LENS DIFFERENTIATION

Crystallin Synthesis in Isolated Epithelia from Calf Lenses

A. J. M. VERMORKEN, J. M. H. C. HILDERINK, W. J. M. VAN DE VEN, and H. BLOEMENDAL

From the Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

ABSTRACT

In the calf eye lens, four morphologically distinct cell types can be detected: three in the epithelial monolayer and one in the cortical part. During differentiation, there is a quantitative change in the synthesis of crystallin subunits. A marked increase in αA-chains and several β-crystallin polypeptides accompanies the transition from epithelial to fiberlike lens cells while synthesis of the noncrystallin proteins diminishes significantly.

KEY WORDS lens · differentiation · protein biosynthesis · crystallins

The lens of the mammalian eye has been explored for study of the typical structural proteins, the crystallins (15, 17, 21, 16, 12, 1), and recently for the investigation of membrane proteins and their biosynthesis (4, 22, 19, 20). The protein biosynthetic events in the lens cortex which contains the bulk of the terminally differentiated fiber cells has been studied intensively (6). However, the question as to which crystallin polypeptide chains are synthesized in the epithelial monolayer has not been answered unequivocally. It would be extremely important to have the answer to this question in order to fruitfully investigate fundamental processes such as regulatory mechanisms in differentiation. Studies have been undertaken to establish the protein pattern of lens epithelia. Papaconstantinou (14) and Van Venrooij et al. (18) claimed the presence of α- and β-crystallins in the lens epithelium; on the other hand, according to Papaconstantinou, γ-crystallin could only be found in lens fibers. The latter finding should, therefore, reflect an aspect of cellular differentiation in the lens. Biosynthesis of α-crystallin in the epithelium has been reported by Delcour and Papaconstantinou (9). In our opinion, in all investigations undertaken so far, contamination of the epithelial preparations with fibers could not be excluded. This is due to the fact that epithelial cells were defined as those cells that adhered to the lens capsule when it was removed from the organ. However, no evidence was provided that fiber cells were completely removed.

In this paper, we describe a study of the synthesis of crystallins in the eye lens epithelium. Microscope observation revealed the absence of fiber cells in our preparations. Evidence is provided that the B_2 chain of α-crystallin is preferentially synthesized in a defined part of the lens epithelium.

MATERIALS AND METHODS

L-[^{35}S]Methionine (sp act 180 Ci/μmol was purchased from the Radiochemical Centre (Amersham, England).

Tissue Preparation

Calf eyes were obtained on ice from the slaughterhouse. They were washed with distilled water and opened at the lateral side, so that the lenses could be removed without adhering iris material. For microscope examination, the epithelia were spread on a microscope slide, as described in Results. The preparations were then fixed in OsO_4 vapor for 3 min and stained according to Giemsa for 30 min (11).

