MYOSIN-LIKE POLYPEPTIDES IN PLASMA MEMBRANE PREPARATIONS

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Received 16 August 1975

1. Introduction

Animal cell plasma membranes contain a heterogeneous collection of polypeptides (reviewed in [1]), a finding which seems consistent with the multitude of functions which the membrane must perform. There is evidence that actomyosin is a constituent of these membranes [1]. In this study, I present data concerning high mol. wt polypeptides in plasma membranes from liver and erythrocytes which indicate that these proteins have several similarities to muscle myosin.

2. Experimental

2.1. *Liver plasma membrane preparation*

Rats of either sex weighing approx. 300 g were anesthetized with Nembutal and injected i.p. with 1000 units of heparin. After 20 min, the liver was perfused via the portal vein with phosphate-buffered saline until blanched. The liver was excised and dissected free of large pieces of connective and vascular tissue, and then fractionated essentially in accordance with the procedure of Ray [2]. Membrane fractions were similarly prepared from freshly slaughtered calf liver, without perfusion.

2.2. *Red blood cell membrane preparations*

Bovine, rat, and rabbit blood was collected into citrate-dextrose solution and stored at 0°C. Ghosts were prepared by conventional means within 5 days [3]. Rabbit reticulocyte membranes, donated by Lettie Lubsen (Harvard University), were prepared from washed reticulocytes by lysing cells with a three-fold dilution with distilled water and collecting the membranes fraction by centrifugation at 27 000 *g* for 15 min. The membranes fraction was washed three times with 15 mM NaCl + 0.5 mM Tris–Cl (pH 7.5), and frozen until used.

2.3. *Sodium dodecyl sulfate polyacrylamide gel electrophoresis*

The system of Shapiro et al. [4] was used, with bromphenol blue as tracker dye. Gels were stained with Coomassie brilliant blue and scanned at 550 nm using a Beckman DU spectrophotometer with a Gilford linear transport attachment and Model 2000 recorder. The sources and subunit mol. wt values [5] of the marker proteins are as follows: bovine carbonic anhydrase (Mann, 29 000), crystalline bovine serum albumin (Pentex, 68 000), β-galactosidase (Boehringer-Mannheim, 130 000), and phosphorylase a (Worthington, 94 000). Myosin (220 000) was prepared from rabbit skeletal muscle by the method of Richards et al. [6]. Actin (46 000) was prepared by the method of Carsten and Mommaerts [7].

2.4. *Amino acid analyses*

Amino acid analyses were performed on a Beckman 120C analyzer by the method of Spackman et al. [8]. Bands cut from polyacrylamide gels were hydrolyzed in 6 N HCl for 24 h in the presence of 0.1% mercaptoacetic acid (Lloyd Waxman, personal communication).

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2.5. *Optical rotatory dispersion*

ORD measurements were performed with a Cary 60 spectropolarimeter.

3. Results

Liver plasma membrane fractions were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and compared to erythrocyte ghosts (fig.1). The major high mol. wt polypeptide of the liver membrane fraction has the same mobility as muscle myosin. The spectrin polypeptides [9] of the ghost preparations have lower mobilities. The high mol. wt liver membrane protein comigrated with myosin in a variety of gel systems in which the mobility varied from 0.1 to 0.5 relative to the tracker dye. Bovine liver membrane fractions had a similar polypeptide distribution. The high molecular weight component accounted for an average of 3.8% of the protein in the membranes, as estimated by scanning stained gels. This component can be extracted in the presence of 0.6 M KCl and ATP and purified further [10].

The high mol. wt spectrin polypeptides were extracted with EDTA solution, as described by Marchesi et al. [9]. Three major polypeptides were extracted, with apparent masses of 250 000, 230 000, and 46 000 daltons. Following ammonium sulfate fractionation [9], only the two high mol. wt components redissolved. The results of polyacrylamide gel electrophoresis and the ORD spectrum of the purified high molecular weight components are illustrated in fig.2. The method of Yang and Doty [11] was used to obtain the value for the parameter b_0 of 304 ± 38.

