CHANGES IN MEMBRANE PROTEIN PATTERN IN RELATION TO LENS CELL DIFFERENTIATION

A. J. M. VERMORKEN, J. M. H. C. HILDERINK, I. DUNIA+, E. L. BENEDETTI+ and H. BLOEMENDAL*

Laboratory of Biochemistry, University of Nijmegen, Geert Grooteplein Noord 21, Nijmegen, The Netherlands and *+Institut de Biologie Moléculaire du CNRS et de l'Université Paris VII, 2, Place Jussieu, 75005 Paris, France*

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1. Introduction

In previous papers we have described that the protein pattern of isolated lens fiber membranes is characterized by a number of polypeptides in different proportions [1–4]. Among these proteins two components with molecular weight of about 26 000 and 34 000 (designated as MP26 and MP34, respectively [2]) become predominant in the protein pattern of a membrane subfraction, enriched in communicating junctions [3]. This type of junctions which connects the lens fibers is assembled in the elongation zone where the epithelial cells differentiate into the lens fibers [4,5]. Moreover, recent observations revealed that polyribosomes derived from the cortical part of the eye lens are able to direct the biosynthesis of membrane proteins even in heterologous cell-free systems [6]. Under special conditions similar results can be obtained with messenger RNA isolated from these polyribosomes [7].

The aim of the present study was to prepare purified plasma membranes from calf lens epithelium and to establish whether the differentiation of lens epithelial cells into fibers is accompanied by the appearance of membrane protein(s) differing from those in the epithelial plasma membranes.

2. Materials and methods

2.1. *Chemicals*

L-[^{35}S]Methionine (spec. act. 200 Ci/mmol) was purchased from the Radiochemical Centre (Amersham, England). Newborn calf serum was obtained from flow.

2.2. *Isolation of lens fiber plasma membranes*

Lens fiber plasma membranes were isolated according to Bloemendal et al. [1] with slight modifications as described [8].

2.3. *Isolation of epithelial plasma membranes*

Calf eyes were obtained on ice from the slaughterhouse. They were washed with water en opened at the lateral side, so that the eye lenses could be removed without adhering iris. A small incision was then made in the lens capsule, after which it could easily be removed from the lens body. About 40% of the epithelial cells remained attached to the lens capsule, as was demonstrated by microscopic examination. About 3000 of these capsules were suspended in 1.5 litres buffer (1 mM $NaHCO_3$ + 1 mM $CaCl_2$) and stirred during 3 h. The capsules, free of cellular material were then removed by decantation. The supernatant was used to isolate membranes employing the procedure mentioned above with the difference that the flotation step was carried out twice.

2.4. *Electron microscopy*

The membrane pellets collected by centrifugation from the gradient layers were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 30 min and postfixed 1% OsO_4 in the same buffer during 1 h. All samples, after dehydration, were embedded in Vestopal. Thin sections were examined, stained with uranyl acetate and lead citrate. Freeze etching of

*To whom reprint requests should be addressed

either unfixed or glutaraldehyde fixed materials was performed in a Balzers apparatus. The fracture and the replicas were performed at −150°C. Some samples were etched for 90 s at −100°C. The electron microscopical observations were made in a Philips EM 300 and 400.

2.5. *Sodium dodecyl sulphate polyacrylamide electrophoresis*

Sodium dodecyl sulphate polyacrylamide electrophoresis was performed according to Laemmli [9] with the modification that a slab gel instead of gel rods was used. The gel was 12 cm long and contained 13% acrylamide, 0.4% methylene–bisacrylamide and 0.1% sodium dodecyl sulphate. Staining and destaining was as described by Weber and Osborn [10].

2.6. *Synthesis of lens protein in vitro*

Calf lens polyribosomes were isolated by procedures described previously [11,12]. Rabbit reticulocytes were prepared as described by Evans and Lingrel [13] and lysed by addition of water. A 30 000 × *g* supernatant fraction of these lysed cells was used as cell-free system. The incubation conditions were as described [7].

2.7. *Immunoprecipitation*

Immunoprecipitation was performed according to the procedure described for viral polypeptides [14]. The antisera were prepared by immunization of rabbits with purified lens fiber plasma membranes, with purified α,β and γ-crystalline and with bovine serum albumin, and tested as described by Van Zaane et al. [15]. The specificity of the antisera against membrane protein was verified by the Ouchterlony technique.

3. Results and discussion

Examination of isolated epithelial plasma membranes reveals that this fraction consists of large membrane profiles having a triple layered structure associated with vesicles of various sizes. Communicating gap junctions which are in most cases short connect the plasma membrane sheets. These and other typical features have been described in detail [4]. On the other hand isolated lens fiber plasma membranes are characterized by extensive junctional segments connecting the general plasma membrane (fig.1).