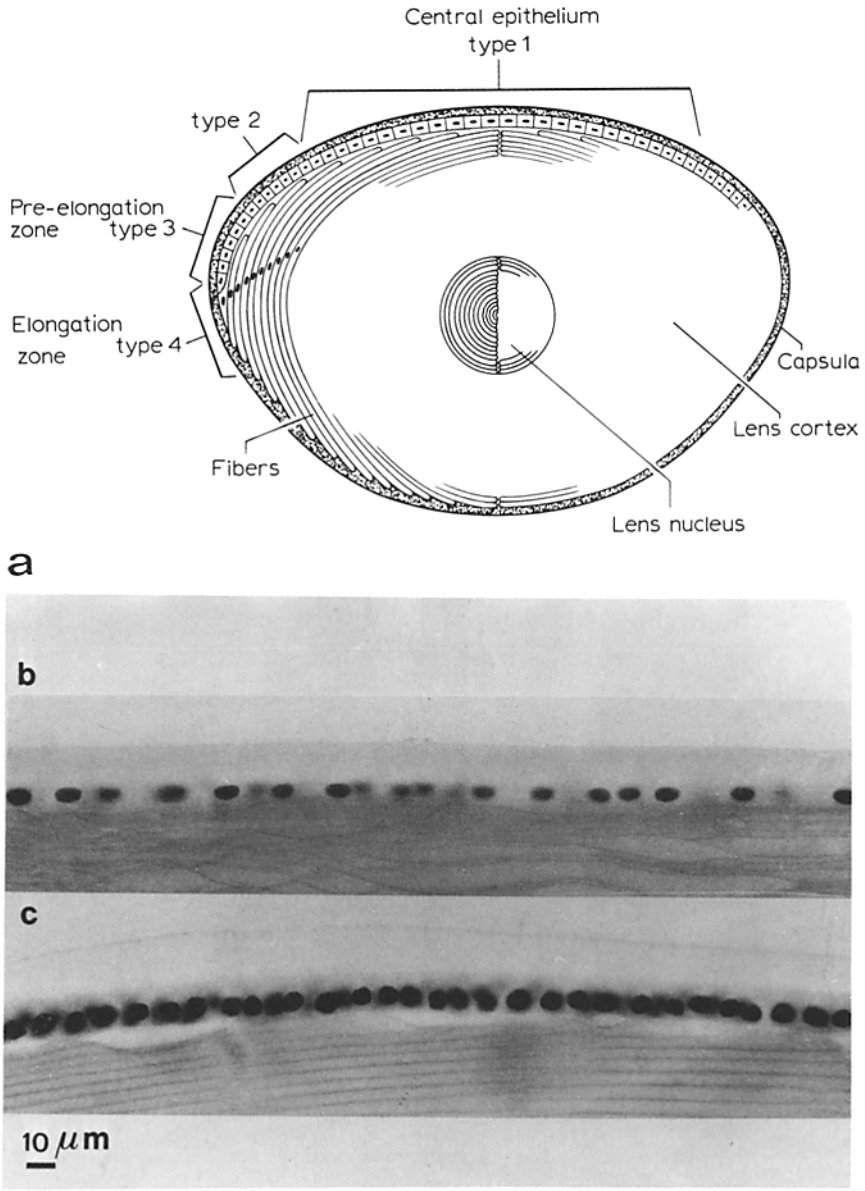


FIGURE 1 (*a*) Schematic drawing of the lens indicating the localization of the four morphologically different cell types. (*b*) Cross section of cells of the central epithelium. Epithelial cells are flat with nuclei spaced unequidistantly (note the few nuclei depicted in the focus of the optical system). Hematoxylin and eosin stains. (*c*) Cross section of cells of the pre-elongation zone. Epithelial cells approach cuboidal forms with almost regular distribution of closely packed nuclei. Hematoxylin and eosin stains.

Labeling of Lens Epithelial Cells

Epithelial cells were labeled for 15 h with L-[^{35}S]methionine in labeling medium (Hanks' basic salt solution supplemented with 10% dialyzed calf serum and amino acids except for methionine). For each incubation, the epithelial cells from ten capsules were used. A maximum of 20% of the added [^{35}S]methionine was incorporated into cellular protein.

Polyacrylamide Gel Electrophoresis

Analyses were performed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis according to

