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Use of a cyanine dye as a probe for albumin and collagen in the extracellular matrix

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Abstract

The aim of this work was to develop a quick method for analysis of macromolecules of the extracellular matrix. Of great interest are soluble components of the extracellular matrix, in particular, carrier proteins, whose variation dynamics can characterize the studied tissue in its development, adult stage, and aging. We suggest the method of analysis of the extracellular matrix to reveal the presence of albumin and collagen by using an anionic cyanine dye as a spectral and fluorescence probe. The method was applied for the analysis of the human vitreous body in the course of its development. Albumin was detected by the appearance of the *trans* monomer absorption and fluorescence bands in the dye spectra, and collagen was detected by the absorption and fluorescence bands of J aggregates. Hyaluronic acid present in the vitreous body does not interfere with the results of the analysis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blot analysis confirmed the presence of albumin in the vitreous body. We suppose that albumin as a protein carrying biologically active macromolecules plays an important role in the processes of differentiation and functional establishment of ocular tissues in the course of their prenatal development.

Keywords: Spectral and fluorescent probe; Cyanine dye; Vitreous body; Albumin; Collagen; SDS–PAGE; Western blot analysis

Studying structural components and soluble proteins of the extracellular matrix is important for characterizing tissues of organisms. To analyze the composition of the extracellular matrix and its variation in the course of the development of an organism, the use of relatively simple and quick methods of detection of the main components of the extracellular medium is of particular importance. Due to the complex composition of the system, the sensitivity and selectivity of the analytical methods applied to the extracellular matrix play large roles.

The vitreous body, which occupies 4/5 of the total volume of the eye, is one of the most important transparent media of organisms [1,2]. Development of the vitreous body of the eye of vertebrates including humans is characterized by significant morphological and biochemical changes [3–5]. At present, the chronology of the appearance and the localization of components of the vitreous body such as hyaluronic acid, glycosaminoglycans, and collagens are intensively studied and abundant data have been obtained [6–10]. At the same time, the characteristics of soluble proteins and the dynamics of their appearance in the course of the vitreous body development remain poorly studied. Albumin, the protein carrying biologically important biomacromolecules (hormones, vitamin A, cytokines, and other compounds), is one of the most important soluble proteins of organisms [11]. This was the reason for studying the presence in the vitreous body of the most abundant serum protein, albumin, in the course of embryonic development of the vitreous body and the dynamics of

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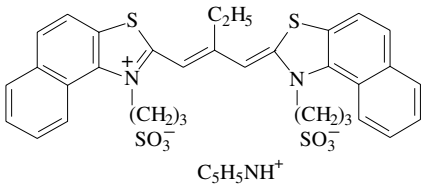
appearance and disappearance of this protein. To analyze the vitreous body, we applied the method of spectral and fluorescent probes. A cyanine dye was used as such a probe, since the photophysical and photochemical properties of cyanine dyes are known to depend on the properties of the surrounding medium [12,13].

We showed earlier that one of cyanine dyes, 3,3′-di(γ-sulfopropyl)-4,5,4′,5′-dibenzo-9-ethylthiacarbocyanine betaine pyridinium salt (DEC), possesses an ability to interact selectively with both collagens, structural proteins of a connective tissue, and albumin, the carrier protein of human serum [14,15].



DEC

We used this unique feature of DEC in the study of the human vitreous body at different stages of its prenatal development.

Materials and methods

Material and tissue preparation

The human vitreous body was studied at the stages from 9.5 to 27/28 weeks of gestation. Eyes were enucleated from human fetuses after legal abortions, which were performed because of social medical indications and obtained by the Research Centre of Obstetrics, Gynecology and Perinatology of the Russian Academy of Medical Sciences, with approval of the Scientific Council, from the licensed institutions of the Ministry of Public Health of the Russian Federation. The age of fetuses was determined by an obstetrician. Under an MBS-9 binocular microscope, the cornea was cut over the limbus, and the vitreous body was taken together with the lens. Then the lens was removed carefully, and the vitreous body was cleaned from residuals of the retina. A small portion of the liquid fraction of the vitreous body was lost upon its extraction. The samples prepared could be kept at −20 °C until the study was performed. For one measurement (spectroscopic, SDS–PAGE, or Western blot analysis), we used samples of the vitreous body of one to four human eyes, depending on eye size.

