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Albumin associated with purified pig lymphocyte plasma membrane

Michael J. OWEN,*† Brian H. BARBER,‡ Ralph A. FAULKES† and Michael J. CRUMPTON*†
†*National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K., and* ‡*Department of Microbiology and Parasitology, University of Toronto, Toronto M5A 1A1, Canada*

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Plasma-membrane preparations purified from pig lymphocytes contained a major polypeptide component of mol.wt. about 68000. This component was identified as pig albumin by the following comparisons with authentic pig serum albumin: (*a*) co-migration when analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis under reducing and non-reducing conditions; (*b*) identical isoelectric points; (*c*) similar 'fingerprints' of arginine-containing tryptic peptides; (*d*) reactivity with anti-(pig albumin) serum. The albumin was bound tightly to the plasma membrane. Biosynthetic labelling of pig lymphocytes under a variety of conditions failed to provide evidence that albumin was synthesized by lymphocytes, suggesting that the plasma-membrane-associated albumin was of extraneous origin. Radiolabelled pig serum albumin, however, failed to bind to the plasma-membrane fraction when added before cell disruption. Although lymphocyte plasma-membrane preparations from other species possessed a polypeptide of about 68000 mol.wt., this was judged not to be albumin on the basis of electrophoretic mobility under non-reducing conditions; also, no polypeptide was precipitated by anti-albumin sera. It is concluded that pig lymphocyte plasma-membrane preparations possess albumin which, although firmly attached, was probably of extraneous origin. This association appeared not to be common to lymphocytes from other species.

Highly purified preparations of pig lymphocyte plasma membrane contain a major Coomassie-Blue-staining component of mol.wt. about 68000, as judged by SDS/polyacrylamide-gel electrophoresis under reducing conditions (Allan & Crumpton, 1971; Chavin & Holliman, 1975; Walsh *et al.*, 1976). On the basis of the relative intensities of Coomassie Blue staining, this protein is present to a similar extent to actin, which has been estimated to represent 5–6% of the total membrane protein (Barber & Crumpton, 1976). It has been detected in the plasma-membrane fraction from mesenteric lymph node, thymus and spleen, and is therefore apparently a major constituent of the surface membrane of both pig B lymphocytes and thymus cells (T lymphocytes). A major Coomassie-Blue-staining band of similar mobility to the pig membrane component and corresponding to a mol.wt. within the range 68000–70000 is also present in plasma-membrane preparations from mouse, human and bovine lymphocytes (Allan & Crumpton, 1972; Ladoulis *et al.*, 1975; Monneron & d'Alayer, 1978).

In the present paper we have investigated the identity of the pig lymphocyte membrane component, and present results which demonstrate that it exhibits a high degree of structural homology with albumin. The results of biosynthetic labelling experiments indicate that the albumin is most likely of extraneous origin. An account of this work has been presented in preliminary form (Owen *et al.*, 1978).

Abbreviations used: SDS, sodium dodecyl sulphate; IgG, immunoglobulin G.

* Present address: Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Materials and methods

Materials

Chemicals were of analytical grade and glass-distilled water was used in all experiments.

L-[^{35}S]Methionine (300–800 Ci/mmol) and Na^{125}I (IMS.30; 13–17 mCi/μg) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Acrylamide (twice recrystallized) and *NN'*-methylenebisacrylamide were purchased from Serva Feinbiochemica, Heidelberg, Germany. SDS (specially pure) was from British Drug Houses,

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Poole, Dorset, U.K., and *NNN'N'*-tetramethylethylenediamine from Koch–Light, Colnbrook, Bucks., U.K. Thin-layer cellulose plates for peptide 'mapping' (Polygram Cel 400) were obtained from Macherey, Nagel and Co., Duren, Germany.

Pig serum albumin (fraction V powder), mouse serum albumin (fraction V powder), human serum albumin (fatty acid-free) and diphenylcarbamoyl chloride ('DPCC')-treated trypsin were products of Sigma Chemical Co., Poole, Dorset, U.K. ^{125}I-labelled pig albumin was prepared by the chloramine-T procedure (Greenwood *et al.*, 1963); a specific radioactivity of about 50 μCi/mg of protein was routinely obtained. Concanavalin A–Sepharose and Sepharose CL-4B were purchased from Pharmacia Fine Chemicals.

Cells

Pig mesenteric lymph nodes were purchased from local commercial abattoirs (British Beef Ltd., Watford, U.K., and Swift Eastern, Toronto, Canada). Suspensions of viable lymphocytes were prepared as described previously, the erythrocytes and dead cells being removed by centrifugation on Isopaque/Ficoll (Perlés *et al.*, 1977). A subculture of the human B lymphoblastoid cell line BRI 8 was given by Searle Diagnostic Ltd., High Wycombe, Bucks., U.K. Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) foetal calf serum (Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.), L-glutamine (2 mM), penicillin (100 units/ml) and streptomycin (50 μg/ml).