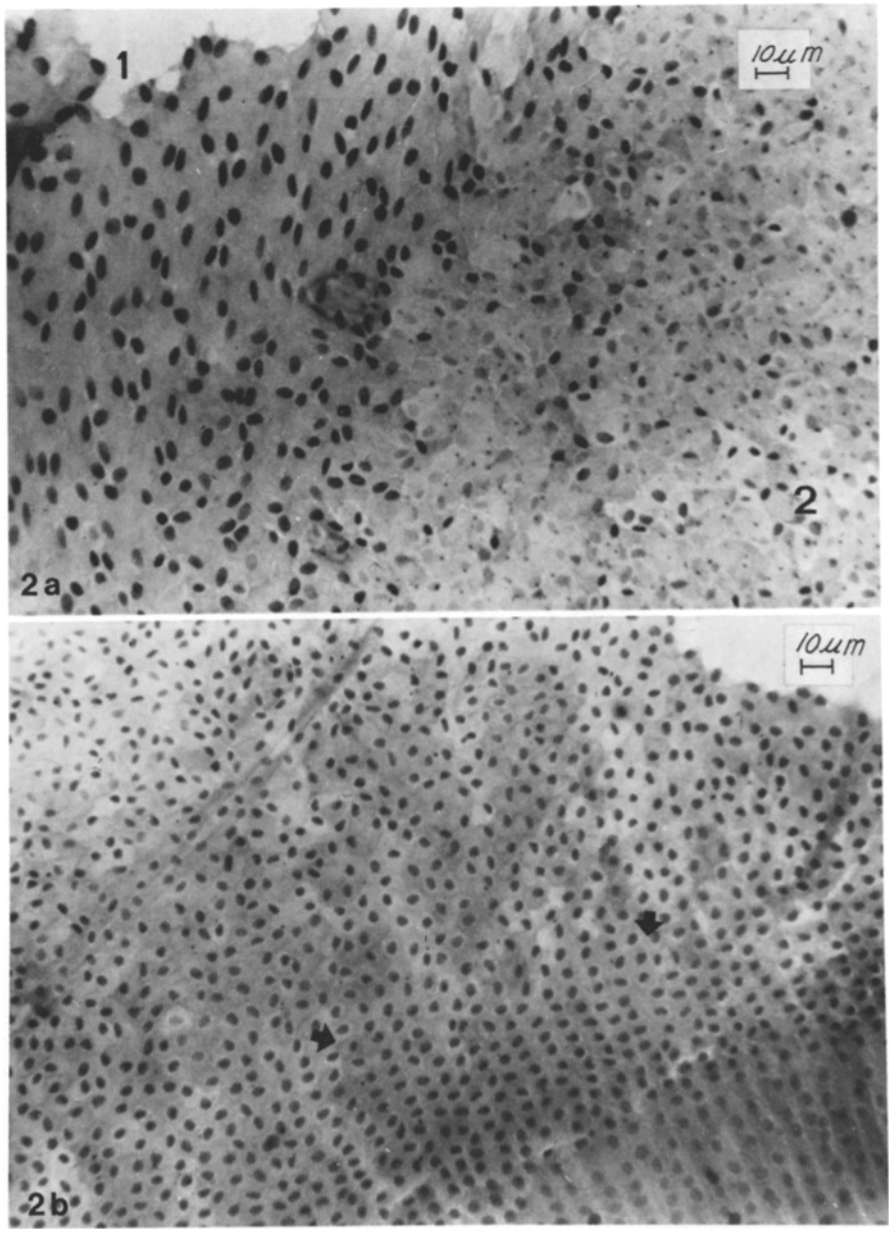


FIGURE 2 Flat mount of calf lens epithelium showing: (*a*) cell types 1 and 2, indicated by black numbers *1* and *2*; and (*b*) the cells of the pre-elongation zone (see arrows).

Laemmli (13), with the modification that a gel slab instead of gel rods was used. The gel was 12 cm long and contained 13% acrylamide, 0.4% methylene-bis-acrylamide, and 0.1% SDS. In this method, a stacking gel was applied. The radioactive samples contained about 10,000 cpm. Staining and destaining were performed as described by Weber and Osborn (23). Gels were processed for autoradiography. For the detection of the labeled proteins the procedure of Bonner and Laskey (7) was used in combination with the drying procedure described by Berns and Bloemendal (2).

Two-Dimensional Electrophoresis

An electrophoretic run was performed in the first direction on polyacrylamide gels containing 6 M urea

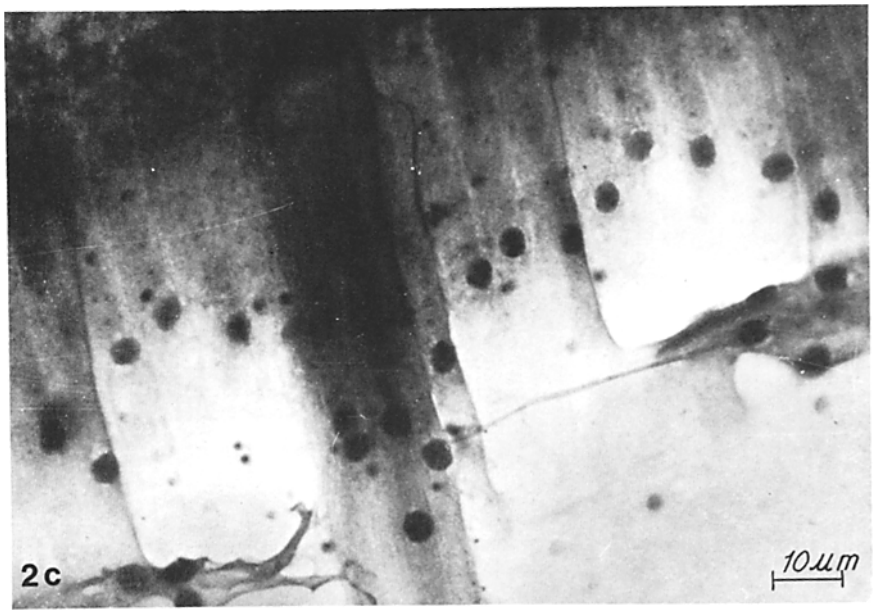
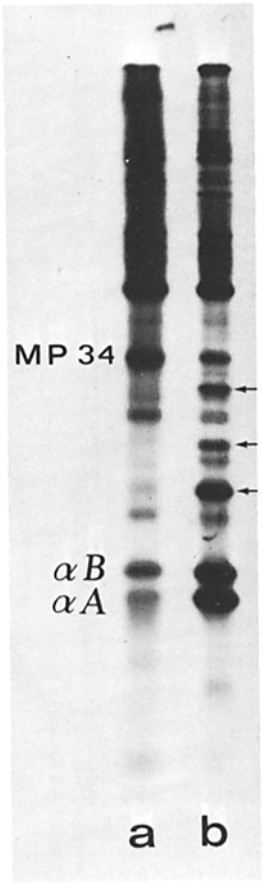


