10. S. K. Clarren and D. M. Bowden, J. Pediat., **101**, No. 5, 819 (1982).
11. S. K. Clarren and D. M. Bowden, Mechanisms of Alcohol Damage in Utero: Ciba Foundation Symposium, London (1984), pp. 157-169.
12. H. M. Murray, L. C. Saland, and E. Reys, Anat. Rec., **199**, No. 3, 178 (1981).
13. C. K. Petito and W. A. Pulsinelli, J. Cerebral Blood Flow Metab., **4**, No. 2, 194 (1984).
14. P. Wainwright and G. Fritz, Exp. Neurol., **89**, No. 1, 237 (1985).

QUENCHING OF LENS PROTEIN FLUORESCENCE IN THE EARLY STAGES OF HEREDITARY CATARACT

M. T. Aitmagambetov, A. I. Deev, E. R. Kostenko, and Yu. A. Vladimirov

UDC 617.741-004.1-055.5/.7-07:617.741-008.939.6-074:593.426

KEY WORDS: cataract; lens; proliferation

Mice of the 10R/Hab strain are distinguished by the fact that they develop a cortical cataract spontaneously at about 2 months of age. Morphologically the cataract in this strain is manifested in two forms: there is either degeneration of the lens fibers followed by their calcification or proliferation of the epithelium of the lens, when the fibers lose their hexagonal shape and are converted into large, ovoid formations [1].

The aim of this investigation was to study possible differences in the physicochemical characteristics of the lens in 10R/Hab mice at the stage preceding the development of opacities, from other strains of mice of the same age, not developing hereditary cataract.

EXPERIMENTAL METHOD

10R/Hab mice were obtained from the collection pool of the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, having been supplied by the National Institute of Oncology and Radiology, Cuba, in 1985, and currently at the 50th inbreeding. Male and female mice aged 4, 6, and 8 weeks, on examination of which with the aid of the SHL-25 slit lamp, no cataract was found, were used in the experiments. For comparison, CBA/J mice from the collection pool of the above-mentioned laboratory, CBA/Lac mice from the Institute of Chemical Physics, Academy of Sciences of the USSR, and (CBA × C57BL/6)F_1 hybrids aged 8 weeks were used for comparison.

The lenses were taken from the animals immediately after decapitation, and if required for investigation the nucleus was separated from the cortex [3]. The capsule was incised and the lens placed in an aliquot (100 μl) of physiological saline containing 0.14 M NaCl, 0.01 M Tris-HCl, pH 7.4, and 1 mM dithiothreitol. The protein concentration was determined by the biuret method [6]. The parameters of quenching of protein fluorescence were determined on a "Hitachi MPF-2" spectrofluorometer in 0.4-ml microcuvettes, the initial solution being diluted 20 times, the protein concentration being 0.1 mg/ml. Quenching parameters were determined by addition of a mixture of 0.5 M solutions of KCl and KNO_3 to the solution, the KNO_3 concentration being varied from 0 to 0.5 M. Fluorescence was excited at 262 nm, i.e., in the region of minimal absorption of KNO_3, allowing for screening, as described previously [2], in accordance with the equation:

$$F = \frac{F_{meas}}{1 - 1.68\,C},$$

N. I. Pirogov Second Moscow Medical Institute. Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 551-553, May, 1991. Original article submitted April 6, 1990.

0007-4888/91/0005-0721$12.50

TABLE 1. GSH and TSH Content and Quenching Parameters of Fluorescence of Lenticular Cortical Homogenates from Different Strains of Mice

Strain of mice	Age of mice, weeks	GSH, mM	TSH, mM	K_a	f_a
CBA/J	8	2,2	68,0	1,4	0,54
CBA/J	12	2,5	59,0	1,6	0,52
F_1 (CBA·C57Bl/6)	8	8,8	98,0	6,6	0,25
CBA	8	6,3	100,8	9,8	0,25
10R/Hab	4	1,9	46,3	10,4	0,30
10R/Hab	6	2,1	58,0	19,4	0,22
10R/Hab	8	1,8	54,7	32,3	0,23

Legend. K_Q) Quenching constant, M^{-1}, f_a) accessibility of fluorophores for quencher.

where F_{meas} is the intensity of fluorescence measured at 335 nm, F denotes fluorescence with a correction for screening of the exciting light, and C denotes the KNO_3 concentration (in M).

Accessibility and the quenching constant of the fluorophores were determined by a modified Stern—Volmer equation [5]:

$$\frac{F_0}{F_0 - F} = \frac{1}{f_a K C} + \frac{1}{f_a},$$

where F_0 denotes fluorescence of fluorophores in the absence of quenching, K is the Stern—Volmer quenching constant, C the concentration of the quencher, and f_a the fraction of fluorophores accessible for the quencher.

The value of $1/f_a$ is determined on the graph of $F_0/F - F$ versus 1/C as the segment intersected on the ordinate (extrapolation to an infinitely large concentration of quencher), and the value of $1/f_aK$ by the slope of the straight line.