Unstimulated (quiescent) pig lymphocytes were labelled biosynthetically with [^{35}S]methionine by incubating the cells (2×10^7/ml) at 37°C for 4–16 h in methionine-free Eagle's medium supplemented with 250 μCi of L-[^{35}S]methionine/ml.

Plasma-membrane preparation

Lymphocyte plasma membrane was prepared from pig mesenteric lymph node, pig spleen and rat spleen as described by Snary *et al.* (1976), and from BRI 8 or pig lymphocyte cell suspensions by using a cell-disrupting pump (Stansted Fluid Power Ltd., Stansted, Essex, U.K.) as described previously (Crumpton & Snary, 1974).

Electrophoretic analysis

SDS/polyacrylamide-gel electrophoresis was carried out in 0.1% SDS on 10% (w/v) acrylamide slab gels by using a 5% (w/v) stacking gel and the Tris/glycine buffer system of Laemmli (1970). Samples (100 μg of membrane protein; Lowry *et al.*, 1951) were solubilized in 100 μl of 2% (w/v) SDS/10% (v/v) glycerol/80 mM-Tris/HCl buffer (pH 8.6)/0.1 M-dithiothreitol [or 5% (v/v) β-mercaptoethanol]/0.02% Bromophenol Blue and were heated at 100°C for 2 min before being layered on the gel. Gels were run at 25 mA per slab until the tracker dye reached the bottom of the gel, and were then stained with 0.01% Coomassie Blue in methanol/water/acetic acid (41 : 52 : 7, by vol.) and destained with the same solvent. Radioautography was performed on the dried gel by using Fuji RX safety film. Alternatively, the dried gel was divided into 1 mm slices and assessed for ^{125}I radioactivity in a gamma counter.

Two-dimensional electrophoresis of [^{35}S]methionine-labelled whole cell lysates was performed by isoelectric focusing in the first dimension and a discontinuous SDS/polyacrylamide-gel system for the second dimension as described by O'Farrell (1975). Lymphocytes were solubilized in 0.5% (w/v) Nonidet P40 at 0°C for 15 min and then centrifuged at 100000 *g* for 1 h. The supernatant was mixed with an equal volume of lysis buffer as described by O'Farrell (1975). The distribution of spots was observed by fluorography with Curix RP1 film (Agfa) as described by Bonner & Laskey (1974). For two-dimensional gel analysis of plasma-membrane samples, the modification of the O'Farrell (1975) procedure outlined by Ames & Nikaido (1976) was used as described for samples solubilized in SDS.

Peptide 'mapping'

Ten tracks each of lymphocyte plasma membrane (100 μg of protein per track) or pig albumin (5 μg per track) were fractionated by SDS/polyacrylamide-gel electrophoresis. The gels were stained with Coomassie Blue, and the albumin and albumin-co-migrating bands were excised. The slices from the tracks were pooled, minced and washed extensively with water before drying under vacuum. NH_4HCO_3 (0.5 ml of 0.25 M solution) containing 25 μg of diphenylcarbamoyl chloride-treated trypsin was added and the mixture incubated at 37°C for 16 h. The supernatant was removed and the gel slices were redigested once with trypsin as described above before extraction of the slices twice with aq. 20% (v/v) pyridine (2×5 ml) for 30 min at 37°C. All extracts were pooled, freeze-dried and then oxidized with 0.1 ml of freshly prepared performic acid at 0°C for 90 min. Water (0.5 ml) was added before freeze-drying, and the residue was redissolved in aq. 20% pyridine (10 μl). A sample (3 μl) was applied to a thin-layer cellulose plate, and chromatography and electrophoresis were performed as described previously (Barber & Crumpton, 1976). The plates were stained for arginine by the method of Yamada & Itano (1966). Controls were included based on slices of polyacrylamide gel, containing no protein, that had been treated in a manner identical with that for the test samples.

Labelling procedures

Plasma membrane (1 mg of protein) was solubilized in 1 ml of 1% (w/v) sodium deoxycholate in 10 mM-Tris/HCl buffer, pH 8.2, and diluted after centrifugation (100000 *g* for 1 h) with 1 ml of 10 mM-Tris/HCl buffer, pH 8.2. Solubilized membrane (1 ml) was labelled with 100 μCi of [^{125}I]-iodide by using chloramine-T (Greenwood *et al.*, 1963). Approx. 20% of the initial radioactivity was incorporated into trichloroacetic acid (equal volume of 10% solution)-precipitable material under these conditions. The surface of intact pig mesenteric-lymph-node lymphocytes (10^7 cells; 95% viable, as judged by eosin dye exclusion) was labelled by lactoperoxidase-catalysed iodination under conditions recommended for vectorial labelling (Hubbard & Cohn, 1976). Iodinated cells were solubilized by incubation at 0°C for 30 min with 0.5 ml of 1% (w/v) Nonidet P40 in phosphate-buffered saline (0.15 M-NaCl/10 mM-sodium phosphate buffer, pH 7.4), followed by low-speed centrifugation (2000 *g* for 5 min) to remove nuclei, and finally centrifuged at 100000 *g* for 1 h. The supernatant was dialysed overnight against phosphate-buffered saline at 4°C and then diluted with an equal volume of the saline to 0.5% (w/v) Nonidet P40.

