DIFFERENCES IN THE SOLUBLE LENS PROTEINS FROM TADPOLE AND ADULT BULLFROGS

Jon Robert Polansky and Thomas Peter Bennett

The Biological Laboratories, Harvard University
Cambridge, Massachusetts, 02138

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SUMMARY. Soluble proteins from the whole lens of Rana catesbeiana at tadpole and adult stages were examined by cellulose acetate electrophoresis. The soluble proteins isolated from the periphery (cortex) and the core (nucleus) of the adult lens were also examined. Electrophoresis for 2 hours showed distinct quantitative differences between the profiles of tadpole and adult frog stages, while a similarity was noted between the whole tadpole lens and the nucleus of the adult lens. Electrophoresis for 50 minutes revealed distinct alpha crystallin fractions, differing in electrophoretic mobility and relative protein content, in adult nucleus, in adult cortex, and in tadpole lens preparations.

During anuran metamorphosis changes in the serum protein pattern and alterations in the enzyme profiles of liver, tail, and kidney have been observed (1, 2, 3). In consideration of these changes, it was of interest to investigate whether alterations in lens proteins were initiated during metamorphosis. Such a study was of particular interest because of the structural and developmental significance attached to lens proteins (4) and because of our desire to establish a model system for studying thyroxine induction of specific proteins. In this communication we report evidence of developmental alterations in the electrophoretic profiles of the soluble lens proteins from R. catesbeiana.

METHODS. Tadpoles and frogs (R. catesbeiana) were obtained from Conn. Valley Biological Supply Co., South Hampton, Mass. Tadpoles were staged according to their leg length to tail length ratio and according to Taylor and Kollros (5) specifications. Frogs were staged according to body weight.

Soluble extracts were prepared from decapsulated lenses that had been carefully removed intact from the eye. The cortex of the lens was operationally defined as those cells which could be readily peeled away from a harder spherical core defined as the nucleus. The lens cells of each preparation were placed in Gelman High Resolution (HR) buffer (.033 ionic strength, Tris-barbital, pH 8.8), homogenized by five passes at setting 4.5 on a TRI-R homogenization motor, and centrifuged at 10,000 x g for one hour.

Quantitative and preparative electrophoresis on cellulose acetate were performed according to standard techniques using the Gelman power supply, electrophoresis chamber No. 51170, Sepraphore III, and HR buffer (Gelman Instrument Co., Ann Arbor, Mich.). For quantitation the cellulose acetate strips were stained with Ponceau-S for ten minutes, cleared with 15% acetic acid in methanol, and scanned with a Joyce-Loebl microdensitometer.

Alpha crystallins were isolated by selecting the major anodal band with the fastest electrophoretic mobility in each protein sample. The top and bottom of each strip were stained to identify the alpha crystallin fraction, which was then eluted with HR buffer. The isolated proteins were concentrated in the Schleicher and Schuell ultrafiltration apparatus in S&S No. 100 collodion bags.

RESULTS. The soluble protein extracts compared in this study were: a) tadpole whole lens, b) frog whole lens, c) frog lens nucleus, and d) frog lens cortex. Electrophoretic strips were examined at 10 to 15 minute intervals up to a 3 hour time period. Two representative times, 50 minutes and 2 hours, were selected to illustrate differences in the various lens extracts. Alpha crystallin fractions could best be compared after 50 minutes, but beta and gamma proteins were not well resolved after this time. After 2 hours of electrophoresis, separation of the beta and gamma crystallins was achieved, but by this time alpha crystallins had migrated off the strip.

Two hour electrophoretic separations of the different preparations are presented in Fig. 1. The profile of the premetamorphic tadpole lens (Fig. 1A) appeared distinctly different from the pattern of the 250 g. adult frog lens (Fig. 1B). A similarity between the profile of the tadpole whole lens and the profile of the frog nucleus may be seen by comparing Fig. 1A and 1C. In the adult frog lens the cortex is markedly different from the nucleus (shown in Fig. 1C and 1D). The relative contributions of the cortical and nuclear fractions to the profile of the whole lens of the adult may be seen by comparing Fig. 1C and 1D with Fig. 1B.

The total protein represented by each fraction in the profiles of Fig. 1 was determined by measuring the area under individual peaks. The percentage of total protein contributed by each peak is presented in Table 1, permitting quantitative comparisons of corresponding peaks in the different preparations.

