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PROTEINS AND GLYCOPROTEINS OF HAMSTER KIDNEY FIBROBLAST (BHK21) PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM

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SUMMARY

1. Hamster fibroblast plasma membranes and endoplasmic reticulum were solubilized by sodium dodecyl sulphate and 2-mercaptoethanol treatment and studied by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. The electrophoretic patterns of plasma membranes and endoplasmic reticulum were different. The amino acid compositions of three major plasma membrane protein bands were determined and were found to differ significantly. Both membranes contained a fast moving component of low apparent molecular weight (< 10000) in sodium dodecyl sulphate–polyacrylamide gel electrophoresis. It could be stained with Coomassie Blue and labelled by amino acids and glucosamine but not by fucose. It was probably lipid since its mobility on polyacrylamide gel electrophoresis corresponded to that of isolated radioactive gangliosides and phospholipids and quantitative amino acid analysis showed it did not contain protein.

2. When cells were labelled with glucosamine or fucose the labels were 9–12 times more concentrated in the plasma membranes than in the homogenate. The apparent molecular weights of the major plasma membrane and endoplasmic reticulum glycoproteins were determined by polyacrylamide gel electrophoresis.

INTRODUCTION

Mammalian cell membranes are mainly composed of lipid, protein and carbohydrate. Detailed analysis has shown that the lipid components of the plasma membrane differ from those of the endoplasmic reticulum[1–4].

Membrane proteins have been difficult to isolate and characterize because of their limited solubility in aqueous media and their tendency to aggregate. GREEN and associates[5,6] and others [7,8] have reported the presence of "structural proteins" as the major protein components of different membranes. The estimated values for the molecular weights of these have varied from 22500 to 65000. Recently LAICO *et al.*[9] claimed that polypeptides with molecular weights around 5000 comprise a major fraction of erythrocyte, mitochondrial and retinal rod outer membranes.

However, there is also evidence that cellular membranes consist of many different proteins with no predominant component[10,11]. Plasma membranes and the membranes of the endoplasmic reticulum, Golgi apparatus and mitochondria give

complex protein patterns on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. The patterns differ from each other[12,13]. The enzyme activities of the membranes also differ characteristically from each other[14,15].

Some membrane proteins are glycoproteins. These have been found in the plasma membranes of red cells[16,17], liver cells[14,18], platelets[19] and fibroblasts[20–23].

Our group is studying the chemical compositions of the cellular membranes of hamster kidney fibroblasts and the biosynthesis of these membranes. We have previously isolated plasma membranes and endoplasmic reticulum from hamster kidney fibroblasts (BHK21) and assessed their purity by enzymatic, immunological and chemical means[24,25]. The lipid composition of these membrane fractions has been studied in detail[26]. In this paper the protein compositions of the plasma membranes and endoplasmic reticulum of BHK21 cells are described.

MATERIALS AND METHODS

Chemicals

Sugar standards for chromatography were D-glucose (British Drug Houses Ltd., Poole, England), D-glucosamine·HCl and L-fucose (Fluka AG, Buchs SG, Switzerland), D-galactose and D-mannose (E. Merck AG, Darmstadt, Germany), *N*-acetyl-D-galactosamine (Sigma Chemical Company, St. Louis, Mo., U.S.A.). Acrylamide (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.) was recrystallized once from chloroform[27]. Nuclear Chicago solubilizer (Amersham Searle, Des Plaines, Ill., U.S.A.). Sodium dodecyl sulphate (Fluka) was 3 times recrystallized from ethanol. Reference proteins: porcine thyroglobulin (Sigma), transferrin (AB Kabi, Stockholm, Sweden), bovine serum albumin (Armour Pharmaceutical Company Ltd., Eastbourne, England), ovalbumin (Sigma) and ribonuclease A (EC 2.7.7.16) (Worthington Biochemical Corporation, Freehold, N.J., U.S.A.). The molecular weights used for the reference polypeptides were: thyroglobulin 165000[28], transferrin 77000[29], bovine serum albumin 68000[30], ovalbumin 44500[30], ribonuclease A 13700[31], Semliki Forest virus envelope protein 50000 and core protein 33000[32]. Radioactive reference proteins were prepared as described by KIEHN AND HOLLAND[33] by methylation with [^{3}H]-dimethyl sulphate. ^{3}H labelling did not change the mobilities of the reference proteins. Radioactive Semlike Forest virus labelled with [^{3}H]phenylalanine was purified as described previously[34]. Neuraminidase (EC 3.2.1.18) (*Vibrio cholerae*) was purchased from Behringwerke AG, Marburg Lahn, Germany.

