Protein Database: Human Retinal Pigment Epithelium

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INTRODUCTION

The retinal pigment epithelium (RPE) is a simple cuboidal epithelium that separates the photoreceptor cells of the retina from their principal blood supply in the choroid. In all vertebrates, the RPE forms an integral part of the blood-retinal barrier and is responsible for vectorial transport of nutrients to rod and cone photoreceptors and removal of waste products to the blood. In addition, the RPE phagocytizes shed photoreceptor outer segments, absorbs scattered light and functions in the retinoid visual cycle and regeneration of bleached visual pigment.
To facilitate studies of retina and RPE in health and disease, we have initiated the development of a human RPE protein database. RPE cells were isolated from normal adult human donor eyes, subcellular fractions prepared and proteins fractionated by electrophoresis. Preliminary proteomic analyses have identified 278 proteins and provide a starting point for building a database of the human RPE proteome. (This report is currently in press at Molecular and Cellular Proteomics).

METHODS

In vivo RPE Samples: Forty-two (42) normal human eyes obtained from the Cleveland Eye Bank within 3-12h postmortem have been used for proteome analyses. Following bisection of the globe behind the limbus, the anterior segment and vitreous were discarded and the retina removed. RPE cells were gently brushed from the eye cup using an artists 7 mm angular paint brush and Ca+2 and Mg+2 free PBS containing 5mM EDTA and 1 mM PMSF (2-3x, 500 µl). The RPE cells were then washed 2-3x by centrifugation in PBS. For select preparations, red blood cells were removed by centrifugation in a Percholl gradient (1.0 - 1.1 g/ml, Amersham/Pharmacia); the pigmented RPE cells float near the top of the gradient and were collected with a pasteur pipette then washed 2x in PBS. Whole cell lysates were prepared by homogenizing RPE cell pellets in isoelectricfocusing (IEF) solvent B (7M urea, 2M thiourea, 4% CHAPS, 0.5% triton X-100, 2% carrier ampholyte, 1% DTT), the solution clarified by centrifugation and protein quantified by a modified Bradford assay. About 95 µg of soluble whole cell RPE protein was recovered per eye (n = 11 eyes).

Subcellular fractionation: Freshly isolated RPE cells were suspended in 1-4 ml of 25 mM Tris-acetate pH 7, 0.25 M sucrose, 1 mM DTT, homogenized with 25-125 manual passes of a glass homogenizer, and clarified in a microfuge 10 min at 1000xg. The clarified RPE lysate was centrifuged at 27,000g/5°C/20 min, yielding the P2 membrane fraction. The supernatant was centrifuged again at 150,000xg for 1h/5°C, yielding the microsomal and cytosolic cell fractions. The microsomal and P2 pellets were resuspended in IEF solvent B. The cytosolic fraction was exchanged into IEF solvent B using Centricon concentrators (Amicon, 10kD MW cut off). Average recovery per eye was about 18 µg cytosolic protein, 16 µg P2 membrane protein and 9 µg microsomal protein (n = 31 eyes) based on the modified Bradford assay.

Electrophoresis: One and two dimensional electrophoresis was performed using the BioRad Mini-Protein II, BioRad Protein IIxi, Pharmacia IPGphor and Pharmacia IsoDalt systems (West et al., 2001; Aulak et al., 2001; Miyagi et al., 2002). Isoelectricfocusing was performed with non-linear pH 3-10 or linear pH 4-7 immobilized pH gradients (18 cm IPG strips, Pharmacia) in 7M urea, 2M thiourea, 4% CHAPS, 0.5% Triton X-100, 2% carrier ampholytes, 1% DTT. Second dimension electrophoresis utilized 23.5 x 18 x 0.1 cm gels (12% acrylamide). Colloidal Coomassie blue (Pierce Code Blue) or silver stained gel patterns were recorded with Quantity One and PDQuest Gel Analysis Software (BioRad). Protein from multiple eyes was utilized for most electrophoretic separations and amounts varied from ~30 to ~500 µg/gel.

Protein Identification by Mass Spectrometry: Gel spots and bands were excised, stain washed away, proteins digested in gel with trypsin and peptides were extracted for mass spectrometric analysis (West et al., 2001; Aulak et al., 2001; Miyagi et al., 2002; Crabb et al., 2002). For peptide mass mapping, peptides were adsorbed onto C18 ZipTips, eluted with acetonitrile and analyzed using a Voyager DE Pro MALDI-TOF mass spectrometer (PE Biosystems). Measured peptide masses were used to query the Swiss-Protein, TrEMBL and NCBI sequence databases for matches using MS-Fit and Profound search programs and a mass tolerance of 50 ppm. For LC MS/MS, tryptic digests were injected onto a 0.3 x 1 mm trapping column (PepMap C18, LC Packings) using a CapLC system (Micromass). Peptides were eluted at 250 nl/min and chromatographed on Biobasic C18 columns (50 µm x 5 cm or 75 µm x 5 cm, New Objectives) directly into a quadrupole time-of-flight mass spectrometer (QTOF2, Micromass). Protein identifications from MS/MS data utilized Micromass software ProteinLynxTM Global Server, MassLynxTM, Ver. 3.5, and the Swiss-Protein and NCBI protein sequence databases.

Practical Recommendations for Building a Protein Databases

Record keeping, bioinformatic analyses and data curating are essential steps in building a protein database. For those contemplating a database project, we offer a few general recommendations. These methods are neither novel nor original-just common sense.

- Define early in the project what information will be included in the database. Before collecting data, construct a summary table with appropriate headings (eg, in Excel) that will include electronic links to the original data (eg, gel scans, mass spectra, search results).
- In separate laboratory records, maintain the details of 1D /2D gels, MALDI TOF MS and LC MS/MS analyses, including sample source, spot/lane identifiers, electrophoresis and MS conditions, etc. Electronically link all gels, spectra and search results used in the database to the summary table as soon as possible after data collection.
- Remove duplications in the database and weak identifications. Check the SwissProt and NCBI annotation pages for evidence of multiple names and accession numbers for each identified protein. Another type of homology search such as FASTS, can also be useful for revealing duplications and detecting weak identifications (e.g. from a single peptide). For the latter, the MS determined sequence is assumed to be accurate, and FASTS used to calculate an Expectation value. The lower the E value, the more statistically significant the sequence alignment.
- Because accession numbers are updated and revised over time, verify and update accession numbers as needed prior to release of the database.

