The incorporation of labeled amino acids into lens protein

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Calf and rabbit lenses cultured in a medium containing a radioactive amino acid incorporate some labeled amino acids into a minor protein fraction, the HL (high labeled) protein, three or four times more rapidly than into any other soluble lens protein. This fraction, isolated by DEAE chromatography, represents about 11 per cent of the total protein but contains about 34 per cent of the total radioactivity. High rates of incorporation into this protein fraction were obtained with C-14 arginine, histidine, and aspartic acid—the three amino acids studied. The rapidly synthesized HL component is clearly not associated with the gamma crystallin group, but, since it is not yet pure, it cannot be related to either the alpha or beta crystallin fractions at the present time. The specific activity of the protein in the equatorial and anterior regions was found to be much greater than that of the protein in the remainder of the lens. The core incorporated little C-14 amino acid although the concentration of labeled amino acid in this section was 65 per cent as high as that in the equatorial region. However, all lens sections examined gave the same over-all pattern of incorporation into the different protein fractions.

In 1950 Merriam and Kinsey[1] demonstrated for the first time that the lens is capable of synthesizing proteins. They were able to show that lens incorporates amino acids into proteins fairly rapidly and estimated their turnover rate at 2.5 to 5 per cent per day. Since that time no new information regarding the synthesis of lens proteins has appeared. An investigation of this basic subject was therefore undertaken with the initial objective of determining which of the three major soluble lens protein fractions, alpha, beta, and gamma crystallins, is synthesized most rapidly. For the incorporation studies, histidine seemed an ideal substrate since this amino acid is found in approximately the same concentration in the three crystallin fractions. The investigation has revealed that there is little difference in the rate of synthesis of the alpha, beta, and gamma groups. However, the significant finding of this study is that one protein fraction, not clearly associated with the three major lens components, incorporates amino acids very rapidly.

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Methods and materials

Calf lenses were removed from eyes approximately an hour after sacrifice and incubated for varying periods in a modified T.C. 199 medium[2] containing the labeled amino acid. Rabbit lenses were removed for experimentation immediately after sacrifice. Following incubation, some of the lenses were frozen with dry ice and dissected into equatorial, anterior, posterior, and core sections. All lens material was stored in a deep freeze until used.

For the determination of radioactivity in the different sections of the lens the material was thawed, homogenized, and then precipitated with 10 per cent trichloroacetic acid. After washing the precipitate three times with 10 per cent trichloroacetic acid, two times with 95 per cent alcohol, and two times with ether, the material was dried and weighed. It was then dissolved with 80 per cent formic acid and counted. All counting was performed with a Packard scintillation counter with vials containing 20 ml. of the counting fluid and 0.5 ml. of the aqueous solution. The counting fluid contained 70 per cent toluene, 30 per cent alcohol, 4 Gm. per liter of 2, 5 diphenyloxazole and 100 mg. per liter of 1, 4-bis-2-2(4-methyl-5-phenyloxazolyl) benzene. Salt solutions greater than 0.1M were first dialyzed before counting. Neutralization of alkaline solutions was also carried out before counting.

For chromatography, the lens material was homogenized with distilled water to give a 10 to 15 per cent (wet weight) solution. The insoluble material was removed by centrifugation for 10 minutes at 20,500 × G. Dialysis of the supernatant was carried out against a hundredfold excess of 0.002M phosphate for 16 hours, and followed by chromatography on DEAE cellulose in the usual manner.[3] Protein elution was followed by absorption at 280 mμ, with use of the automatic scanning system previously described.[3] Aliquots of 0.5 ml. were then taken for counting.