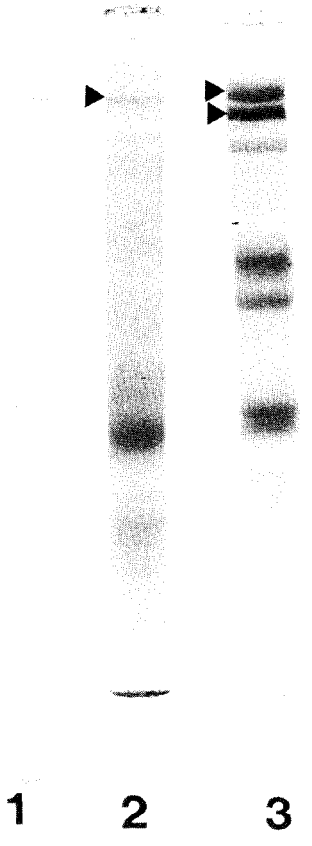

Fig.1. Plasma membrane fractions analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Gels contain 5% acrylamide, 0.135% methylenebisacrylamide, but were run separately, so the bands are not aligned. (1) Myosin and serum albumin, (2) rat liver plasma membrane, and (3) bovine erythrocyte ghosts. The 220 000 dalton polypeptide of the liver plasma membrane and the spectrin polypeptides are indicated.

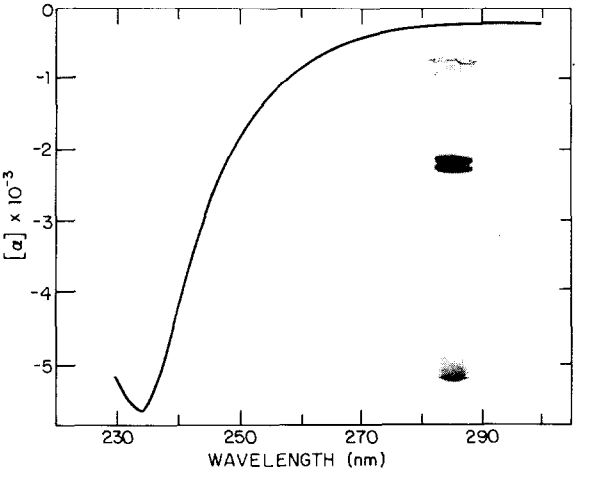

Fig.2. ORD and polyacrylamide gel electrophoresis of bovine spectrin. The protein was dialyzed against 0.3 M NaCl, 1 mM EDTA, 50 mM Tris–Cl (pH 8.6). The spectrum was recorded at 22°C, with a protein concentration of 0.31 mg/ml (determined by amino acid analysis), and a path length of 1 cm. The spectrum obtained with the dialysis buffer was used as baseline, and is not shown. The specific rotation at 233 nm was −5935.

This figure is indicative of an α-helix content of 48 ± 6%, compared to the value of 60% reported for muscle myosin [12]. When acetone-treated, dried ghosts were extracted with a buffer containing ATP and ascorbate [7] the 46 000 dalton polypeptide was extracted selectively. This result is consistent with previous descriptions of actin-like proteins associated with erythrocyte ghosts [13,14].

The procedures of Carsten and Mommaerts [7] were also followed to extract proteins from membrane fractions of rabbit reticulocytes. Fig.3 illustrates the results of extracting acetone-treated or untreated membrane fractions. In the case of untreated membranes, the ATP-ascorbated extract was similar in polypeptide composition to the EDTA extract from erythrocyte ghosts, with the addition of an 80 000 dalton polypeptide. Extracts of acetone-treated membranes, however, were virtually devoid of the high mol. wt polypeptides. When the extracts were treated under conditions used for the polymerization of muscle actin [7], the 46 000 dalton polypeptide became sedimentable (100 000 *g* 3 h). The pellet was depleted of the 80 000 dalton polypeptide. The high mol. wt polypeptides were present in the pellets from extracts prepared from untreated membranes. The nature of the 80 000 dalton component was not investigated.