Radiochemicals

Chlorella ^{14}C-labelled protein hydrolysate (52 mC/matom carbon), D-[1-^{14}C]-glucosamine (55 mC/mmole), L-[4,5-^{3}H]leucine (34.1 C/mmole), DL-[G-^{3}H]isoleucine (524 mC/mmole), L-[2,3-$^3H_2$]valine (1.5 C/mmole). D-[1-^{3}H]galactose (500 mC/mmole), [^{3}H]dimethyl sulphate (167 mC/mmole), DL-[G-^{3}H]phenylalanine (1590 mC/mmole) were from the Radiochemical Centre, Amersham, England. [1-^{14}C]-Palmitic acid (20 mC/mmole) from Calatomic, Los Angeles, Calif., U.S.A. L-[G-^{3}H]-Fucose (4.3 C/mmole) from New England Nuclear, Boston, Mass., U.S.A. [^{32}P]-Orthophosphate from Institutt for Atomenergi, Kjeller, Norway.

Cells

BHK21, clone Wi-2, a continuous cell line derived from baby hamster kidney cells, was grown in monolayers as described previously[34]. The cells were harvested before they had reached saturation density.

Membrane isolation

Plasma membrane, endoplasmic reticulum and soluble fractions were isolated by a slight modification of the method of WALLACH AND KAMAT[35]. The procedure has been described previously[24]. The endoplasmic reticulum probably contains both smooth membranes and rough surfaced membranes from which most of the ribosomes have been stripped.

Radioactive labelling

The cells were grown for 2 days in normal BHK21 culture medium before labelling. For amino acid labelling the cells were washed twice in EAGLE's[36] minimal essential medium containing 0.2% bovine serum albumin, 200 I.U./ml penicillin, 100 μg/ml streptomycin, 25 I.U./ml Mycostatin but one-tenth the normal amount of the amino acids that were used for labelling. Incorporation was performed in 5 ml of this medium per bottle under continuous gentle shaking at 37°. Carbohydrates and lipids were incorporated with the same technique as amino acids, but the incubation medium contained normal amounts of amino acids.

To study the distribution of radioactivity between proteins and lipids, cells were precipitated with 10% trichloroacetic acid, the precipitates washed with 5% trichloroacetic acid and lyophilized. Lipids were extracted with chloroform–methanol as described previously[26]. Three fractions were obtained: the residual "protein" fraction, the "lipid" fraction and the "ganglioside" fraction. The lipid fractions were evaporated to dryness in scintillation bottles and counted in toluene. The protein fractions were hydrolyzed in 0.5 ml 0.2 M piperidine and counted in BRAY's[37] solution. Radioactivity of the three fractions was expressed as counts/min of the fractions obtained per mg cell protein used for lipid extraction.

Identification of sugar labels

As carriers, 2.5 μg each of D-glucose, D-galactose, D-mannose, D-glucosamine, *N*-acetyl-D-galactosamine and L-fucose were added to aliquots of [^{14}C]glucosamine or [^{3}H]fucose labelled plasma membranes. Hydrolysis was performed as for amino acid analysis but in 4 M HCl and for only 4 h. The HCl was then neutralized with silver carbonate, the samples centrifuged and the supernatant applied to Whatman 1 filter paper and chromatography run for about 20 h with ethyl acetate–pyridine–water (10:4:3, v/v/v). The sugar spots were stained weakly with alkaline silver nitrate[38] and the chromatography strip cut into 1 cm pieces and incubated with 0.5 ml 0.2 M piperidine by shaking at 37° for 2 h and measured in Bray's solution.

[^{14}C]Glucosamine labelled plasma membranes were also treated with neuraminidase in 1 ml of the medium of DULBECCO AND VOGT[39] containing 25 I.U. of neuraminidase at 37° for 1 h. 50 μg bovine serum albumin was added and the protein precipitated with trichloroacetic acid and counted on Millipore filters in toluene-based scintillation fluid.

Polyacrylamide gel electrophoresis

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis was performed as described by WEBER AND OSBORN[40]. Gels were always prerun with 0.1 % 2-mercaptoethanol for at least 1 h before applying the sample. Membrane samples were delipidated for electrophoresis according to SCANU *et al.*[41]. Sodium dodecyl sulphate and 2-mercaptoethanol to final concentrations of 1 % (w/v) and 5 % (v/v), respectively, were added to membrane samples and these incubated at 37° for 2 h.

Radioactive gels were sliced into 2-mm slices with a gel slicer with a series of parallel fine gauge wires. The radioactivities were measured in Nuclear Chicago solubilizer–toluene[42] with the channels adjusted so that no ^{3}H counts were found in the ^{14}C channel. The extent of ^{14}C overlap in the ^{3}H channel was set to 10 %.

Chemical determinations

Protein was measured with bovine serum albumin as standard in 0.1 % sodium dodecyl sulphate by the method of LOWRY *et al.*[43].

Amino acid analysis was performed as described previously[34].

Essentially the procedure of BUTLER[44] was used to determine the amino acid composition of proteins from polyacrylamide electrophoretograms. Neither methionine nor its oxidation products could be determined exactly.

Isolation of radioactive lipids

To isolate gangliosides, cells were labelled with [^{14}C]palmitic acid and [^{3}H]-galactose for 16 h. The plasma membranes were isolated and the gangliosides purified as described[26]. A radioactive phospholipid fraction was obtained after incubating cell cultures for 24 h with [^{32}P]orthophosphate. Phospholipids were isolated from whole cells as described[26].

