A CHANGE IN THE STOICHIOMETRY OF ASSEMBLY OF BOVINE LENS α-CRYSTALLIN SUBUNITS IN RELATION TO CELLULAR DIFFERENTIATION*

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SUMMARY: Incorporation of [^{3}H]leucine into oligomeric α-crystallin via individual subunits has been measured in epithelial cells and cortex fiber cells from adult bovine lenses in vitro. Our data show that the ratio of [^{3}H]leucine incorporation via subunits αB_2 and αA_2 is shifted from a value of about 1:2 in epithelial cells to a value of about 1:3 in fiber cells. Thus, in this system, cellular differentiation is accompanied by a change in the stoichiometry of assembly of individual subunits to form the oligomeric α-crystallin molecule. These results indicate possible changes in the rates of synthesis of individual α-crystallin subunits.

α-Crystallin is an oligomeric structural protein that is found in both epithelial cells and fiber cells of the bovine lens (1) and has a molecular weight of approximately 1×10^6 (2, 3). Treatment with urea and mercaptoethanol dissociates the oligomer into its polypeptide subunits, all of which have single molecular weights of about 25,000 (4). These subunits can be resolved into two acidic proteins (αA_1 and αA_2), which have isoelectric points of 5.6 and 5.9, respectively, and two basic proteins (αB_1 and αB_2), which have isoelectric points

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of 7.07 and 7.42, respectively (5–8). Thus, α-crystallin is composed of four qualitatively different subunits, each molecule containing a total of 40 subunits.

In recent reports from this laboratory we have shown that the relative proportions of the four subunits within the intact oligomer changes significantly in both epithelial cells and fiber cells. It was shown that the acidic subunit αA_1 accumulates in both cell types during aging, and it was suggested that this subunit is formed by posttranslational modification of the primary structure of subunit αA_2 (5, 9, 10). In addition, the ratio of the αA_2 and αB_2 subunits, which are direct products of the translation of specific mRNAs, increases significantly in the highly differentiated fiber cell, as compared to the epithelial cell (9–11).

It appears therefore that several changes in the macromolecular architecture of α-crystallin occur in both cell types. Such changes can be mediated by alteration in the rates of synthesis or assembly of individual subunits. To determine whether this is actually the case, we studied the rate of incorporation of [^{3}H]leucine into α-crystallin via the individual subunits in lens epithelial cells and fiber cells. These cell types represent two specific stages of cellular differentiation, and the data presented in this communication indicate that there is a significant shift in the stoichiometry of assembly of αA_2 and αB_2 subunits, from a ratio of 2:1 in the epithelial cells to a ratio of 3:1 in the fiber cells.

MATERIALS AND METHODS

Preparation of Radioactive α-Crystallin

Adult bovine lenses were carefully removed from the eyes of freshly slaughtered animals and placed in cold Hanks' salts solution. Approximately 80 lenses were used in each experiment. Incubation was performed during 4 hours under continuous shaking at 37° C in a modified Eagle's minimal essential

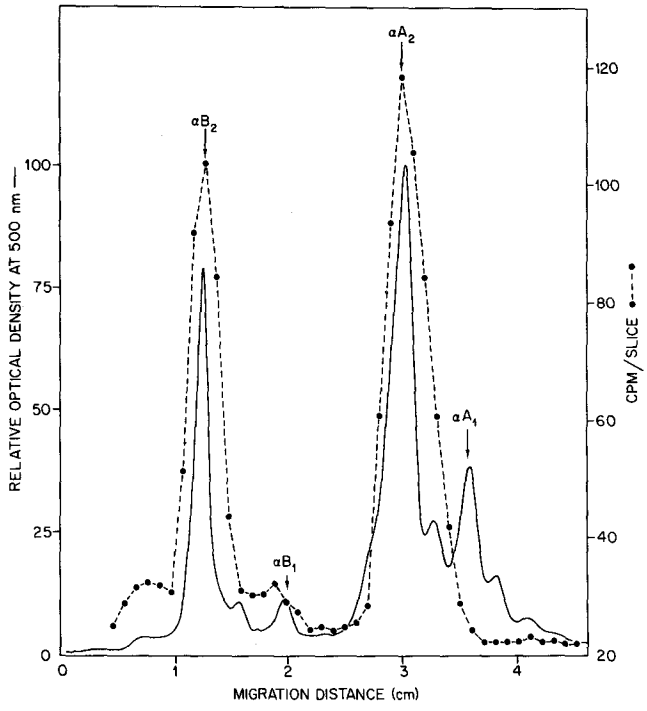

Figure 1. Resolution of the α-crystallin subunits by acrylamide gel electrophoresis. The α-crystallin was prepared from adult epithelial cells. Optical density (——) and radioactivity (●--●) are shown.

medium supplemented with 15% dialyzed calf serum (Microbiological Associates). L-(4, 5-^{3}H) leucine (58 Ci per mmole; Schwarz BioResearch) was added to the medium. A final specific activity of 50 μCi per ml of culture medium was used. At successive incubation time intervals, samples (8 to 10 lenses) were removed from the medium and immediately immersed in cold Hanks' solution. The collagenous capsule to which epithelial cells tightly adhere was then carefully removed, and capsules (epithelial cells) and fiber cells were frozen separately at -20° C.

Homogenization

Epithelial cells and cortex fiber cells were homogenized in 0.005 M sodium phosphate buffer (pH 7.0), using 4 ml buffer per lens cortex and 0.5 ml buffer

per lens capsule. The homogenate was cleared by centrifugation at 10,000 X g for 10 min, then ribosomes were removed by centrifugation at 105,000 X g for 90 min. The ribosome-free supernatant contained all the buffer-soluble material, the major part of it being the crystallins.