TABLE 1

In Vivo Human RPE Proteins

Protein	Accession Number[a]	MALDI TOF MS Matches (Error ppm)[b]	MALDI TOF MS Coverage %	LC MS/MS Matches	LC MS/MS Peptides Identified[c]	Cell Fraction[d]	1D Gel[e] Cell Fraction - MW (kD)	2D Gel[f] Cell Fraction / Spot No.	MW (kD) / pI 2D Gel Observed	MW (kD) / pI Calculated
Acetyl-CoA Acetyltransferase 1	P24752	5	19	7		C, WC	C 40	45/7.3		45.20 / 8.98
Acetyl-CoA Acyltransferase 2	P42765			4		C	C 43	40/5.3		41.91 / 8.50
Acid Ceramidase	Q13510			3		C	C 38			44.64 / 7.52
Acidic Ribosomal 60S Protein P0	P05388	6 (18)	28			M		40/5.8	40/7.4	34.27 / 5.72
Acidic Ribosomal 60S Protein P2	P05387			3		WC		11/4.5	40/6.5-7.2	11.66 / 4.42
Aconitate Hydratase	Q99798	11	21	16		C, WC	C 97	94/6.9	38-40/7.6-7.8	85.42 / 7.36
Actin 1, Alpha	P02568	8	34	10		P	P 43	45/5.2		41.83 / 5.30
Actin, Beta	P02570	9	35	6		M, C, WC		44/5.1-5.3, 41.5/5.3	40/5.5	41.74 / 5.29
Actin, Gamma	P02571	8	34	4		C,P,WC	C 43	84/5.1	68/5.8	41.60 / 5.45
Acyl-CoA Dehydrogenase, C2 To C3 Short Chain	P16219			2		C	C 39		41/5.4	44.30 / 8.13
Acyl-CoA Dehydrogenase, Medium-Chain Specific	P11310			4		C	C 40, C43		44/6.8	46.58 / 8.61
Acyl-CoA Dehydrogenase, Very-Long-Chain Specific	P49748			7		C	C 70			70.39 / 8.92
Acyl-CoA Oxidase 1	Q15067			2		C	C 50			74.44 / 8.35
Adenylate Kinase Isoenzyme 1	P00568	5 (21)	31			C		27/7.8	11/6.2	21.67 / 8.40
ADP, ATP Carrier Protein	P05141			5		P	P 33		40/6.4, 38/7.7	32.83 / 9.84
Aflatoxin B1 Aldehyde Reductase	O43488			1	FFGNSWAETYR	C	C 36			33.06 / 9.78
Aldehyde Dehydrogenase, E2 Isozyme	P05091			12		C	C 55			56.38 / 6.63
Aldehyde Dehydrogenase, E3 Isozyme	P49189			6		M, C	C 54		50 / 6.2	53.53 / 6.01
Aldolase A, Fructose-bisphosphate	P04075	10 (7)	29			WC		42/7.5	11/4.5	39.28 / 8.39
Aldolase C, Fructose-bisphosphate	P09972	10	40	2		M, C, WC	C 35	43/6.5, 43/6.9, 43/6.6	41/5.1	39.44 / 6.41
Alpha-1-Antichymotrypsin	P01011			3		C		65/4.4		25.18 / 5.64
Alpha-1-Antitrypsin	P01009	11	33	6		C, WC		65/5.0	82/5.9	46.73 / 5.37
AMBP Protein Precursor	P02760			1	MTVSTLVLGEGATEAEISMTSTR	WC		41/7.1	43/5.4, 44/5.3	38.98 / 6.20
Aminobutyrate Aminotransferase	P80404			2		C	C 53		97/6.4	56.55 / 7.56
Annexin II	P07355			5		C		38/7.4		38.45 / 8.00
Annexin IV	P09525			1	GLGTDEDAIISVLAYR	C	C 22			35.75 / 5.85
Annexin V	P08758			4		C	C 22		52/5.8	35.94 / 4.94
Annexin VI	P08133			4		C	C 75			75.74 / 5.42
Antioxidant Protein 2	P30041			3		WC		46/6.4		24.89 / 6.30
Antiquitin	P49419			5		M	C 55	60/6.5		55.36 / 6.44
Apolipoprotein A-I	P02647	11	44	7		M		30/5.2	43/6.3	30.77 / 5.56
Arrestin C	P36575	6 (13)	21			M		51/5.5	48/6.5	42.76 / 5.53
Asparate Aminotransferase	P00505	9	20	13		C, WC	C<19	46/8.4		47.47 / 9.14
Ataxin 1	P54253			1	EASPSTLNDK	WC		30/7.5		84.03 / 8.90
ATP Synthase Oligomycin Sensitivity Conferral Protein	P48047			2		P	P 29		27/6.9, 27/7.0	23.33 / 10.2
ATP Synthase, Alpha	P25705	8	23	12		M, P, WC		57/7.8	36/6.4	59.75 / 9.16
ATP Synthase, Beta	P06576	16	39	13		M, C, P, WC		49/5.1, 31/5.0, 62/5.5	48/7.4, 46/7.5	56.55 / 5.26
ATP Synthase, Delta	P30049			2		WC		11/4.5	32/6.5	17.47 / 5.50
Brain Acid Soluble Protein 1	P80723			15		WC		46/4.6	66/6.2	22.50 / 4.70
Cadherin 3	P22223			1	STGTISVISSGLDR	C	C 65			90.75 / 4.64
Calgranulin B	P06702			1	LGHPDTLNQGEFK	WC			65/5.5	13.22 / 6.00
Calmodulin-like Protein	Q9NZT1			2		C		C 29	38 / 7.3	15.90 / 4.3
Calnexin	P27824			3		P	P 100			67.57 / 4.47
Calreticulin	P27797	7	27	5		C, WC	C 50		66/4.0	48.14 / 4.29
Calumenin	O43852	8 (12)	3			WC			28/5.5	37.11 / 4.47
