ACTIN ASSOCIATED WITH PURIFIED LYMPHOCYTE PLASMA MEMBRANE

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1. Introduction

Actin is now recognized as a major cytoplasmic component of many non-muscle cells [1]. Its functional role, both in a polymeric (i.e. microfilaments) and monomeric form, has, however, yet to be clearly defined. In the case of lymphocytes, microfilaments have been implicated in various cell surface-mediated phenomena including the capping of surface immunoglobulin [2–5], lectin-induced mitogenicity [6,7] and lymphocyte-mediated cytotoxicity [8,9]. One possible explanation for the apparent linkage between cell surface events and cytoplasmic microfilaments would be a specific interaction between the filaments and the cell surface (plasma) membrane. We have investigated this possibility by examining purified lymphocyte plasma membrane preparations for the presence of actin. It was reasoned that if a specific association existed within the cell, then it may well survive the isolation of the plasma membrane. In this paper we present results which indicate that actin, or a protein exhibiting a high degree of homology with actin, is a major component of extensively purified lymphocyte plasma membrane from pig and human sources.

2. Materials and methods

2.1. *Materials*

Actin was purified from pig muscle [10]. Polyacrylamide gel electrophoresis in SDS of the purified protein revealed a single band which comigrated with an authentic sample of rabbit muscle actin. Diphenylcarbamyl chloride-treated trypsin, highly polymerized DNA and Lubrol PX were products of Sigma Chemical Co. DNase I (RNase free; 2450 units/ml) was obtained from Worthington Biochemicals and the thin-layer cellulose plates (Polygram Cel 400) from Macherey-Nagel and Co.

2.2. *Lymphocyte plasma membrane*

Plasma membrane was prepared from pig mesenteric lymph node lymphocytes as described previously [11] except that a larger model of the tissue press was used [12], and the plasma membrane recovered from the discontinuous sucrose gradient was washed twice by resuspending with a Pasteur pipette in 10 mM Tris-HCl buffer, 0.15 M NaCl, pH 7.4. Suspensions of pig mesenteric lymph node lymphocytes and of cultured human lymphoblastoid cells (cell line BRI 8) were broken by using a cell-disrupting pump (Stansted Fluid Power Ltd, Stansted, Essex). The plasma membrane fractions were separated by differential and sucrose gradient centrifugation as described previously [11]. The BRI 8 membrane was, however, washed by resuspending once in a Dounce homogenizer with 10 mM Tris-HCl, pH 7.4 and once with distilled H_2O. According to various criteria the purified plasma membranes were not contaminated to a significant extent by other subcellular components [11].

Plasma membrane (2 mg of protein/ml) was solubilized in 1% Lubrol PX by incubating at 22°C for 30 min, followed by centrifugation at 100 000 *g* for 1 h.

2.3. *SDS-polyacrylamide gel electrophoresis*

Polyacrylamide gel electrophoresis was performed in 0.1% SDS on 7.5% (w/v) acrylamide slab gels (8 × 10 × 0.1 cm) using a 3% (w/v) stacking gel and the Tris-glycine buffer system of Laemmli [13].

Membrane samples were dissolved in 2% (w/v) SDS, 10% (v/v) glycerol, 80 mM Tris-HCl, pH 8.6, by heating at 100°C for 2 min and were stored at −20°C. Immediately prior to analysis, dithiothreitol and Bromophenol blue were added to 0.1 M and 0.02% respectively, and the samples placed at 100°C for a further 2 min. Gels were stained with 0.01% Coomassie blue in methanol–water–acetic acid (41:52:7, by vol.).

2.4. *Peptide mapping*

Lymphocyte plasma membrane (about 1 mg of protein in 1 ml) was fractionated by SDS-polyacrylamide gel electrophoresis using a single slab gel. The gel was fixed, stained, and the band comigrating with purified pig muscle actin excised. Protein was extracted and iodinated as described previously [14], with the exception that phenylmethylsulphonyl fluoride was omitted. A sample of purified pig muscle actin was iodinated in an identical manner and carried through the mapping procedure in parallel with the gel-extracted proteins. The iodinated samples were left at 0°C for 15 min, diluted with 1 ml containing 1 mg of bovine serum albumin, and precipitated with 0.2 ml of 50% (w/v) trichloroacetic acid. After 15 min at 0°C, the precipitates were recovered by centrifugation, washed once with acetone containing 0.1 M HCl, once with acetone and dried. The pellets were oxidized at 0°C for 90 min in 100 μl of performic acid (prepared by reacting 0.1 ml of 30% H_2O_2 with 1.9 ml of formic acid at 22°C for 2 h) and dried overnight. The residues were resuspended in 0.1 ml of 0.1 M NH_4HCO_3, digested twice at 37°C for 2.5 h with diphenylcarbamyl chloride treated trypsin (20 μl each of a 1 mg/ml solution), dried and dissolved in 20 μl of H_2O. The two samples to be compared were loaded on either side of the midline of a thin-layer cellulose plate (20 × 20 cm) pre-wetted with pyridine–acetic acid–water (111:3.7:1000, by vol, pH 6.5). Electrophoresis was carried out in the same buffer for 4.5 h at 3000 V and 4°C. The plate was air-dried, cut along the midline, and subjected to chromatography (solvent: H_2O–acetic acid–pyridine–butanol, 143:50:143:204.