Immunological procedures

Anti-(pig albumin) serum was raised in rabbits by using commercial pig albumin (fraction V) that had been further purified by adsorption to DEAE-Sephadex A50 (Pharmacia), elution with 0.5 M-KCl, followed by gel filtration on Ultrogel AcA34; the peak tube was used directly. Two rabbits were immunized, each with 1 mg of purified albumin in 1 ml of Freund's Complete Adjuvant injected at multiple sites. Serum was collected weekly for 3 weeks, commencing 4 weeks after injection; the samples from each rabbit were pooled. Normal serum was collected from each animal before immunization.

Immune precipitation was carried out by using fixed *Staphylococcus aureus* (Cowan 1 strain) organisms to precipitate antigen–antibody complexes (Kessler, 1975). Solubilized plasma membrane or cell lysates (1 ml) were incubated with 50 μl of either anti-(pig albumin) serum or normal rabbit serum. After 30 min at 20°C, 200 μl of a 10% (w/v) suspension of fixed *Staph. aureus* was added and the mixture incubated for 4 h at 4°C. The bacteria were then washed twice either with 0.5% (w/v) sodium deoxycholate in 10 mM-Tris/HCl buffer, pH 8.2 (plasma membrane), or with 0.5% Nonidet P40 in phosphate-buffered saline (cell lysates), and the washed pellet was heated at 100°C for 2 min with 100 μl of SDS sample buffer. The suspension (50 μl) was analysed by SDS/polyacrylamide-gel electrophoresis. The gel was stained with Coomassie Blue, dried, divided into 1 mm slices and the radioactivity counted in a gamma counter.

Coupling of IgG to Sepharose

An IgG fraction was prepared from anti-(pig albumin) serum and normal (i.e. preimmune) serum by precipitating with 18% (w/v) and then 14% (w/v) Na_2SO_4 (Kekwick, 1940). It was coupled (10 mg of protein/ml of gel sediment) to CNBr-activated Sepharose CL-4B (CNBr/Sepharose ratio of 100 mg/ml) in 0.1 M-$NaHCO_3$, pH 8.5, at 4°C for 15 h; excess reactive groups were blocked with 0.1 M-ethanolamine/HCl, pH 8.5.

Results

Purified pig lymphocyte plasma membrane contains albumin

When highly purified plasma membrane prepared from pig mesenteric-lymph-node lymphocytes was analysed by SDS/polyacrylamide-gel electrophoresis under reducing conditions, a prominent band of mol.wt. 68000 was detectable by Coomassie Blue staining (Fig. 1, track A). Densitometric scanning of the Coomassie-Blue-stained gel revealed that the band comprised about 5% of the total membrane protein (results not shown). This band appeared to co-migrate with an authentic sample of reduced pig serum albumin (Fig. 1, track F). SDS/polyacrylamide-gel electrophoresis under non-reducing conditions also failed to reveal any detectable difference in mobility between the pig plasma-membrane component and pig serum albumin (Fig. 1, tracks C and D). Under non-reducing conditions the apparent molecular weight of the pig membrane component decreased to 55000 (Fig. 1, tracks B and C), as estimated by reference to the mobilities of reduced standard proteins. The observed sigmoidicity in track (B) is presumably a result of diffusion of the reducing agent from track (A) to track (B) during the electrophoresis procedure. Pig albumin exhibited identical behaviour (Fig. 1, tracks D–E). This large difference in mobility between the reduced and unreduced states reflects the particular distribution of intramolecular disulphide bonds in albumin, which contains 17 disulphide bridges, resulting in a severely restricted domain structure (Geisow, 1977). The identical effects of reducing agents on the electrophoretic mobility of pig albumin and the pig membrane component argue strongly that they possess similar extensively disulphide-bond-stabilized domain structures. Further, since such large differences in apparent molecular weight between the reduced and unreduced states is a relatively rare event (see Fig. 1, tracks A and C), this result argues strongly in support of the pig membrane component being albumin.