FIGURE 2c Flat mount of calf lens epithelium showing short fiber cells adhering to the lens capsule.



in 13-cm tubes as described by Bloemendal (3). Thereafter, the gel was sliced longitudinally with an apparatus described by Berns and Bloemendal (2). The inner slice was used as sample gel for the run in the second dimension on an SDS polyacrylamide gel. This slice was kept in position by pouring a stacking gel solution (5% acrylamide) on top of the gel slab.

RESULTS

To investigate whether elongating fibers adhere to and contaminate the epithelial preparations, the collagenous capsule of the lens was opened at the postequatorial region by means of a few radial incisions. Then, the anterior part of the capsule was spread on a microscope slide. Microscope examination reveals four morphologically distinct types of cells showing different size and arrangement (Fig. 1*a*). Type 1 cells are located in the center of the lens epithelium and consist of relatively large polygonal cells (Figs. 2*a* and 1*b*). Type 2 cells, consisting of much smaller epithelial cells, are found in a ring around this central part of the epithelium (Fig. 2*a*). Most

FIGURE 3 Autoradiograph of an SDS gel electrophoretic pattern of: (*a*) products synthesized in the central epithelium after incubation with [^{35}S]methionine. (*b*) products synthesized in the combined zones 2, 3, and 4 of the epithelium after incubation with [^{35}S]methionine; the upper arrow indicates βB_{1a}, the middle one βB_5, and the lowest one βB_p.

