INCREASED ACCESSIBILITY OF MOUSE LENS PROTEIN FLUOROPHORES DURING RADIATION CATARACT DEVELOPMENT

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One of the most important structural changes in the lens during cataract development is oxidation of aromatic and sulfur-containing amino acids of the lens proteins (crystallins) and the formation of macromolecular aggregates on account of disulfide cross-linkages, such aggregates being regarded as one of the chief causes of the increased scattering of light in the lens. It has been suggested that chemical modification of the amino acids of crystallins is connected with the unrolling of protein globules in the initial stages of cataract formation [9]. However, the formation of intramolecular disulfide and bityrosine cross-linkages, on the other hand, would be more likely to lead to a more compact arrangement of the protein globules. To determine the trend of structural changes in crystallins during cataract formation, in the investigation described below accessibility of the basic protein fluorophores, namely tryptophanyls, for water molecules was assessed; the analysis was based on the effect of increased fluorescence of tryptophanyls during replacement of H_2O by D_2O [5].

EXPERIMENTAL METHOD

A cataract induced by total γ-irradiation of (CBA × C57BL/6)F_1 hybrid mice in a sublethal dose (5 Gy) was used as the model [2]. At a certain stage of cataract development the animals were killed by decapitation and the lenses extracted and homogenized in 0.14 M NaCl − 0.01 M Tris-HCl buffer, pH 7.4, using 3 ml of buffer solution per lens. The protein concentration in the homogenates, determined by the biuret method [7], was 0.4-0.5 mg/ml depending on the weight of the lens The homogenate was mixed with the corresponding buffer solution in the ratio of 1:3. Two buffer solutions were used in the work: 0.01 M Tris-HCl in H_2O and D_2O (95%), the pH and pD values of these solutions being identical, at 7.4. The pD of the solutions was measured by the equation $pD = pH_{meas} + 0.4$, where pH_{meas} denotes pH values measured by a glass electrode in D_2O solutions [6]. The final concentration of D_2O in the samples with heavy water was thus 70%. Fluorescence was recorded on a Hitachi MPF-4 spectrofluorometer in microcuvettes (diameter 4 mm). Fluorescence was excited at 295 nm and recorded at 335 nm.

EXPERIMENTAL RESULTS

The criterion of accessibility of the tryptophanyls used was the ratio between intensities of fluorescence at the peak of the emission spectrum in the sample with H_2O (F_{H_2O}) and 70% D_2O (F_{D_2O}). During cataract development, the ratio F_{D_2O}/F_{H_2O} increased (Table 1), evidence of increased accessibility of the tryptophanyls for water.

The fraction of tryptophanyls accessible for D_2O can be estimated by using the following approximate model. As a first approximation trytophanyls can be divided into two groups: those completely accessible for D_2O (their fraction may be called α) and those completely inaccessible for D_2O (their fraction will be $1 - \alpha$). Let us also assume that dependence of the intensity of fluorescence on D_2O for tryptophanyls of group 1 is the same as for free tryptophan. It was shown previously than an increase in the fluorescence of L,D-tryptophan (y) as a function of the D_2O fraction by volume is described by the empirical equation [1]:

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TABLE 1. Effect of D_2O on Fluorescence of Mouse Lens Homogenates at different Stages of Development of Radiation Cataract (M ± m)

Stage of radiation cataract	F_{D_2O}/F_{H_2O}	α
Transparent lenses of unirradiated animals (control) (16)	1,10±0,03	0,14
Formation of discrete punctate opacities in the lens (16)	1,13±0,02	0,18
Puncatate opacities merged to form small opaque disk (16)	1,17±0,04	0,24
Disk enlarged, with rays running from it to the periphery (16)	1,30±0,07	0,28

Legend. Number of lenses given in parentheses.

$$y = I_{D_2O}/I_{H_2O} = 1{,}13X^{1{,}22} + 1,$$

where I_{D_2O} and I_{H_2O} denote the intensity of fluorescence in the corresponding 0.01 M Tris-HCl (pH, pD = 7.4)-buffer of D_2O (100%) and H_2O (100%) respectively (excitation 295 nm, emission 335 nm); X denotes the D_2O fraction by volume.