RESULTS

Incorporations of amino acids and sugars

The kinetics of [^{3}H]valine *plus* [^{3}H]leucine *plus* [^{3}H]isoleucine incorporation into whole cell protein, lipid and ganglioside fractions are shown in Fig. 1A. Most of the activity was found in the protein fraction, but the lipid fraction contained about 5 % of the label. The incorporations of [^{3}H]fucose and [^{14}C]glucosamine are shown in Figs. 1B and 1C. Fucose was only incorporated into protein, but about 11 % of the glucosamine label was incorporated into the lipid fraction and 2.5 % into the ganglioside fraction. An 8-h labelling period was subsequently used for membrane protein labelling for polyacrylamide gel electrophoresis.

Paper chromatography showed that the fucose label was recovered from labelled plasma membranes only in the spot migrating as fucose. Of the glucosamine label approximately 70 % was identified as glucosamine, 20 % as galactosamine and about 10 % was in sialic acids (released by neuraminidase treatment). Chemical analysis of BHK21 lipids did not reveal glucosamine, galactosamine or fucose[26]. The amounts of radioactivity per μg protein in the cell fractions are shown in Table I. The carbohydrate labels were concentrated in the plasma membrane fraction. The relative activity of the fucose label in the plasma membranes was 12 times that of the homogenate and 4 times that of the endoplasmic reticulum. The concentration

of the glucosamine label in the plasma membranes was 9 times greater than in the homogenate and 2.2 times greater than in the endoplasmic reticulum.

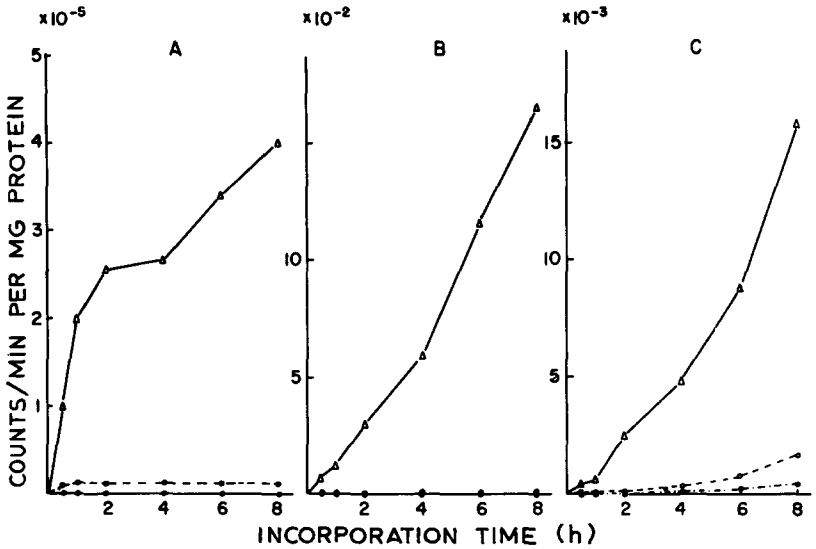

Fig. 1. Incorporation of radioactive precursors into whole cell protein (△), lipid (○) and ganglioside (●) fractions. Counts/min per mg cell protein used for lipid extraction. One bottle was used for each determination point. A. Incorporation of [^{3}H]leucine + [^{3}H]isoleucine + [^{3}H]-valine. Cells were labelled for each incorporation time with 1.6 μC/ml of each of the amino acids. B. Incorporation of [^{3}H]fucose. 4 μC/ml [^{3}H]fucose was used for labelling for each incorporation time. C. Incorporation of [^{14}C]glucosamine. 2 μC/ml [^{14}C]glucosamine was used for labelling for each incorporation time.

TABLE I

RADIOACTIVE LABELS OF BHK21 CELL FRACTIONS

2 or more experiments performed with closely identical results. Cells from 10 tissue culture bottles were labelled with 2.5 μC [^{14}C]-labelled chlorella protein hydrolysate per ml, 3 μC [^{14}C]glucosamine per ml or 17.6 μC [^{3}H]fucose per ml for 8 h.

	Counts/min per μg protein				*Relative radioactivity (fraction/homogenate)*		
	H	*S*	*ER*	*PM*	*S/H*	*ER/H*	*PM/H*
[^{14}C]amino acids	411	405	520	528	0.98	1.26	1.28
[^{14}C]Glucosamine	74	16	293	650	0.22	3.95	8.80
[^{3}H]Fucose	20	11	60	240	0.55	3.00	12.00

Abbreviations: H, homogenate; S, soluble fraction; ER, endoplasmic reticulum; PM, plasma membranes.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of unlabelled membranes

