A CHANGE IN α-CRYSTALLIN SUBUNIT COMPOSITION IN RELATION TO CELLULAR DIFFERENTIATION IN ADULT BOVINE LENS[1]

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SUMMARY

The vertebrate lens is composed of two distinct cell types, the epithelial cell and the fiber cell. α-Crystallin, a structural protein found in both cell types, is composed of four subunits (αA_1, αA_2, αB_1, and αB_2). In the epithelial cells the α-crystallin consists mainly of αA_2 and αB_2 with trace amounts of αA_1 and αB_1. In the fiber cell there is a large increase in the amount of αA_1 and αB_1 subunits. This quantitative increase in two specific subunits is related to the process of cellular growth and differentiation in the vertebrate lens.

INTRODUCTION

α-Crystallin, a tissue-specific structural protein of the vertebrate lens, has a molecular weight of approximately 1×10^6 (1, 2) and is an aggregation of polypeptide subunits whose molecular weights are approximately 25,000 (3). These subunits are not covalently linked and may be easily disaggregated by several denaturing agents (4-6). By column chromatography (4), isoelectric focusing (7), and acrylamide gel electrophoresis (5), the mixture of polypeptide subunits can be resolved into two acidic species having isoelectric points of 5.6 (αA_1) and 5.9 (αA_2) and two basic species having isoelectric points of 7.07 (αB_1) and 7.42 (αB_2). (The nomenclature used for α-crystallin subunits is that suggested by S. G. Waley (8).)

Recent studies have shown that the subunit composition of fiber cell α-crystallin changes during embryonic growth and development (4, 5, 9, 10). More precisely, it was

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shown that fiber cell α-crystallin of embryonic lenses lacks one of the acidic subunit species (αA_1), and that this subunit accumulates very slowly during postembryonic life. Furthermore, the appearance of this subunit is a result not of gene activation but of the chemical conversion of acidic subunit αA_2 to acidic subunit αA_1 (10).

In this paper we present evidence which shows that the changes in subunit composition of α-crystallin may be related specifically to cellular differentiation, i.e. to fibrogenesis, in adult bovine lenses. It will be shown that α-crystallin isolated from adult lens epithelial cells consists mainly of one basic subunit (αB_2) and one acidic subunit (αA_2), whereas α-crystallin of the more highly differentiated adult lens fiber cell is composed of two basic subunits (αB_2 and αB_1) and two acidic subunits (αA_2 and αA_1).

MATERIALS AND METHODS

Preparation of α-crystallin. — Adult bovine lenses obtained from freshly slaughtered cows were immediately placed on ice. The epithelial cells, which adhere tightly to the external collagenous capsule, were separated manually from the cortex fiber cells by careful removal of the capsule. The two cell types were separately homogenized in 0.005 M phosphate buffer (pH 7.0), and the homogenate was cleared by centrifugation at 10,000 g for 10 min. The supernatant was centrifuged at 105,000 g for 90 min to eliminate ribosomes. An aliquot of this supernatant (2–20 mg protein in a volume no greater than 3 ml) was layered over 30 ml of a 5–20% sucrose gradient and centrifuged for 18 hr at 24,000 rpm (SW25.1 rotor). A highly purified α-crystallin fraction may be separated from the rest of the lens protein by this procedure (11). One-ml fractions were collected. The α-crystallin fractions were pooled, exhaustively dialyzed against 0.005 M phosphate buffer (pH 7.0), and lyophilized.

Analysis of α-crystallin subunits. — Lyophilized α-crystallin was dissolved in 0.005 M phosphate buffer (pH 7.0) containing 40% sucrose, 7 M urea, and 0.003 M mercaptoethanol. Aliquots containing approximately 50 μg of α-crystallin were analyzed by acrylamide gel electrophoresis (12). A 7.5% separating gel with 7 M urea was used. Electrophoresis was carried out for 1 hr at 4 mA/tube with Tris-glycine buffer (pH 8.5). After the run the gels were stained for 2 hr with Amido-Schwartz in 7.5% acetic acid and destained electrophoretically. The gels were then scanned in a Gilford Recording Spectrophotometer Model 240 at 500 nm. The area under each protein peak was determined with a planimeter.

