A Polyacrylamide-Gel Electrophoretic Study of Human Tear Proteins

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Abstract. The protein composition of normal and pathological tears was studied by polyacrylamide-gel (disc) electrophoresis. Polyacrylamide-gel electrophoresis was shown to detect at least 14 fractions in 2–10 µl of native tears. A comparison was made between the protein composition of normal tears and serum. The most characteristic bands in the tear-protein pattern were identified by parallel electrophoresis of tears, serum, human milk and purified egg-white lysozyme. The identified fractions were specific tear prealbumin, serum albumin, transferrin, lactoferrin and lysozyme. An unidentified major tear-protein component was also described. The tear-protein pattern was divided into six zones: (1) prealbumin zone; (2) post-albumin zone; (3) post-transferrin zone; (4) macroglobulin zone; (5) basic globulin zone; (6) prelactoferrin zone. A significant rise in transferrin-Zone, and post-transferrin zone, was observed in tears from cases of acute catarrhal conjunctivitis. The optimal circumstances were discussed under which major and minor tear components and basic and acidic tear proteins can be determined simultaneously. Polyacrylamide-gel electrophoresis is recommended as a useful method to study the various diseases of the anterior segment of the eye.


Introduction

Human tears are a mixture of the secretory products of the main and accessory lacrimal glands, the conjunctival goblet cells and the Moll's, Zeis and meibomian glands. Tears are an aqueous solution of various electrolytes, low molecular-weight organic compounds (such as glucose, urea, amino acids and lipids) and contain a number of different proteins (Tapasztó 1973, 1976; Gachon et al. 1979).

Tear proteins have always been in the focus of general interest, being involved in various diseases of the outer eye. The basic aim of all tear-protein investigation is to get information about the processes in the anterior segment of the eye under both normal and pathological conditions. Changes in tear-protein composition occur in the diseases of the lacrimal gland, as well as in the inflammatory, allergic and degenerative processes of the cornea and the conjunctiva. Tear-protein determinations are of diagnostic value in the dry-eye syndromes, especially in the diagnosis and differential diagnosis of Sjögren's and Stevens-Johnson syndromes. The detection of immunoglobulins and other immunologically active substances may be useful in the study of local immunological reactions following corneal transplantation (Krause 1959; Tapasztó 1973, 1976; Liotet et al. 1980).

Tear-protein composition was determined by different types of electrophoresis (paper, agar, agarose and cellulose-acetate membrane electrophoresis) under normal and various pathological conditions (Krause 1959; Francois and Rabaei 1960; Tapasztó and Vass 1965; Tapasztó 1973; Liotet 1979). Relatively large samples (30–300 µl) are needed for the generally used electrophoretic methods; much more than the normal tear volume of the eye. Another disadvantage is that the sensitivity of these methods is not sufficient. A number of minor tear-protein components of clinical importance are rarely, or never detected by conventional electrophoretic procedures.

Polyacrylamide-gel electrophoresis is a highly sensitive microanalytical method generally used in the investigation of small samples of dilute and complex protein solutions.
Fig. 1a, b. Polyacrylamide-gel electrophoretic pattern of normal tears a in the electrophoretic system of Ornstein and Davis and b in the system of Reisfeld.

Fig. 2a, b. Polyacrylamide-gel electrophoretic patterns of a 10 μl ten-fold diluted normal serum and b 10 μl normal tears (Ornstein-Davis system, 7% acrylamide gel, TRIS-glycine buffer pH 8.9, stained by Coomassie Brilliant Blue). Abbreviations: A, albumin; T, transferrin; H, haptoglobins; STP, specific tear prealbumin; U, unidentified major tear protein.

Fig. 3a–c. Polyacrylamide-gel electrophoretic patterns of a 10 μl 0.1% solution of purified egg-white lysozyme, b 10 μl native tears, and c 10 μl native human milk (Reisfeld system, 7% acrylamide gel β-alanine-acetic acid buffer pH 4.5, stained by Coomassie Brilliant Blue). Abbreviations: LYS, lysozyme; L, lactoferrin.

(Maurer 1971). It has been shown to be especially suitable for study of the protein composition of various ocular fluids such as tears, aqueous humor and subretinal fluid (Mukai 1968; Lang et al. 1975; Riebel et al. 1975; Tapasztó 1976).

The aims of this study were as follows: (1) to determine the optimal volume and protein content of tear samples for polyacrylamide-gel electrophoresis; (2) to find the optimal operational circumstances and staining techniques for the study of major and minor tear proteins; (3) to determine the number and nature of bands which can be detected in normal tears; (4) to demonstrate the differences between tear and serum samples with similar protein concentrations; (5) to show the changes in protein pattern caused by the increased permeability of conjunctival vessels; and (6) to identify the most characteristic bands in normal and pathological tear-protein patterns.

Materials and Methods

Tear Samples. Tears were obtained from normal healthy subjects and from patients suffering from different types of conjunctivitis. Following the nasal instillation of a drop of 80% ethanol the lower eyelid of the patient was slightly pulled down, so that the drainage of tears could be stopped through the inferior lacrimal canaliculus. Tears accumulating in the lower conjunctival sac were collected by microcapillary tubes. Tears were centrifuged at 500 g for 10 min so as to eliminate cells. The supernatant was either examined without delay or stored at −20°C for a maximum of 48 h.

Determination of Protein Concentration. The total protein concentration of tears was determined by a modified Lowry procedure (Daughaday et al. 1952). Bovine serum albumin was used as a standard. Serum (obtained from the same patients) was diluted by a physiologic solution of sodium chloride (generally ten-fold) to equal the total protein concentrations of the corresponding tear samples.

Polyacrylamide-Gel (Disc) Electrophoresis. Gels were cast in 150-mm glass tubes with an inner diameter of 5 mm. The spacer gel was 5 mm, and the separation gel 75 mm high. Separation gels contained 7% acrylamide monomer. TRIS-glycine buffer (pH 8.9) was used when gels were prepared according to Ornstein (1964) and Davis (1964), and β-alanine-acetic acid buffer (pH 4.5) when gels were prepared by the method of Reisfeld et al. (1962). Samples of 2–10 μl were mixed with 50 μl 40% saccharose solution and applied directly on the top of the spacer gel. A vertical electrophoretic instrument (type 69, Reanal, Budapest, Hungary) and a DC power supply (type OQ-49, Labor Műszerszopar Művek, Budapest, Hungary) were used for the electrophoresis. Runs were carried out at 2 mA/tube for 30 min and continued at 5 mA/tube for 75 min. As indicators, 5 μl 0.1% aqueous solution of bromphenol blue and 5 μl 0.1% methyl green solution were applied. Purified egg-white lysozyme (grade 1; crystallized, dialyzed and lyophilized three times; Sigma, St. Louis, 110, USA) was used for electrophoresis. All other chemicals were of analytical grade and purchased from Reanal (Budapest, Hungary).

Staining Procedures. Gels shown in Figs. 1–3 were stained by Coomassie Brilliant Blue by the method of Chrambach