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INCORPORATION IN VITRO OF LENS MEMBRANE PROTEIN INTO RETICULOCYTE MEMBRANES

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SUMMARY: Upon *de novo* synthesis of lens proteins in a reticulocyte lysate membrane- and cytoskeleton-specific lens polypeptides are detected in membranous structures derived from the heterologous system. The results strongly suggest that an onset of membrane and cytoskeleton assembly takes place *in vitro*.

INTRODUCTION

Lens cells represent not only a convenient tool for investigations on *de novo* synthesis of the structural protein (crystallins)(1), but provide also a suitable system to study the *in vitro* biosynthesis and assembly of plasma membranes. Those studies are facilitated by the fact that lens plasma membranes can be isolated either by density gradient centrifugation (2) or by a method based upon their insolubility in water and urea (3). This allows detection of possible aspecific adsorption inherent in the isolation procedure.

The present paper provides evidence that, upon *de novo* synthesis in a reticulocyte lysate, from the bulk of newly synthesized lens protein predominantly MP34, MP45, and MP55 are found in heterologous membranous structures, as judged both by density and solubility criteria. Furthermore, it appeared that MP34 and MP45 are preferentially detected as urea-insoluble components, whereas MP55 and α-crystallin polypeptides (αA_2) can be extracted by urea treatment.

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MATERIALS AND METHODS

All methods applied have been described previously. The reticulocyte lysates were prepared according to Evans and Lingrel (4) Lens fiber plasma membranes were isolated as reported elsewhere either by flotation in a discontinuous sucrose gradient (2) or by water and urea extraction of a lens homogenate (3). Translation of isolated lens polysomes in the reticulocyte lysate has been published earlier (5). Urea-polyacrylamide gel electrophoresis was done as published elsewhere (6).
Sodium dodecylsulfate polyacrylamide gel electrophoresis was carried out as described by Laemmli (7). 2D-electrophoresis were performed as reported by Kibbelaar and Bloemendal (8). Scintillation autoradiography of the dried gels was done after Bonner and Laskey (9).

RESULTS AND DISCUSSION

Fig. 1 shows that the protein patterns of lens fiber plasma membranes are virtually identical after application of either urea extraction of lens homogenates or isolation of the water-insoluble lens fraction that concentrates at the 1.14-1.16 g/cm^3 interface of a discontinuous sucrose density gradient. Therefore, if newly synthesized proteins can be found in these patterns they represent either internal or external membrane components.

We demonstrated previously that not only crystallin but also lens plasma membrane polypeptides can be synthesized *in vitro* (5), and that specific messengers for these non-crystallin proteins can be isolated (10). One of the characteristic lens plasma membrane constituents is a polypeptide with an apparent molecular weight of 34,000, designated MP34 (11) (compare fig. 1). Its biosynthesis *in vitro* was proven by immunoprecipitation with specific antisera after cell-free incubation (12). However, it remained to be established if this newly synthesized polypeptide would be assembled *in vitro* together with other membrane constituents or whether it required the intact cell for assembly. To answer this question isolated lens polyribosomes were translated in a reticulocyte lysate as described previously (10). A portion of the incubation mixture was analyzed directly by sodium dodecylsulfate polyacrylamide gel electrophoresis followed by scintillation autoradiography. It can be seen that the bulk of newly synthesized protein

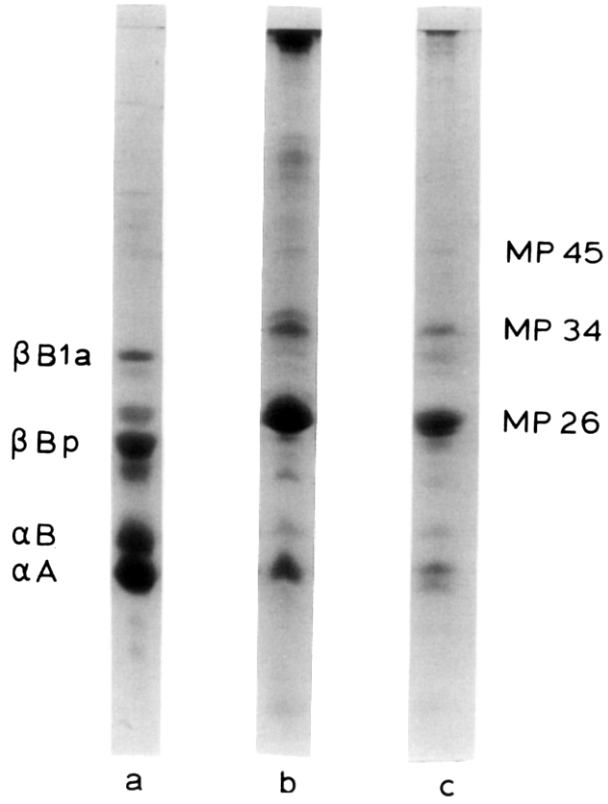

Fig. 1 Sodium dodecylsulfate polyacrylamide gel electrophoresis of proteins from lens plasma membranes. The fractions were isolated as described elsewhere (12,20).
a) For comparison the water-soluble lens proteins (crystallins) are shown.
b) The lens fraction that is insoluble both in water and urea. (membranes prepared by a method based on insolubility criteria).
c) Proteins from lens plasma membranes (isolated by buoyant density gradient centrifugation).