The equation thus obtained shows clearly that on complete replacement of H_2O by D_2O the fluorescence of tryptophan is increased by 2.13 times, which is virtually the same as was obtained in [4], when at pH 6.0, the ratio of the quantum yield in D_2O to the quantum yield in H_2O was found to be 2.10, with an accuracy of ±10%. In the case of proteins whose tryptophanyls can be divided into those completely accessible (fraction a) and those completely inaccessible for water and D_2O, the increase in fluorescence of protein in D_2O (y_{pro}) will evidently be:

$$y_{pro} = \alpha(1.1x^{1.22} + 1) + (1 - \alpha),$$

from which it is possible to calculate α, knowing the value of y_{pro} from the experiment:

$$\alpha = (y_{pro} - 1)/1.1x^{1.22}.$$

for the case of 70% D_2O (x = 0.7) we have $\alpha = (y_{pro} - 1)/0.718$. The results also are given in Table 1. They show clearly that in the initial stage of cataract formation there is a marked increase in the accessibility of the tryptophanyls for water. The increase in the number of groups in the protein exposed to water may in turn lead to an increase in the oncotic pressure within the lens cells, which, as we know, contain a high concentration of proteins (35% of the wet weight). In this connection it is interesting to note that the initial stages of cataract development are accompanied by swelling of the fibrous cells of the lens [8]. In the case of human senile cataract some increase (by 13%) in the H_2O content in lenses at the stage of the initial and unripe cataract also was found by the NMR-spin echo method [3].

The increase in accessibility of tryptophanyls for water observed during cataract development is evidence that the early stages of this process are accompanied by unrolling of the proteins and the formation of high-molecular-weight aggregates, which scatter light, from these proteins. The possibility likewise cannot be ruled out that the accessibility of trytophanyls for water may be enhanced by proteolysis, for we know that during cataract development high-molecular-weight protein aggregates resistant to the action of proteases are formed [10].

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PANCREATIC TISSUE GLUTAMATE DEHYDROGENASE ACTIVITY IN ACUTE EXPERIMENTAL PANCREATITIS AND ITS RESPONSE TO SODIUM THIOSULFATE

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The study of glutamate dehydrogenase (GDH; EC 1.4.1.3) is of great interest in connection with the central role of this enzyme in amino acid and nitrogen metabolism, and also with the high biosynthetic activity of the pancreas. The study of GDH activity under the influence of sodium thiosulfate, a substance whose high therapeutic efficacy is documented by morphological and functional studies, is likewise of great importance. The intracellular localization of GDH and the character of its changes in foci of reactive inflammation and tissue destruction is another interesting topic.

GDH synthesis is known to take place on microsomal membranes, connections with which are effected through phosphatidylserine; it subsequently undergoes intracellular migration and is located in the mitochondria; molecules of cardiolipin, moreover, facilitate its transport through the membrane into the mitochondrial matrix [5].

The aim of this investigation was to study GDH activity and its subcellular distribution in different segments of the pancreas under normal conditions, in acute experimental subtotal pancreatic necrosis, and after administration of sodium thiosulfate.

EXPERIMENTAL METHOD

Experiments were carried out on 220 rats with a body weight of 200-250 g. The animals were divided into the following groups: control, mock operation (laparotomy + general anesthesia), with acute pancreatitis, and receiving or not receiving sodium thiosulfate. Acute hemorrhagic (subtotal) pancreatic necrosis was induced by cooling the splenic segment of the pancreas with ethyl chloride to −30°C [2]. Sodium thiosulfate was injected into the peritoneal cavity during the operation in the form of a 30% aqueous solution, after which a 1.5% solution was given instead of drinking water at the rate of 25 mg/100 g body weight. Splenic (subjected to direct injury) and duodenal (affected by reactive inflammation) segments of the pancreas were studied separately 3, 24, and 72 h and 7, 14, and 30 days after establishment of the disease. Enzyme activity was determined in mitochondrial (MCh) and microsomal (MS) fractions of pancreatic tissue obtained by differential centrifugation of a 10% tissue homogenate in 0.1 M sodium-phosphate buffer (pH 7.8) at 12,000*g* and 105,000*g* respectively; the supramicrosomal (cytosolic, CS fraction) also was studied. Enzyme activity was determined [6] in two directions: in the reaction of oxidative deamination of

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