STUDY OF SOLUBLE LENS PROTEINS OF NORMAL MICE AND MICE WITH CATARACT

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UDC 617.741-004.1-092.9.07:617.741-008.939.6-074

KEY WORDS: cataract, lens, soluble proteins

Fiber formation in the lens is accompanied by synthesis of crystallins, which ultimately are transformed into a stable protein matrix. The proteins of the lens account for about 35% of its total mass and are divided into soluble and insoluble [4, 6]. In the normal adult human lens the proportion of soluble proteins is about 83%. The quantity of soluble proteins changes with age and differs in different animals. During the development of senile cataract the fraction of soluble protein falls to 50% [1].

Changes in the fraction of soluble proteins during cataract development depend on the stage of the disease and the type of cataract. Soluble fractions are obtained by the use of detergents, chaotropic agents, or sonification. The results obtained by different methods, however, differ substantially [3, 7, 9-11].

The aim of this investigation was to determine relative changes in concentration and to study the integral characteristics of the secondary structure of the soluble proteins extracted from homogenates of lenses of normal mice and mice with cataracts.

EXPERIMENTAL METHOD

Mice with hereditary cataract, of the $Cat^{Fr}Cat^{Fr}$ and Balb lines were decapitated at the age of 6 months and the lenses removed, homogenized four at a time in 1.5 ml of distilled water (glass — glass), kept at 20°C for a further 5 min, and centrifuged for 2 min at 3000 rpm. Aliquots of 0.5 ml were taken from the supernatant and their absorption spectra recorded on a DU-50 spectrophotometer in a cuvette 0.2 cm thick [5].

To determine the relative concentration and relative degree of aggregation of the molecules of extracted protein, together with the method of absorption spectrophotometry we also used the method of measuring the limiting concentration of UV-induced radicals (the LCR method) [2].

In accordance with the LCR method solutions of extracted proteins were diluted with an equal volume of ethylene-glycol and 10 μl of this solution was applied to a quartz plate (4 × 6 mm), and then cooled to —196°C. These solutions readily vitrify, while remaining true. The sample was irradiated at —196°C with focused light from a very high pressure DRSh-500 mercury vapor lamp (500 W) through a UFS-2 light filter (260-400 nm) until a limiting concentration of free radicals stable under these conditions had formed in the sample, twice as high as the protein concentration in a true solution. During aggregation of the protein molecules the limiting concentration of radicals decreased in proportion to the degree of aggregation [2]. EPR spectra were recorded at —196°C on an RÉ-1306 radiospectrometer.

Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. E. Vartanyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 6, pp. 609-610, June, 1991. Original article submitted December 22, 1990.

 0007-4888/91/0006-0798$12.50

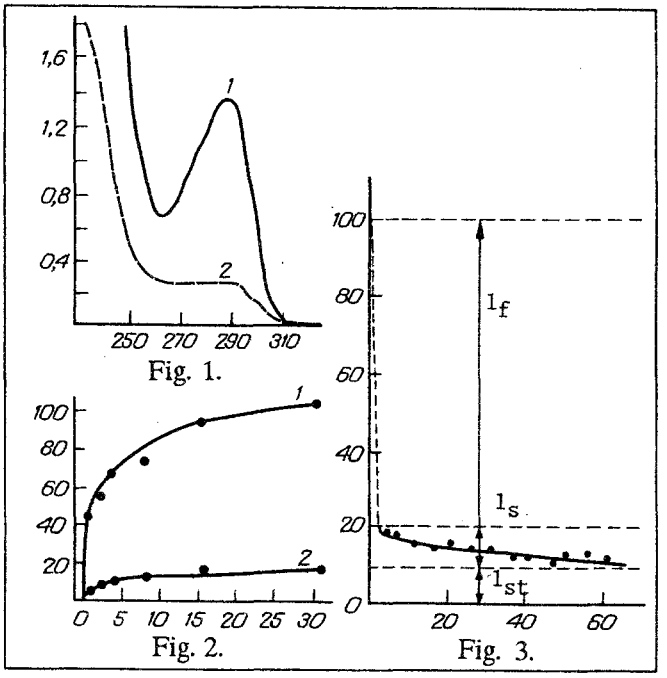

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 1. Absorption spectra of aqueous extracts of homogenates of lenses of normal (1) mice and mice with cataract (2). Abscissa, wavelength, nm; ordinate, optical density.