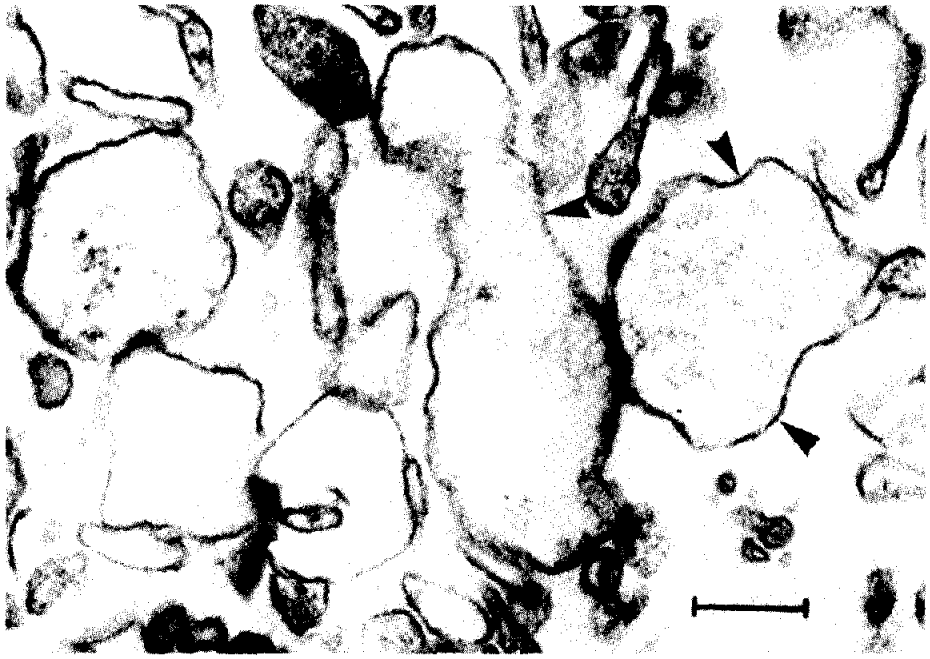

Fig.1. Electron micrograph of purified BRI 8 plasma membrane. The membrane was fixed as previously described [11]. Thin sections were stained with uranyl acetate and lead citrate prior to observation in a Phillips 300 microscope. The arrowheads indicate apparent points of contact between filaments and the membrane bilayer. Magnification: bar represents 0.2 μm.

by vol.) perpendicular to the electrophoresis direction. Autoradiograms were prepared with Kodirex AP54 film by exposure for 1 to 5 days.

2.5. *DNase I inhibition assay*

DNase I activity was assayed as described by Lindberg [15] except that the DNA substrate was made up in 0.5% Lubrol PX. Samples (5 to 50 μl) of DNase I in 5 mM $CaCl_2$, 0.5% Lubrol PX, pH 7.5, were added to the DNA solution (1 ml), and the change in optical density at 260 nm measured. The initial slope of the change at 260 nm was linear over the range 0.04 to 0.4 μg of DNase I. The inhibitory capacity of 1% Lubrol-solubilized plasma membrane was determined by measuring the amount of membrane required to give the same inhibition as that obtained with a known amount of purified pig actin in 0.5% Lubrol. The actin or solubilized membrane was incubated with DNase I for 5 min before initiating the reaction by the addition of substrate. Protein concentrations were determined by the method of Lowry et al. [16].

3. Results

3.1. *Plasma membrane preparations contain filamentous material*

Electron micrographs of BRI 8 plasma membrane (fig.1) revealed that many vesicles contained filaments whose diameters (50 – 80 Å) resembled those of microfilaments [1]. The plasma membrane had been extensively washed under hypotonic conditions during its preparation and, as assessed by lactate dehydrogenase activity, was not significantly contaminated by cytoplasm [17]. This suggests that the filamentous material is intimately associated with the membrane. This view is supported by the observation that points of contact appear to exist between the filaments and the membrane bilayer (fig.1). Similar results have been obtained for plasma membrane isolated from pig lymph node tissue and pig lymphocyte suspensions.