The polyacrylamide gel electrophoresis patterns of stained plasma membranes and endoplasmic reticulum are shown in Fig. 2. The proteins of the plasma membranes and endoplasmic reticulum are designated by their apparent molecular weights and the letters P and E, respectively. In Figs. 2A and 2B are 5 % gels of delipidated plasma

membranes and endoplasmic reticulum. The resolution was good for the high molecular weight components but proteins with an apparent molecular weight < 70000 gave diffuse patterns. The major components in the plasma membrane fraction were P96 and P76 and in the endoplasmic reticulum fraction E95 and E78. The greatest

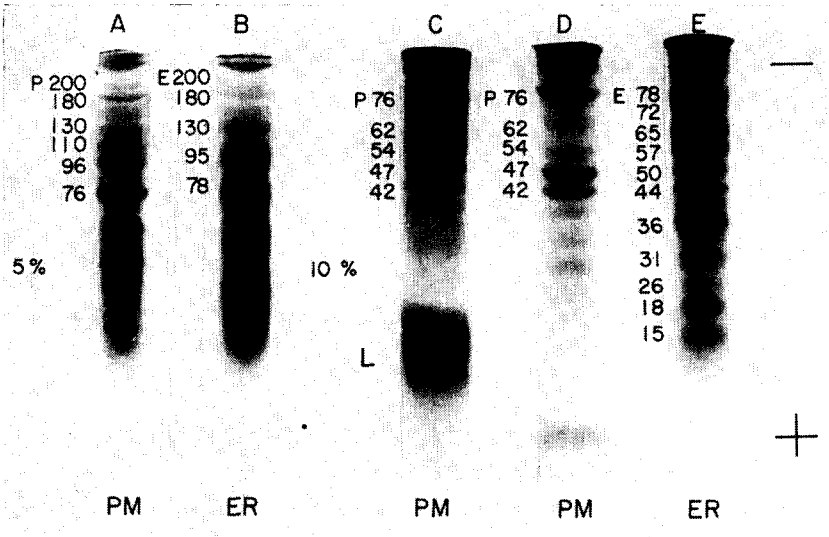

Fig. 2. Polyacrylamide gel electrophoresis patterns of Coomassie Blue stained plasma membranes (PM) and endoplasmic reticulum (ER). A. Delipidated plasma membranes run on a 5% gel. B. Delipidated endoplasmic reticulum run on a 5% gel. C. 10% gel of plasma membranes before delipidation. D. 10% gel of plasma membranes after delipidation. E. 10% gel of delipidated endoplasmic reticulum.

difference between plasma membranes and endoplasmic reticulum was that a band corresponding to P110 was missing from endoplasmic reticulum. Figs. 2D and 2E show 10% polyacrylamide gels of delipidated plasma membranes and endoplasmic reticulum. Fig. 2D shows that proteins P47 and P42 were also main components of plasma membranes. Proteins E65, E57 and E36 were main endoplasmic reticulum components (Fig. 2E). It is clear that plasma membrane differs from endoplasmic reticulum. The resolution was better with delipidated samples. Fig. 2C shows a 10% gel of plasma membranes before delipidation. The most important difference between delipidated and lipid-containing samples is a fast moving component called the L-band which is present in the latter. This band had a low apparent molecular weight ($<$ 10000). Delipidation by ethanol–ether removed less than 5% of the plasma membrane protein by quantitative amino acid analysis of the lipid extract. I tried to dissociate the BHK21 membrane proteins further with 6 M urea and 1% sodium dodecyl sulphate, or using 1–12% sodium dodecyl sulphate and heating to 70°. These treatments did not produce any clearcut differences from the patterns shown in Fig. 2.

The apparent molecular weights of the plasma membrane and endoplasmic reticulum proteins are shown in Table II. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate separates soluble proteins according to their molecular weights[45,40]. It should be stressed, however, that determinations of molecular weights of membrane proteins by sodium dodecyl sulphate–polyacrylamide gel

electrophoresis must be regarded with caution. Membrane proteins may bind more sodium dodecyl sulphate than soluble proteins[32]. In some instances the carbohydrate part may also greatly influence the mobilities observed on polyacrylamide gels[46]. It is also possible that not all proteins are dissociated completely into their polypeptide chains.

TABLE II

APPARENT MOLECULAR WEIGHTS OF PLASMA MEMBRANE AND ENDOPLASMIC RETICULUM PROTEINS

Stained plasma membrane proteins *		*Amino acid labelled plasma membrane proteins* **		*Stained endoplasmic reticulum proteins* *		*Amino acid labelled endoplasmic reticulum proteins* **	
P200	200000***		—	E200	200000***		—
P180	180000***		—	E180	180000***		—
P130	130000***	M130	130000***	E130	130000***	R128	128000***
P110	110000***	M110	110000***		—		—
P96	96000	M98	98000	E95	95000	R96	96000
P88	88000		—	E92	92000		—
P76	76000	M72	72000	E78	78000	R72	72000
	—		—	E72	72000		—
P62	62000	M63	63000	E65	65000	R60	60000
P54	54000		—	E57	57000		—
P47	47000	M48	48000	E50	50000	R49	49000
P42	42000	M41	41000	E44	44000	R41	41000
P32	32000	M34	34000	E36	36000		—
P28	28500	M28	28500	E31	31000	R28	28500
P24	24000	M26	26000	E26	26000	R26	26000
	—	M20	20000	E18	18000	R20	20000
	—		—	E15	15000		—

* Mean of 10 determinations. Calculated from 10% gels if not otherwise indicated.
** Mean of 3 determinations.
*** Calculated from 5% gels.