Carbonic Anhydrase I	P00915	7 (10)	38			C		C 16	32/6.6	28.87 / 6.59
Carbonic Anhydrase II	P00918	5	29	3		C, WC		WC 18	33/6.7	29.11 / 6.86
Carnitine O-Palmitoyltransferase II	P23786			5		C	C 74			73.77 / 8.38
Catalase	P04040			6		M	M 67			59.76 / 6.90
Cathepsin A	P10619			1	DLECVTNLQEVAR	M	M33, M 34			54.49 / 6.16
Cathepsin B	P07858			1	NGPVEGAFSVYSDFLLYK	M	M 35			36.66 / 5.69
Cathepsin D	P07339	13	54	10		M,C,WC	C 100	WC 7, M 44, C 22	35/5.3-5.5, 34/5.5, 32/5.4	44.55 / 6.10
Cathepsin Z	Q9UBR2			1	NVDGVNYASITR	C	C 65			33.85 / 7.1
CD36 Antigen 2	Q14108			1	TSLDWWITDK	C	C 44, M 44			54.29 / 5.00
Cell-Surface Antigen Heavy, 4F2	P08195			1	VAEDEAEAAAAAK	P	P 100			57.94 / 5.20
Cellular Retinaldehyde-Binding Protein	P12271	16	48	6		M, C, P, WC	P 35	WC 8, M 45, C 18	39/5.0, 38/5.0, 28-30/5.4	36.47 / 4.98
Cellular Retinol-Binding Protein	P09455			1	ALDVNVALR	M	M 20			15.70 / 5.11
Centractin, Alpha	P42024	6 (11)	26			M		M 11	46/6.4	42.61 / 6.19
Centractin, Beta	Q13841	6 (16)	24			M		M 19	45/6.1	37.10 / 6.19
Chaperonin GroEL	A32800			8		M			64/5.2	61.04 / 5.80
Chymotrypsin Inhibitor	1313184C			3		C			48/4.5	48.64 / 5.79
Citrate Synthase	O75390			2		C	C 44, M 44			51.70 / 8.13
Clathrin Heavy Chain 1	Q00610			8		P	P >97			191.61 / 5.48
Clusterin	P10909			1	EILSVDCSTNNPSQAK	WC			38/5.4	52.48 / 6.20
C-Myc Promotor Binding Protein	P22712			2		C		C 20	36/5.6	37.07 / 7.20
Cofilin Non-muscle	P23528			3		C		C 30	22/7.4	18.54 / 8.50
Complement C1	Q07021			3		C	C 21, C33			31.36 / 4.74
Complement C3	P01024			1	VFSLAVNLIAIDSQVLCGAVK	WC			44/6.5	187.15 / 6.30
Complement C9	P02748			1	GTVIDVTDFVNWASSINDAPVLISQK	WC			44/6.4, 56/5.8	63.16 / 5.50
Creatine Kinase A	1QK1A			1	GTGGVDTAAVGGVFDVSNADR	WC			45/5.0	42.72 / 6.30
Creatine Kinase Ubiquitous Mitochondrial	P12532			5		M		M 48	45/7.5	47.02 / 8.7
Creatine Kinase, B Chain	P12277	18	50	6		M, C, WC	M 43	WC 28, M 48, C 25	47/5.3, 46/7.4, 44/5.3	42.64 / 5.34
Creatine Kinase, M chain	P06732			1	LEKGQSIDDMIPAQK	C		C 25	40/5.5	43.08 / 7.20
Crystallin A Chain, Alpha	P02489			1	FVIFLDVK	M	M 25			19.74 / 5.78
Crystallin B Chain, Alpha	P02511	6 (7)	30			P, WC		WC 16	27/6.6	20.16 / 6.76
Crystallin Zeta	Q08257			5		C	C 35			35.21 / 8.56
Cytochrome B5	P00167	5 (11)	69			WC			13/5.0	11.27 / 5.02
Cytochrome C Oxidase Polypeptide Vb	P10606			1	GLDPYNVLAPK	P	P 19			11.65 / 9.9
Delta 1-Pyrroline-5-Carboxylate Dehydrogenase	P30038	9	22	5		C, WC	C 60	WC 23, C8	61/6.7, 59/7.0	61.75 / 8.25
Delta 3,5-Delta 2,4-Dienoyl-CoA Isomerase	Q13011			2		C	C 34			35.99 / 6.61
Dermcidin	P81605			1	ENAGEDPGLAR	M			66/7.4	11.27 / 6.50
Dienoyl-CoA Reductase	Q16698			1	VAGHPNIVINNAAGNFISPTER	C	C 22			36.06 / 9.35
Dihydroflavonal 4-Reductase, Putative	O22617 (F)			1	LLEHGYTVR	WC			30/6.2	38.08 / 6.70
Dihydrolipoamide Dehydrogenase	P09622	7	17	3		M	M 65	M 26	5/6.9	54.18 / 7.59
Dihydrolipoamide S-Succinyltransferase	PN0673	7	22	3		M	M 55	M 43	54/5.7	48.69 / 9.01
DNA Polymerase III, Alpha	P57332 (B)	7		1	LTLLASTQEGYKNLILLISR	M	M 34			132.78 / 9.2
DNA-directed RNA Polymerase II	P24928	7		1	NSVSQVIQLR	WC	C 45			14.57 / 6.9
EGLN2 Protein	Q96KS0	7		1	LLIFWSDR	WC			46/6.6	43.65 / 8.20
Electron Transfer Flavoprotein, Alpha	P13804	7		8		M, C	C 22			35.07 / 8.62
Electron Transfer Flavoprotein, Beta	P38117	7		4		WC			46/6.6	27.83 / 8.50
Elongation Factor, Tu	P49411	7		2		C		C 31	38/6.8	49.54 / 7.26
Elongatoin Factor, Putative	P15252 (T)			2		C	C 80		26/5.1	14.59 / 5.04
Emmprin	P35613			2		P	P 50			29.22 / 5.41
Endoplasmic Reticulum Protein	P30040	7	30	2		M, WC	C 54		34/5.2	28.99 / 6.77