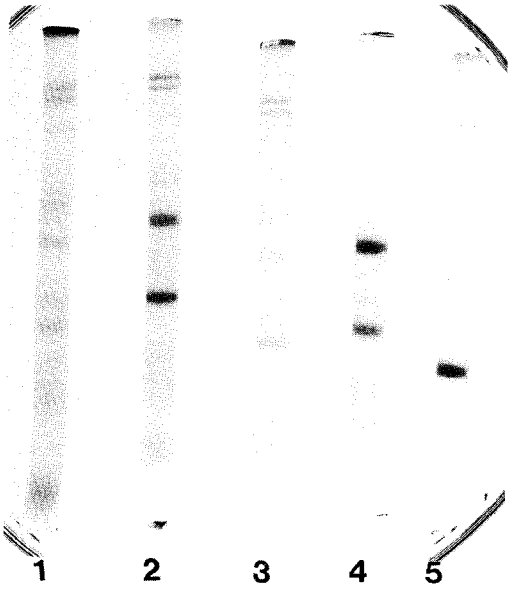

Fig.3. Extraction of reticulocyte membrane proteins. Membranes were treated with 9 vol of acetone at 0°C, collected by centrifugation, and dried at room temperature. The membranes, treated and untreated, were suspended in 0.2 mM ATP, 0.2 mM ascorbic acid, 1 mM histidine (pH 7.0), incubated for 30 min at 0°C, then centrifuged at 95 000 *g* for 1 h. The supernatants were adjusted to a final concentration of 0.1 M KCl and 0.1 mM $MgCl_2$. After standing 8 h at 0°C, the 'polymerized' fraction was collected by centrifugation at 95 000 *g* for 3 h. Fractions analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis: (1) reticulocyte membranes, (2) ATP-ascorbate extract, (3) polymerized fraction, (4) ATP-ascorbated extract from acetone-treated membranes, and (5) polymerized fraction from acetone-treated membranes. Gels contain 6% acrylamide, 0.81% methylenebisacrylamide.

Amino acid analysis can be used to identify proteins which have unusual amino acid compositions. In the case of muscle myosin heavy chain, the content of glutamic acid is 20 mol percent [15] and is therefore distinguishable from most proteins on this basis. The high mol. wt polypeptides from liver and erythrocytes were cut from polyacrylamide gels and subjected to acid hydrolysis. Table 1 shows only those values which can reliably be estimated by this method. There is a striking similarity among the liver, erythrocyte, and muscle polypeptides, which is especially significant in the case of glutamic acid.

4. Discussion

The data presented above indicate that polypeptides related to muscle myosin and actin are present in plasma membrane preparations from two nonmuscle tissues. Although the mode of attachment to the membrane has not been investigated, the disposition of these components may be similar to the arrangement of the actin-like filaments of *Acanthamoeba castellanii*, which are attached to the cytoplasmic face of the plasma membrane [16].

The functions of actomyosin in nonmuscle cells has not been established unequivocally, but it seems likely to be important in regulating membrane function. There is compelling evidence for the involvement of actin is cytokinesis, since filaments which bind heavy meromyosin comprise the contractile ring beneath the cleavage furrow in dividing HeLa cells [17,18]. Evidence which favors the view that intracellular components (though not necessarily actomyosin) influence the distribution of surface components has been obtained in a number of cell types [19,20,21]. Additional support for the view that actomyosin-like pro-

Table 1
Amino acid composition data

Amino Acid	Spectrin	RBC A	RBC B	Liver	Myosin
Aspartic acid	16.8	15.1	16.1	19.3	16.4
Glutamic acid	31.7	30.0	30.2	29.1	30.5
Alanine	14.2	10.9	12.2	12.7	15.1
Valine	7.3	7.4	7.6	7.8	8.4
Isoleucine	6.2	6.7	6.9	8.5	8.2
Leucine	19.2	24.5	21.6	17.8	15.8
Phenylalanine	4.6	5.4	5.6	5.1	5.7

The data are expressed as mol. % of (aspartic acid + glutamic acid + alanine + valine + isoleucine + leucine + phenylalanine). The data for spectrin were calculated from the data of Marchesi et al. [9]. The data for myosin were calculated from the data of Lowey et al. [15]. RBC A and B are the spectrin polypeptides with apparent masses of 250 000 and 230 000 daltons, respectively, from bovine ghosts. The liver protein is the 220 000 dalton polypeptide from rat liver.

teins may be involved in plasma membrane function comes from studies of red blood cell ghosts. The distribution of 8.5 nm intramembranous particles and surface components is altered under conditions which affect the spectrin component, such as treatment with anti-spectrin antibodies [22].

Acknowledgements

I wish to thank my colleagues in the laboratory of Guido Guidotti for their assistance and helpful comments. This work was supported by grants from the National Institutes of Health and the National Science Foundation to Guido Guidotti and by a predoctoral training grant from the National Institute of Health, administered by the Department of Biological Chemistry, Harvard University.

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