Fig. 2. Dependence of concentration of paramagnetic centers on duration of action of UV-light on aqueous — ethylene-glycol solutions of aqueous extracts of proteins from lenses of normal mice (1) and mice with cataract (2). Abscissa, time (in min); ordinate, concentration of free radicals (in %).

Fig. 3. Death (at —30°C) of free radicals (UV-induced at —196°C) in aqueous extract of proteins from homogenate of normal mouse lenses. 1_f, 1_s, 1_{st}) quantity of radicals taking part in fast and slow reaction and remaining stable at —30°C respectively. Abscissa, time (in min); ordinate, concentration of free radicals (in %).

To determine the integral characteristics of the secondary structure of the extracted proteins, a recombination-kinetic (RK) method [8] was used. By means of this method, the fraction of the peptide chain of the protein molecule consisting of one particular secondary structure (β-structure or α-helix) we determined from the kinetic curve of death of free radicals, UV-induced at —196°C. The fraction of all peptide chains present in a spatially regular form can be determined by the RK-method in a mixture of different globular proteins.

EXPERIMENTAL RESULTS

Absorption spectra of aqueous extracts from homogenates of normal lenses and lenses with cataracts are given in Fig. 1. The maximum of absorption at λ = 280 nm corresponds to absorption of tryptophan-containing proteins [5]. Composition of optical density values at this wavelength shows that in the presence of a developed cataract the concentration of extracted protein was reduced about tenfold.

The limiting concentration of UV-induced free radicals in a vitrified aqueous-ethylene-glycol solution for extract from lenses with cataract also was reduced about tenfold compared with normal (Fig. 2). Coincidence of the results obtained by absorption spectrophotometry and the LCR method shows that 90% of water-soluble protein of the normal lens is bound in the lens during cataract development.

It can also be concluded that the oligomeric composition of the water-soluble proteins in the normal lens and lens with cataract is roughly identical. If the oligomeric composition was changed, the ratio between values of limiting concentrations of radicals would be increased in the case of an increase in the content of protein aggregates and reduced in the case of a decrease. The results obtained by absorption spectrophotometry were unchanged depending on the degree of aggregation of the extracted proteins. Thus the combined use of absorption spectrophotometry and the LCR method enables the relative quantity of water-soluble proteins to be determined and changes in the oligomeric composition to be monitored at different stages of development of cataract and in different types of cataract, and with these considerations in mind the method is convenient for assessing activity of anticataract agents.

The kinetic curve of death of UV-induced radicals extracted from normal lenses is shown in Fig. 3. The kinetic curve for lenses with cataract is exactly the same in appearance. The course of the curve is determined by two reactions. Reaction 1 ends after 5 min, reaction 2 continues for more than 1 h. About 10% of the original free radicals die in the slow reaction. It was shown in [8] that for any protein the fraction of radicals involved in the slow death reaction is always equal to the fraction of the peptide chain which is in an irregular form. Thus about 90% of the peptide chain of water-soluble proteins extracted from normal lenses and lenses with cataract is in a spatially-regular, and evidently α-helical form.

LITERATURE CITED

1. I. L. Golenza, Vopr. Med. Khim., **16**, No. 5, 498 (1970).
2. K. M. L'vov and B. M. Bekmurzaev, Biofizika, **35**, No. 3, 421 (1990).
3. J. Bours, Curr. Eye Res., **3**, 691 (1984).
4. Z. Dischee, Biochemistry of the Eye, Basel (1968), pp. 413-428.
5. R. Itzhaki and D. M. Gill, Analyt. Biochem., **9**, 401 (1964).
6. A. Kamel and J. Horwitz, Invest. Ophthal., **26**, 229 (1985).
7. K. M. L'vov and Yu. A. Kim, Biopolymers, **14**, 83 (1975).
8. G. J. van Kamp and H. J. Hoenders, Exp. Eye Res., **17**, 417 (1973).
9. B. J. Ortwerth, P. R. Olesen, and Krishna Sharma, Exp. Eye Res., **43**, 955 (1986).
10. M. A. Riflelaar and H. Bloemendal, Exp. Eye Res., **29**, 679 (1979).
11. D. Roy and A. Spector, Exp. Eye Res., **26**, 429 (1978).