Fig.1. Isolated lens fiber plasma membranes showing that this fraction, in a large proportion, consists of junctions (arrows).

The protein pattern of lens fiber plasma membranes analysed on SDS–polyacrylamide gel electrophoresis differs from that of the epithelial membranes. Figure 2 shows that the polypeptide with mol wt 26 000 which is one of the predominent components in lens fiber plasma membranes, is virtually absent in the pattern of epithelial plasma membrane proteins. In the low molecular weight region (below 30 000) components belonging to the crystallins are less pronounced in the latter profile. Two sharp bands in the region of 50 000 are more prominent in the epithelial plasma membranes than in the gel pattern of fiber plasma membranes, whereas a 45 000 component is present apparently in the same proportion in both preparations.

The differences between the protein profiles of epithelial and fiber plasma membranes reflect changes

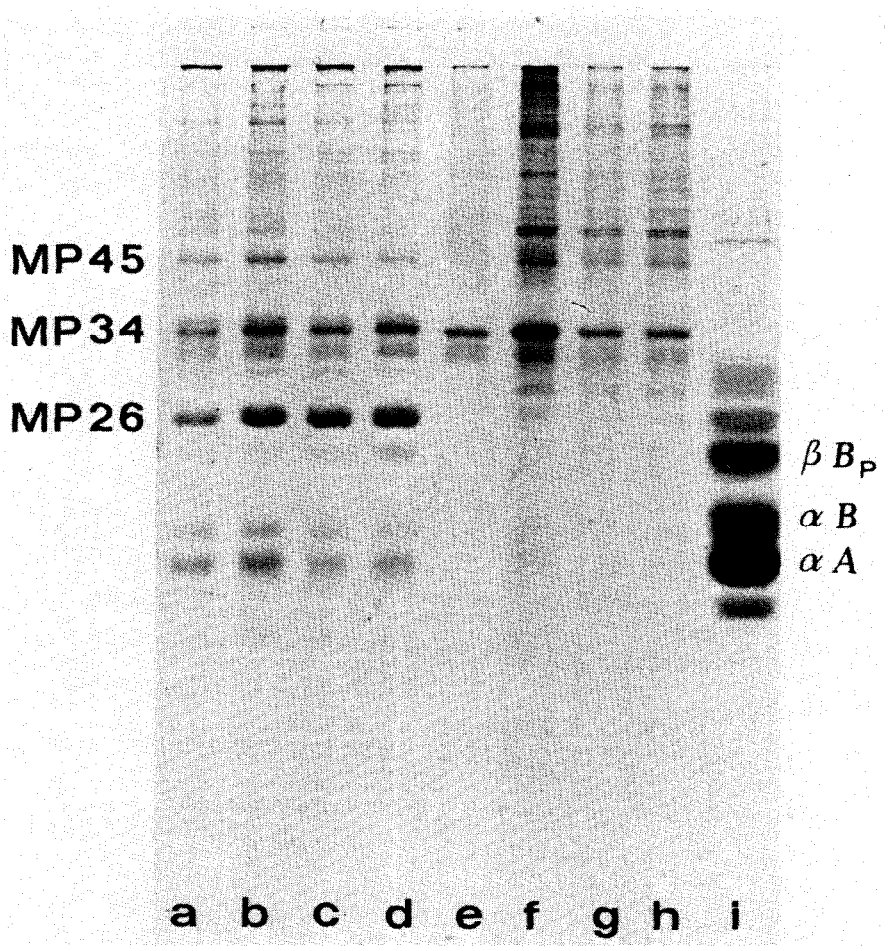

Fig.2. Sodium dodecyl sulphate gel electrophoresis of lens plasma membrane proteins: (a) Isolated fiber plasma membranes gathered at the interface between d 1.20 and d 1.22. (b) Isolated fiber plasma membranes gathered at the interface between d 1.18 and d 1.20. (c) Isolated fiber plasma membrane gathered at the interface between d 1.16 and d 1.18. (d) Isolated fiber plasma membranes gathered at the interface between d 1.14 and d 1.16. (e) Isolated epithelial plasma membranes gathered at the interface between d 1.14 and d 1.16. (f) Isolated epithelial plasma membranes gathered at the interface between d 1.16 and d 1.18. (g) Isolated epithelial plasma membranes gathered at the interface between d 1.18 and d 1.20. (h) Isolated epithelial plasma membranes gathered at the interface between d 1.20 and d 1.22. (i) For comparison the pattern of water-soluble lens crystallins is shown.

in molecular organization of the plasma membranes during differentiation, the most remarkable difference being the accumulation of MP26 in the fiber plasma membrane fraction. Therefore, the process of differentiation does not involve merely quantitative variations in the protein profile but a pronounced qualitative difference, which can be correlated with the formation of junctions.