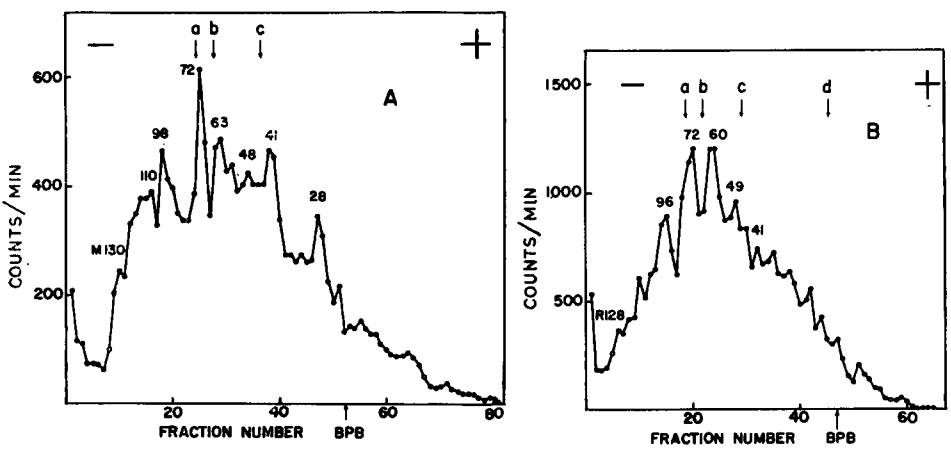

Fig. 3. 5% polyacrylamide gel electrophoresis patterns of delipidated amino acid labelled plasma membranes (A) and endoplasmic reticulum (B). Migrations of the ^{3}H-labelled molecular weight marker proteins are indicated. a. transferrin; b, bovine serum albumin; c, ovalbumin; d, ribonuclease A. Cells from 10 tissue culture bottles were labelled with 2.5 μC/ml [^{14}C]-labelled chlorella protein hydrolysate for 8 h. BPB = bromphenol blue.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of amino acid labelled membranes

Membrane proteins labelled by ^{14}C-labelled chlorella protein hydrolysate were run on polyacrylamide gel electrophoresis together with internal ^{3}H-labelled reference proteins. Plasma membranes and endoplasmic reticulum were electrophoresed on 5% polyacrylamide gels (delipidated, Figs. 3A and 3B) and on 10% polyacrylamide gels (not delipidated, Figs. 4A and 4B). The protein peaks of the plasma membranes and endoplasmic reticulum are designated by their apparent molecular weights and the letters M and R, respectively. Components corresponding to most of those of stained gels are also found here. The apparent molecular weights of radioactive plasma membrane and endoplasmic reticulum proteins are given in Table II.

Both membranes contained strong L-peaks, which were absent from delipidated membranes. The electrophoretic position of the radioactive L-peak (Fig. 4A) corresponded to the L-band stained with Coomassie Blue in Fig. 2C. When [^{14}C]leucine

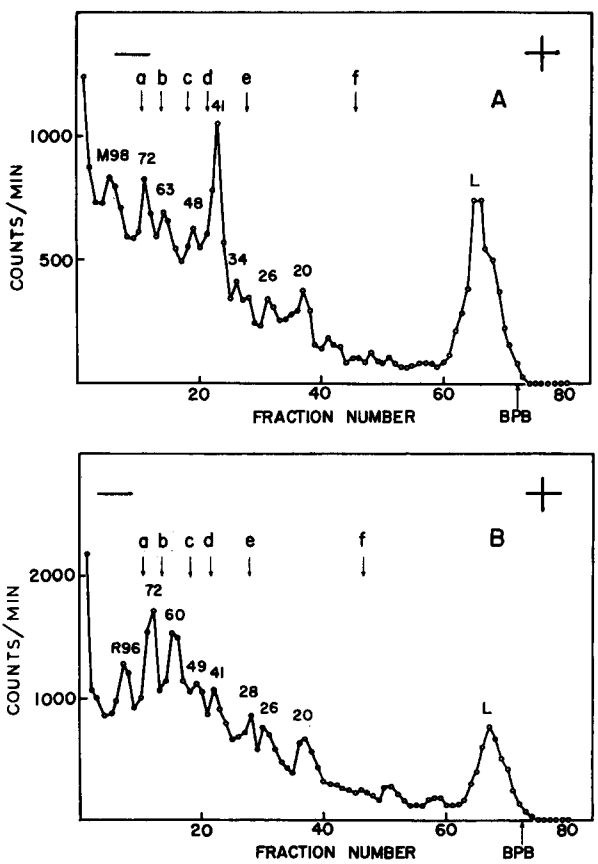

Fig. 4. 10% polyacrylamide gel electrophoresis patterns of amino acid labelled plasma membranes (A) and endoplasmic reticulum (B). Membranes were not delipidated. Migrations of the molecular weight marker proteins are indicated. a, transferrin; b, bovine serum albumin; c, Semliki Forest virus envelope protein; d, ovalbumin; e, Semliki Forest virus core protein; f, ribonuclease A. Cells from 10 tissue culture bottles were labelled with 2.5 μC/ml [^{14}C]-labelled chlorella protein hydrolysate for 8 h.

instead of chlorella protein hydrolysate was used to label the membranes no L-peaks were seen.