Arginine was determined by a modified Sakaguchi reaction.[4] Protein samples were prepared for amino acid analysis by hydrolysis in a sealed tube under nitrogen, with constant boiling HCl at 110° for 20 hours. The amino acid analysis was carried out with a Phoenix amino acid analyzer according to the procedure of Piez and Morris.[5]

Results

The incorporation of radioactive amino acid into different regions of the calf lens was first investigated. After a 22 hour in vitro incubation with C-14 histidine, the lens was divided into equatorial, anterior, posterior, and core sections. The results shown in Table I indicate that the incorporation of histidine is approximately 3 to 4 times greater into the equatorial region than into the anterior section, 15 to 30 times greater than into the posterior sec-

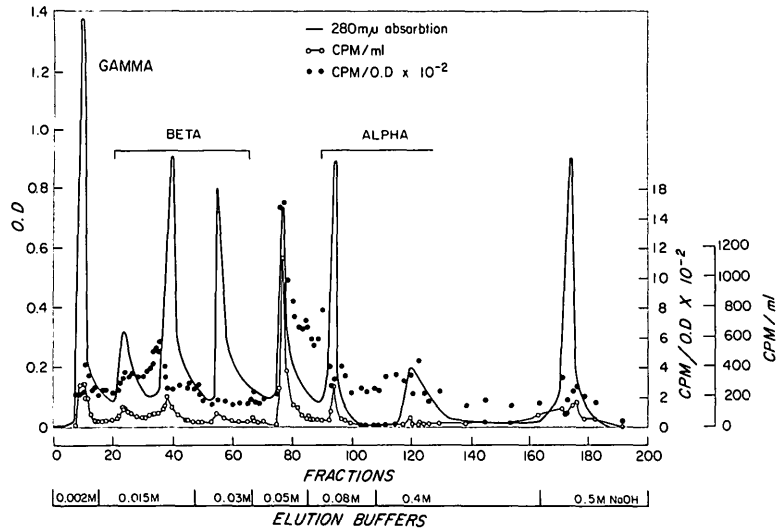

Fig. 1. The calf lens protein was fractionated on a 15 Cm. DEAE column by stepwise elution. All buffers were prepared by the dilution of 0.500M (NaH_2PO_4,K_2HPO_4), pH 6.85 ± 0.05. Fractions were collected in 15 ml. aliquots, and 0.5 ml. aliquots were used for counting.

Table I. Incorporation of C-14 histidine into various regions of the calf lens

Region	*Equatorial*			*Anterior*			*Posterior*			*Core*		
Experiment	1	2	3	1	2	3	1	2	3	1	2	3
Wet weight (mg.)	217	190	168	164	125	135	120	93	90	445	541	515
Dry weight protein (c.p.m./mg.)	765	980	910	293	220	350	20	69	56	10	20	22
Free histidine (c.p.m./c.c.) lens H_2O*	47,000	42,000	49,000	38,000	33,000	45,000	40,000	39,000	43,000	32,000	28,000	37,000

*Based on 67 per cent H_2O.

Table II. Summary of the fractionation of calf lens protein on DEAE cellulose

Fraction	*Per cent of counts recovered from DEAE column*	*Per cent of absorbancy recovered from column*	*Specific activity (c.p.m./O.D.)*
0.002M	12.0	16.1	246
0.015M (1)	9.0	8.6	350
(2)	18.3	21.6	290
0.03M	8.2	16.8	170
0.05M (HL)	34.0	10.8	1,050
0.08M	8.0	8.2	330
0.4M	3.1	3.1	340
NaOH	9.8	13.0	230

The fractions of each peak eluted from the DEAE column (Fig. 1) were pooled and the total protein and radioactivity determined.

tion, and from 40 to 80 times greater than into the core. Thus the core and posterior sections, which together comprise more than 60 per cent of the total lens by weight, incorporate histidine very slowly during the 22 hour period. These results cannot be explained on the basis of the availability of free labeled amino acid, for even the core region has about two thirds of the C-14 histidine concentration of the equatorial section. It is of interest to note that although the equatorial region has the highest rate of protein synthesis, the anterior section is also very active. The variation in weight and specific activity of any given section is undoubtedly due to the subjectivity involved in separating the lens into the various regions.

To determine the distribution of histidine incorporated into the lens protein, it was necessary to separate the protein fractions by DEAE column chromatography. Our ability to detect differences in the rate of synthesis of the lens proteins fractions was increased by using only the equatorial region. By this procedure the bulk of the nonlabeled protein was removed and the specific activity of the remaining protein concomitantly increased.