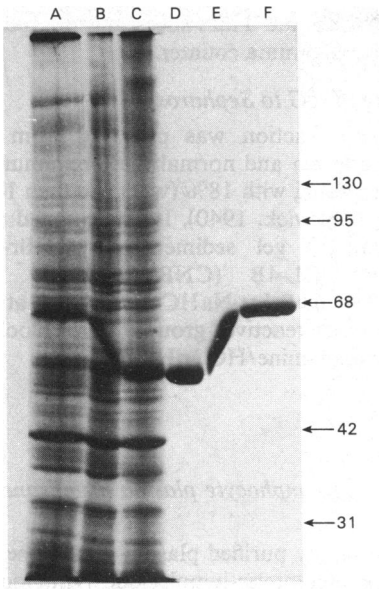

Fig. 1. *SDS/polyacrylamide-gel electrophoretic analysis of pig mesenteric-lymphocyte plasma membrane and pig albumin*

Plasma membrane (100 μg) or pig albumin (5 μg) was electrophoresed after solubilization in 100 μl of SDS sample buffer [2% (w/v) SDS, 10% (v/v) glycerol, 80 mM-Tris/HCl buffer, pH 8.6, and 0.02% Bromophenol Blue] in the presence or absence of 5% (v/v) β-mercaptoethanol. The tracks shown are: (A), plasma membrane, reduced; (B) and (C), plasma membrane, unreduced; (D) and (E), pig albumin, unreduced; (F), pig albumin, reduced. The molecular-weight markers (molecular weight $\times 10^{-3}$) refer to the mobilities of the following reduced standard proteins (molecular weights in parentheses): β-galactosidase (130000); phosphorylase *b* (95000); bovine serum albumin (68000); actin (42000); deoxyribonuclease 1 (31000).

Two-dimensional polyacrylamide-gel-electrophoretic analysis of purified pig mesenteric-lymphocyte plasma membrane is shown in Fig. 2. The Coomassie-Blue-stained profile (Fig. 2*a*) revealed a major component of mol.wt. about 68000 (Fig. 2*a*; indicated by an arrow). The Coomassie-Blue-stained profile of an identical sample to which pig albumin was added before electrophoresis is shown in Fig. 2(*b*). The intensity of the Coomassie-Blue-staining component of 68000 mol.wt. increased, whereas the staining intensities of other spots remained unchanged. This result demonstrated that pig albumin and the pig plasma-membrane component were indistinguishable in both molecular weight and isoelectric point.

The co-migration of the pig membrane component and pig albumin in both dimensions indicates that they possess a close structural homology. Direct evidence for such an homology was obtained from comparisons of tryptic-peptide 'fingerprints' of the respective polypeptides eluted from a polyacrylamide gel after electrophoresis. Fig. 3 shows that the distributions of arginine-containing peptides, detected by using phenanthraquinone, were closely similar. For each protein 13 major spots were detected, one of which was found after incubating polyacrylamide-gel slices containing no protein with trypsin (labelled 'b' in Fig. 3). Each major spot detected on tryptic-peptide 'maps' of pig albumin was correlated with a spot of similar position on 'maps' of the 68000-mol.wt. component. This result suggested that a close primary-sequence similarity exists between these two proteins.

Immunological cross-reactivity between the 68000-mol.wt. pig membrane component and pig albumin was demonstrated by using an antiserum prepared against purified pig albumin. Immunoprecipitation of deoxycholate-solubilized chloramine-T-iodinated pig plasma membrane with anti-(pig albumin) serum revealed a labelled band of 68000 mol.wt. on SDS/polyacrylamide gels (Fig. 4*a*). This band was not, however, immunoprecipitated when preimmune serum was used (result not shown) or when solubilized plasma membrane purified from lactoperoxidase-iodinated pig mesenteric lymphocytes was immunoprecipitated with anti-(pig albumin) serum (Fig. 4*b*).

Occurrence in lymphocytes of other species

The characteristic change in the electrophoretic mobility of albumin on reduction (see Fig. 1) was used to screen for the presence of albumin in lymphocyte plasma-membrane preparations other than from pig mesenteric lymph node. By this criterion, albumin was clearly identified in plasma membranes purified from pig spleen (Fig. 5*a*, tracks A–C). It was not, however, detected in plasma membranes purified from rat spleen (Fig. 5*b*, tracks D–F), the human B lymphoblastoid cell line BRI 8 (Fig. 5*c*, tracks A–C), or mouse spleen (results not shown), in spite of the respective reduced membranes having a clearly detectable band in the region of 68000 mol.wt. The characteristic change in electrophoretic mobility of pig albumin (Fig. 1, tracks D–F) was, however, observed with human (Fig. 5*c*, tracks D–F) and mouse (Fig. 5*b*, tracks A–C) serum albumins; rat serum albumin was not available as a marker, but in view of the similar behaviour of pig, mouse and human albumin it appears reasonable to assume that it would occupy similar positions to those of the other species in both the unreduced and reduced states. According to the above analysis, mouse, rat and human lymphocyte plasma membranes contain little albumin, although the possibility that an amount too small to be detected was present cannot be categorically ruled out.

