ISOLATION AND PROTEIN PATTERN OF EYE LENS FIBER JUNCTIONS

I. DUNIA, C. SEN GHOSH* and E. L. BENEDETTI
Institut de Biologie Moléculaire du CNRS et de l'Université Paris VII, France

and

A. ZWEERS and H. BLOEMENDAL**
Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

Received 4 June 1974

1. Introduction

The membranous constituents in bovine lens fibers are mainly represented by the plasma membrane. In fact during the process of differentiation the other membranous cytoplasmic organella vanish. Previous electron microscopic observations have revealed that the fibers in the lens cortex are interconnected by a special type of intercellular junctions [1]. The latter has only a few morphological features in common with the 'gap' junction or nexus observed in other animal tissues [2]. In contrast to the general plasma membrane, nexuses have been found to be almost insoluble by detergents (3–5). Hence an attempt has been made to isolate fiber junctions from the lens cortex using deoxycholate or Sarcosyl solubilization followed by equilibrium density gradient centrifugation.

In the present paper the method of isolation is described. Furthermore some evidence is provided that the fraction enriched in intercellular junctions is characterized by rather simple protein pattern as compared to the general lens plasma membrane protein profile.

2. Materials and methods

The isolation of lens plasma membranes has been described previously [1]. For the fractionation of junctions isolated plasma membranes (protein content 1 mg per ml) have been treated for 15 min at room temperature with sodium deoxycholate to a final concentration of 1% [3]. The insoluble fraction was collected by velocity centrifugation at 25 000 rpm for 15 min in an SW25 rotor of a Spinco ultracentrifuge. The pellet was resuspended in 0.001 M bicarbonate, layered on top of a linear sucrose gradient (20–60%) and centrifuged for 18 hr in an SW41 rotor at 40 000 rpm. The junctions were collected at a density of about 1.14–1.15 g/ml.