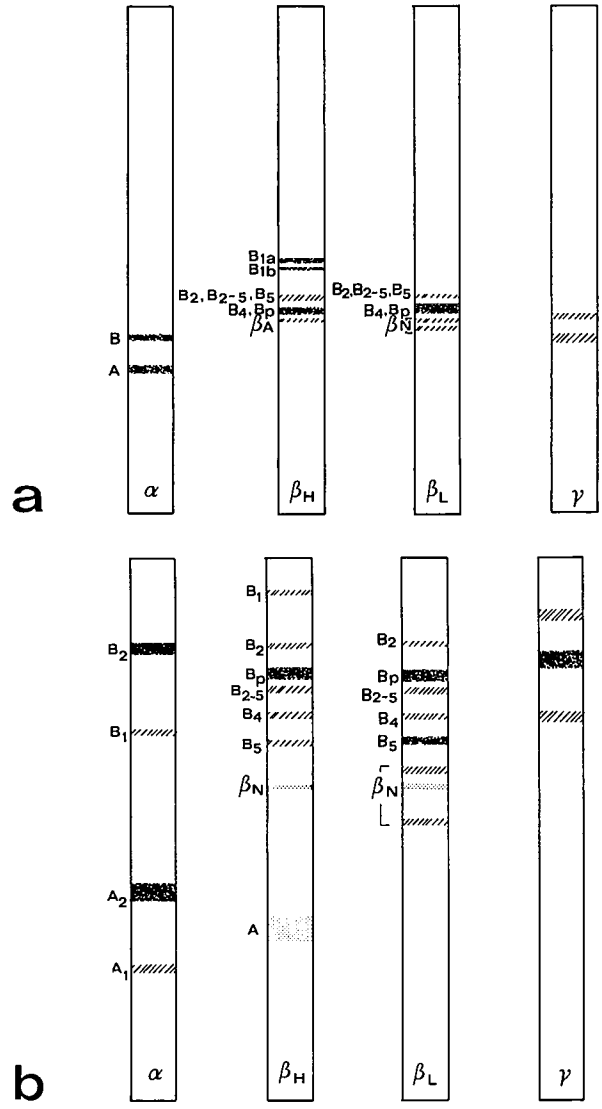


FIGURE 4 (*a*) Schematic drawing indicating the nomenclature of the crystallin chains on a sodium dodecyl sulfate gel. (*b*) Schematic drawing indicating the nomenclature of the crystallin chains on an alkaline urea gel.

striking is the very regular arrangement, and the relatively large size of the cell nuclei in the area close to the elongation zone (type 3, Figs. 2*b* and 1*c*). Fig. 2*c* shows fiberlike cells of the elongation zone (type 4). Although these fiberlike cells are relatively short, they are definitely not epithelial cells. The occurrence of these cells in the preparation demonstrates that the epithelium cannot be removed from the lens free from fibers merely by removing the capsule.

About 9 mm of the inner part of the epithelium was isolated by careful cutting with scissors. These preparations containing cells of type 1 are fiber-free but are occasionally contaminated with cell type 2. Incubation of this part of the epithelium with [^{35}S]methionine followed by electrophoretic analysis of the newly synthesized polypeptides reveals a pattern which is quite different from that obtained after incubation of the combined cell types 2, 3, and 4 (Fig. 3). In the crystallin region of the electrophoretic pattern of the central lens epithelium, label is present where

