLLHDIIHTK
	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
40	Fibroblast growth factor-3	26 868	843.9	145–151	RQPSAER
			1066.3	152–160	LWYVSVNGK
			1278.7	193–204	QLQSGLPRPPGK
	Distrophin-associated glycoprotein	97 562	679.9	303–308	KPPLPK

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
			1281.2	360–371	DPVPGKPTVTIR
			1664.2	374–389	GAIIQTPTLGPIQPTR
			2129.6	283–302	EGAMSAQLGYPVVGWHIANK
	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1315.4	19–31	VNVDEVGGEALGR
			1835.3	42–57	TYFPHFDLSHGSAQVK
	Transthyretin*	13 742	1367.6	22–34	GSPAINVAVHVFR
			1523.7	35–48	KAADDTWEPFASGR
41	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
42	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
			1835.2	42–57	TYFPHFDLSHGSAQVK
	Cystatin C*	15 781	686.2	72–77	ALQVVR
			1081.2	63–71	ASNDMYHSR
			1227.3	52–62	ALDFAVGEYNK
			1793.2	81–96	QIVAGVNYFLDVELGR
	Transthyretin*	13 742	1367.6	22–34	GSPAINVAVHVFR
			1523.7	35–48	KAADDTWEPFASGR
43	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
	Cystatin C*	15 781	686.2	72–77	ALQVVR
			1081.2	63–71	ASNDMYHSR
			1227.3	52–62	ALDFAVGEYNK
	Transthyretin*	13 742	1367.6	22–34	GSPAINVAVHVFR
			1523.6	35–48	KAADDTWEPFASGK
44	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
	Hemoglobin α*		1379.6	122–133	EFTPPVQAAYQK
		15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
	Transthyretin*	13 742	1368.2	22–34	GSPAINVAVHVFR
			1523.6	35–48	KAADDTWEPFASGK
45	Thrombospondin-3	104 183	1550.2	107–121	VHAVNLQQAGLADGR
			1756.3	48–63	TALLTAGDIYLLSTFR
			3317.6	8–37	GALALLLLCFFTSASQDLQVIDLLTVGESR
	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR

Table 1. Continued

Band	Protein	Mol. mass	[M+H]+	Residue	Identified peptides, sequence from database
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
	Transthyretin*	13 742	1367.6	22–34	GSPAINVAVHVFR
			1523.6	35–48	KAADDTWEPFASGK
46	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	819.0	94–100	VDPVNFK
			1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
	β2-Microglobulin*	13 696	753.3	33–39	HPAENGK
			1123.3	102–111	VNHVTLSQPK
			1149.4	69–78	VEHSDLSFSK
47	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
	β2-Microglobulin*	13 696	753.3	33–39	HPAENGK
			1123.3	102–111	VNHVTLSQPK
			1149.4	69–78	VEHSDLSFSK
48	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
	Apo A-II*	11 157	1089.4	70–78	EQLTPLIKK
			1157.4	68–77	SKEQLTPLIK
			1200.4	52–62	VKSPELQAEAK
			2386.9	79–100	AGTELVNFLSYFVELGTQPATQ
49	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
			1379.6	122–133	EFTPPVQAAYQK
	Hemoglobin α*	15 239	1072.5	33–41	MFLSFPTTK
			1530.7	18–32	VGAHAGEYGAEALER
50	Hemoglobin β*	15 964	1127.3	97–105	LHVDPENFR
			1275.8	32–41	LLVVYPWTQR
	Hemoglobin α*	15 239	819.2	94–100	VDPVNFK
			1671.1	68–83	VLGAFSDGLAHLDNLK