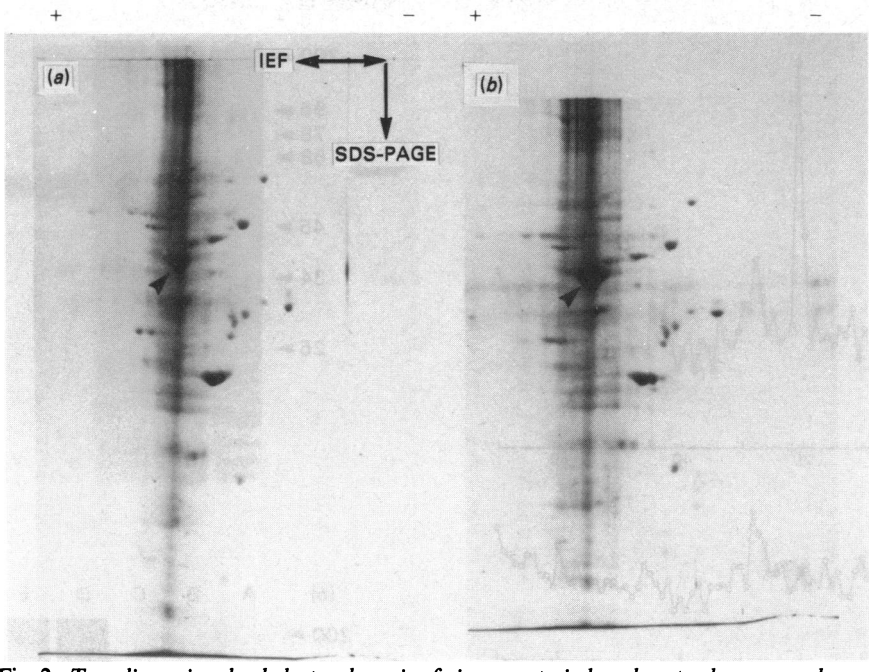

Fig. 2. *Two-dimensional gel electrophoresis of pig mesenteric-lymphocyte plasma membrane*

Plasma membrane was solubilized and electrophoresed with no additions (*a*) or after addition of 5 μg of pig albumin (*b*). The first dimension was isoelectric focusing (IEF). After electrophoresis in the second dimension ('SDS-PAGE'), the gels were stained with Coomassie Blue. The arrows refer to the position of the major Coomassie-Blue-staining spot of 68 000 mol.wt.

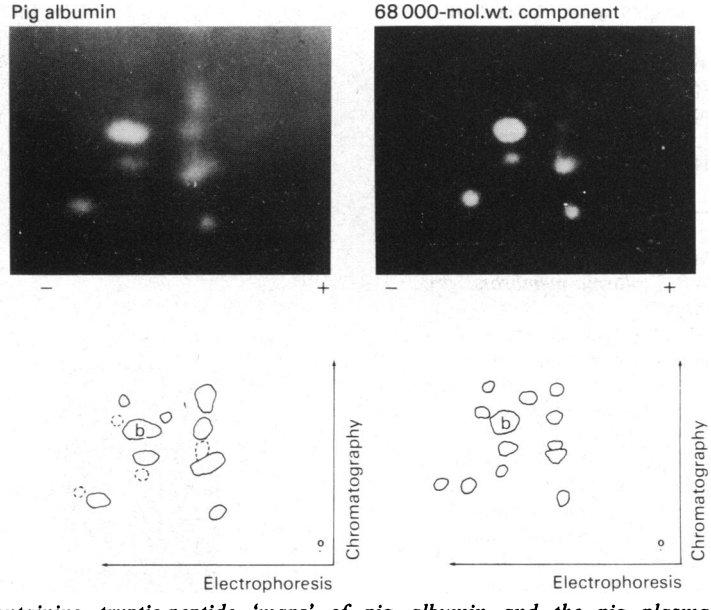

Fig. 3. *Arginine-containing tryptic-peptide 'maps' of pig albumin and the pig plasma-membrane component of 68 000 mol.wt.*

After SDS/polyacrylamide-gel electrophoresis, bands were excised from the gel, digested with trypsin and applied to thin-layer cellulose plates. Separation was in two dimensions, by electrophoresis (pH 6.5) and chromatography in the first and second dimensions respectively. Fluorescence of arginine-containing peptides is shown after phenanthraquinone staining. The schematic representations depict tracings from the photographs. The peptide labelled 'b' was also detected in control 'fingerprints' of gel slices containing no protein that had been treated in an identical manner with the test samples. Abbreviation: o, origin.

