PROPERTIES OF A CRYOPROTEIN IN THE OCULAR LENS*

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Previous studies have indicated that the "cold cataract" phenomenon observed in the lenses of young animals is due to a specific protein fraction, which decreases in concentration as the lens ages (Zigman and Lerman 1964 and 1965, Lerman and Zigman 1965). The "cold cataract" cannot be demonstrated in lenses of older animals but aqueous extracts of lens proteins (from all age groups) are capable of being precipitated at temperatures below 10° C., provided that the concentration of the so-called cold precipitable protein fraction is above 0.3%.

The total soluble lens proteins were obtained by homogenizing groups of rat lenses in distilled water and removing the insoluble protein fraction by centrifugation at 600 x G for 20 minutes. The supernatant was then centrifuged at 105,000 x G for 1 hour to remove the particulate matter and the total soluble protein fraction thus obtained was cooled to 0° C. The resulting precipitate was designated as the cold-precipitable protein fraction and the supernatant was designated as the cold-soluble protein fraction. Separation of the cold-soluble protein fraction and the cold-precipitable protein fraction of five to six week old rat lenses using DEAE-cellulose

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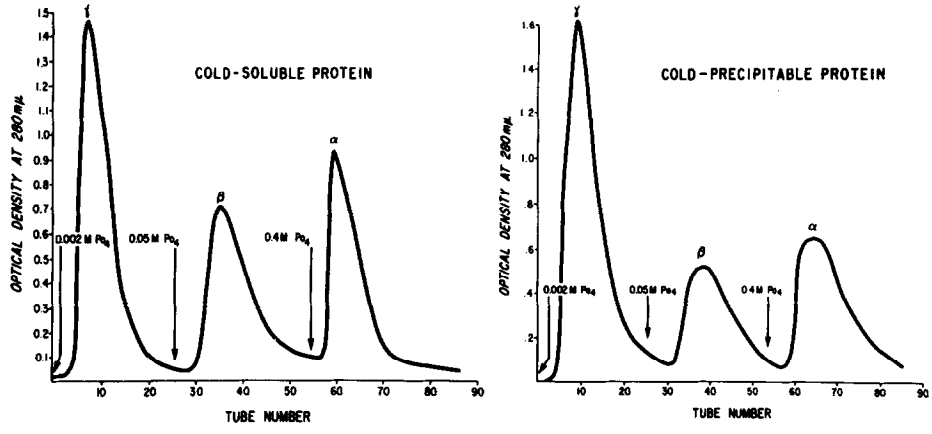
chromatography (Spector 1964) revealed that the gamma crystallin is the major component of both fractions (figure 1). The cold-precipitable fraction contained approximately 65% gamma crystallin, whereas the cold-soluble fraction contained 40% (table 1).



Separation of rat lens cold-precipitable and cold-soluble protein fractions by DEAE-cellulose column chromatography (Spector 1964). Column size 22 x 1.1 cm. and stepwise elution with phosphate buffer (concentrations given above); 2 ml. samples per tube were collected.

TABLE 1

COMPOSITION OF COLD-SOLUBLE AND COLD-PRECIPITABLE PROTEIN FRACTIONS IN 3 WEEK OLD RAT LENS*

	cold-precipitable %	cold-soluble %
α -Crystallin	22.50	31.25
β -Crystallin	12.25	27.50
γ -Crystallin	65.25	41.25

* Separation of crystallins on DEAE cellulose column.

Only the gamma crystallin behaved as the cryoprotein and its ability to precipitate in the cold could be influenced by a variety of factors (table 2).

TABLE II

FACTORS INFLUENCING COLD PRECIPITATION OF GAMMA CRYSTALLIN

1.	TEMPERATURE:	Must be $< 10^{o}C$.
2.	CONCENTRATION:	Must be $>$ 3 mg/ml.
3.	PH:	Maximum pp'n at pH 6.7 - 6.8 Very little ppt'n $<$ 5 or $>$ 9.
4.	UREA:	0.25 M urea inhibits ppt'n.
5.	SH BLOCKERS SUCH AS N-ETHYLMALEIMIDE AND IODOACETAMIDE:	No effect at 0.011 M.
6.	OTHER PROTEINS IN SOLUTION (ALPHA AND BETA CRYSTALLIN):	Inhibit cold ppt'n when concentration exceeds that of gamma crystallin.

The amino acid composition of gamma crystallin (table 3) shows that it is a basic protein containing a considerable number of amino acid residues with nonpolar side-chains. Its behaviour in the cold and with urea, suggest the possibility that some of these apolar groups might be involved under these conditions.

TABLE III

AMINO ACID COMPOSITION OF GAMMA CRYSTALLIN

AMINO ACID	MOLE A A PER MOLE OF PROTEIN
Lysine	2.6
Histidine	4.0
Arginine	13.8
Aspartic Acid	12.8
Threonine	3.4
Serine	8.9
Glutamic Acid	15.6
Proline	4.6
Glycine	10.4
Alanine	3.2
Half Cystine	3.5
Valine	4.6
Methionine	3.6
Isoleucine	3.8
Leucine	8.2
Tyrosine	10.5
Phenylalanine	6.2
Ammonia	30.0

The cold precipitation phenomenon is not due to the aggregation of smaller proteins into larger molecular weight units as may occur with the serum macroglobulins. The gamma fraction contains the smallest of the soluble lens proteins, with a molecular weight of approximately 20,000 and a sedimentation rate of 4S. When the cold-precipitable protein fraction is dissolved in acid media (pH 4.0) it aggregates to form a 17S fraction and the ability to precipitate below 10^{o} C. is lost. Similar acid-pH aggregation effects have been noted with TMV protein (Lauffer 1958 and Ansevin 1964). The inhibition of cold precipitation may be due to aggregation resulting in a reduced number of exposed hydrophobic groups. The hypothesis that exposed hydrophobic groups are responsible for the tendency of gamma crystallin to precipitate in the cold is consistent with the observation that low concentrations of urea (0.3M) prevent this phenomenon. It is possible that the alpha and beta crystallins tend to keep the gamma crystallin in solution in the normal lens by a similar mechanism (hydrophobic bonding). If either alpha and/or beta crystallin are present in a concentration above that of gamma crystallin (as in older animals) that "cold cataract" phenomenon is no longer observed. Hence this phenomenon can only be demonstrated in the lenses of young animals in which gamma crystallin constitutes the major portion of the soluble lens proteins. The concentration of gamma crystallin in soluble lens proteins derived from rat lenses of different ages is shown in table 4.

TABLE IV

CONCENTRATION* OF ALPHA, BETA AND GAMMA CRYSTALLIN IN RAT LENSES.

AGE	ALPHA	BETA	GAMMA
5-6 weeks	20	20	60
6 months	38	42	20
11 months	41	46	13

* The concentration is expressed as a per cent of the total soluble protein.

It is interesting to speculate that the foregoing phenomenon might be an example of protein interaction which, in the lens, may be of some importance in maintaining transparency and proper optical function.

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