Spectroscopic analysis

The anionic cyanine dye, 3,3′-di(γ-sulfopropyl)-4,5,4′,5′-dibenzo-9-ethylthiacarbocyanine betaine pyridinium salt (provided by the Niikhimfotoproekt Research Center, Moscow, Russia), which efficiently interacts with albumin and collagens [14–16], was used as a probe. The absorption spectrum of DEC in aqueous solution at a low concentration (1×10^{-6} to 5×10^{-6} M) represents a band with $\lambda_{max}=535$ nm and a shoulder at ~570 nm accounted for by the light absorption by the dimers and *cis* monomers of the dye, respectively, which are in equilibrium [17] (Fig. 1, curve 1). Neither *cis* monomers nor dimers of DEC fluoresce. When we added albumin to the solution, the intensity of the original absorption spectrum of the dye decreased and the band of the *trans* monomer bound to albumin appeared with $\lambda_{max}=612$ nm (Fig. 1, curve 2). The *trans* monomer is formed as a result of *cis–trans* isomerization when DEC interacts with strongly binding sites of albumin; this monomer is characterized by intense fluorescence [14]. The addition of collagens to the DEC aqueous solution causes J aggregation of the dye on collagens resulting in a long-wavelength absorption band of J aggregates ($\lambda_{max}=642$–650 nm) with relatively weak fluorescence [15]. DEC J aggregates are formed from DEC dimers by noncovalent binding of a few dimeric units to one another following the "head-to-tail" pattern. Their absorption spectra are shifted to the red side with respect to the spectrum of the initial dye. The collagen molecule serves as a template for ordering the dye units in the formation of J aggregates. We used the phenomena described above to detect albumin and collagen in the vitreous body of the developing human eye.

Absorption spectra were measured in a 1-cm cuvette on a Shimadzu UV-3101PC spectrophotometer, and fluorescence and fluorescence excitation spectra were measured on an Aminco–Bowman spectrofluorimeter. To avoid the reabsorption and inner filter effects [18], the absorbance of solutions used in spectrofluorimetric measurements was below 0.1. The native vitreous body of the eye was introduced into the aqueous solution of DEC (0.7×10^{-6} to 1.3×10^{-6} M, which corresponded in many cases to an

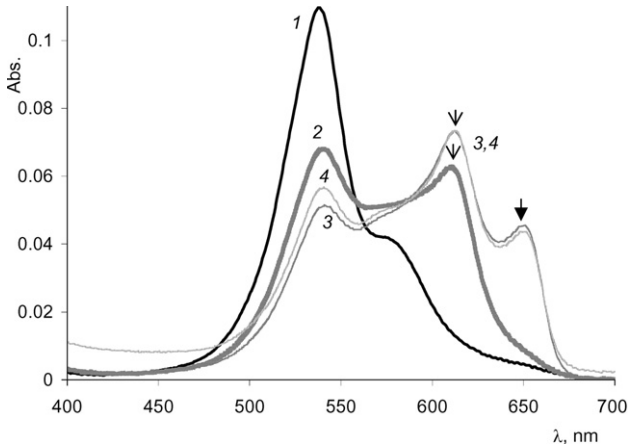


Fig. 1. Absorption spectra of aqueous solutions of DEC (1.6×10^{-6} M) (1) in the absence and (2–4) in the presence of (2) human serum albumin (5.8×10^{-7} M), (3) human serum albumin (5.8×10^{-7} M) + collagen type I (0.45 mg/L), and (4) human serum albumin (5.8×10^{-7} M) + collagen type I (0.45 mg/L) + hyaluronic acid (1.4 g/L). Bold arrow marks the J aggregate band, and fine arrows mark the *trans* monomer bands.