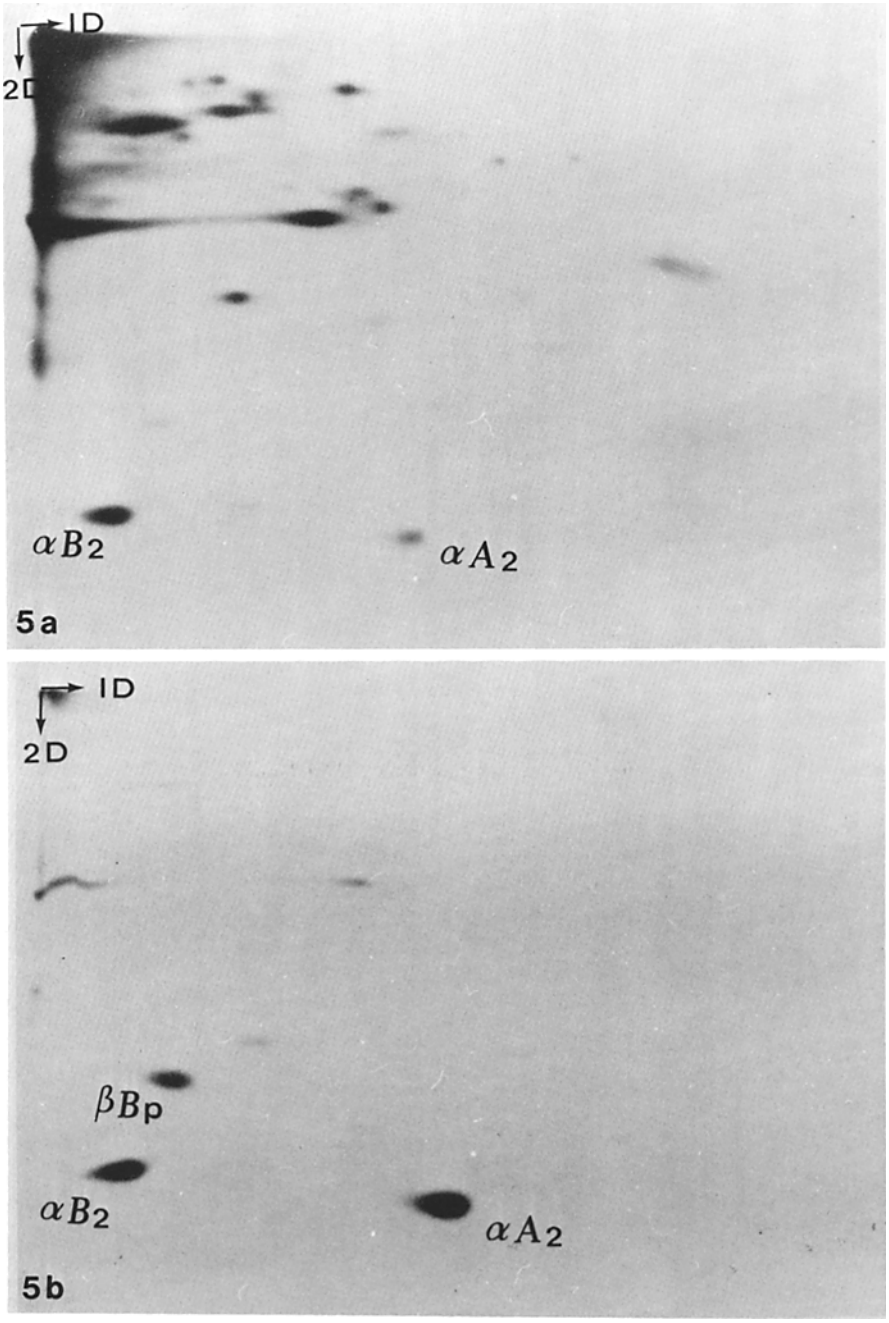


FIGURE 5 Two-dimensional polyacrylamide gel electrophoresis of newly synthesized protein in calf lens epithelial cells. (*a*) Autoradiograph of products synthesized de novo in the central part of the lens epithelium. (*b*) Autoradiograph of the products synthesized de novo in the combined zones 2, 3, and 4 of the lens epithelium.

α-crystallin is located and virtually absent where the β-crystallin chains βB_p, βB_2, βB_{2-5}, βB_5, and βB_{1a} are found (see Fig. 4 for a schematic drawing of the nomenclature of the β-crystallin chains). When the combined zones 2, 3, and 4 are incubated, a polypeptide pattern is obtained,

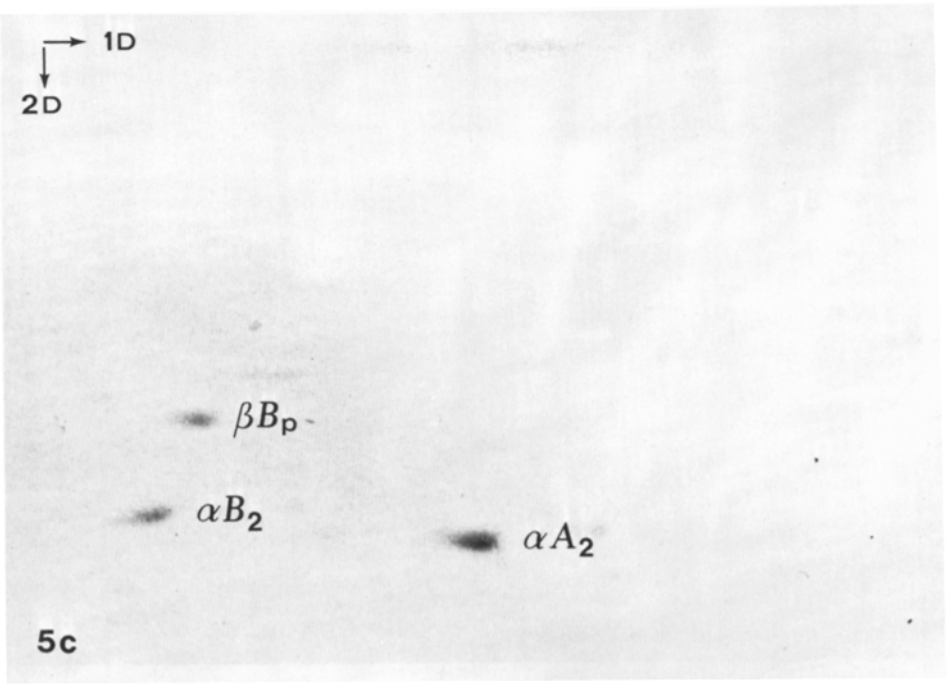


FIGURE 5c Two-dimensional polyacrylamide gel electrophoresis of the major crystallin polypeptides. Stained pattern of isolated native α-crystalline chains and the main polypeptide of β-crystallin βB_p.

characterized mainly by an increase of the A chain of α-crystallin, the major chain B_p of β-crystallin and by the appearance of the B_5 and B_{1a} chains of β-crystallin (compare Fig. 3*a* and *b* with Fig. 5*a* and *b*. For nomenclature, see Fig. 4). On the other hand, the synthesis of the membrane constituent MP 34 (5, 22) and of the noncrystallin polypeptides is diminished. This can be concluded by comparison of Figs. 5*a* and *b*. The two electropherograms have been "normalized" by application of an amount of radioactivity so that the number of counts in the αB_2-spots are virtually equal.