Sodium dodecyl sulphate–polycrylamide gel electrophoresis of carbohydrate labelled membranes

Cells were simultaneously labelled with [^{14}C]glucosamine and [^{3}H]fucose, then plasma membranes were isolated and run on 5% polyacrylamide gel electrophoresis (Fig. 5A). The apparent molecular weights of the main plasma membrane glycoproteins are listed in Table III. These are designated by PG and the apparent molecular weight. In plasma membranes the fucose and glucosamine labels were found together. The major plasma membrane components were PG128, PG108, PG88, and PG48. Fig. 5B shows the electrophoretic pattern of the endoplasmic reticulum fraction isolated from the same cell sample as the plasma membranes of Fig. 5A. The concentrations of [^{14}C]-glucosamine and [^{3}H]fucose in endoplasmic reticulum were 2.2. and

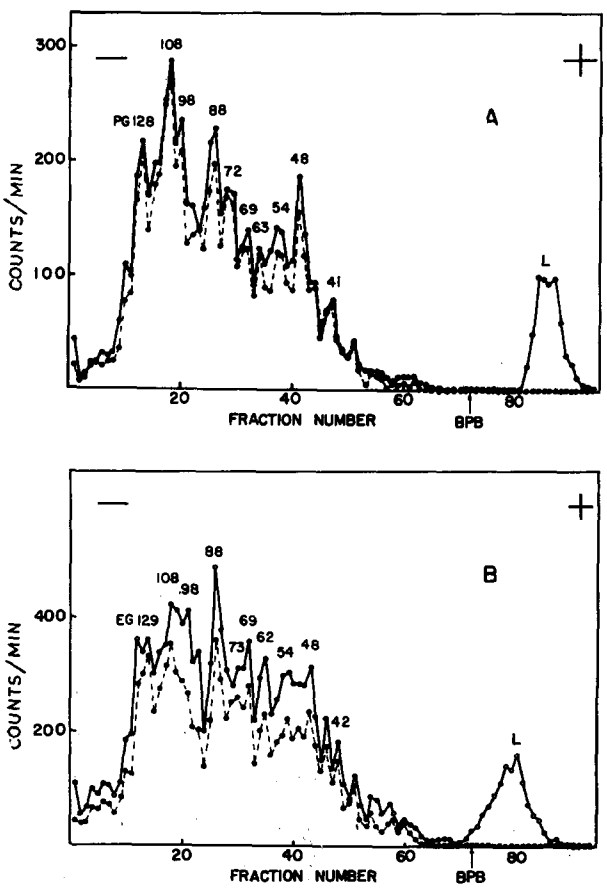

Fig. 5. 5% polyacrylamide gel electrophoresis patterns of [^{14}C]glucosamine (○—○) and [^{3}H]fucose (●—●) labelled membranes, not delipidated. A. plasma membranes, B. endoplasmic reticulum. Cells from 10 tissue culture bottles were simultaneously labelled with 2 μC [^{14}C]glucosamine and 12 μC [^{3}H]fucose per ml for 8 h. 80 μg plasma membrane protein and 376 μg endoplasmic reticulum protein were electrophoresed.

TABLE III

APPARENT MOLECULAR WEIGHTS OF MEMBRANE GLYCOPROTEINS

Mean of 3 determinations. Calculated from 5% gels.

Plasma membrane	*Molecular weight*	*Endoplasmic reticulum*	*Molecular weight*
PG128	128000	EG129	129000
PG108	108000	EG108	108000
PG98	98000	EG98	98000
PG88	88000	EG88	88000
PG72	72000	EG73	73000
PG69	69000	EG69	69000
PG63	63000	EG62	62000
PG54	54000	EG54	54000
PG48	48000	EG48	48000
PG41	41000	EG42	42000

4 times, respectively, less than in plasma membranes, so more endoplasmic reticulum protein was applied. The pattern resembled that of plasma membranes. The components marked EG129-42 had similar mobilities to the corresponding plasma membrane glycoproteins (Table III). There was generally a clearly lower ratio of fucose to glucosamine label in the endoplasmic reticulum glycoproteins.

L-peaks with glucosamine label were found in both membranes. The L-peaks did not contain fucose label.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of radioactive gangliosides and phospholipids

A purified BHK21 ganglioside (*N*-acetylneuraminyllactosylceramide) labelled with [^{14}C]palmitic acid and [^{3}H]galactose was run on polyacrylamide gel electrophoresis (Fig. 6). The migration corresponded to the L-peaks of Figs. 5A and 5B. On polyacrylamide gel electrophoresis ^{32}P-labelled BHK21 phospholipids had mobilities identical to this ganglioside.