Isolation of Oligomeric α-Crystallin

Homogenates were exhaustively dialyzed against 0.005 M sodium phosphate buffer (pH 7.0) to remove the free amino acids. Protein concentration was measured spectrophotometrically by the method of Warburg and Christian (12), and an aliquot was removed for each sample for total protein radioactivity counting. A sample containing 5 to 20 mg of protein was then layered over a 5 to 20% sucrose gradient (in 0.005 M sodium phosphate buffer, pH 7.0) and centrifuged for 18 hours at 24,000 rpm (SW 25.1 rotor). By this procedure a highly purified α-crystallin fraction was separated from the rest of the lens proteins (11, 13). Fractions of 1 ml each were collected, and those containing α-crystallin were pooled, exhaustively dialyzed against 0.005 M phosphate buffer (pH 7.0), and lyophilized.

Isolation and Analysis of Radioactive α-Crystallin Subunits

Lyophilized α-crystallin was dissolved in 0.005 M sodium 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 (11, 14). The gels were stained with Amido Schwarz and scanned at 500 nm. The stained gels were then either sliced in 1-mm slices (epithelial cells) or whole bands were cut off (fiber cells), placed in scintillation vials, dried, and then solubilized by incubation overnight in 0.4 ml of hydrogen peroxide at 60°C. The Hyamine-toluene-fluors mixture was added to the vials, and the radioactivity was measured by scintillation counting.

RESULTS

Resolution of the acidic subunits (αA_2 and αA_1) and the basic subunits (αB_2 and αB_1) of epithelial cell α-crystallin by acrylamide gel electrophoresis is shown in Fig. 1. The optical density and radioactivity profiles clearly show that [^{3}H]leucine is actively incorporated into both αA_2 and αB_2 subunits. Some radioactivity is also associated, although poorly resolved, with αB_1 subunit. On the contrary, no significant label is present in the αA_1 region of the profile, indicating that this subunit is not a direct product of mRNA translation. [This phenomenon has been described in detail (9, 11)]. Qualitatively similar profiles are obtained by electrophoresis of α-crystallin from cortex fiber cells.

We have compared the amount of radioactivity associated with αB_2 and αA_2 subunits in epithelial cells and cortex fiber cells from several batches of adult bovine lenses cultured for various time intervals ranging from 1 to 7 hours (epithelial cells) and from 4 to 6 hours (cortex fiber cells). As the leucine contents of αA_2 and αB_2 subunits are nearly identical (8.7 and 9.1 mole percent, respectively, see ref. 17), the relative rates of incorporation of labeled leucine into the subunits provide a direct measure of their stoichiometry of assembly. The results are given in Table I.

It can be seen that fiber cell differentiation in the bovine lens is accompanied by a change in the stoichiometry of subunit assembly. Thus, in epithelial cells about 1 molecule of αB_2 subunit is incorporated into the aggregate together with 2 molecules of subunit αA_2. In cortex fiber cells, the corresponding ratio is approximately 1:3. This change in the stoichiometry of assembly of αA_2 and αB_2 subunits parallels a corresponding modification in the relative proportions of those two subunits within the polymeric α-crystallin. Indeed, the proportion of acidic subunits is higher in fiber cells than in epithelial cells (6, 10).

TABLE I

Percentage of [^{3}H]leucine incorporated into oligomeric α-crystallin via αB_2 and αA_2 subunits in epithelial cells and cortex fiber cells from adult bovine lenses.[2] Data are expressed as the means of several samples. The figures in parentheses indicate the number of gels analyzed. The confidence limits at the 0.95 level are also given for epithelial cells.

	αB_2	αA_2	$\frac{\alpha A_2}{\alpha B_2}$
Epithelial Cells			
Experiment 1 (7)	31.0 ± 2.3	57.1 ± 3.9	1.84
Experiment 2 (6)	29.7 ± 3.5	64.0 ± 3.2	2.12
Cortex Fiber Cells			
Experiment 1 (3)	23.6	69.0	2.92
Experiment 3 (1)	22.9	71.9	3.13

DISCUSSION

Our data clearly show that the stoichiometry of assembly of αA_2 and αB_2 subunits into the oligomeric α-crystallin molecule changes with cellular differentiation. We have seen that the ratio of incorporation of [^{3}H]leucine via αB_2 and αA_2 subunits changes from 1:2 in epithelial cells to 1:3 in cortex fiber cells. This shift towards the preferential incorporation of labeled leucine via the αA_2 subunit in cortex fiber cells has been consistently found in several experiments involving adult lenses of various ages.

Preliminary investigations have shown that free subunit pools, if present, are relatively small and equilibrate with the amino-acid pool in less than 1 hour (11). Thus it seems highly unlikely that the observed shift towards a reduced incorporation of [^{3}H]leucine via subunit αB_2 in fiber cells would result from a decrease in the specific activity of αB_2 subunit itself due to some increase in its pool size.

It seems reasonable, therefore, to suggest that the observed changes in the stoichiometry of assembly of individual α-crystallin subunits might reflect concomitant changes in the balance of synthesis of individual polypeptide chains. For example, we know from our previous studies that mRNA templates coding for the synthesis of these subunits are stabilized in the fiber cell (15, 16). The change in ratio seen in the fiber cell α-crystallin might be the result of a differential stabilization of these templates. Thus, these data would predict that αA_2 templates are more stable (or have a longer half-life) than αB_2 templates. We have made preliminary observations that indicate different stabilities for these templates. Details of those studies will be published in a separate communication.

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