is crystallin, whereas MP34 is present in minute amounts only (fig. 2a). The remainder of the incubation mixture was subjected to the membrane isolation procedure based on bicarbonate washings and density gradient centrifugation (13). Part of the pellet obtained after repeated bicarbonate washing was run on a sodium dodecylsulfate polyacrylamide gel. The pattern is shown in fig. 2b. Obviously, most of the crystallins have been removed. However, the αA chain is still present, while MP34,

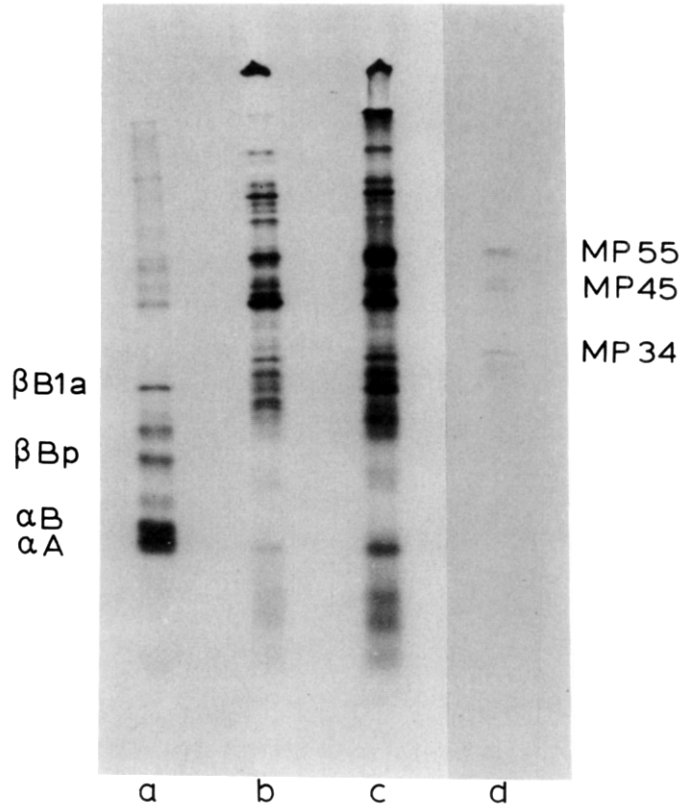

Fig. 2 Autoradiography as described by Bonner and Laskey (9) of the translation products obtained after incubation of calf lens polysomes in a reticulocyte lysate and separated on sodium dodecylsulfate polyacrylamide gels. The methodology of translation has been reported previously (5,10).
a) Newly synthesized polypeptides after addition of lens polysomes.
b) Pattern obtained from the pellet that remains after repeated bicarbonate extraction. Note that most of the soluble crystallins are removed.
c) The pellet obtained in b) was subjected to sucrose density gradient centrifugation. The proteins shown are derived from the sediment.
d) Proteins isolated at a density of 1.14-1.16 g/cm^3 of the gradient mentioned under c).

MP45, and MP55 are enriched. The remaining portion of the pellet was then analyzed by density gradient centrifugation. In fig. 2c the protein pattern of the sediment is depicted, whereas fig. 2d shows that at density 1.14 - 1.16 g/cm^3, (which is specific for purified lens plasma membranes) MP34, MP45, and MP55 are found almost exclusively.

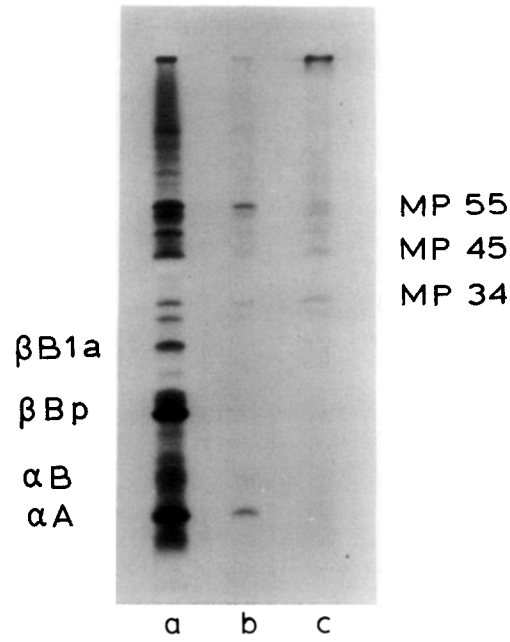

Fig. 3 Autoradiography of the sodium dodecylsulfate polyacrylamide gels of translation products after incubation of calf lens polysomes in a reticulocyte lysate. The methodology of translation has been reported previously (5,10).
a) For comparison the translation products after addition of lens polysomes are shown.
b) Pattern of the polypeptides from the water-insoluble lens fraction.
c) Pattern of the polypeptides from the urea-insoluble lens fraction.