We have shown [3] that lens fiber junctions, purified by detergent solubilization and sucrose gradient centrifugation, are characterized by the two major components MP26 and MP34. Freeze fracture experiments show that the differentiation of the communicating junctions existing between elongating and terminally differentiated fibers is characterized by an accumulation of intramembranous particles of protein nature

(fig.3). The molecular mechanism of the junctional assembly may either rely on de novo synthesis of membrane constituents or lateral displacement within the plane of the plasma membrane of a preexisting pool of juntional constituents. Since there is a continuous and slow accumulation of newly synthesized plasma membrane lipids and protein during the process of elongation it is more likely that the assembly of communicating cortical junctions is associated with the insertion of newly synthesized protein species. The appearance of MP26 in the fibers reinforces this assumption.

For the understanding of the biogenesis of the fiber junctions it would be important to know

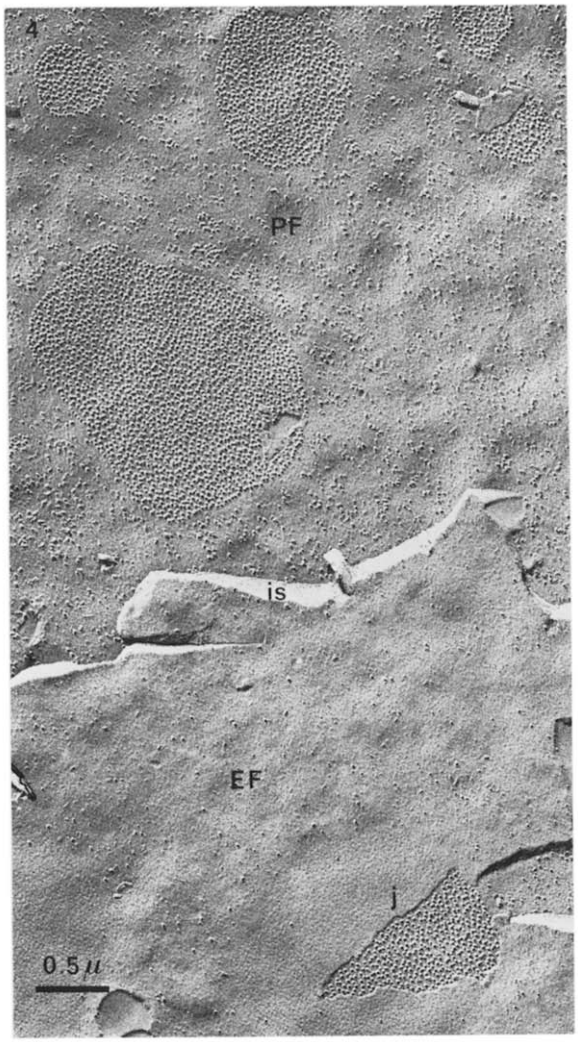

Fig.3. Replica of freeze-fractured elongating lens epithelium plasma membranes. The junctional assembly is characterized by the multicentric accumulation of intramembranous 9 nm particles. PF indicates the protoplasmic fracture face, EF the external one. j indicates a junctional domain, the intracellular space is indicated by 'is'.