3.2. *Identification of actin in plasma membrane preparations*

Fig.2 shows the SDS-polyacrylamide gel electrophoresis patterns of the purified plasma membrane

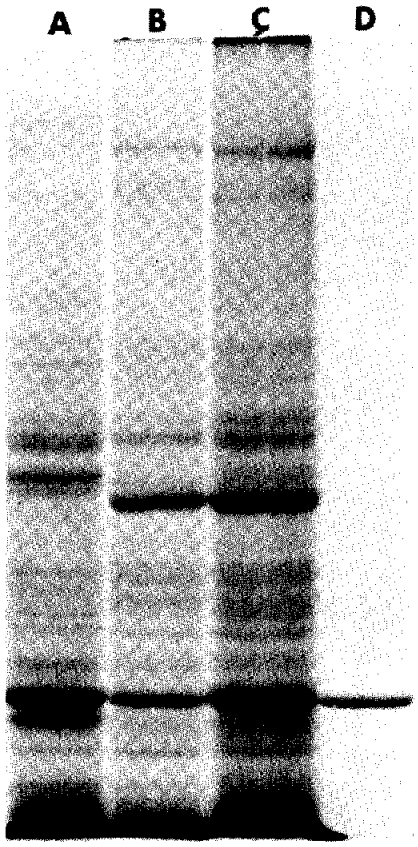

Fig.2. SDS-polyacrylamide gel electrophoresis patterns stained with Coomassie blue. Plasma membranes from (A) BRI 8 cells, (B) pig mesenteric lymph node and (C) suspension of pig lymphocytes. Gel D represents purified pig muscle actin.

preparations. Each pattern possessed a prominent band comigrating with pig muscle actin. This band represents the major Coomassie blue staining component of the BRI 8 plasma membrane and the second most intensely-staining band of the pig membrane. Preliminary experiments utilizing chloramine T-catalysed iodination [14] of pig lymphocyte plasma membrane solubilized in 2% sodium deoxycholate indicate that the actin-comigrating band accounts for 5 to 6% of the protein-bound ^{125}I. This result suggests that actin represents about 5% of the membrane protein, assuming a uniform distribution of accessible tyrosine residues among the membrane proteins.

The extent of homology between actin and the

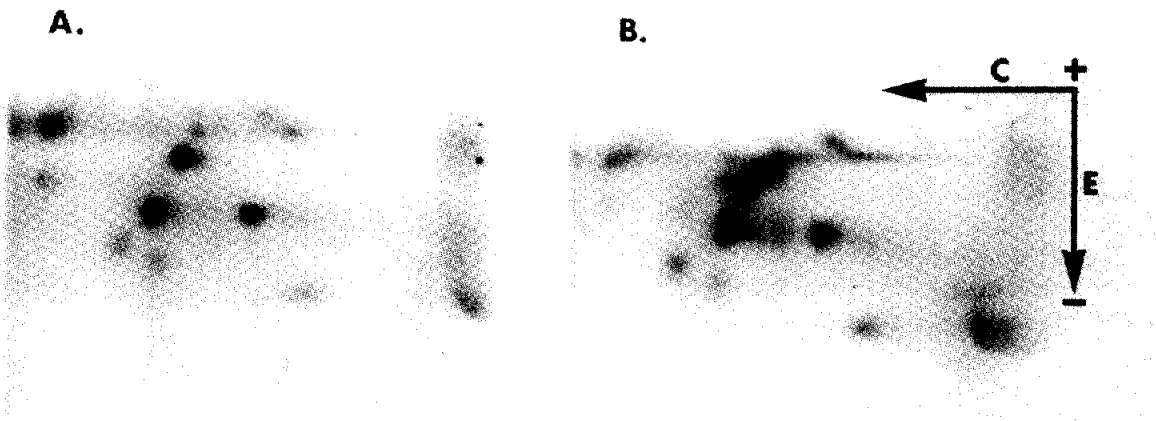

Fig.3. Tryptic peptide maps of ^{125}I-labelled peptides of the actin-comigrating band of BRI 8 plasma membrane (A) excised from a polyacrylamide gel after electrophoresis in SDS, and purified pig muscle actin (B). E represents the direction of electrophoresis in the first dimension and C the direction of chromatography in the second dimension.

actin-comigrating bands was assessed by comparing their tryptic peptide maps. Fig.3 shows the maps obtained for the actin-comigrating band of BRI 8 plasma membrane and purified pig muscle actin. Similar maps have been obtained for the actin-comigrating band from polyacrylamide gels of pig lymphocyte plasma membrane. Although individual experiments often differed in the degree of resolution and 'tightness' of the spots, in each case the relative distribution of spots for purified actin and the membrane protein samples was closely similar. At least 13 spots were coincidental and no additional spots were detected in the membrane bands that were not also apparent in the authentic actin preparation. In a similar study of adrenal medulla, Phillips and Slater [18] also reported finding 13 major spots for I^{125}-labelled actin.