$[M+H]^+$, m/z used for CID MS. Residue, numbers from N-terminal cited from database.
*Found in plasma and listed on database (3).
Proteins identified previously (1) but not in the present paper: DNA binding protein, aquapolin-CHIP, thyroid receptor interaction protein, uracil-DNA glycosylase, fatty acid coenzyme A ligase, SnoN2, EFT-ubiqunone oxidoreductase, SP100-B, angiotensin-converting enzyme*, nuclear receptor subfamily I, dystrophin/utrophin-associated protein, guanine nucleotide binding protein, S100 calcium-binding protein, phosphoglycerate mutase, syntaxin5, GOS28/P28 protein, prostaglandin D2 synthase*, apotosis inhibitor hiap2, CGI-180 protein, glycerol-3-phosphate dehydrogenase, signal recognition particle protein, ER81 protein, alpha-actinin, FYVE-finger protein EIP1, guanine nucleotide exchange factor, lipoprotein GlnI, 27 kDa prosomal protein, indolethylamine *N*-methyltransferase, liver-specific BHLH-ZIP transcription factor, desrin, gene pp21 protein, putative HLA-associated protein.

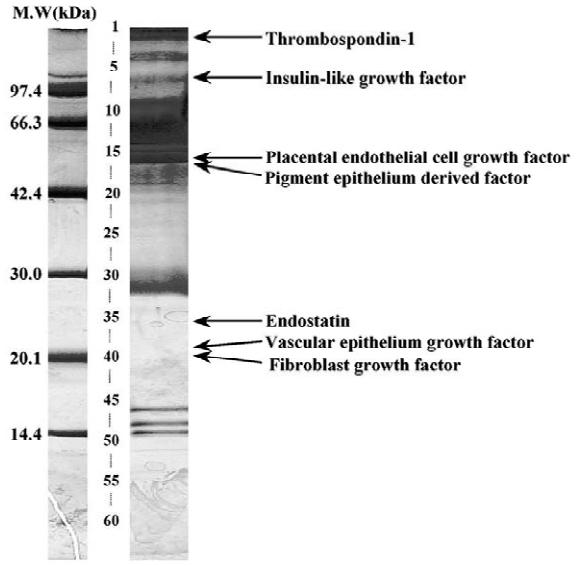

Fig. 1. One-dimensional SDS–polyacrylamide gel electrophoresis of proteins in VH derived from a patient with diabetic retinopathy. The position of seven angiogenesis regulating factors are shown by arrows. Factors were identified by blind extraction, digestion, and MS analysis. Migration positions of size markers are shown on the left. Proteins were not identified with stained gel but the profile of stained gel is shown to exhibit the migration position of each protein.

giogenic factors. Proteins were not identified by stained gel but the profile of stained gel is shown in the figure to exhibit the migration position of each protein. The profiles of the gel electrophoresis in repeated analyses were essentially same. The identified proteins from different gels were reproducible, although the relative recovery (signal intensity of MS spectra) of the peptides was different among running.

3.2. CID spectra of protein identification

Fig. 2a–e shows CID spectra of the tryptic digests of spots (#2, #7, #16, #17, #36) in VH derived from a patient with diabetic retinopathy. The CID spectra obtained from spot #2 matched the sequences of three peptides of a protein, thrombospondin-1 (TSP-1). One of three spectra is shown in Fig. 2a. These spectra covered 4% of the total TSP-1 sequence, which strongly support its identification. Other angiogenic modulated factors also identified by CID spectra and database search. Fig. 2b–e show each typical CID spectra of the tryptic digests of spots (#7, #16, #17 and #36). The CID spectra matched the sequences of three, four, four and three peptides of proteins, insulin-like growth factor 1a, platelet endothelial cell growth factor, pigment epithelium-derived factor (PEDF) and endostatin, respectively. These spectra covered 21, 11, 12 and 16% of the protein sequences, respectively, which strongly support these identifications.

3.3. A catalogue of proteins in human vitreous humor

As listed in Table 1, 84 different proteins were clearly identified from the patient with proliferative diabetic retinopathy. Previously we reported 51 proteins in VHs by 2D-PAGE, and five high isoelectric point (p*I*) proteins by ion-exchange column chromatography. These 84 included 24 of the 51 different proteins which we previously found by 2D-PAGE [1]. Sixty proteins were identified only by the present method, these are listed in the table caption. In total, we found 116 different proteins. In addition, we found five proteins seen at the genomic level only. These are listed in Table 2.