Enolase, Alpha	P06733	8	32	6		M, C, WC	C 45	WC 26, M 47, C20	57/6.6, 53/6.9, 55/6.7	47.10 / 6.36
Enolase, Beta	P13929			1	AAVPSGASTGIYEALELR	C		C 20	36./5.6	46.81 / 8.00
Enolase, Gamma	P09104	19	50	11		M, C, WC	C 45	M 3, C 27	49/4.9, 51/4.7	47.15 / 4.94
Enoyl-CoA Hydratase	P30084			4		M, C	M 32, C32			31.37 / 8.34
Epoxide Hydrolase 1	P07099			4		M, C	M 45, C35			52.94 / 6.77
Esterase D	P10768			2		C	C 35			31.44 / 7.0
Estradiol 17 Beta-Dehydrogenase 4	P51659			1	VVLVTGAGAGLGR	C	C 35			79.68 / 8.96
Estradiol 17 Beta-Dehydrogenase 8	Q92506			1	SALALVTGAGSGIGR	C	C 32			26.97 / 6.09
Fatty Acid-binding Protein	Q01469			1	FEETTADGR	WC			28/6.5	15.16 / 6.60
Fibrinogen Beta	P02675			1	EGVNDNEEGFFSAR	C	C 73			12.73 / 6.16
Fibroinogen, Gamma	P02679			1	ANQQFVYCEIDGSGNGWTVFQK	WC			44/6.5	51.48 / 5.40
Fibronectin	P02751			1	TGLDSPTGLDFSDITANSFTVHWIAPR	WC			44/6.8	137.42 / 5.00
Flagellar Biosynthetic Protein, putative	O24978 (H)			1	IAIGFVGIILIASAIMGRFK	C		C 32	46/6.9	27.99 / 6.50
Flavin Reductase	P30043			1	TVAGQDAVIVLLGTR	C		C 29	40/7.4	21.98 / 7.00
FMS-related Tyrosine Kinase 1	P53767 (R)			1	YSVHGYSLIIK	M		M 49	70/5.6	150.23 / 8.7
Fodrin Alpha	S01091 (X)			2		P	P >97			51.94 / 5.7
Fumarate Hydratase	P07954			4		C	C 44, M 44			54.63 / 8.85
Gelsolin	P06396			6		C	C 100		98/5.4-5.6	85.70 / 5.90
Glial Fibrillary Acidic Protein	P14136	11	28	3		C, WC	C 35	WC 29	60/5.3	49.88 / 5.42
Glucosamine N-Acetyl-6-Sulfatase	P15586			1	IQEPNTFPAILR	C	C 20			62.08 / 8.60
Glucose Transporter 1	P11166			2		P	P 100			53.99 / 9.16
Glucose-Regulated Protein 75 kD	P38646	11	2	10		M, P, WC	P 74	WC 10, M 5	79/5.3, 78/5.4	73.78 / 5.97
Glucose-Regulated Protein 78 kD	P11021	24	43	15		M, C, P, WC	C 75, P 70	M 16, P 3	80/5.0, 83/5.0	72.33 / 5.07
Glucose-Regulated Protein 94 kD	P14625			14		C, P	C 100		98/5.0, 90/5.1	92.51 / 4.78
Glutamate Dehydrogenase 1	P00367	9	24	7		M, C, WC	M 32, C 55	M 35	58/7.1	61.39 / 7.66
Glutathione S-Transferase P	P09211	9	49	2		M, C, WC	M 23	C 28	28/5.5	23.30 / 5.96
Glyceraldehyde-3-Phosphate Dehydrogenase	P04406	8	48	8		M, C, P, WC	M 35, C 35, P 35	WC 9, M 4, C18, P 1	42/7.4-7.8, 40/7.8-8.5, 40/66-7.0	35.92 / 8.58
Glycogen Phosphorylase	P06737			1	VLYPNDNFFEGK	C	C 95			97.13 / 7.1
GTP-Binding Protein Rab1	TVHUYP			1		M	M 31			22.47 / 5.47
GTP-binding Protein yptV5	P36864 (V)			2		M	M 29			23.04 / 5.8
Guanine Nucleotide Binding Protein, Alpha 2	P29777			1	IGAADYQPTEQDILR	C	C 38			39.96 / 5.59
Guanine Nucleotide Binding Protein, Beta 1	P04901			4		P	P 34			37.38 / 5.60
Guanine Nucleotide Binding Protein, Beta 2	P11016	8	23	4		M, WC, P	P 35	M 34	39/5.2-5.4	37.33 / 5.6
Heat Shock Cognate 71 kD Protein	P11142	13	26	15		M, C, WC	M 70, C 70	WC 6, C 2	76/5.2, 31/7.0	70.89 / 5.37
Heat Shock Protein 105 kD	Q92598			1	GCALQCAILSPAFK	C	C 100			96.15 / 5.38
Heat Shock Protein 27 kD	P04792	6	33	5		M, P, WC	M 34, P 32	M 37	30/5.9	22.78 / 5.95
Heat Shock Protein 60 kD	P10809	12	3	11		M, WC	M 65		66/5.6	61.05 / 5.70
Heat Shock Protein 70 B2	P41827 (A)			3		C	C 72			70.14/5.8
Heat Shock Protein 70 kD Protein 1	P08107			3		C	C 72			69.52 / 5.00
Heat Shock Protein 90 kD, Alpha	P07900			5		C	C 96		98/4.8	84.66 / 4.94
Heat Shock Protein 90 kD, Beta	P08238			5		C	C 95		98/4.8	83.13 / 4.97
Heat Shock-related 70 kD Protein 2	P54652			5		C	C 75			70.00 / 5.7
Hemoglobin, Beta	P02023			8		C, P	C 100, P 20			15.86 / 6.81
Hemoglobin, Delta	P02042			3		C	C 45			15.92 / 7.97
Heterogeneous Nuclear Ribonucleoproteins A2/B1	P22626			1	IDTIEIITDR	M	M 36			37.42 / 8.97