The most noticeable differences between the profiles of the tadpole whole lens and the frog whole lens were a decreased concentration of peak 1 and peak

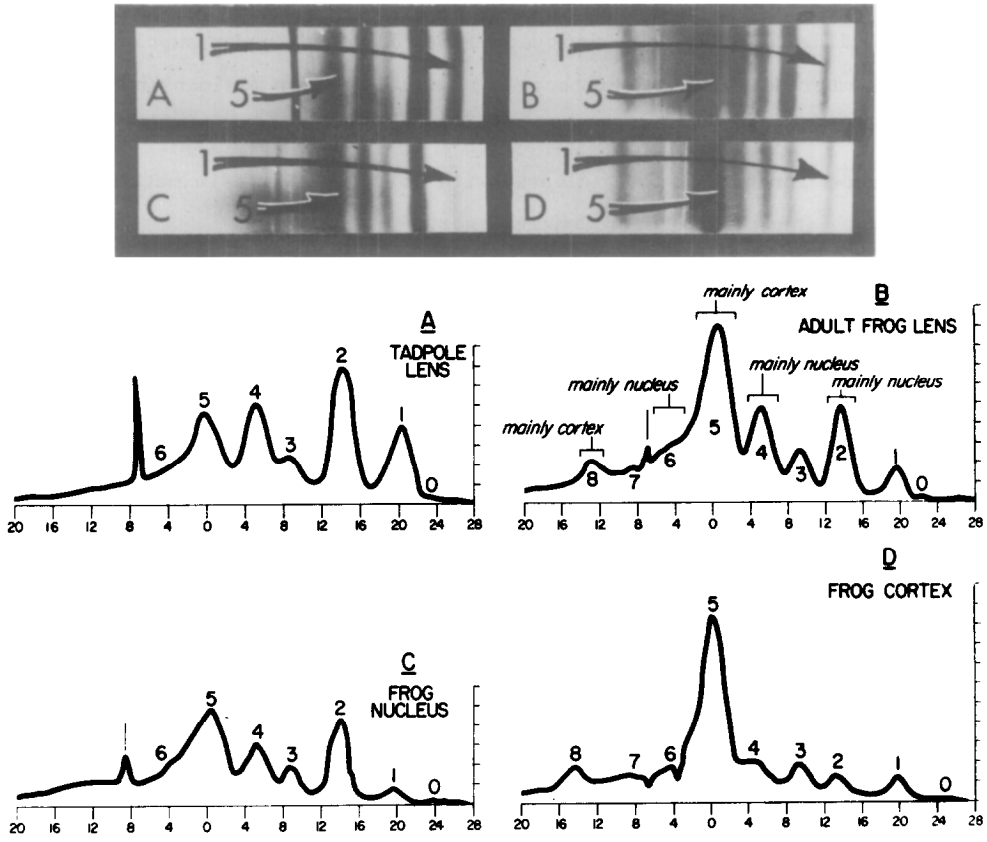

Figure 1. 2 hour electrophoresis of soluble lens proteins. (A) Tadpole whole lens, (B) frog whole lens, (C) nucleus of frog lens, and (D) cortex of frog lens. Separations of beta and gamma crystallins have been achieved, but alpha crystallin, (1)*, has migrated off the strip in the anodal direction. The presence of a small amount of insoluble protein, visible as a sharp, narrow peak to the left of peak 6, marks the origin in (A), (B), and (C). An arbitrary scale was employed in this and other figures. Peak 5 was used as a reference point ("0" on the abscissa). The anode is at the left.

2, an enhanced amount of peak 5, and the appearance of peaks 7 and 8 in the adult. Peak 1 occurred in small amounts in both the cortex and nucleus of the cells of the adult (Fig. 2D). Similarly, in studies to be published (8), T(1)* is shown to be a cortical protein.

The differences in relative mobility between the C(1)*, T(1)*, and N(1)* alpha crystallins were subjected to statistical analysis (Table 2). The calculation of relative mobilities was facilitated by using peak 5 as a standard peak

TABLE 1

The percentage of the total protein (excluding alpha crystallin) contributed by each soluble protein fraction in the long term electrophoretic separations of Fig. 1 are presented in (A), (B), (C), and (D) of this table. The amount of protein under each peak was determined after extrapolation to the baseline.

Percent Total Protein of Individual Fractions

LENS SAMPLE	8	7	6	5	4	3	2	1
A. Tadpole lens			7.1	20.2	17.6	9.2	23.5	12.9
B. Frog lens	8.9	6.0	11.8	35.8	14.6	6.9	12.4	3.6
C. Frog nucleus			11.3	30.7	14.6	6.8	16.1	2.6
D. Frog cortex	10.7	8.7	6.0	46.3	10.0	8.4	6.0	3.9

TABLE 2

Ratios of migration for each alpha crystallin were calculated by dividing the distance between peak (1)* and peak 5 by the distance from peak 1 to peak 5 (see Fig. 2A,B) in a number of electrophoretic separations. A characteristic relative mobility for C(1)*, T(1)*, and N(1)* was determined by averaging the ratios of migration (Average Ratio) for each of the alpha crystallins. These relative mobilities were shown to be significantly different by the t-test ($P \ll .001$). The table indicates the number of independent cellulose acetate strips analyzed (Trials) and the standard deviation (SD) from the Average Ratio.