Some of the proteins we have reported previously and some in the present report were also found in plasma and listed on database [3], which are marked in the table. Previously we reported 35 non-plasma proteins in VH, and now we added 40 non-plasma proteins. By comparing the 2D-PAGE profiles obtained from VH proteins with those of plasma reported in literature, the specific proteins in VH were located at p*I* values between 5.0 and 9.0, and at a molecular mass between 20 000 and 65 000 u. In the present report, many non-plasma proteins were found between 20 000 and 100 000 u.

3.4. Angiogenic and anti-angiogenic factors

The identified proteins include seven angiogenesis-regulating proteins [4–10], five of which were not found by extraction from stained 2D-PAGE. The analysis by the method described here was not quantitative, but we may very roughly estimate the amount by the intensity of the peptide signals. We estimate the order of the concentration of these

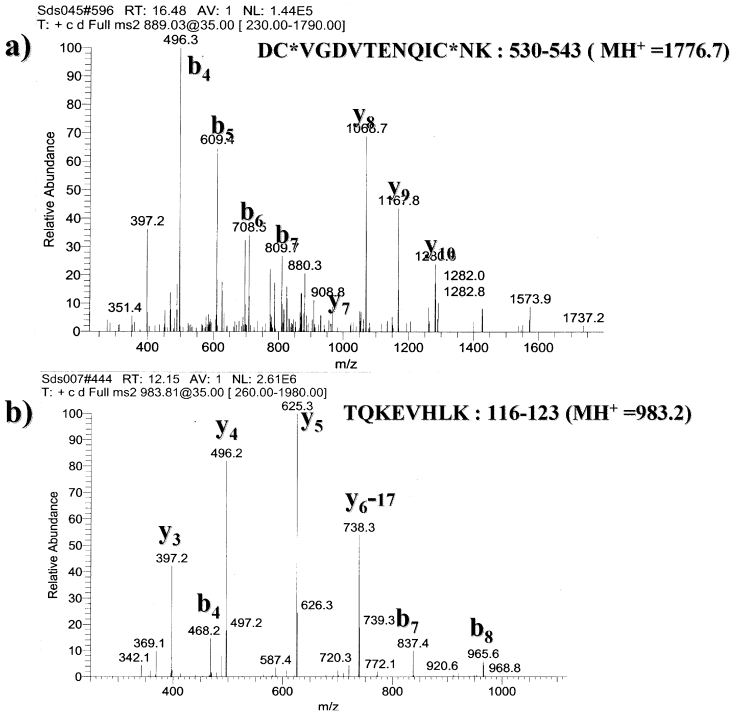

Fig. 2. ESI-MS–MS spectra of peptides from the in-gel digest of 1D gel spots (#2, #7,#16, #17, #36) from a diabetic retinopathy patient. Five angiogenesis regulating factors of the resulting CID spectra are shown here, along with the database sequence of peptides. Peaks representing y and b series ions are marked.

angiogenic and anti-angiogenic factors is similar to that of various proteins, excluding plasma proteins. We assume the concentrations of these factors are relatively high in vitreous. It may reflect the important role of these factors in vitreous.

Further quantitative analyses are important to elucidating the role of these factors is generating angio-proliferative retinopathy. We preliminarily analyzed PEDF in VH obtained from five patients with diabetic vascular proliferative retinopathy and from five with macular hole, which is retinopathy without vascular proliferation, by SDS–PAGE and Western blotting. The average density of the PEDF band showed no difference between the groups with diabetic retinopathy and macular hole (R. Koyama et al., unpublished data). More precise quantification is needed, but it is noteworthy that anti-angiogenic factor was not at a lower level in angio-proliferative state.

These data are important for examining quantitative and structural changes of these factors in the vitreous with diabetic retinopathy, and may be useful in the study of pathology of various eye diseases.