The results obtained from fractionating the soluble protein from the equatorial region of a number of calf lenses incubated with C-14 histidine are shown in Fig. 1. When the counts per milliliter were determined for the column fractions, the aliquots eluted with the 0.05M buffer were found to contain the greatest activity. It can be clearly seen in Fig. 1 that the radioactivity contained in tube 78 far exceeds any other fraction. This observation becomes even more striking when it is noted that the total protein in the peak eluted with 0.05M PO_4 is less than in three other peaks. This is reflected by the fact

that the protein eluted with the 0.05M buffer has a specific activity more than 3 times greater than that of any other protein component. Little variation in synthetic rate is evident among the other components. The results are summarized in Table II. The peak eluted with 0.05M PO_4 contains 33 per cent of the activity but only 11.2 per cent of the protein, thus indicating that this is the most rapidly synthesized protein fraction. Henceforth in this discussion, for the sake of brevity, this protein fraction eluted with 0.05M PO_4 will be referred to as the highly labeled or HL protein.

Previous work has made it possible to assign the DEAE column fractions to the classical alpha, beta, gamma crystallins.[3] The experiments reported suggest that the incorporation of C-14 histidine into alpha, beta, and gamma crystallin is of the same order of magnitude (Fig. 1). It may be of some significance that the HL protein does not clearly fall into either the beta or alpha crystallin groups.

To ascertain whether the radioactivity is truly associated with lens protein, the total soluble lens protein and the HL fraction were treated in various ways. Exhaustive dialysis against 0.5M KCl at neutral and acid pH and against 6M urea was carried out. Ammonium sulfate precipitation and extraction with trichloroacetic acid at 90° were also performed. In no case was it possible to alter the specific activity significantly.

The observed high rate of synthesis of the HL protein may be due to a proportionately higher histidine concentration or a much lower 280 mμ extinction coefficient in this component in comparison to the other lens proteins. In order to check these possibilities, amino acid analysis was performed on the alpha, beta, and gamma crystallins and the HL protein. The pertinent results in Table III show that the histidine content of the HL protein is about the same as the alpha fraction and about 20 per cent greater than the beta and gamma fractions. Thus the high rate of incorporation into the HL protein is not due to the presence of a disproportionate amount of histidine. Only phenylalanine, tyrosine, and tryptophan absorb light appreciably at 280 mμ. The total content of phenylalanine and tyrosine does not vary by more than plus or minus 10 per cent in these protein fractions. Tryptophan was not determined but it is highly unlikely that the variation in tryptophan alone can explain the observed results. That this is the case is further confirmed by the observation that the 280 mμ extinction coefficients of alpha, beta, and gamma crystallins are similar.

Table III. Amino acid analysis of lens protein fractions

	Amino acid residue (per cent)			
Amino acid	*Alpha*	*Beta*	*Gamma*	*HL*
Histidine	4.1	3.4	3.5	4.3
Tyrosine	3.2	4.4	8.6	2.2
Phenylalanine	7.3	4.4	4.1	7.0
Arginine	6.7	5.8	10.3	6.9
Aspartic acid	8.8	7.8	9.9	6.6

To determine whether the same pattern of incorporation could be shown for amino acids other than histidine, a basic amino acid, arginine, and a acidic amino acid, aspartic acid, were chosen. The aspartic acid content of the HL protein is about 15 to 30 per cent lower than that of the other fractions. The arginine content of the HL protein is the same as the alpha fraction, 35 per cent lower than the gamma fraction, and 20 per cent higher than the beta fraction.

A comparison of the results obtained after incubation with either C-14 arginine, aspartic acid, or histidine is shown in Table IV. Again in every experiment the HL protein contains from 2½ to 3 times more activity than any of the other protein fractions. It should be noted that in these experiments elution with 0.015M and 0.08M buffers was omitted. Thus the 0.03M and the 0.4M fractions represent the bulk of the beta and alpha protein, re-

spectively. Again with aspartic acid and arginine the degree of incorporation into the alpha, beta, and gamma fractions appears relatively similar. Although the specific activity of the three amino acids in the incubation medium was comparable, a much lower rate of incorporation into the lens proteins was observed with aspartic acid. The low rate of incorporation of aspartic acid in contrast to histidine and arginine may be due to a proportionately slower rate of uptake of this amino acid by the lens.