Heterogeneous Nuclear Ribonucleoproteins C1/C2	P07910			1	GFAFVQYVNER	C	C 36			33.29 / 5.11
Hexosaminidase A	P06865			1	GLETFSQLVWK	C	C 50, C55			60.69 / 5.04
Hexosaminidase B	P07686			3		WC			30/5.9	63.09 / 6.70
High Modility Group Protein 1	P09429			1	IKGEHPGLSIGDVAK	WC			46/6.6	24.76 / 5.70
Histone H2A.x	P16104			1	VTIAQGGVLPNIQAVLLPK	M	M 30			14.05 / 10.87
Histone H2B.n	P23527			1	ESYSIYVYK	M	M 20			13.80 / 10.32
Histone H4	P02304			3		P	P 19			11.24 / 11.36
Hydroxyacyl-CoA Dehydrogenase	Q16836			7		C	C 23	C 33	38/7.7	34.28 / 8.88
Hydroxyisobutyrate Dehydrogenase	P31937			1	DFSSVFQFLR	M	M 35			35.32 / 8.38
Hydroxymethylglutaryl-CoA Lyase	P35914			2		C	C 23			34.39 / 8.81
Hydroxymethylglutaryl-CoA Synthase	P54868			8		C	C 50			56.63 / 8.40
Hypothetical Protein ypsB	P50839 (J)			1	LSNLEKHVFGSK	M		M 14	41/5.1	11.57 / 5.9
Hypothetical Protein 16.3 kD	O15113			1	VLEEALLSR	C	C 46			97.32 / 4.9
Hypothetical Protein 68.9 kD	Q8T845 (D)			1	IDLDSLPPSLK	WC		WC 27	66/7.1	68.85 / 7.0
Hypothetical Protein MJ0677	Q58090(N)			1	KFYANRYAELAK	M		M 49	70/5.6	35.84 / 5.1
Hypothetical Protein ykgB	O34499 (J)			1	ALSEPKLAAK	C	C 16			38.39 / 5.4
Ig Gamma-1 Chain C Region	P01857			3		M		M 42	55/7.9	36.09 / 8.60
Inorganic Pyrophosphatase	Q15181			1	YVANLFPYK	C	C 70			32.84 / 5.27
Intermediate Filament Protein ON3	P18520 (C)			3		C	C 40			57.78 / 5.15
Interphotoreceptor Retinoid-Binding Protein	P10745	15	23	9		M, C, WC	C 100	WC 21, M 8, C 35	>97/4.7, >97/4.8	135.36 / 4.98
Intracellular Chloride Channel Protein p64H1	Q96NY7			1	YLNNAYAR	WC			97/4.4	64.90 / 4.20
Isocitrate Dehydrogenase [NAD], Alpha	P50213			2		C	C 45			39.57 / 6.9
Isocitrate Dehydrogenase [NADP]	P48735			3		C	C 43			58.74 / 8.89
Isopentenyl Diphosphate, Delta-Isomerase 1	Q13907			1	AFSVFLFNTENK	C	C <19			26.31 / 5.93
Junction Plakoglobin	P14923			1	TLVTQNSGVEALIHAIIR	C		C 29	40/7.4	81.48 / 6.30
Keratin 19	P08727			3		M, C			40/6.5-7.2	44.09 / 5.10
Keratin 8	P05787			8		M, C			38-40/7.6-7.8	54.30 / 5.60
Lactate Dehydrogenase A, LDH-A	P00338			3		C	C 64, 33			36.69 / 8.44
Lactate Dehydrogenase B, LDH-B	P07195	8	28	6		C		C11	40/5.5	36.64 / 5.71
Lamin B2	Q03252	12 (11)	18			WC			68/5.8	59.00 / 5.90
Laminin Binding Protein	P08865	6 (14)	26			WC			41/5.4	31.79 / 4.80
Leucine Rich Protein	Q9UGS3			1	LIYLYLSDNQLAGLSAAALEGAPR	WC			44/6.8	85.60 / 9.20
Lysosome Membrane Protein II	Q14108			1	TSLDWWITDK	C	C 80			54.15 / 5.00
Lysosome-Associated Membrane Glycoprotein 2	P13473			3		C	C 95			44.96 / 5.35
Lysozyme C	P00695			1	STDYGIFQINSR	C			11/6.2	15.44 / 9.28
Malate Dehydrogenase	P40926	5	25	9		C, WC	C 35	WC 25, C 6	40/6.4, 38/7.7	35.53 / 8.92
Methylmalonate-Semialdehyde Dehydrogenase	Q02252			6		C	C 69			57.82 / 8.9
Methylmalonyl Coenzyme A Mutase	P22033			2		C	C 85			83.10 / 6.40
Motor Protein	Q16891			2		WC			50 / 6.2	83.66 / 6.4
Myosin Light Chain Alkali	P16475			1	EAFQLFDRT	WC			11/4.5	16.78 / 4.60
NAD Dependent Malic Enzyme, 65 kD Isoform	P37224 (L)			1	SLDILQDPWFNK	M		M 14	41/5.1	69.67 / 5.8
NADH Dehydrogenase Fe-s Protein 1	P28331			5		P	P 72			79.57 / 5.80
NADH-Ubiquinone Oxidoreductase 42 kD Subunit	O95299	10	25	2		P, WC	P 38		82/5.9	39.26 / 6.52
Nucleoside Diphosphate Kinase A	P15531			3		C		C 26	43/5.4, 44/5.3	65.85 / 8.30
Oxoglutarate Dehydrogenase E1 Component	Q02218	12	16	2		M, P	P 100	M 27	97/6.4	113.47 / 6.62
Oxoisovalerate Dehydrogenase 2, Beta	P21953			1	LGVSCEVIDLR	C	C 35			43.12 / 5.89
Oxoisovalerate Dehydrogenase, Alpha	P12694			1	VDGNDVFAVYNATK	C	C 56			50.37 / 8.14