RESULTS

The differences between the subunit compositions of α-crystallins from epithelial

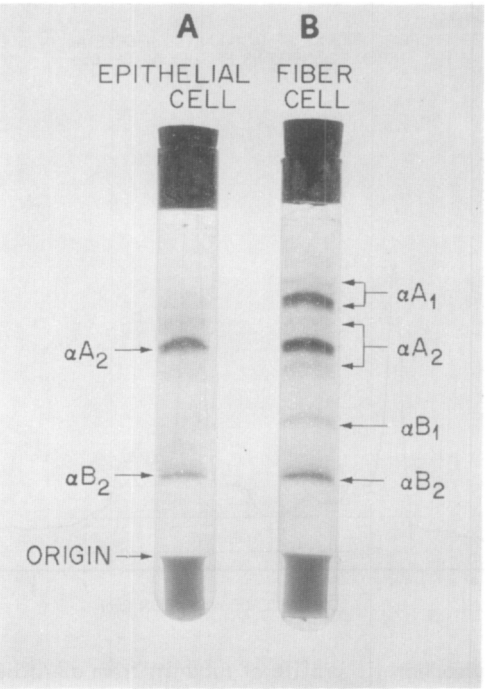

Fig. 1. Acrylamide gel electrophoresis of α-crystallin subunits from (A) epithelial cells and (B) fiber cells.

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cells and from cortex fiber cells are shown by the electrophoretic patterns in Figure 1. Treatment of α-crystallins from the fiber cells with 7 M urea–0.003 M mercaptoethanol resulted in the dissociation of the molecule into four main subunits: two basic subunits (αB_2 and αB_1) and two acidic subunits (αA_2 and αA_1). Similar electrophoretic analysis of urea–mercaptoethanol-treated α-crystallin from the epithelial cells shows that this α-crystallin is composed mainly of two of the four subunits found in the fiber cell, namely, αB_2 and αA_2 with trace amounts of αB_1 and αA_1.

Scanning profiles of these acrylamide gels are shown in Figures 2 and 3. It can be seen that the electrophoretic patterns of the dissociated α-crystallin from epithelial cells and fiber cells are qualitatively similar in that very small amounts of subunits αB_1 and αA_1 are detectable. The differences, therefore, between epithelial cell and fiber cell α-crystallin are quantitative, and minor subunit constituents (αB_1 and αA_1) of the former cell type become major constituents of α-crystallins in the fiber cell. A comparison of the amount of subunits αB_1 and αA_1 in epithelial cell and fiber cell α-crystallin is shown in Table 1. These data show a highly significant difference in α-crystallin

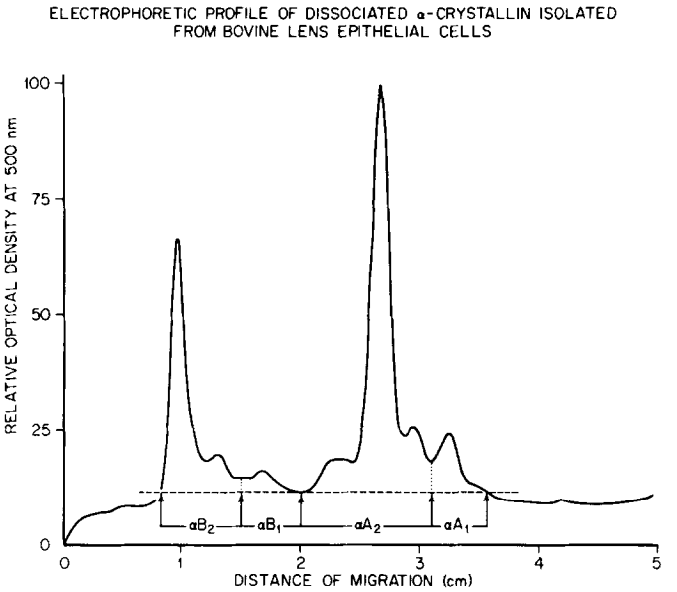

Fig. 2. An electrophoretic profile of subunits from adult bovine lens epithelial cell α-crystallins.