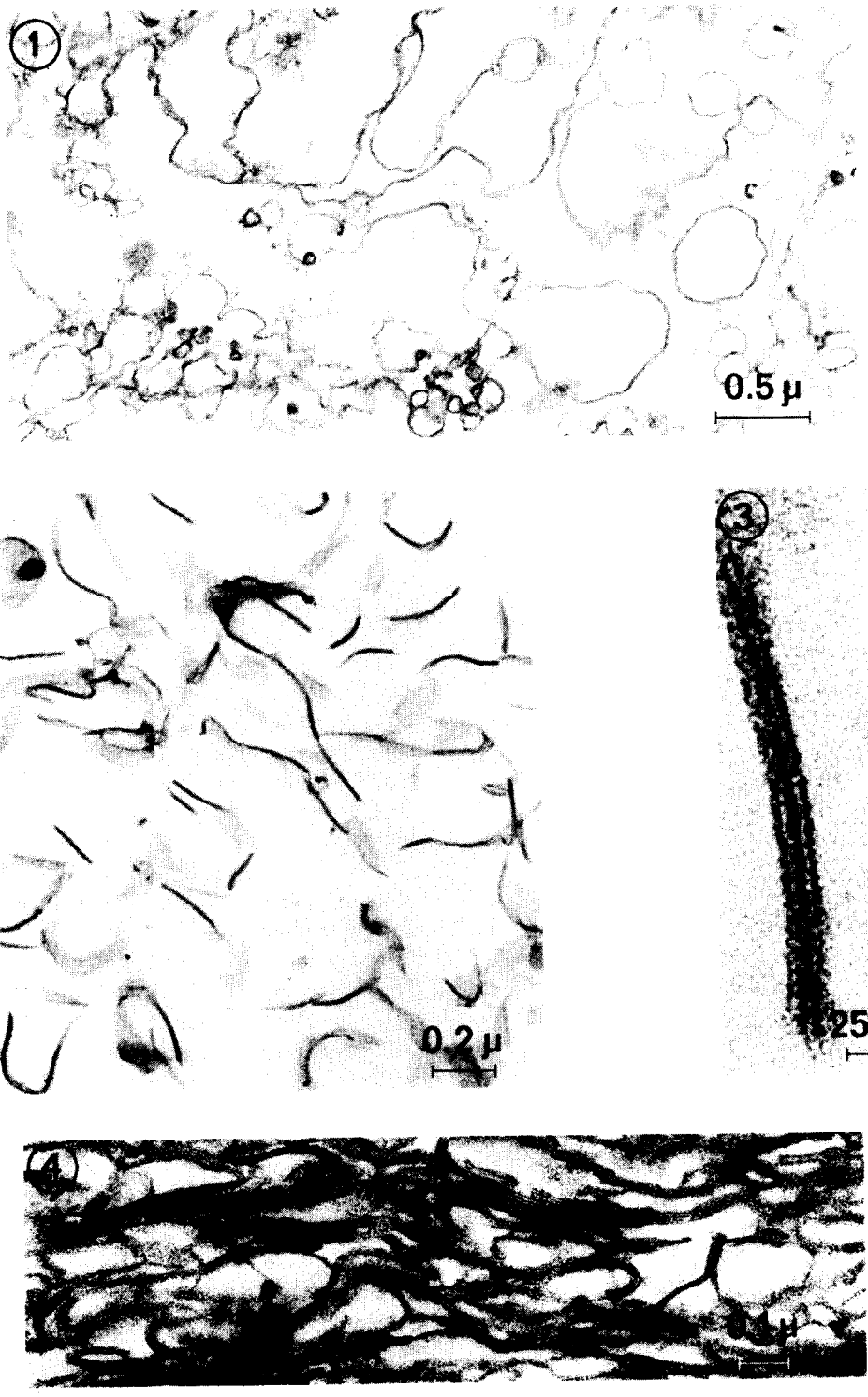
In a number of experiments the isolated membranes were pretreated with trypsin–chymotrypsin [1] or/and hyaluronidase and collagenase [5]. In other experiments the isolation of the junctions was performed according to the method of Goodenough and Stoeckenius [5]. The junctions were gathered at a density of 1.14–1.15 g/ml.

Electron microscopy was done on isolated fractions fixed for 1 hr in 4% gluteraldehyde in 0.2 M cacodylate buffer, pH 7.2 and post-fixed in 1% osmium tetroxyde in the same buffer.

The fixed samples were embedded in Vestopal W. Freeze etching of the isolated fractions was performed as described earlier [1].

Gel electrophoresis was carried out in 13% polyacrylamide containing 0.1% sodium dodecyl sulfate according to Laemmli [6]. The electrophoretic run

* Present address: Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India.
** To whom requests for reprints should be addressed.



Figs. 1–4. Electron micrographs of eye lens plasma membranes and junctions: 1) isolated lens fiber membranes; 2) isolated lens fiber junctions, obtained after solubilization by Sarcosyl; 3) this fraction shows rather short pentalaminar segments; 4) packed pellet of isolated junctions obtained after deoxycholate treatment of lens plasma membranes.

was for 1 hr at 6 V/cm and 2.5 hr at 12 V/cm in 13 cm gel tubes.

Staining was achieved in Coomassie brilliant Blue R for 2 hr at 37°C and destaining in a solution containing 7.5% acetic acid and 5% methanol in water for 24 hr at 37°C.

The samples under investigation were solubilized by boiling in a mixture of 4% sodium dodecyl sulfate, 10% mercaptoethanol and 20% glycerol.

3. Results and discussion

The lens fiber plasma membranes appear in the isolated fraction as extensive membranous profiles interconnected by pentalaminar structures representative for the junctional devices (fig. 1).

In junction rich fractions obtained after Sarcosyl solubilization the short pentalaminar segments are concentrated (figs. 2 and 3). In this fraction residual trilaminar membranes are very scarse. However, some amorphous or vesicular material is occasionally found mixed with the pentalaminar segments.