In a control incubation without lens polyribosomes no radioactivity at all could be detected in this region.

Our findings so far do not prove that MP34 and MP45 are actually incorporated into membrane fragments. If the newly synthesized products were hydrophobic, it might be that they are attached by hydrophobic interaction to pre-existing membrane fragments of the reticulocyte lysate. In order to exclude this possibility, we washed the incubation mixture with water and subsequently with 6 M urea solutions. After the water washing all soluble crystallins, with exception of αA, were removed while MP55 appeared to be enriched (fig. 3b). The remaining αA chains and most of the MP55 disappeared after the urea washings, leaving behind

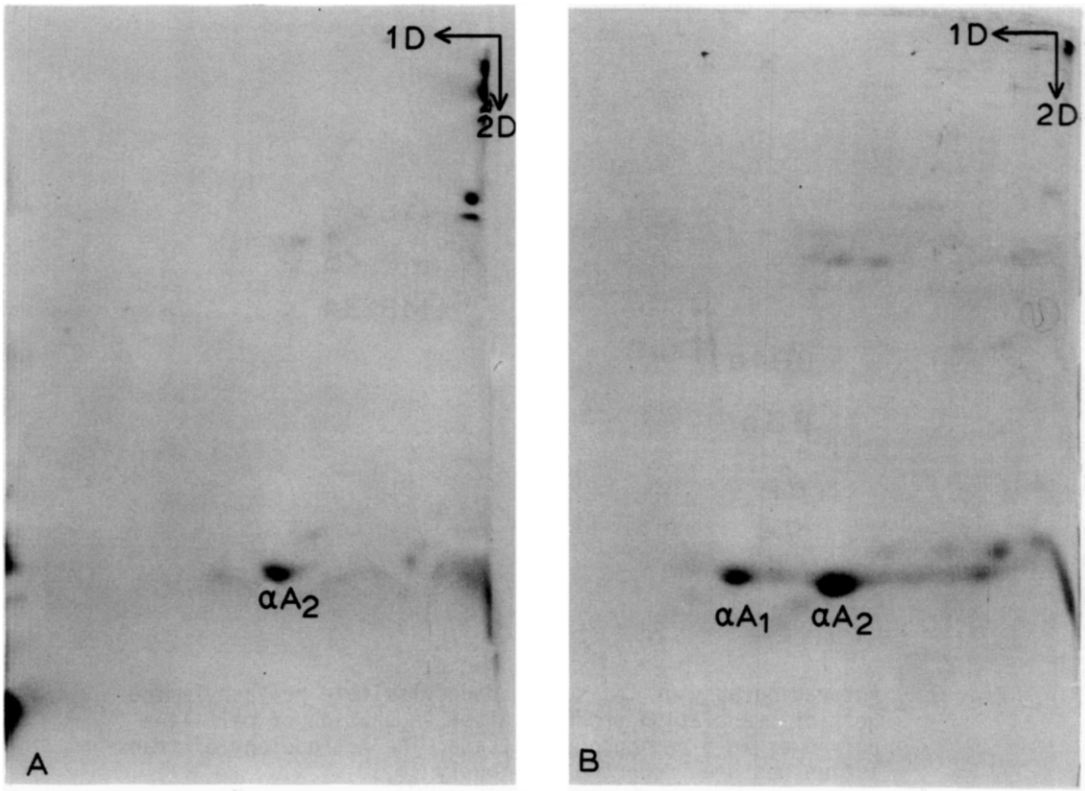

Fig. 4 Two-dimensional electrophoresis of newly synthesized polypeptides in the water-insoluble lens fraction.
A) Autoradiograph of the newly synthesized polypeptides.
B) Stained pattern of the unlabeled water-insoluble fraction to which αA-chains have been added (for comparison). First dimension: urea-polyacrylamide gel electrophoresis, second dimension: dodecylsulfate polyacrylamide gel electrophoresis.

MP34 and MP45 (fig. 3c). This strongly suggests the idea that both polypeptides interact *in vitro* with the core of the lipid bilayer.