In the experiments involving the incorporation of C-14 arginine into lens protein, the arginine content of each fraction was determined and the specific activities calculated. The results were essentially the same as those based on 280 mμ absorption, again emphasizing that the HL protein has a specific activity of 2½ to 3 times greater than the other fractions.

It was of interest to compare the specific activity of the different protein fractions isolated from different sections of the lens (Table V). The same general pattern of incorporation was found for all sections of the lens; thus the HL protein always had a specific activity 2 to 4 times greater than any other fraction. Experiments with rabbit lens gave similar results, indicating that the HL protein is an unusually active metabolic component in rabbit as well as calf lens.

Table IV. Specific activity of lens protein fractions isolated from the equatorial region after incubation with C-14 amino acid

	c.p.m./O.D.°		
Fraction	*Arginine*	*Aspartic acid*	*Histidine*
0.002M	2,600	85	570
0.03M	1,980	83	750
HL	7,600	262	3,060
0.4M	3,120	128	870
NaOH	1,820	150	—
Soluble lens protein	3,400	192	1,140

The material was fractionated on DEAE cellulose under conditions similar to those used in Fig. 1. However, elution with the 0.015M and 0.08M buffers was omitted.

°The specific activities of the fractions were corrected to an initial specific activity of the labeled amino acid in the medium equal to 1×10^6 c.p.m./mμ.

Discussion

It is now clear that the HL protein component incorporates some amino acids much more rapidly than any other lens protein. However, its role in the over-all metabolism of the lens is still obscure. Preliminary attempts to demonstrate a precursor relationship between the HL protein fraction and other lens proteins have been unsuccessful. However, our present inability to show a precursor relationship for this protein does not rule out such an important role for this protein.

The HL protein is not only present in

Table V. Specific activity of protein fractions isolated from different sections of the lens

	Calf				*Rabbit*			
	Equatorial section°		*Anterior section*°		*Whole lens*†		*Whole lens*°	
Fraction	*c.p.m./O.D.*	*Ratio*‡	*c.p.m./O.D.*	*Ratio*‡	*c.p.m./O.D.*	*Ratio*‡	*c.p.m./O.D.*	*Ratio*‡
0.002M	1,360	0.35	182	0.36	220	0.25	1,360	0.41
0.03M	1,020	0.26	140	0.28	290	0.32	940	0.28
HL	3,900	1.0	500	1.0	900	1.0	3,300	1.0
0.4M	1,600	0.41	148	0.30	296	0.33	1,700	0.51
Total soluble protein	1,700	0.44	250	0.50	480	0.54	1,550	0.47

°Arginine labeled.

†Histidine labeled.

‡Specific activity of indicated fraction / Specific activity of HL fraction

all parts of the lens but contains relatively more label in all parts of the lens as well. Furthermore, fractionation of cattle lens indicates no diminution in the relative quantity of this protein. These observations would suggest that this component may continue to carry out its metabolic role in the lens after the animal has matured.

Although the HL protein does not appear to be associated with the gamma crystallin group, at present, it is not possible to relate it to the classical alpha or beta crystallin fractions. The amino acid composition of the HL protein more closely resembles alpha crystallin than beta crystallin, but it has an S_{20w} of 14 which is in the range of the heaviest beta component.* The fact that the HL protein cannot be clearly identified with the classical crystallins may be significant. Possibly this rapidly metabolizing protein is not a part of the structural lens protein.

It is now obvious that the HL material is not a pure protein. This was first indicated by the variation in the specific activity of the HL fractions isolated by DEAE chromatography (Fig. 1). Further purification of this material has revealed a contamination of more than 50 per cent. Thus the specificity of the HL protein is even higher than that reported, further emphasizing the discrepancy in the rate of amino acid incorporation into this protein relative to the other lens proteins. Such contamination makes it difficult to relate clearly this protein fraction to the alpha or beta crystallin without further purification.

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*Unpublished results.