3.3. *Inhibition of DNase I by detergent-solubilized plasma membrane*

Lazarides and Lindberg [19] recently demonstrated that native monomeric actin (G-actin) is a specific inhibitor of DNase I. The application of this reaction to membrane-associated actin required prior solubilisation of the membrane. A number of detergents were screened for their compatibility with the DNase I assay. Lubrol PX satisfied the optical requirements for the assay and at a concentration of 0.5% had a minimal affect upon DNase I activity. Thus, purified pig muscle G-actin in 0.5% Lubrol caused 50% inhibition of activity at an actin to DNase I molar ratio of 3:1. When small aliquots of pig lymphocyte plasma membrane solubilised in 1% Lubrol PX were added to the DNase I assay mixture in 0.5% Lubrol, the inhibition was proportional to the amount of membrane added. Approx. 100 μg of membrane protein in 100 μl caused 60% inhibition of the DNase I activity, equivalent to the addition of 0.5 μg of purified actin under the same conditions. The inhibition appeared to be specific, in that no reduction of activity was observed when equivalent amounts of bovine serum albumin in 1% Lubrol were added. This result indicates the presence of native 'actin-like' material in solubilized lymphocyte plasma membrane. Unfortunately, the DNase I inhibition assay could not be used to provide a quantitative estimate of the 'actin-like' material, because 1% Lubrol solubilised only 50–60% of the total membrane protein and, as assessed by SDS-polyacrylamide gel electrophoresis, the actin band was poorly represented in the soluble fraction.

4. Discussion

It has recently been proposed [4,20] that cytoplasmic microfilaments play a key role in the regulation of lymphocyte cell surface receptor mobility. Our results indicate that a protein having a high degree of homology with muscle actin is a major constituent of highly purified preparations of human and pig lymphocyte plasma membrane. The most compelling evidence in support of this conclusion is the marked similarity between the tryptic peptide maps for ^{125}I-labelled pig muscle actin and the actin-comigrating band of lymphocyte plasma membrane. The inhibition of DNase I activity by Lubrol-solubilized plasma membrane is also consistent with the presence of an 'actin-like' protein, and suggests that at least a fraction of the putative membrane-associated actin is in the native configuration. On the other hand, the possibility that some differences exist between muscle and membrane actin cannot be ruled out [21], since no attempt was made to observe the total complement of tryptic peptides.

Plasma membranes isolated from pig mesenteric lymph node, suspensions of pig lymphocytes, and cultured human lymphoblastoid BRI 8 cells have been examined. Although the pig mesenteric lymph node membrane may conceivably have been contaminated by actin from non-lymphocyte sources (e.g. fibroblasts or macrophages), such contamination is clearly ruled out in the case of the cultured BRI 8 cells. In view of the apparently similar actin contents of the three different plasma membrane preparations (fig.2), it seems unlikely that non-lymphocyte actin represents a major source of contamination in the pig membrane.

The question of whether or not the apparent association between actin and the purified plasma membrane accurately reflects the situation in the intact cell must also be considered. As two independent methods of disrupting pig lymphocytes (see Materials and methods) gave rise to plasma membrane with similar actin contents, it appears unlikely that the association results from a particular method of cell rupture. In a similar study of synaptosomal membrane, Blitz and Fine [22] approached the problem of non-specific association by adding radiolabelled actin to the cell homogenate. They found that the purified membrane possessed essentially no radioactivity and concluded that the membrane-associated actin was 'intrinsic to the synaptosomes'. These results, and the apparent tenacity of actin binding as indicated by its persistence throughout the washing steps, suggest that the association of actin with lymphocyte plasma membrane is unlikely to be an adventitious consequence of cell disruption.

In whole lymphocytes, microfilaments are found preferentially underlying the cell surface membrane [4,20] and in surface protrusions such as microvilli [20]. Electron micrographs of the purified plasma membrane vesicles revealed membrane-adherent filamentous material similar in appearance to microfilaments (fig.1). This result suggests that a significant fraction of the membrane-associated actin may be present as filaments, and strengthens the arguments that a functional association exists between the cytoplasmic microfilaments and the lymphocyte cell surface membrane [4,20]. The unequivocal assignment of this filamentous material to microfilaments must, however, await its definitive identification by such techniques as heavy meromyosin labelling [1].

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