3.5. Hypothetical proteins in VH

Five proteins identified in VH so far were not seen in protein database and seen at the genomic level only, i.e., hypothetical proteins. These are listed in Table 2. One of them, MJ0781 (accession No. Q58191) was first identified from *Methanococcus jannaschii* and the function of this protein was suggested to be cleavage signal translocating protein, a major constitute of the membrane-localized translocation channel, formation of ribonucleoprotein complex, a receptor to signal recognition particle [11]. KIAA0112 (No. Q15050) showed homology to yeast ribosome biogenesis regulatory protein and the full-length cDNA clones was isolated from size-fractionated cDNA libraries of human immature myeloid cell line KG-1 [12]. An unknown protein (No. 14726525) was similar to T-cell-derived inducible

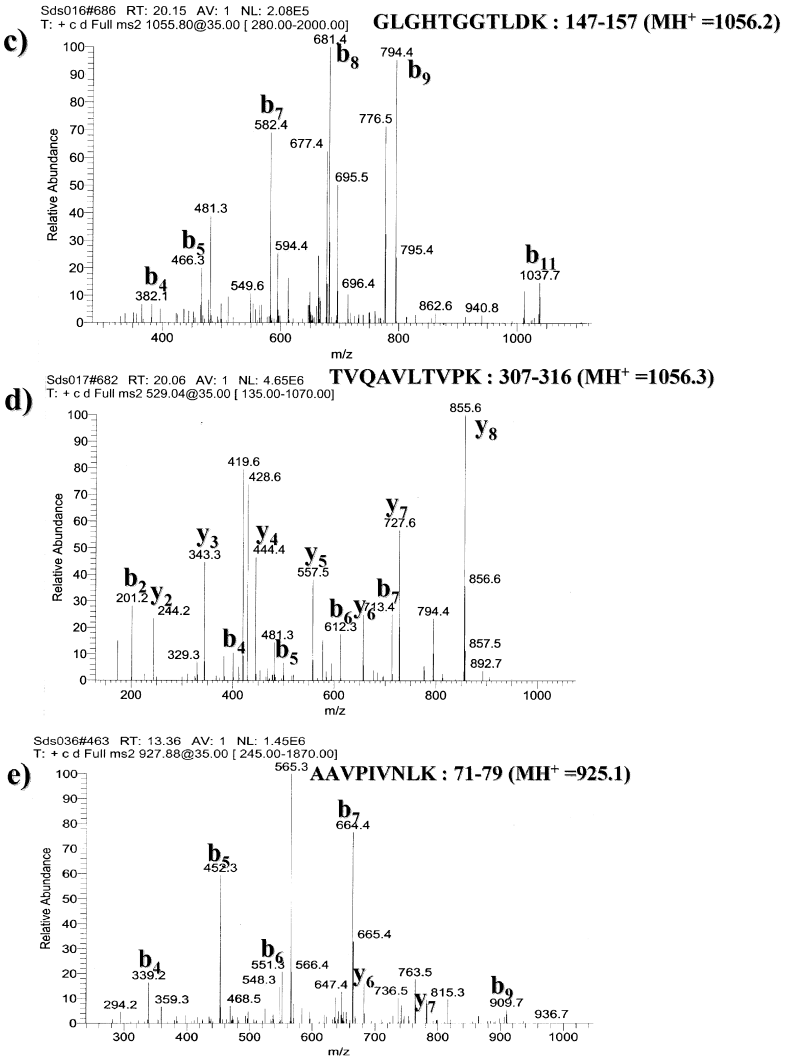

Fig. 2. (*continued*)

factor α (TIFA), a novel tumor necrosis factor-receptor associated factor 6 (TRAF6) binding protein. TRAF6 is a critical mediator of signal transduction by the viral oncogene latent membrane protein 1 [13]. The others (Nos. 4200222 and 15929862) were only identified the sequence of the messenger RNA.

Proteins which have homologies to them were not found yet.

The identification of these 121 proteins in VHs may be an important initial step to investigate pathological changes in retinopathies.