	Average Ratio	Trials	SD	t-Test
C(1)*	1.50	24	± .033	≪.001
T(1)*	2.03	26	± .033	
N(1)*	2.37	11	± .030	≪.001

of reference to obtain ratios of migration which were independent of buffer flow across the electrophoretic strip and which were independent of the amount of peak separation that occurred. These migration ratios were used to define a relative mobility (Average Ratio) characteristic of each alpha crystallin. The relative mobilities for C(1)*, T(1)*, and N(1)* are shown to be significantly different by the t-test (Table 2).

The electrophoretic patterns shown in Fig. 3A-C provide supporting evidence that the alpha crystallins T(1)*, C(1)*, and N(1)* are distinct proteins. This

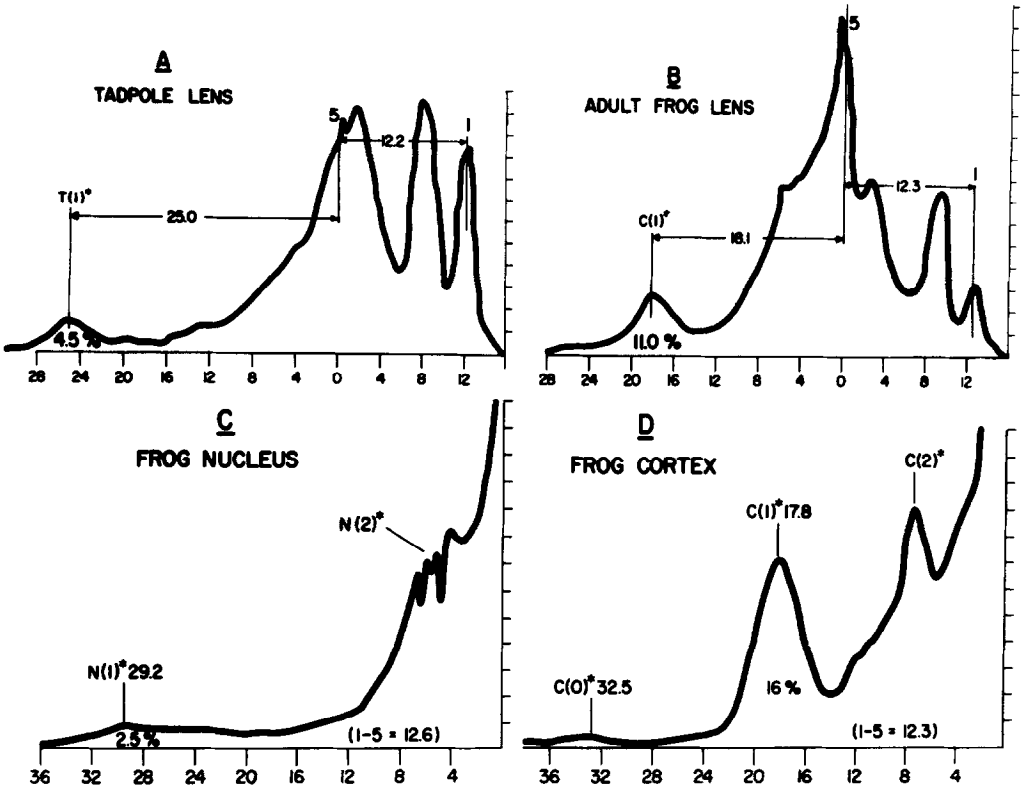

Figure 2. 50 minute electrophoresis of soluble lens proteins. (A) Tadpole whole lens, (B) frog whole lens, (C) nucleus of frog lens, and (D) cortex of frog lens. The alpha crystallin region is designated (1)*; the percentage of the total protein in each profile contributed by the (1)* proteins is indicated under each alpha crystallin peak. The distance that peak 1 and the distance that peak (1)* migrated relative to peak 5 are indicated on the profiles. (C) and (D) show in detail the soluble proteins anodal to peak 5 for the frog nucleus and frog cortex, respectively. Although the densitometer has been adjusted to show enlarged views of the alpha crystallin region, the abscissae for (C) and (D) correspond to the abscissae for (A) and (B) as well as those for Fig. 1 and Fig. 3. In (C) and (D), the distance that the (1)* protein migrated is indicated above the appropriate fraction. The distance between peak 1 and peak 5 (1-5) is shown in parenthesis, although peak 1 and peak 5 are not shown in these figures. The (2)* fraction probably consists of unresolved beta crystallins (see peaks 7 and 8 of 2 hour separation), and the C(0)* probably represents what has been commonly referred to as "pre-alpha." The anode is at the left.