After deoxycholate treatment the insoluble fraction mainly consists of pentalaminar junctional segments of various length (fig. 4). Occasionally at the ends of the isolated junctions very short trilaminar membranous fragments are visible. In the freeze-etched preparation the main structural feature of the isolated junction appeared to be preserved.

The protein profile of the isolated lens fiber plasma membranes is characterized by a number of components. The approximate molecular weight of the major bands is indicated in fig. 5B. In the region from the top of the gel to 41 000 dalton several bands are detected. The intensity of these bands is reduced when the membranes have been pretreated with hyaluronidase and collagenase. Moreover the main bands in this region are virtually abolished by trypsin treatment (fig. 5C).

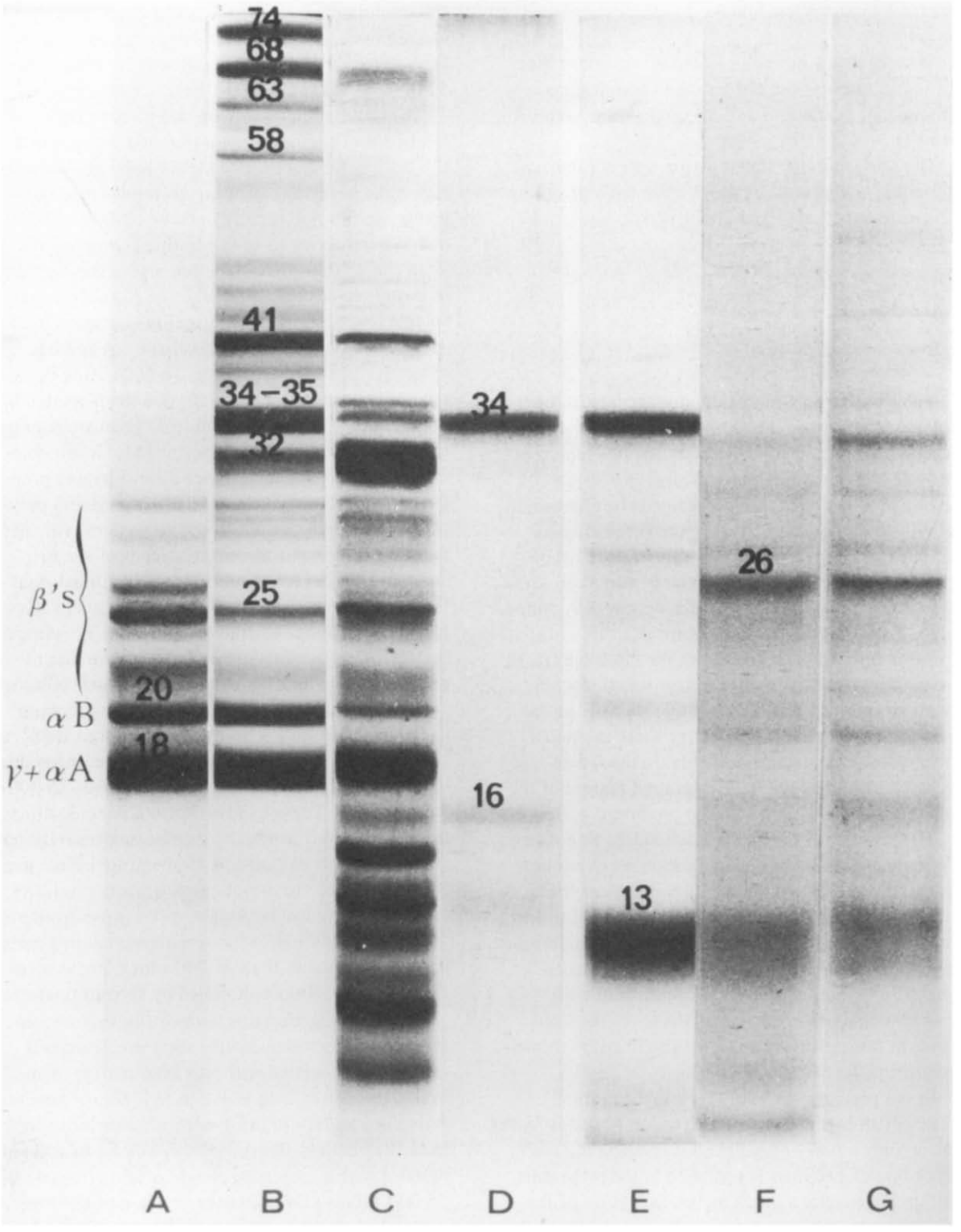
In our previous studies [1] using the method of Weber and Osborn [7] in the region of about 38 000 dalton only a fuzzy broad band was detected. The procedure of Laemmli [6] applied in the present investigation enables a much higher resolution of the membrane protein components. In comparison with the results shown earlier the broad main component located in the 38 000 dalton region is now well resolved into three major components, ranging from 32 000–41 000 dalton (fig. 5B).