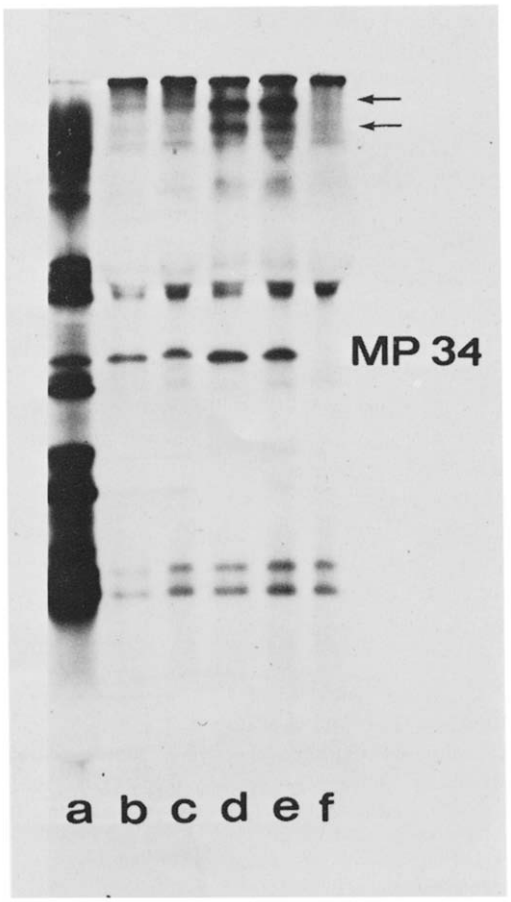

Fig.4. Autoradiograph of the sodium dodecyl sulphate gel electrophoretic patterns of protein, immunoprecipitated with different antisera. (a) For comparison the [^{35}S]methionine labeled proteins, synthesized in the reticulocyte cell-free system, supplemented with lens polyribosomes are shown. (b) and (c) Two different antisera directed against lens epithelial plasma membranes were used for immunoprecipitation. (d) and (e) Two different antisera directed against lens fiber plasma membranes were used for immunoprecipitation. (The high molecular weight products, not precipitated with antisera directed against epithelial plasma membranes are indicated with arrows.) (f) An antiserum directed against bovine serum albumin was used as a control.

whether MP26 and MP34 are synthesized in the lens cortex. In order to verify this possibility, polysomes from lens cortex were added to a reticulocyte lysate. After incubation, immunoprecipitation was carried out with antiserum directed against the cortical fiber membranes. Analysis of the precipitate on sodium dodecyl sulphate polyacrylamide gels revealed that MP34 has been synthesized (fig.4). Analysis of precipitates from control experiments, using antisera directed against bovine serum albumin, and α,β and γ-crystallin showed no radioactivity in the 34 000 region. Therefore, formation of fiber junctions seems to be associated with de novo synthesis of the MP34 component. Unfortunately, so far we were unable to demonstrate that isolated fiber polysomes can also direct the synthesis of MP26. It cannot be excluded that this negative result may be due to low antigenicity of MP26. As a consequence newly synthesized MP26 would escape detection in the immunoprecipitation technique. Alternatively, MP26 may interact in the lysate with reticulocyte components and may be found as a band of higher molecular weight upon sodium dodecyl sulphate gel electrophoretic analysis. The observation that a component of mol. wt $> 100\,000$ is precipitated with antisera directed against fiber membranes, whereas sera against epithelial membranes do not precipitate this component (fig.4) favors this assumption, which, however, needs further experimental support.

It has been suggested that α-crystallin is associated with lens fiber plasma membranes [1,16]. Actually even in our highly purified lens fiber membrane preparations this protein is consistently found. It even resists deoxycholate treatment but can almost completely be removed by repeated urea washings (fig.5). Conversely in the isolated epithelial plasma membranes, α-crystallin is virtually absent in spite of the fact that this protein is synthesized in the epithelium even in vitro [17]. It is noteworthy that plasma membranes from epithelium cultured in vitro also lack completely MP26 (in preparation). In conclusion it may be proposed that the association of crystallin polypeptides to the plasma membranes as integral components is dependent upon the presence of a specific protein in the bilayer. It will be a subject of further investigation whether MP26 is responsible for this association.

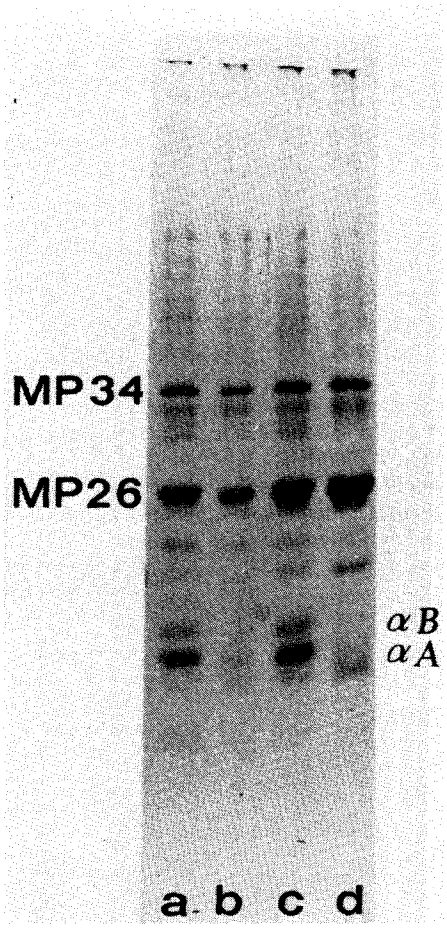

Fig.5. Sodium dodecyl sulphate gel electrophoretic patterns of plasma membrane proteins from lens fibers. (a) Untreated membranes. (b) Membranes, repeatedly washed with 6 M urea. (c) Membranes, washed with deoxycholate. (d) Membranes, washed both with deoxycholate and 6 M urea.

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