Cutting out of the radioactive αA_2 and αB_2 spots from the electropherogram followed by liquid scintillation counting reveals a ratio of 1:3 for the central lens epithelium and 3:2 for the combined zones 2, 3, and 4, respectively.

That the radioactivity in the low molecular weight region is indeed due to α-crystallin chains is demonstrated by two-dimensional coelectrophoresis of the newly synthesized polypeptides and a mixture of isolated unlabeled α-crystallin polypeptides and βB_p (compare Fig. 5*a* and *b* with Fig. 5*c*). It can be seen that radioactivity and Coomassie Blue staining coincide. This means that the polypeptides synthesized *de novo* and the native chains have both identical net charges and identical molecular weights.

DISCUSSION

The problem concerning the synthesis of crystallins in lens epithelium is interesting since, during the processes of differentiation and aging, changes in their subunit composition and physical properties occur (6). Moreover, it has been shown that α-crystallin is intimately bound to the lens fiber membranes (4, 8), whereas epithelial membranes seem to contain much less α-crystallin (22). Our present results show that the B_2 chain of α-crystallin is preferentially synthesized in a defined region of the lens epithelium.

Delcour and Papaconstantinou (10) reported a change in stoichiometry of the A_2 and B_2 chains of α-crystallin during differentiation from 2:1 (in epithelium) to 3:1 (in fibers). Our results with "fractionated" epithelia demonstrate that in the earliest stage of α-crystallin synthesis the ratio of A_2 to B_2 is 1:3. Increased synthesis of A_2 chains occurs in the region close to or in the elongation zone. Futhermore, our findings demonstrate that the onset of fiber differentiation is also accompanied by the formation of the β-crystallin chains B_{1a}, B_5, and B_p.

In conclusion, it can be stated that lenticular cell differentiation is paralleled by sequential changes in polypeptide synthesis. Since we were able to "separate" different areas of the calf lens epithelium and have observed different activities in these areas with respect to the synthesis of α- and β-crystallin subunits, promising avenues for further research have been opened. In view of our observations, the previously reported absence of α-crystallin in epithelial plasma membranes cannot be explained by the absence of α-crystallin synthesis, since we clearly demonstrated that αA and αB chain formation does take place in this part of the lens. Therefore, the absence of α-crystallin has either to be ascribed to intrinsic properties of the epithelial membranes resulting in a different binding capacity or, alternatively, to a difference between the "older" α-crystallin in the fibers and the "younger" α-crystallin in the epithelium. The latter interpretation would be in agreement with experiments of Bracchi et al. (8).

The authors are grateful to Prof. C. Jerusalem for his help with the histological work, and to Mr. A. A. Groeneveld and Mr. J. van der Heyden for skillful technical assistance.

The present investigations were carried out partly under the auspices of the Netherlands Foundation for Chemical Research (S. O. N.) and with financial aid from The Netherlands Organization for the Advancement of Pure Research (Z. W. O.).

Received for publication 7 March 1977, and in revised form 5 July 1977.