The latter components are still present after trypsin treatment of the membranes (fig. 5C). Moreover after this enzymatic digestion in the 34 000–35 000 dalton region the heavy band appears to resolve into two bands and in some experiments the 32 000–33 000 dalton band seems to split into three components.

In the 18 000–30 000 dalton region the majority of protein bands coincides with the so-called water-soluble lens proteins; the crystallins (compare fig. 5A). The two classes of α-crystallin chains αA and αB, respectively, are located in the 18 000–20 000 dalton region. Between 22 000 and 30 000 the β-crystallin polypeptide chains are distributed, while γ-crystallin, in contrast to an earlier statement [1], almost coincides with the αA chains. Since a considerable proportion of crystallin chains, especially α-crystallin polypeptides, are still present in the gel pattern, even after repeated washing or trypsin treatment of the isolated membranes (compare fig. 5C), it is likely that these components are intrinsic constituents of the plasma membrane. Whether or not these 'membrane crystallins' reflect the linkage between the plasma membrane core and the structural lens fiber protein awaits elucidation. The existence of such a linkage has been suggested previously by Bracchi et al [8].

Trypsin treatment also gives rise to the appearance of a number of polypeptides in the 10 000–15 000 dalton region. These bands are also observed, although to a lesser extent, when the membrane preparation is stored for a few days at low temperature before performing the electrophoretic experiment. It is likely that the low molecular weight species arise from proteolytic degradation of the components with a molecular weight higher than 41 000 which are, as mentioned before, almost abolished by trypsin treatment.

In this connection it is noteworthy that trypsin treatment removes microfilaments which are still intimately associated with lens fiber plasma membranes even after their isolation [1]. Microfilaments found in a variety of cell types including lens epithelium [9] and lens fibers probably consist of actin-like proteins with a molecular weight ranging from 45 000–55 000 [10,11]. Whether the deletion of the high molecular weight components after trypsin treatment


74
68
63
58
41
34–35
32
25
20
18
34
16
26
13
β's
αB
γ+αA
A
B
C
D
E
F
G

of the isolated lens plasma membrane also reflects the degradation of microfilament constituents is under investigation now.

One of the major proteins whose molecular weight varies in different experiments from 32 000–34 000 seems to be of particular interest. In fact this component is always found as a main lens fiber plasma membrane constituent. Moreover in a number of experiments this component is enriched upon solubilization of the membranes by detergents (see fig. 5D, E). Since the insoluble membrane fraction mainly consists of lens fiber junctions it can be postulated that this major protein band corresponds to a proper component of this structure.

Goodenough and Stoeckenius [5] and Goodenough [12] claimed that in mouse liver 'the gap junction contains one major protein with a molecular weight between 13 000 and 23 000 and two minor proteins'.