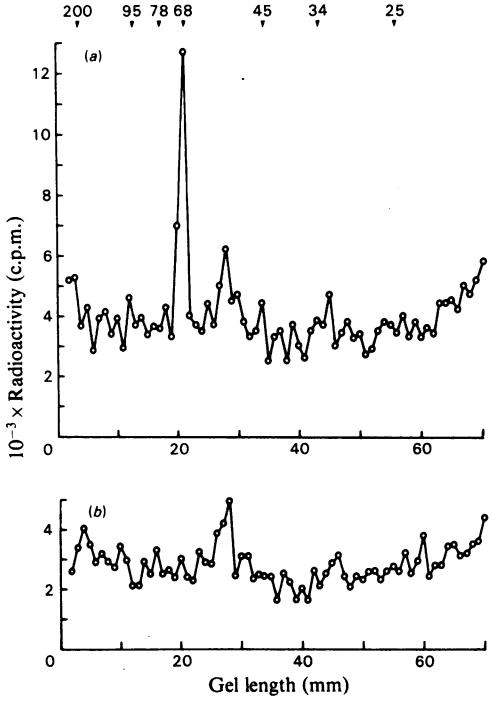

Fig. 4. *Immunoprecipitation of pig mesenteric-lymphocyte plasma membrane or cell lysate with rabbit anti-(pig albumin) serum*

Plasma membrane was radioiodinated by the chloramine-T procedure after solubilization in sodium deoxycholate (*a*). A cell lysate was prepared from lymphocytes iodinated by the lactoperoxidase procedure (*b*). Immunoprecipitates were analysed after reduction by SDS/polyacrylamide-gel electrophoresis and the dried gel was divided into 1 mm sections before assessment of radioactivity.

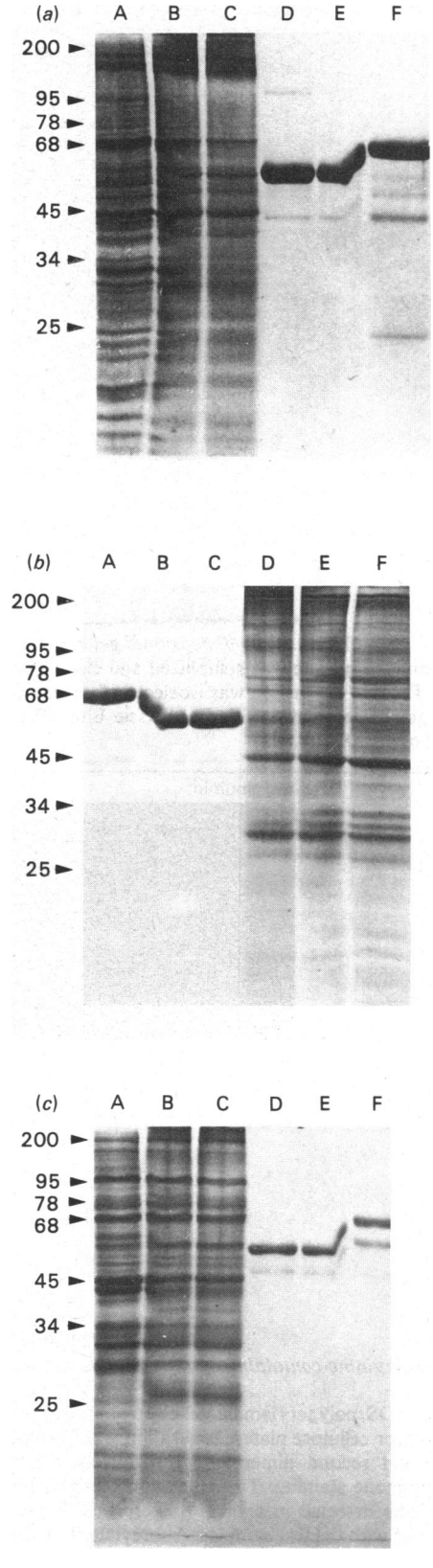

Fig. 5. *SDS/polyacrylamide-gel electrophoresis of pig spleen, rat spleen and BRI 8 plasma-membrane preparations*

Pig spleen plasma membrane (gel *a*): track A, plasma membrane, reduced; tracks B and C, plasma membrane, unreduced; tracks D and E, pig albumin, unreduced; track F, pig albumin, reduced. Rat spleen plasma membrane (gel *b*): track A, mouse albumin, reduced; tracks B and C, mouse albumin, unreduced; tracks D and E, plasma membrane, unreduced; track F, plasma membrane, reduced. BRI 8 plasma membrane (gel *c*): arrangement of tracks as in gel (*a*); human albumin was used in tracks D–F. Solubilization and reduction were as described in the legend to Fig. 1. The gels were stained with Coomassie Blue. The mobilities of the following reduced standard proteins are indicated (subunit molecular weights in parenthesis): myosin (200000); phosphorylase *b* (95000); transferrin (78000); bovine serum albumin (68000); ovalbumin (45000); glyceraldehyde 3-phosphate dehydrogenase (34000); IgG light chain (25000).