REFERENCES

1. BERNS, A. J. M., V. V. A. M. SCHREURS, M. W. G. VAN KRAAIKAMP, and H. BLOEMENDAL. 1973. Synthesis of lens protein *in vitro*. Translation of calf-lens messenger in heterologous systems. *Eur. J. Biochem.* **33**:551–557.
2. BERNS, A. J. M., and H. BLOEMENDAL. 1974. Translation of mRNA from vertebrate eye lenses. *Methods Enzymol.* **30**:675–694.
3. BLOEMENDAL, H. 1963. Zone Electrophoresis in Blocks and Columns. Elsevier Publishing Co., Amsterdam. 55–101.
4. BLOEMENDAL, H., A. ZWEERS, F. VERMORKEN, I. DUNIA, and E. L. BENEDETTI. 1972. The plasma membranes of eye lens fibers. Biochemical and structural characterization. *Cell Differ.* **1**:91–106.
5. BLOEMENDAL, H., A. J. M. VERMORKEN, M. KIBBELAAR, I. DUNIA, and E. L. BENEDETTI. 1977. Nomenclature for the polypeptide chains of lens plasma membranes. *Exp. Eye Res.* **24**:413–415.
6. BLOEMENDAL, H. 1977. Biosynthesis of eye lens protein. Biochemistry of Cell Differentiation, MTP International Review of Science. Butterworth, University Park Press. **15**:195–225.
7. BONNER, W. M., and R. A. LASKEY. 1974. A film detection method for tritium-labeled protein and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**:83–88.
8. BRACCHI, P. G., F. CARTA, P. FASELLA, and G. MARAINI. 1971. Selective binding of aged α-crystallin to lens fiber ghosts. *Exp. Eye Res.* **12**:151–154.
9. DELCOUR, J., and J. PAPACONSTANTINOU. 1972. Biochemistry of bovine lens protein. IV. Synthesis and aggregation of α-crystallin subunits in differentiating lens cells. *J. Biol. Chem.* **247**:3289–3295.
10. DELCOUR, J., and J. PAPACONSTANTINOU. 1974. A change in the stoichiometry of assembly of bovine lens α-crystallin subunits in relation to cellular differentiaton. *Biochem. Biophys Res. Commun.* **31**:257–261.
11. GIEMSA, G. 1905. Bemerkungen zur Färbung der Spirochaeta pallida. *Dtsch. Med. Wochenschr.* **31**:1026–1027.
12. HERBRINK, P. 1976. The polypeptide composition of β-crystallin. Ph.D. thesis, University of Nijmegen, Nijmegen, The Netherlands. Chap. 3.
13. LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. **227**:680–685.
14. PAPACONSTANTINOU, J. 1967. Molecular aspects of lens cell differentiation. *Science (Wash. D. C.)* **156**:338–346.
15. SPECTOR, A., and J. H. KINOSHITA. 1964. The incorporation of labelled amino acids into lens protein. *Invest. Ophthalmol.* **3**:517–522.
16. STROUS, G. J. A. M., H. VAN WESTREENEN, and H. BLOEMENDAL. 1973. Synthesis of lens protein *in vitro*. N-terminal acetylation of α-crystallin. *Eur. J. Biochem.* **38**:79–85.
17. STROUS, G. J. A. M., H. VAN WESTREENEN, J. VAN DER LOGT, and H. BLOEMENDAL. 1974. Synthesis of lens protein *in vitro*. The lens cell-free system. *Biochim. Biophys. Acta.* **353**:89–98.
18. VAN VENROOIJ, W. J., A. A. GROENEVELD, H. BLOEMENDAL, and E. L. BENEDETTI. 1974. Cultured calf lens epithelium. I. Methods of cultivation and characteristics of the cultures. *Exp. Eye Res.* **18**:517–526.
19. VERMORKEN, A. J. M., J. M. H. C. HILDERINK, W. J. M. VAN DE VEN, and H. BLOEMENDAL. 1975. Further studies on the lens cell-free system. *In vitro* synthesis of the non-crystallin lens proteins. *Biochim. Biophys. Acta.* **414**:167–172.

20. VERMORKEN, A. J. M., J. M. H. C. HILDERINK, W. J. M. VAN DE VEN, and H. BLOEMENDAL. 1976. Isolation and translation of non-crystallin messenger RNA from calf lens. *Biochim. Biophys. Acta.* **454**:447–456.
21. VERMORKEN, A. J. M., P. HERBRINK, and H. BLOEMENDAL. 1977. Synthesis of lens protein *in vitro*. Formation of β-crystallin. *Eur. J. Biochem.* **78**:617–622.
22. VERMORKEN, A. J. M., J. M. H. C. HILDERINK, I. DUNIA, E. L. BENEDETTI, and H. BLOEMENDAL. 1977. Change in membrane protein pattern in relation to lens cell differentiation. *FEBS Lett.* **83**:301–306.
23. WEBER, K., and M. OSBORN. 1969. The reliability of molecular weight determination by dodecyl sulphate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**:4406–4412.