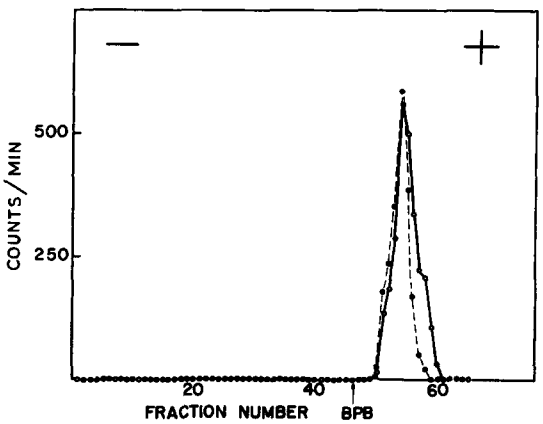

Fig. 6. 5% polyacrylamide gel electrophoresis of BHK21 gangliosides. 10 cell bottles were labelled with a total of 45 μC [^{14}C]palmitic acid (○—○) and 450 μC [^{3}H]galactose (●—●). Gangliosides were isolated from plasma membrane fraction and dissolved in 1% sodium dodecyl sulphate–5% 2-mercaptoethanol and electrophoresed.

TABLE IV

AMINO ACID COMPOSITION OF BHK21 PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM

Mean of 5 determinations from different samples. Moles/1000 moles (half-cysteine and tryptophan not included).

Amino acid	*Plasma membranes*	*Endoplasmic reticulum*
Lysine	71.5	74.5
Histidine	22.2	21.6
Arginine	48.8	54.7
Aspartic acid	95.6	90.8
Threonine	60.8	52.9
Serine	67.8	65.6
Glutamic acid	118.4	114.9
Proline	55.8	54.1
Glycine	76.6	84.2
Alanine	77.3	78.8
Valine	67.1	70.9
Methionine	19.0	22.2
Isoleucine	51.9	48.7
Leucine	95.6	94.4
Tyrosine	28.5	29.5
Phenylalanine	43.0	42.1

TABLE V

AMINO ACID COMPOSITION OF PLASMA MEMBRANE PROTEINS P96, P76, AND P42

Mean of 4 determinations. Moles/1000 moles (tryptophan, cysteine and methionine not included).

Amino acid	*P96*	*P76*	*P42*
Lysine	71.3	81.2	73.3
Histidine	25.8	25.6	30.2
Arginine	48.9	41.8	43.7
Aspartic acid	101.8	93.7	93.0
Threonine	53.0	56.2	61.0
Serine	69.2	103.7	85.0
Glutamic acid	139.9	118.1	113.9
Proline	55.0	50.6	49.3
Glycine	75.4	97.4	89.9
Alanine	80.1	81.2	81.3
Valine	60.4	59.3	60.3
Isoleucine	49.6	43.7	54.8
Leucine	97.1	83.7	88.1
Tyrosine	31.2	26.9	35.1
Phenylalanine	41.4	36.2	41.3

Amino acid analysis

The amino acid compositions of the plasma membranes and the endoplasmic reticulum are given in Table IV. The amino acid compositions were strikingly similar. In fact they resembled the amino acid compositions of erythrocyte ghosts[10], mitochondrial structural protein[47] and the major protein component from sarcotubular membranes[48].

The L-band from Coomassie Blue-stained polyacrylamide gel electrophoretograms was analyzed for amino acids. It did not contain detectable amounts of amino

acids, whereas over 60% of the applied protein was recovered as amino acids from the gel between the origin and the L-band. The amino acid compositions of three main plasma membrane protein bands (P96, P76, P42) from 5% gels were analyzed (Table V). These proteins had significantly different amino acid compositions.

DISCUSSION

The plasma membrane and endoplasmic reticulum of BHK21 cells have been characterized by enzymatic, immunological and chemical methods[24–26]. The concentrations of (Na^+–K^+)-activated ATPase (EC 3.6.1.4), surface antigens, sphingoglycolipids and free cholesterol are 10–20 times greater in the plasma membrane fraction than in the homogenate and 4–5 times greater than in the endoplasmic reticulum fraction. The endoplasmic reticulum fraction contains higher concentrations of NADH diaphorase (EC 1.6.99.2) and β-glucuronidase (EC 3.2.1.31) than the plasma membranes. The NADH diaphorase has been used as marker for the endoplasmic reticulum fraction. We have estimated that the endoplasmic reticulum fraction contains 10–20% plasma membrane contamination[24]. The degree of endoplasmic reticulum contamination in the plasma membrane fraction is estimated to approximately 25%[24], but may be less[35,49].