P115 Protein, Putative	O51074 (Q)			1	SELNLDGLSK	WC			52/5.8	95.84/5.7
Palmitoyl-Protein Thioesterase 1	P50897			2		M	M 34			34.19 / 6.07
Peptidyl-prolyl Cis-trans Isomerase A	P05092			2		P	P 21			17.76 / 8.46
Peptidyl-prolyl Cis-trans Isomerase B	P23284			7		P	P 23			23.74 / 9.42
Peroxin-1	O43933			1	GMMKELQTK	M, C		C 37	43/6.3	42.85 / 6.20
Peroxiredoxin 6	1PRXA			3		WC			48/6.5	24.90 / 6.30
Peroxisomal Bifuctional Enzyme	Q08426			2		C	C 78			79.20 / 9.22
Phosducin	P20941			1	FSLDVLPTLLIYK	C	C 20			28.23 / 5.1
Phosphatidylethanolamine Binding Protein	P30086	7	58	4		M, C, P, WC	M 29	WC 15, C 3, P 10	27/6.9, 27/7.0	20.76 / 7.43
Phosphatidylinositol Transfer Protein, Alpha	Q00169	5	25	1	VILPVSVDEYQVGQLYSVAEASK	M, C	C 34	M 38	36/6.4	31.67 / 6.13
Phosphoglycerate Kinase 1	P00558	9	33	1	ACANPAAGSVILLENLR	C, WC	C 43	WC 24, C 5	48/7.4, 46/7.5	44.59 / 8.3
Phosphoglycerate Mutase 1	P18669	5	27	1	ALPFWNEEIVPQIK	C, WC	C 31	WC 12	32/6.5	28.67 / 6.75
Phospholipase C, Beta-1	Q9NQ66			1	QEQYYSEK	M		M 33	66/6.2	138.7/6.1
Programed Cell Death Protein 8	O95831			3		C	C 70			66.90 / 9.04
Prohibitin	P35232	5	23	1	DLQNVNITIR	WC, P	P 34		35/5.8	29.80 / 5.57
Proteasome, Component C2	P25786	4 (2)	22			M		M 21	34/6.4	29.55 / 6.15
Proteasome, Epsilon Chain	P28074	6 (12)	3			M		M 24	26/7.9	22.89 / 8.66
Proteasome, IOTA Chain	P34062	6 (13)	24			M		M 22	30/6.4	27.40 / 6.35
Proteasome, Subunit Alpha Type 2	P25787	5	25	2		M	P 30	M 23	28/6.9	25.76 / 7.12
Proteasome, Subunit Beta Type 1	P20618	6 (5)	31			M		M 39	29/7.5	26.48 / 8.27
Proteasome, Subunit P40.5, 26S	Q9UNM6	5 (10)	19			M		M 25	42/5.6	42.95 / 5.53
Proteasome, Subunit XAPC 7	O14818	4 (7)	28			M		M 40	30/8.2	27.89 / 8.60
Protein 14-3-3 Delta/Zeta	P29312			2		C	C 33			27.74 / 4.73
Protein 14-3-3 Epsilon	P42655			1	LICCDILDVLDK	C	C 34			29.17 / 4.63
Protein Disulfide Isomerase, A1	P07237	8	27	9		C, WC	C 55, C60		55/4.5	57.11 / 4.76
Protein Disulfide Isomerase, A3	P30101	23	43	8		M, C, P, WC	C55, C 60	WC 2, M 1, C 23	64/5.4, 57/5.6, 64/5.5, 66/5.2	56.78 / 5.98
Protein Disulfide Isomerase, A4	P13667			7		C	C 43			72.93 / 4.96
Protein Disulfide Isomerase, A6	Q15084			5		C	C 50			48.12 / 4.95
Protein Kinase C Substrate, 80 kD	P14314			2		C	C 95			59.28 / 4.4
Puromycin-Sensitive Aminopeptidase	P55786			1	LGLQNDLFSLAR	C	C 100			103.30 / 5.49
Pyruvate Dehydrogenase E1, Alpha	P08559			2		M	M 80			43.30 / 8.35
Pyruvate Dehydrogenase E1, Beta	P11177	7 (10)	27			M, WC		M 31	40/5.4	36.49 / 5.38
Pyruvate Kinase, M1 Isozyme	P14618	9	24	11		M, WC, C	M 60, C60	WC 27, M 9, C 7	65/7.1, 57/7.4-7.6, 60/6.8	57.74 / 7.57
Radixin	P35241			3		P	P 70			68.45 / 5.81
Ras - Related Protein Rab-7	P51149			4		M	M 32			23.51 / 6.39
Recoverin	P35243	6	44	9		C, WC		C 14	28/5.0-5.1	23.13 / 5.06
Reticulocalbin 1	Q15293			5		WC			40/5.1	38.87 / 4.90
Reticulon Protein 3	O95197			1	LFLVEDLVDSLK	M	M 32			25.61 / 8.67
Retinal Pigment Epithelium 65	Q16518	7	17	17		M, C, P	C 66, P 65	M 18	63/5.9	60.95 / 6.05
Retinol Dehydrogenase, 11-cis	Q92781			6		M	M 34, P 32			34.98 / 9.47
Rhodopsin	P08100			2		P	P 100			38.89 / 6.21
Ribophorin I	P04843			1	VTAEVVLAHLGGGSTSR	P	P 70			160.99 / 5.31
Ribosomal 60S Protein L12	XP_060130			1	VAPEEPGPGPSPVR	WC			47/4.6	38.05 /10.7
RPE-Retinal G Protein-Coupled Receptor	P47804			1	GIWQCLSPQK	C	C dye front			31.87 / 8.35
Salivary Peptide, Low MW	0712205B			1	GPPPPPGKPQ	WC			50/5.1	0.95/9.4
Saposin Precursor	SAHUP			2		M	M 20			58.48 / 5.03