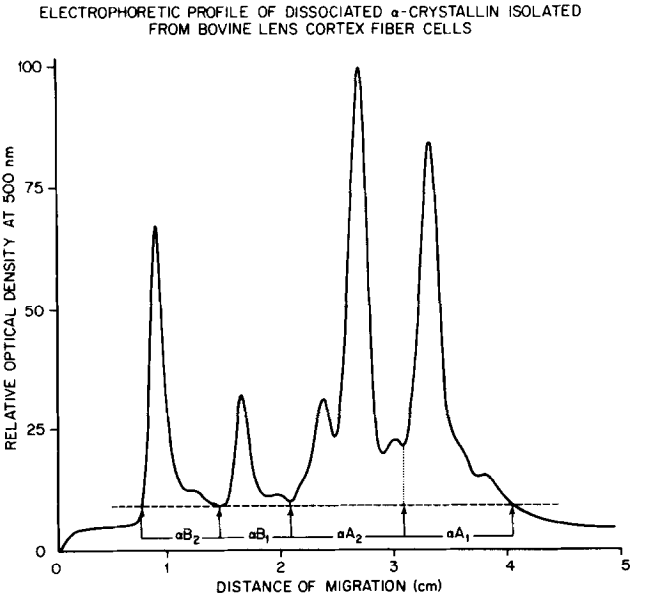

Fig. 3. An electrophoretic profile of subunits from adult bovine lens fiber cell α-crystallins.

TABLE 1

A Comparison of the Amount of Subunits αB_1 and αA_1 in Adult Lens Epithelial Cell and Fiber Cell α-crystallin

	$\frac{\alpha B_1 (100)}{(\alpha B_2 + \alpha B_1)}$		$\frac{\alpha A_1 (100)}{(\alpha A_2 + \alpha A_1)}$	
Epithelial cells	10.8		6.8	
	5.4		7.7	
	9.4	8.5 ± 2.1	16.6	10.4 ± 4.2
Fiber cells	26.4		46.1	
	31.6		43.6	
	30.6	29.5 ± 2.1	39.0	42.9 ± 2.6

These data are the results of three separate experiments.

subunit composition between epithelial and cortex fiber cells in adult bovine lenses. The obvious differences in structure of the two cell types may be related to these observed differences in α-crystallin.

DISCUSSION

In the present studies we have shown that α-crystallin from lens epithelial cells consists mainly of an aggregation of subunits αA_2 and αB_2. Small but significant amounts of subunits αA_1 and αB_1 are also found in epithelial cell α-crystallin. In the fiber cell, however, subunits αA_1 and αB_1 become major constituents of the α-crystallin molecule. These major changes in subunit composition are detected in postembryonic lens cortex fiber cells.

In a previous study we showed that subunit αA_1 is not detectable in α-crystallin extracted from fetal lenses (10). In addition, it was demonstrated that subunit αA_1 gradually appears as a constituent of α-crystallin during postembryonic development. This polypeptide chain is not a direct product of genetic translation, and it was concluded from these studies that a posttranslation conversion of $\alpha A_2 \rightarrow \alpha A_1$ is responsible for the enrichment of subunit αA_1 in the cortex fiber cells.

With respect to the accumulation of subunit αB_1 in cortex fiber cell α-crystallin, our preliminary data indicate that this subunit is a direct product of genetic translation.

This is indicated by the high specific activity of the isolated subunit, as determined by [^{3}H]leucine incorporation studies (13).

We conclude from these studies that the α-crystallin molecule of adult lens epithelial cells consists mainly of subunits αA_2 and αB_2 with trace amounts of αA_1 and αB_1. The composition of the fiber cell α-crystallin also consists of subunits αA_2 and αB_2, but the amount of αA_1 and αB_1 increases sharply in these cells.

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