The previously described junctional polypeptide MP26 (14) is absent in the autoradiograph of the membrane fraction. This is in accordance with our earlier observations that this polypeptide is not found among the translation products of lens fiber polyribosomes after immunoprecipitation with an antiserum directed against total lens cortex membrane protein (12). Evidence has been provided that this component is synthesized on plasma membrane-bound polyribosomes (14,15).

Some α-crystallin synthesized *de novo* also becomes part of the water-insoluble fraction. This is rather striking, since it was thought hitherto that only aged α-crystallin would be water-insoluble (16). Our experiments demonstrate that exclusively the αA_2 chain is present in the water-insoluble fraction showing that transition into αA_1, the deamidated form of this polypeptide (17), has not occurred yet (see fig. 4). Therefore, aging of α-crystallin *per se* does not seem to be a prerequisite for binding to the membranes.

MP45 and MP55 have been shown to be major constituents of the lens cytoskeleton. MP45 is identical to actin (18) whereas MP55 has common features with a protein from other tissues designated desmin or skeletin.

Moreover, it has been concluded that there is complex formation between lens plasma membranes and the cytoskeleton (19), as MP45 and MP55 are consistently found in membrane structures. Our experiments, therefore, also suggest that an onset of cytoskeleton assembly may take place *in vitro* and that membrane assembly is determined only by the structural features of the constituent polypeptides.

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REFERENCES

1. Bloemendal, H. (1977), Science 197, 127-138.
2. Bloemendal, H., Zweers, A., Vermorken, A.J.M., Dunia, I. and Benedetti, E.L. (1972), Cell Diff. 1, 91-106.
3. Benedetti, E.L., Dunia, I., Bentzel, C.J., Vermorken, A.J.M., Kibbelaar, M.A. and Bloemendal, H. (1976), Biochim. Biophys. Acta 457, 353-384.
4. Evans, M.J. and Lingrel, J.B. (1969), Biochemistry 8, 829-831.
5. Vermorken, A.J.M., Hilderink, J.M.H.C., Ven, W.J.M. van de and Bloemendal, H. (1975), Biochim. Biophys. Acta 414, 167-172.
6. Bloemendal, H. (1967), in Electrophoresis Vol. 2 8 379-423 Ed. Bier Academic Press, New York.
7. Laemmli, U.K. (1970), Nature 227, 680-685.
8. Kibbelaar M.A. and Bloemendal, H. (1976), INSERM, 60, 59-70.
9. Bonner, W.J. and Laskey, R.A. (1974), Eur. J. Bioch. 46, 83-88.

10. Vermorken, A.J.M., Hilderink, J.M.H.C., Ven, W.J.M. van de and Bloemendal, H. (1976), Biochim. Biophys. Acta 454, 447-456.
11. Bloemendal, H., Vermorken, A.J.M., Kibbelaar, M.A., Dunia, I. and Benedetti, E.L. (1977), Exp. Eye Res. 24, 413-415.
12. Vermorken, A.J.M., Hilderink, J.M.H.C., Dunia, I., Benedetti, E.L. and Bloemendal, H. (1977), FEBS Letters 83, 301-306.
13. Vermorken, A.J.M., Waal, R. de, Ven, W.J.M. van de, Bloemendal, H. and Henderson, P.Th. (1977), Biochim. Biophys. Acta 496, 495-506.
14. Bloemendal, H., Benedetti, E.L., Ramaekers, F.C.S., Dunia, I. and Kibbelaar, M.A. Proc. 26th Coll. 1978, Brussels Ed. H. Peeters Oxford Pergamon Press, in the press.
15. Bloemendal, H., Benedetti, E.L., Ramaekers, F.C.S., Dunia, I., Kibbelaar, M.A. and Vermorken, A.J.M. (1979), Mol. Biol. Exp. 5 no. 1 in the press.
16. Bracchi, P.G., Carta, F., Fassela, P. and Maraini, G. (1971), Exp. Eye Res. 12, 151-154.
17. Bloemendal, H., Berns, A.J.M., Ouderaa, F. van der, and Jong, W.W. de (1972), Exp. Eye Res. 14, 80-81.
18. Kibbelaar, M.A., Selten-Versteegen, A.M.E., Dunia, I. and Benedetti, E.L. (1979), Eur. J. Bioch. in the press.
19. Benedetti, E.L., Dunia, I., Cartaud, U., Hatae, T., Farvard - Sereno, C., Bentzel, C., Kibbelaar, M.A. and Bloemendal, H. (1977) in: IId Colloquium: Hormones and cell regulation (Dumont, J.E. & Nunez, J., eds.) Elsevier North Holland, vol. 2., pp. 305-328.
20. Dunia, I., Sen Ghosh, C., Benedetti, E.L., Zweers, A. and Bloemendal, H. (1974), FEBS Letters 45 139-144.