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figure presents mixture studies in which the mobilities of the three alpha crystallins are compared, two at a time. Lens preparations were mixed and then electrophoresed for 35 minutes. All mixtures showed evidence of two alpha crystallin peaks, whereas 35 minute electrophoretic separations of individual preparations

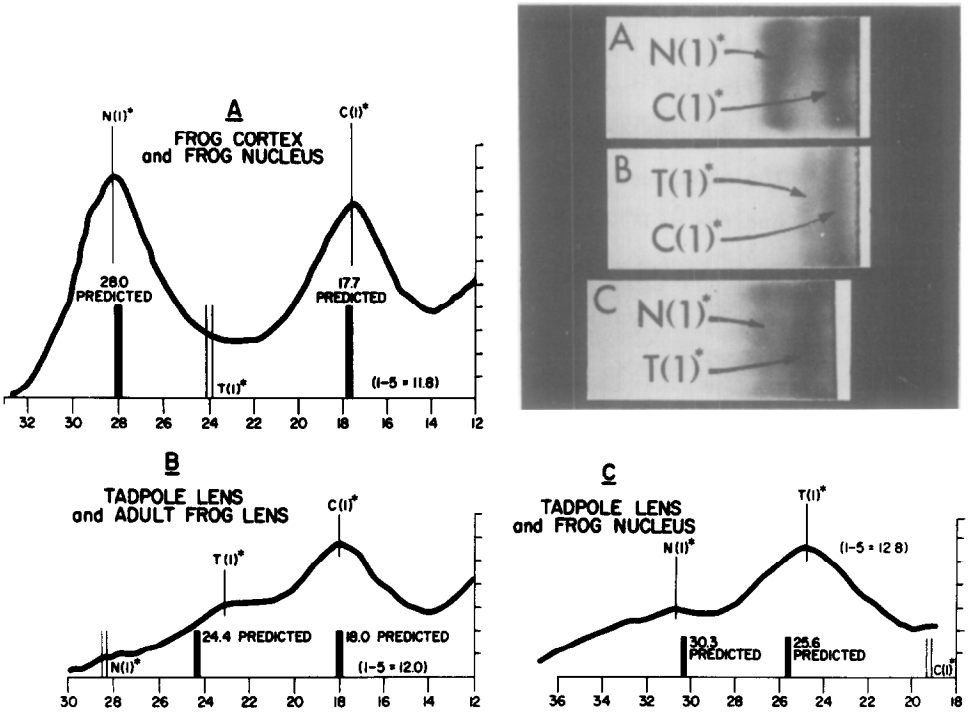

Figure 3. 35 minute electrophoresis of mixtures of individual lens preparations in Methods. (A) Cortex of frog lens and nucleus of frog lens, (B) tadpole whole lens and frog whole lens, and (C) tadpole whole lens and nucleus of frog lens. Densitometric tracings show only the alpha crystallin region, but the abscissa and "0" point correspond to those employed in Fig. 1 and Fig. 2. Each alpha crystallin, (1)*, has a distinct mobility that can be readily distinguished. The dark rectangles in each profile predict the distance a given (1)* fraction should have migrated according to the characteristic "Average Ratios" in Table 2 and the observed individual peak 1-5 distances (indicated in parentheses). The anode is at the left.

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resulted in a single (1)* peak. Each (1)* protein identified in the mixture studies was verified by calculating the predicted distance a given alpha crystallin should have moved from peak 5. The predicted values were determined by multiplying the characteristic "Average Ratios" from Table 2 by the individual 1-5 distances. The congruence between the actual positions of the (1)* peaks with the predicted (1)*-5 distances (shown in the dark rectangles of each profile) further supported the existence of three distinct alpha crystallins.

DISCUSSION. The soluble lens proteins may be provisionally classified according to their relative migration in an electric field. Although there may be considerable flux of buffer across paper and cellulose acetate, the data ob-

tained from free electrophoresis (9), paper electrophoresis (10), and cellulose acetate electrophoresis (9, 11) agree that the most anodal major fraction represents alpha crystallin, the least anodal represents gamma crystallin, with beta crystallin having an intermediate mobility. Under this general scheme, fraction (1)* contributes the alpha proteins, peaks 1 and 2 probably contribute the gamma components (11), and the middle peaks contribute the beta proteins.