The plasma membrane fraction isolated by our method contains a high level of lipids (1.5 mg lipid per mg protein). BHK21 plasma membranes isolated by the method of WARREN *et al.*[50] contained only 31% lipid by weight[3]. In their procedure the surface membranes were fixed before isolation, and therefore some proteins might be nonspecifically attached to the membranes. Another possibility is that we lose some loosely bound "epiproteins" of the membranes during the isolation procedure (*cf.* HINMAN AND PHILLIPS[51]).

Both stained and radioactive plasma membrane and endoplasmic reticulum electrophoretograms show constant differences between the two fractions. The different protein compositions of plasma membrane and endoplasmic reticulum fractions cannot be due to the presence of ribosomes in the endoplasmic reticulum fraction. Preliminary experiments using extensively washed ribosome-free membranes[51] still show the differences between plasma membranes and endoplasmic reticulum. YOUNGHANS *et al.*[13], who studied stained polyacrylamide electrophoresis patterns of liver membrane fractions, also found differences between the fractions. However, KIEHN AND HOLLAND[33,52], did not find clear differences between the radioactive proteins of the plasma membranes and microsomes of cultured fibroblasts. They used the method of WARREN *et al.*[50] to isolate the plasma membranes and did not estimate the purity of the membrane fractions. The plasma membrane and endoplasmic reticulum fractions of BHK21 cells also differ in that the plasma membrane fraction contains higher levels of protein-bound carbohydrates.

Proteins P96, P76 and P42 are main plasma membrane components. Amino acid analysis of these proteins shows that they have different amino acid compositions, and cannot be aggregates of an identical smaller subunit. One further difference between these proteins is evident from the glycoprotein studies. These show that P96 and P76, which have similar apparent molecular weights as PG98 and PG72 (Fig. 7A), respectively, are glycoproteins, whereas very little carbohydrate label is found in PG41.

The apparent molecular weights of most plasma membrane proteins closely

resemble those of the endoplasmic reticulum. Some of these proteins could be the same, but this has to be proved by chemical analysis. Which of these are due to cross contamination and which are intrinsic components of the membranes is not known. Amino acid analysis of the endoplasmic reticulum proteins was not performed because of the complexity of the endoplasmic reticulum electrophoretograms.

One further similarity between the plasma membranes and the endoplasmic reticulum is the presence of the L-band. This band has some common features to the "miniproteins" studied by LAICO *et al.*[9] in that it has a high mobility on polyacrylamide gels, stains with Coomassie Blue, and is present only in membranes that have not been delipidated. It also labels with radioactive chlorella protein hydrolysate and glucosamine, but not with leucine or fucose. However, amino acid analysis did not show any protein in this band. On sodium dodecyl sulphate–polyacrylamide gel electrophoresis radioactive phospholipids and gangliosides have mobilities corresponding to this band. Therefore the L-band must be primarily composed of lipid (*cf.* ref.53). LENARD[54] has also found such a band by polyacrylamide gel electrophoresis of erythrocyte membranes. The band was thought to be glycolipid.

In keeping with the knowledge that glycoproteins are concentrated in the plasma membranes[23,55]. I found that radioactive fucose is as good a marker for the plasma membranes as (Na^+–K^+)-activated ATPase, gangliosides and surface antigens. Radioactive glucosamine is also concentrated in the membranes but the plasma membrane/endoplasmic reticulum ratio is less than for fucose. Some of the fucose and glucosamine derived radioactivity in the endoplasmic reticulum may be due to contaminating plasma membranes.

The apparent molecular weight of the major BHK21 plasma membrane glucosamine–fucose-containing component in sodium dodecyl sulphate–polyacrylamide gel electrophoresis is 108000. This corresponds to the results of LENARD[56] for the major glycoprotein of red cell membranes from different species, and may be of more general significance. EVANS[18] estimated the molecular weight of the major rat liver plasma membrane glycoprotein to be 140000. Thus the main membrane glycoproteins seem to have rather high apparent molecular weights.

Most of the glucosamine labelled BHK21 glycoproteins had similar mobilities on polyacrylamide gel electrophoresis whether in the plasma membrane or the endoplasmic reticulum fraction. The glycoproteins, however, contained more fucose relative to glucosamine when they were isolated from the plasma membranes than from the endoplasmic reticulum. Fucose in oligosaccharide chains is always terminal[57]. It has been proposed that the most internal sugars of the oligosaccharide chains in glycoproteins destined for export are linked to the polypeptide backbone on the ribosomes[58], after which sugars are sequentially added when the protein migrates from the endoplasmic reticulum to the Golgi apparatus[59,60]. Whether the plasma membrane glycoproteins also are made on the endoplasmic reticulum is not known. BOSMANN *et al.*[61] showed that [^{14}C]glucosamine is incorporated into membrane glycoproteins on the smooth membranes of HeLa cells (probably identical with the Golgi apparatus). Subsequently the label migrates to the plasma membranes. If glycoproteins found in the BHK21 cell endoplasmic reticulum are the same as those of the plasma membranes, my results support the possibility that terminal sugars, like fucose, are added later in the synthesis of membrane glycoproteins and that sugars may be added on different cellular membranes.

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