The concentration of reduced glutathione (GSH) and the total content of thiol groups (TSH) were determined with the aid of Ellman's reagent by the method in [2]. The content of GSH and TSH was expressed per milligram wet weight of lens.

EXPERIMENTAL RESULTS

Lowering of the glutathione level is known to be an early sign of cataract formation. In mice of different strains but of the same age (8 weeks) the GSH content differed significantly (Table 1). However, in mice of the 10R/Hab strain the glutathione content was close to the corresponding values for the CBA/J strain, in which cataract was found not more often than in the other strains. Moreover, in 10R/Hab mice aged 8 weeks no significant change was observed in levels of reduced glutathione and total thiols.

Comparative analysis of conformations of the cortical crystallins was carried out by the method of quenching of protein fluorescence by nitrate anions (Fig. 1). Accessibility of protein fluorophores for the quencher (f_a), measured by this method, enables the degree of exposure of fluorophores into the aqueous phase to be judged. As the data show, high values of f_a were observed in CBA/J mice, whereas the value of f_a was similar in all the other strains tested. It follows from the results that constants of quenching of protein fluorophores by nitrate anions increase significantly in 10R/Hab mice with age, to reach 32.3 M^{-1} by 8 weeks. Measurement of the quenching constant of free L-tryptophan under these conditions gave a value of 27 M^{-1}, close to the value of 30^{-1} obtained previously at pH 6.0 in [4].

The value of the quenching constant of protein fluorophores in lenses from different strains of mice except 10R/Hab at the age of 8 weeks are thus lower than values of the quenching constant corresponding to neutrally charged tryptophan. This reduction of the quenching constant may be due both to steric factors and to the presence of a negative charge on the surface of the protein. If the second of these alternatives is adopted, the most likely candidate for the role of groups with a high negative charge phosphates. Recently, cAMP-dependent phosphoryllation of the principal lens protein (α-crystallin) relative to serine residues [9], has been found [7, 8]. It was discovered that in human lenses affected by

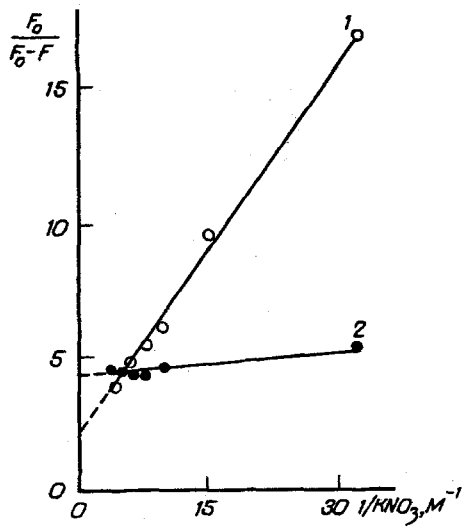

Fig. 1. Typical curves of quenching of fluorescence of protein fluorophores of lenticular cortical homogenates from CBA/J (1) and 10R/Hab mice (2). Protein concentration 0.1 mg/ml. Medium: 0.14 M NaCl, 0.5 M KCl + KNO_3, 0.01 M Tris-HCl, pH 7.4. Age of mice 8 weeks.

cataract the level of phosphoryl ation is significantly depressed, whereas in lenses of healthy subjects with no sign of cataracts, and between 30 and 60 years of age, the level of phosphoryllation is not depressed [9]. It can be tentatively suggested that phosphoryl ation of proteins maintaining a high negative charge prevents their aggregation, which is one cause of opacity.

It can accordingly be postulated that the progressive increase in the quenching constant of protein fluorescence produced by nitrate in 10R/Hab mice is connected with a decrease in the density of negative charges on the crystallins, caused by depression of their phosphoryl ation.

LITERATURE CITED

1. E. Kh. Abdrashitova, R. Castillo, and M. Puig, Current Problems in Standardization of Laboratory Animals for Medico-Biological Research [in Russian], Part 2, Moscow (1988), pp. 100-101.
2. M. T. Aitmagambetov, A. I. Deev, A. N. Kabachenko, and Yu. A. Vladimirov, Byull. Éksp. Biol. Med., No. 3, 347 (1989).
3. T. V. Birich, N. P. Poznyak, and I. M. Bateva, Oftal'mol. Zh., No. 7, 388 (1985).
4. E. A. Burshtein, Progress in Science and Technology. Series: Biophysics [in Russian], Vol. 7, Moscow (1977), pp. 1-84.
5. J. R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum, New York (1983).
6. A. Darbre (Ed. 3, Practical Protein Chemistry, Wiley, New York (1986).
7. R. Chiesa, M. A. Gavinowich-Kolks, N. J. Kleiman, and A. Spector, Exp. Eye Res., **46**, 199 (1988).
8. A. Spector, R. Chiesa, J. Sredy, and W. Gorner, Proc. Nat. Acad. Sci. USA, **82**, 4712 (1985).
9. J. Sredy and A. Spector, Exp. Eye Res., **39**, No. 5, 653 (1984).