Acknowledgements

This work was supported by a 2001–2002 Grant-in-Aid for Scientific Research (C) (14572190) from

Table 2
Hypothetical proteins identified in our study, 2D-PAGE and ion-exchange chromatography

Band #	Protein		Rel. mol. mass	$[M+H]^+$	Residue	Identifies peptides, sequence from database
10	MJ0781 (Acc.No. 21431847)	1D	82 724	984.4	632–631	ILGIVEIVK
				1202.5	642–651	TTLYEYNGLK
				1676.2	599–612	IMLTALNFIINQQR
13	KIAA0112 (434779)	1D	44 427	1628.9	148–160	WQQFARLKGIRPK
	Unknown protein (15929862)	2D	52 266	1882.2	314–330	QTVSWAVTPK
				2248.8	186–203	LHPVLHKEEKQHLERLNK
				2284.5	91–108	QICGTHRQTKKMFCDMDK
				2704.8	313–336	RGPLNSDRSDYFAAWGARVFSFGK
	Hypothetical protein (14726525)	2D	21 445	1045.6	93–101	KTNLIVDSR
				1307.7	119–128	FGEYQFLMEK
				1475.8	39–51	EKLPSSEVVKFGR
				1707.8	64–77	QVSRVQFSLQLFKK
	Hypothetical protein (4200222)	2D	29 502	1052.8	205–212	IQELEHQR
				1795.0	1–14	MRESQLQQEDPMDR
				2211.3	106–124	KTTAIIAEYKQICSQLSTR

Rel. mol. mass, relative molecular mass shown in data base, in which carbohydrate was not included. $[M+H]^+$, m/z used for CID MS. Residue, numbers from N-terminal cited from database.

the Ministry of Education, Science and Culture of Japan.

References

[1] T. Nakanishi, R. Koyama, T. Ikeda, A. Shimizu, J. Chromatogr. B 776 (2002) 89.
[2] R.J. Simpson, L.M. Connolly, J.S. Eddes, J.J. Pereira, R.L. Moritz, G.E. Reid, Electrophoresis 21 (2000) 1707.
[3] http://www.expasy.ch/ch2dothergifs/publi/plasma-acidic and basic.gif
[4] K. Suzuma, H. Takagi, A. Otani, H. Oh, Y. Honda, Am. J. Pathol. 154 (1999) 343.
[5] S. Shigematsu, K. Yamauchi, K. Nakajima, S. Iijima, T. Aizawa, K. Hashizume, Endocr. J. 46 (Suppl.) (1999) S59.
[6] D.J. Hill, A. Flyvbjerg, E. Arany, F.F. Lauszus, J.G. Klebe, J. Clin. Endocrinol. Metab. 82 (1997) 1452.
[7] J. Spranger, M. Osterhoff, M. Reimann, M. Mohlig, M. Ristow, M.K. Francis, V. Cristofalo, H.P. Hammes, G. Smith, M. Boulton, A.F. Pfeiffer, Diabetes 50 (2001) 2641.
[8] H. Noma, H. Funatsu, H. Yamashita, S. Kitano, H.K. Mishima, S. Hori, Arch. Ophthalmol. 120 (2002) 1075.
[9] A.N. Witmer, J. van den Born, G.F. Vrensen, R.O. Schlingemann, Curr. Eye Res. 22 (2001) 190.
[10] J. Spranger, H.P. Hammes, K.T. Preissner, H. Schatz, A.F. Pfeiffer, Diabetologia 43 (2000) 1404.
[11] C.J. Bult, O. White, G.J. Olsen, L. Zhou, R.D. Fleischmann, G.G. Sutton, J.A. Blake, L.M. FitzGerald, R.A. Clayton, J.D. Gocayne, A.R. Kerlavage, B.A. Dougherty, J.F. Tomb, M.D. Adams, C.I. Reich, R. Overbeek, E.F. Kirkness, K.G. Weinstock, J.M. Merrick, A. Glodek, J.L. Scott, N.S. Geoghagen, J.C. Venter, Science 273 (1996) 1058.
[12] T. Nagase, N. Miyajima, A. Tanaka, T. Sazuka, N. Seki, S. Sato, S. Tabata, K. Ishikawa, Y. Kawarabayasi, H. Kotani, DNA Res. 2 (1995) 37.
[13] U. Schultheiss, S. Puschner, E. Kremmer, T.W. Mak, H. Engelmann, W. Hammerschmidt, A. Kieser, EMBO J. 20 (2001) 5678.