Origin of association of albumin with pig plasma membrane

The structural evidence presented above indicated that a protein that is most probably identical with albumin is associated with purified pig lymphocyte plasma membrane. This association can be explained by interaction of exogenous albumin with the surface of intact cells or with the plasma-membrane fraction during cellular disruption, or by synthesis of albumin by pig lymphocytes. The possibility of association of exogenous albumin with pig lymphocyte plasma membrane was assessed by adding radioiodinated pig albumin either to pig lymphocytes before disruption or to purified pig lymphocyte plasma membrane. The results shown in Table 1 indicate that essentially no radioactivity was associated with the purified or washed plasma membranes respectively. Moreover, when the plasma-membrane preparations were analysed by SDS/polyacrylamide-gel electrophoresis, no radioactive band of 68000 mol.wt. was observed on radioautography of the dried gel, although unlabelled albumin was shown to be associated with the plasma-membrane fraction as assessed by the characteristic change in electrophoretic mobility when unreduced (results not shown). On the basis of these results, it appears likely that the albumin was not derived from its interaction with the plasma membrane during the isolation procedure.

In order to assess the possibility that albumin was actually synthesized by lymphocytes, pig mesenteric lymphocytes were labelled biosynthetically for 4h with [^{35}S]methionine, and the distribution of labelled polypeptides was assessed by two-dimensional gel electrophoresis. The Coomassie-Blue-staining pattern of the two-dimensional gel (Fig. 6*a*) shows the positions of added unlabelled albumin and actin. The positions of these markers are also indicated by arrows in the radioautogram of the same polyacrylamide gel (Fig. 6*b*). No labelled component co-migrated with the added pig albumin, whereas an intensely labelled spot co-migrating with added pig actin was observed. This result indicated that albumin was probably not synthesized by pig lymphocytes. This result was confirmed by immunoprecipitation with anti-(pig albumin) serum, which failed to precipitate an albumin-co-migrating band from biosynthetically labelled pig lymphocytes (results not shown). Similar experiments with pig mesenteric lymphocytes labelled for 16h, mouse lymphocytes, mitogen-stimulated lymphocytes and cultured human lymphoblastoid cells also failed to provide any direct evidence for biosynthesis of albumin by lymphocytes.

Discussion

Evidence has been presented which demonstrates that a protein of close structural and immunological similarity to pig albumin is a major component of purified preparations of pig lymphocyte plasma membrane. Most importantly, albumin and the pig membrane component possess identical isoelectric points, indistinguishable mobilities on SDS/polyacrylamide gels under reducing and non-reducing conditions, and a closely similar spectrum of arginine-containing tryptic peptides; the pig membrane component also reacts with an antiserum raised against an authentic sample of pig serum albumin. Taken together these data present a strong, if not overwhelming, case that the pig membrane component is structurally identical with pig albumin. Albumin was present in the plasma-membrane preparations from pig thymus and spleen as well as from mesenteric lymph node, and was independent of whether the plasma membrane was prepared directly from tissue or from dispersed cell suspensions. This association appeared to be unique to the pig, since, although plasma-membrane preparations from other species (human, mouse, rat) as well as from both cultured and normal cells possessed a major polypeptide component of about 68000 mol.wt. when analysed by SDS/polyacrylamide-gel electrophoresis under reducing conditions, no direct evidence was obtained that this band represented albumin. The association of albumin

Table 1. *Binding of ^{125}I-labelled pig albumin to pig lymphocyte plasma membrane*

^{125}I-labelled pig albumin (10μg) was added to 10^8 pig mesenteric-lymph-node lymphocytes (viability 95%) in 1ml of phosphate-buffered saline. After incubation at 20°C for 30min the cells were disrupted and plasma membranes prepared. The radioactivity associated with purified plasma membrane was determined. Alternatively, purified pig lymphocyte plasma membrane (1mg of protein in 1ml of 10mM-Tris/HCl buffer, pH7.4) was incubated with 50μg of ^{125}I-labelled pig albumin at 20°C for 30min and then washed three times with 10mM-Tris/HCl buffer. Radioactivity in the pellet was determined.

	Initial radioactivity (c.p.m.)	Final radioactivity (c.p.m.)	Binding (% of initial c.p.m.)
Cells	141000	520	0.37
Plasma membrane	697000	930	0.13