S-Arrestin	P10523	6	27	8		M, C, P, WC	C 45, P 43	C 38, M 10	49/6.1	45.09 / 6.14
Serum Albumin	P02768	8	34	17		M, C, P, WC	C 70	M 49, P 6,WC 31	70/5.6-5.8, 55/5.0-5.1	69.36 / 5.92
SNF2- like Helicase	Q9XBI4 (J)			1	KVIVWGMFVK	M		M 33	58/6.2	73.34 / 6.5
Spectrin, Alpha, Non-erythroid 1	Q13813			7		P	P >97			284.28 / 5.22
Statherin Precursor	P02808			1	DSSEEKFLR	C		C 39	70/4.6	5.19 / 8.70
Succinate Semialdehyde Dehydrogenase	P51649			1	LAGLSAALLR	C	C 55			57.21 / 8.62
Succinyl-CoA Ligase, Beta Chain	Q96I99			2		C	C 40			45.13 / 5.46
Succinyl-CoA:3-Ketoacid-CoA Transferase	P55809			4		C	C 60			56.15 / 7.13
Superoxide Dismutase	P04179	5	34	1	GDVTAQIALQPALK	M, C	M 25	C 13	28/6.7	24.72 / 8.35
T-Complex Protein 1, Alpha	P17987	10 (10)	24	6		M		M 17	65/5.7-5.8	60.34 / 5.80
T-Complex Protein 1, Beta	P78371	7 (10)	18	6		M		M 33	57/6.1	57.49 / 6.01
T-Complex Protein 1, Epsilon	P48643	6	17	5		M, WC		M 36	62/5.3	59.67 / 5.45
T-Complex Protein 1, Gamma	P49368	9 (20)	2			M		M 32	64/6.1	60.33 / 6.23
T-Complex Protein 1, Theta	P50990	7 (18)	16			M		M 28	55/5.3	59.62 / 5.42
T-Complex Protein 1, Zeta	P40227			1	VATAQDDITGDGTTSNVLIIGELLK	M		M 50	65/6.5	58.00 / 6.6
Thioredoxin Dependent Peroxide Reductase 1	P30048			3		M, C	M 32	C 15	30/5.8-5.9	27.69 / 7.68
Thioredoxin Dependent Peroxide Reductase 2	Q06830	10	41	6		WC, C, P	P 31	WC 1, C 1	27/7.4, 28/6.6, 39/7.5	22.11 / 8.27
Thioredoxin Peroxidase 1	P32119	5	31	2		M, WC			25/5.9	21.89 / 5.66
Thiosulfate Sulfurtransferase	Q16762			2		C	C 22			33.16 / 6.78
Transaldolase	P37837			3		C	C 36			37.54 / 6.36
Trans-Enoyl-CoA Isomerase	P42126			2		C	C <19			32.81 / 8.80
Transferrin	P02787	9	15	2		M, C, WC	C 80	WC 20, M 7	86/6.2, 80/6.5	77.04 / 6.81
Transitional Endoplasmic Reticulum ATPase	P55072			4		C	C 100			89.32 / 5.14
Transketolase	P29401			1	LDNLVAILDINR	C	C 70			67.87 / 7.58
Transthyretin	P02766	5 (10)	51			C		C 12	37/5.3	12.60 / 5.33
Trifunctional Enzyme, Alpha	P40939			3		C, P	C79, P 90			83.10 / 9.28
Triosephosphate Isomerase	P00938	9	38	5		C, WC	C <19	WC 17, C4	30/6.5, 29/6.0-6.6	26.53 / 6.51
Tripeptidyl Peptidase I	O14773	9	23	6		M, C, P, WC	C 44, M 44	P 6	54/5.0	69.12 / 5.97
Tubulin Beta-2	P05217	23	59	9		M, C, P, WC	P 45, C 50	W WC 5, M 2	61/4.7, 60/4.6	49.83 / 4.79
Tubulin, Alpha, Ubiquitous	P05209	12	37	11		M, P, WC	P 50	M 15	57/5.0	50.15 / 4.94
Tumor Rejection Antigen-1	P14625			14		P	P 60			92.47 / 4.76
Ubiquinol-Cytochrome C Reductase Core Protein I	P31930	10	22	7		WC			54/6.1	52.62 / 5.94
Ubiquitin	P02248			1	TITLEVEPSDTIENVK	M	M 30			8.56 / 6.56
Ubiquitin-Activating Enzyme E1	P22314			1	LAGTQPLEVLEAVQR	C	C >97			117.78 / 5.57
Unknown Protein AF007134	AF007134	5 (17)	24			C		C8	35/6.4	27.97 / 6.08
Unknown Protein AAH08028	Q96HW2			6		M		M 42	51/7.8	49.40 / 9.4
Unknown Protein AAH17450	Q8WVW5			12		WC			46/6.6, 38/6.3	40.49 / 6.1
Unknown Protein MGC:3932	Q96E76			3		M		M 51	65/7.6	57.94 / 8.6
Unknown Protein MGC:8116	Q922U2 (M)			3		WC		WC 5	60/4.8	61.75 / 8.1
Vacuolar ATP Synthase A, Ubiquitous Isoform	P38606			4		C	C 70			68.26 / 5.35
Vacuolar ATP Synthase, Brain Isoform	P21281	7	17	2		M, C	C 50	M 29	52/5.3	56.57 / 5.66
Villin 2	P15311			14		P	P 70			69.40 / 5.94
Vimentin	P08670	14	39	3		C, P, WC	C 40, P 40	WC 3	60/5.0	53.65 / 5.06
Vitronectin Receptor, Alpha	P06756	11	17	16		M, P, WC	P >97	M 30	>97/4.4	116.05 / 5.45
Voltage Dependent Anion Channel 1	P21796	7	38	6		M, P, WC	M 35, P 35	WC 14, M 6, P 2	38/7.8, 36/8.2, 40/8.0	30.64 / 8.63
Voltage Dependent Anion Channel 2	P45880			4		P	P 35			31.59 / 7.50