According to this identification, the larger amount of gamma crystallin in adult, while it was present in a high concentration in the tadpole. Peak 2 was prominent in the tadpole and in the adult nucleus but not in the adult cortex. The change noted in peak 5 was probably due to the high concentration of peak 5 protein in the cortical cells of the adult. Peak 8 appeared as a distinct band in the cortex but not in the nucleus nor in the tadpole lens.

Electrophoresis for 50 minutes, shown in Fig. 2A-D, provided a rapid means of separating alpha crystallin from the other soluble proteins.

The major fraction that migrated farthest toward the anode in each preparation was tentatively identified as alpha crystallin and was designated (1)*. This identification was verified by repeating the isoelectric precipitation method of Bloemendal to obtain alpha crystallin (6) and showing that this isolated fraction corresponded to the fastest anodal component. In addition, the fastest anodal component was isolated from electrophoretic separations of the different lens preparations. These presumptive alpha crystallins were then tested by immunoelectrophoresis in agarose. The position (7) of the resulting precipitin arcs provided further evidence that all the different (1)* proteins represented alpha crystallins.

When the anodal fractions of each preparation were compared by 50 minute electrophoresis (Fig. 2), alpha crystallins could be distinguished in adult nucleus, adult cortex, and in tadpole lens preparations which differed in their electrophoretic mobilities. These may be seen by comparing the 50 minute pattern from the tadpole whole lens, which shows T(1)*, with patterns from the frog nucleus and frog cortex, which show N(1)* and C(1)*, respectively. The alpha crystallin extracted from the adult whole lens showed a migration identical to cortical alpha crystallin but did not show the nuclear alpha crystallin which was present in a much smaller concentration. C(1)* extracted from lenses of 250 g. frogs migrated slower toward the anode than did T(1)* from premetamorphic tadpoles (Fig. 2A,B). N(1)* (Fig. 2C) had a faster anodal mobility than either C(1)* or T(1)*.

Fig. 2 also shows that a quantitative increase in the relative amount of alpha crystallin occurs between the tadpole and frog (see estimates of percentage of total protein in Fig. 2A and B). This quantitative increase in the frog lens was also observed when we compared cortical cells from frog and tadpole lens preparations (8). Further, the C(1)* of the frog is contributed by the cortical the larval tadpole lens compared to the adult frog lens is in agreement with studies which have shown that gamma crystallins are the predominant embryonic lens proteins in bovines (12). The higher proportion of gamma crystallin in the core of R. catesbeiana lens relative to the peripheral cells also agrees with distribution studies from bovine lenses (9) as does the localization of the fast moving alpha crystallin in the cortical cells. The observed shift in mobility between embryonic and adult alpha crystallins in R. catesbeiana was opposite in direction from that of bovine alpha crystallin (13, 14) but in the same direction as that of the hamster (14).

The differences reported here in the soluble lens proteins between tadpoles and frogs are the first to our knowledge concerning alterations of lens proteins during amphibian metamorphosis.

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REFERENCES

1. Bennett, T.P. and Frieden, E. (1962). In "Comparative Biochemistry", (M. Florkin and H.S. Mason, eds.), vol. 4, Academic Press, N.Y., 483.
2. Cohen, P.P. (1966). "Harvey Lectures", Ser. 60, 119.
3. Weber, R. (1966). In "The Biochemistry of Animal Development", (R. Weber, eds.), vol. 2, Academic Press, N.Y., 227.
4. Bloemendal, H. (1969). Exptl. Eye Res., 8, 227.
5. Taylor, A.C. and Kollros, J.J. (1946). Anat. Record, 94, 7.
6. Hoenders, H.J., Schoenmakers, J.G.G., Gerding, J.J.T., Tesser, G.I., and Bloemendal, H. (1968). Exptl. Eye Res., 7, 291.
7. Clayton, R.M., Campbell, J.C., and Truman, D.E.S. (1968). Exptl. Eye Res., 7, 11.
8. Polansky, J.R. and Bennett, T.P. (1970). To be published.
9. Cobb, B.F. and Koenig, V.L. (1968). Exptl. Eye Res., 7, 581.
10. Spector, A. (1964). Invest. Opthal., 3, 182.
11. Campbell, J.C., Clayton, R.M., and Truman, D.E.S. (1967). Exptl. Eye Res., 7, 4.
12. Papaconstantinou, J. (1965). Biochem. Biophys. Acta, 107, 81.
13. Mehta, P.D. and Maisel, H. (1968). Exptl. Eye Res., 7, 265.
14. Rabaey, M. (1965). Invest. Opthal., 4, 573.