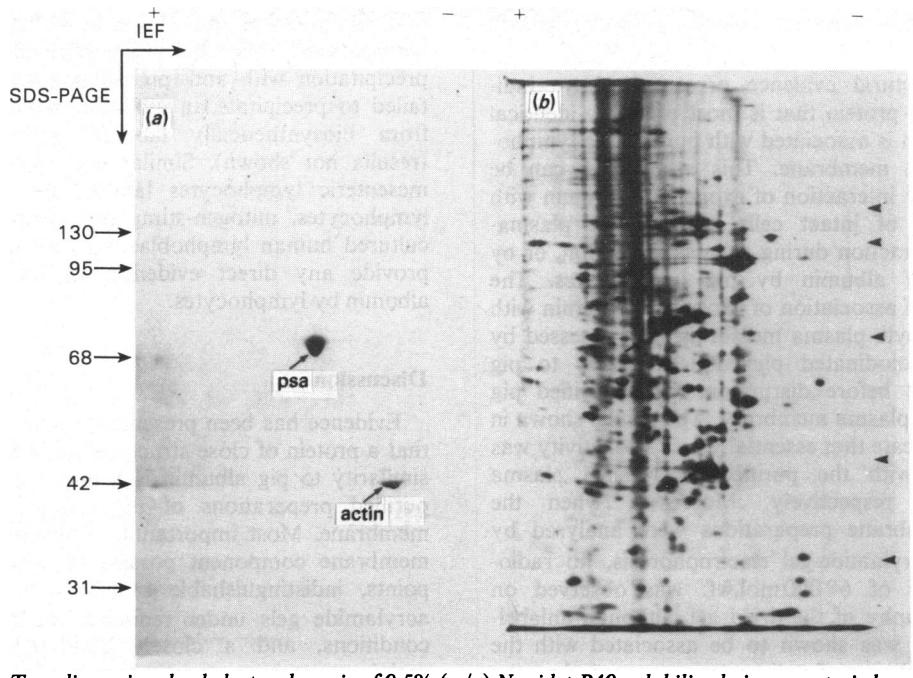

Fig. 6. *Two-dimensional gel electrophoresis of 0.5% (w/v) Nonidet-P40-solubilized pig mesenteric lymphocytes*
Cells were labelled biosynthetically with [^{35}S]methionine. After solubilization, pig albumin ('psa') and actin (5 μg each) were added before electrophoresis. The Coomassie-Blue-stained gel is shown in (*a*) (the amount of lysate protein added was insufficient to detect by Coomassie Blue staining) and the fluorograph of the same gel is shown in (*b*). The upward-pointing arrows in (*b*) refer to the positions of actin and pig albumin. The reduced standard proteins are those described in the legend to Fig. 1. Other abbreviations: IEF, isoelectric focusing (first dimension); SDS-PAGE, SDS/polyacrylamide-gel electrophoresis (second dimension).

with pig lymphocyte plasma membrane is therefore unlikely to be of major biological importance.

There are several possible explanations for the presence of albumin in a lymphocyte membrane preparation. In particular, its association could result from non-specific adsorption during the plasma-membrane preparation procedure. This possibility was rendered unlikely by the failure of albumin to bind to the plasma-membrane fraction when added to the cell suspension before disruption, although it is conceivable that the plasma-membrane binding sites were saturated before preparation of a cell suspension from pig mesenteric lymph node. Alternatively, albumin may be synthesized by pig lymphocytes, although its generally accepted site of synthesis is the liver. Indeed, Teodorescu *et al.* (1977) have claimed that albumin is synthesized and secreted by rabbit lymphoid tissue. Another serum protein, namely α-acid glycoprotein (orosomucoid), that is synthesized by the liver is also synthesized by human lymphocytes as a membrane-bound polypeptide that is subsequently cleaved and released in a soluble form (Gahmberg & Andersson, 1978). Biosynthetic labelling studies using [^{35}S]methionine failed, however, to demonstrate that the pig membrane component is synthesized by lymphocytes, as judged by two-dimensional gel-electrophoretic analysis and by immunoprecipitation with anti-(pig albumin) serum. Similar experiments with mouse and human lymphocytes, including cells activated with non-specific mitogens, were also negative in spite of using labelling periods of up to 16h. Biosynthesis of albumin by pig lymphocytes was therefore considered to be extremely unlikely.

This interpretation in turn suggests that the pig plasma-membrane-associated albumin is probably of extraneous origin. Compelling evidence in support of this suggestion, is, however, lacking, and some results appear to be more consistent with alternative explanations. Thus, the albumin is apparently strongly bound to the plasma membrane or is occluded within plasma-membrane vesicles, since it is not dissociated by the breakage, homogenization and extensive washing procedures employed during membrane preparation and it does not exchange with added ^{125}I-labelled albumin. It is, however, quantitatively released by non-ionic detergents (Barber & Allore, 1979). One possible explanation for the association of extraneous pig albumin with the plasma membrane is that albumin enters the cytosol as a result of endocytotic activity and that high-

affinity binding sites exist for albumin at the membrane's cytoplasmic face. As albumin is known to contain hydrophobic binding sites (Rudman & Kendall, 1957), an association between albumin and the hydrophobic regions of bilayer-associated proteins would appear feasible. The marked increase in serum albumin uptake which occurs when endocytotic activity of pig lymphocytes is stimulated by incubation with the mitogen phytohaemagglutinin (E. Yakobson, R. Lyall & M. J. Crumpton, unpublished work) is consistent with this possibility.

The significance of pig lymphocyte plasma-membrane-associated albumin, if any, remains to be established. As some lymphokines and α_2-macroglobulin are associated with albumin (Dumonde *et al.*, 1969; Rocklin, 1975), it is conceivable that albumin acts as a carrier for molecules involved in pig lymphocyte function. This possibility is, however, not very compelling in view of its probable exogenous origin and its apparent absence in lymphocytes of other species.

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