The discrepancy between the finding of these authors and our results may reflect some differences in tissue and type of junctions, or the consequence of a selective degradation by the enzymatic digestion or by the proper action of different detergents. The latter possibility is compatible with the fact that in addition to the 34 000 dalton component found in both deoxycholate and Sarcosyl insoluble fractions (compare fig. 5D and 5E), a diffuse band in the 13 000 dalton region is clearly detected on the gel pattern of the Sarcosyl insoluble fraction, whereas in the junction isolated after deoxycholate solubilization, but submitted to the same enzymatic treatment, at most a very faint band occasionally appears in the same region. In a few experiments the gel pattern of the detergent-insoluble fraction is characterized by the appearance of a major component in the 25 000–26 000 dalton region while the 34 000 band only remains as a rather faint band (see fig. 5F, G). Since the morphological feature of the isolated junctional fraction is virtually identical in all experiments, it is hard to determine whether or not the 26 000 component in fact arises also from degradation. The interpretation is even more complicated by the fact that the 26 000 dalton band is found almost in the region where the main β-crystallin chain βBp [13] is located.

In conclusion, the finding that the lens fiber junctions are characterized by a rather simplified protein patern as compared with the general plasma membrane allows the postulate that the junctional assembly might consist of a convergent accumulation of particulate entities formed by very few equivalently related proteins. However, at present it remains difficult to relate these proteins to the complex structural architecture of the junction.

Addendum. After our manuscript was completed a recent publication of Goodenough [14] provided additional information on the variation of the gel pattern of isolated mouse liver gap junction protein. In contrast to his previous observation [5,12] now the occurrence of a 34 000 band has been reported in striking accordance with our present results.

Furthermore Goodenough claims that in the presence of reducing agents the protein pattern is characterized primarily by a doublet at the 10 000 dalton position. Since in all our experiments either mercaptoethanol or dithiothreitol at appropriate concentration was used and the 34 000 band remained, it is hard to decide whether the presence of low molecular components is due to degradation rather than to a meaningful dissociation.

Fig. 5. SDS gel electrophoresis of lens plasma membrane proteins: A) structural lens proteins (for comparison); B) proteins from purified membranes; C) proteins from membranes purified after trypsin treatment; D) proteins from membranes purified after treatment with trypsin, hyaluronidase and collagenase, followed by deoxycholate (DOC) treatment, E) as D instead of DOC Sarcosyl was used; F) occasionally instead of the result depicted under E the pattern shown here was observed; G) same as F. but trypsin only was used as enzyme. (The numbers on the bands indicate the molecular weights of the corresponding polypeptides in 1000 daltons.)

References

[1] Bloemendal, H., Zweers, A., Vermorken, F., Dunia, I. and Benedetti, E. L. (1972) Cell Diff. 1, 91–106.
[2] Benedetti, E. L., Dunia, I. and Diawara, A. (1973) Europ. J. Cancer 9, 263–272.
[3] Benedetti, E. L. and Emmelot, P. (1968) J. Cell Biol. 38, 15.
[4] Evans, W. H. and Gurd, J. W. (1972) Biochem. J. 128, 691–699.
[5] Goodenough, D. A. and Stoeckenius, W. (1972) J. Cell Biol. 54, 641
[6] Laemmli, U. K. (1970) Nature 227, 680–685.
[7] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4413.
[8] Bracchi, P. G., Carta, F., Fassela, P. and Maraini, G. (1971) Exp. Eye Res. 12, 151–154.
[9] Rafferty, N. S. and Esson, E. A. (1974) J. Ultrastruct. Res. 46, 239–253.
[10] Tilney, L. G. and Mooseker, M. (1971) Proc. Natl. Acad. Sci. U.S. 68, 2611–2615.
[11] Fine, R. and Bray, D. (1971) Nature New Biology 234, 115–118.
[12] Herbrink, P. and Bloemendal, H. (1974) Biochim. Biophys. Acta 336, 370–382.
[13] Goodenough, D. A. (1973) Membrane Research, First ICN-UCLA Symposium on Molecular Biology (C. Fres Fox ed.) 337–346. Acad. Press, New York and London.
[14] Goodenough, D. A. (1974) J. Cell Biol. 557–563.