a. Swiss-Protein database accession numbers are shown in plain font. Links to Swiss-Protein accession numbers use the EXPASY server at http://us.expasy.org/sprot/. NCBI accesssion numbers are in italics. Links to NCBI accession numbers use the Entrez server at http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=Protein. Parenthesis following accession numbers designate identifications based on homology with other species: A=Anopheles albimanus, B=Buchuera aphydicola, C=Carassius auratus, D= Dictyostelium discoideum, F= Fragaria x ananassu, H= Helicobacter pylori, J= Bacillus, L= Amaranthus hypochondriacus, M= Mus musculus, N=Methanocaldococcus jannaschii, Q= Borrelia burgdorferi, R= Rattus norvegicus, T= Hevea brasiliensis, V=Volvox carter, X=Xenopus laevis.

b. Average error of measured peptide masses is shown for identifications based soley on peptide mass mapping.

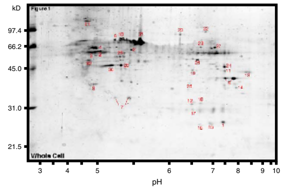
c. Peptide sequences are shown for identifications based on a single peptide (underlined residues were determined).

d. Cell fractions in which the protein was found: WC, whole cell; C, cytosolic; P, P2 membrane; M, microsomal.

e. SDS-PAGE are shown for C, P2 and M cell fractions in electronic Figure 5. Identified proteins were excised from the indicated MW region.

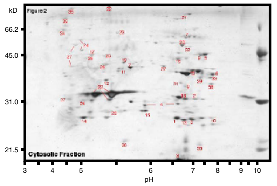
f. Representative 2D PAGE profiles for WC, C, P2 and M cell fractions are shown in electronic Figures 1-4, respectively. Identified proteins were excised from the numbered spots in the figures and/or from the indicated MW and pI range in other 2D gels.

FIGURE 1



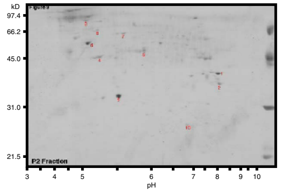
Human RPE Whole Cell Lysate. Approximately 150 µg protein was applied to 2D gel analysis (75,202 Vh IEF) and the gel stained with colloidal Coomassie Blue. Numbered spots were excised for protein identification (Table 1).

FIGURE 2



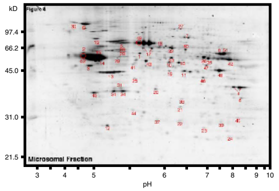
Human RPE Cytosol. Approximately 150 µg RPE cytosolic protein was applied to 2D gel analysis (87,550 Vh IEF) and the gel stained with silver. Numbered spots were excised for protein identification (Table 1).

FIGURE 3



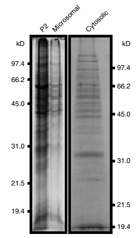
Human RPE P2 Membrane Subcellular Fraction. Approximately 80 µg RPE P2 membrane protein was applied to 2D gel analysis (87,550 Vh IEF) and the gel stained with silver. Numbered spots were excised for protein identification (Table 1).

FIGURE 4



Human RPE Microsome Subcellular Fraction. Approximately 190 µg RPE microsomal protein was applied to 2D gel analysis (88,400 Vh IEF) and the gel stained with colloidal Coomassie Blue. Numbered spots were excised for protein identification (Table 1).

FIGURE 5



SDS-PAGE Human RPE Subcellular Fractions. One dimensional SDS-PAGE was performed with the RPE P2 membrane (~30 µg) and microsomal fractions (~30 µg) on amini-gel (10 x 8 cm) and with the RPE cytosolic fraction (~40 µg) on a 20 x 20 cm gel. Gels were stained with colloidal Coomassie Blue and consecutive, ~1 mm bands excised from the top to the bottom of the lane for protein identification (Table 1). A total of 65 bands were cut and analyzed from each of the P2 and microsomal sample lanes and 80 bands from the cytosolic sample lane.

CONCLUSIONS

- A total of 278 human RPE proteins are listed in Table 1. The 1D and 2D PAGE methods were complimentary, each contributing unique protein identifications. One hundred sixty (160) proteins were identified following 2D PAGE, 180 proteins following 1D PAGE, and 62 proteins were identified from both 1D and 2D gels.
- Less than half (44%) of the proteins recently identified from in vitro cultures of rat RPE-J cells (West et al., 2001) were also observed in the present analysis of human in vivo RPE.
- Ten of the 278 identified proteins are currently designated hypothetical or of unknown function and ~6% were identified based on homology with other species.
- Despite efforts to purify RPE cells free of extracellular debris and other cell types, low level blood contamination (eg, hemoglobin) was detected in three gels. Photoreceptor specific proteins such as phosducin, recoverin and rhodopsin were also identified but may be in the RPE due to phagocytosis.
- A number of proteins were identified that are known to be associated with specialized functions of the RPE. These included proteins associated with retinoid metabolism and the visual cycle such as cellular retinaldehyde-binding protein, cellular retinol-binding protein, retinal pigment epithelium 65, 11-cis-retinol dehydrogenase 5, retinal G protein-coupled receptor and interphotoreceptor-retinoid binding protein (IRBP). The RPE is the most active phagocytic tissue in humans. Identified proteins involved in macromolecular degradation included cathepsins B, D and Z, lysozyme and several proteasome components. The photooxidative environment in the retina and active phagocytic processing provide abundant reactive oxygen species to the RPE. Identified anti-oxidant proteins included thioredoxin dependent peroxide reductase 1 and 2, catalase, peroxiredoxin 6, superoxide dismutase, glutathione S-transferase and thioredoxin peroxidase.

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ACKNOWLEDGEMENTS

This work was supported in part by NIH grants EY06603, EY02362, EY13160, EY014239 and EY014240, a Research Center grant from The Foundation Fighting Blindness, and funds from the Cleveland Clinic Foundation.