ANALYSIS OF THE ULTRASTRUCTURE OF VITROSIN FIBERS ON THE BASIS OF THE POLARIZATION OPTICAL METHOD

by

A. GEDEON MATOLTSY

Department of Dermatology, Harvard Medical School, and Dermatological Laboratories, Massachusetts General Hospital, Boston 14, Massachusetts (U.S.A.)

A previous paper described the isolation and properties of a viscous thixotropic fibrous protein, named vitrosin, of the vitreous body of the eye[4]. In the present study fibers were prepared from vitrosin, and their form and crystalline double refraction were investigated; from these data conclusions were drawn about the shape and molecular structure of the submicroscopic particles of vitrosin.

Preparation of vitrosin fibers

Vitrosin was prepared from the vitreous body of 50 fresh cattle eyes according to a method described in a previous paper[4]. The precipitated vitrosin was dialyzed for 48 hours against distilled water at 5° C. After this, fibers were pulled from the sticky precipitate with the help of glass hooks. The fibers were placed on a celluloid grid and dried in a desiccator at room temperature. Ten fibers were selected, each of which showed intense uniaxial double refraction and measured 80–120 μ in diameter. These fibers were used in the present study.

Determination of form and crystalline double refraction of vitrosin fibers

Both form and crystalline double refraction of vitrosin fibers were determined according to AMBRONN's immersion technique[1]. The fibers were immersed in various solutions of different refractive indices, *viz.*: water: 1.33, ethanol: 1.36, chloroform: 1.44, *o*-xylene: 1.50, and aniline: 1.58. After a 24-hour immersion, the double refraction was determined with a Leitz polarization microscope. The retardation ($\Delta\lambda$) was measured with the Berek compensator in monochromatic light of 540 mμ wave length. Thickness (d) was measured by focusing the microscope first on the top, then on the bottom of the fiber, and reading the difference on the micrometer scale. The double refraction ($n_{\parallel} - n_{\perp}$) was calculated from the equation:

$$(n_{\parallel} - n_{\perp}) = \frac{\Delta\lambda}{d}$$

The average values of the double refraction in the various solutions were plotted as ordinates against the respective refractive indices of the solutions (n_2). A hyperbolic curve was obtained, as shown in Fig. 1.

The value of crystalline double refraction of the fiber was derived from the minimum of the curve at about $n_2 = 1.50$. At this point the form double refraction is eliminated because the refractive index of the submicroscopic particles is equal to that of the

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immersion fluid. The residual double refraction is then equal to the crystalline double refraction, which is $14 \cdot 10^{-4}$ (Fig. 1).

By difference, the form double refraction in water is $9 \cdot 10^{-4}$.

Interpretation of the results

The fact that vitrosin fibers show crystalline as well as form double refraction indicates that their submicroscopic particles must have considerable regularity of structure and orientation. According to WIENER's theory[6], the positive form double refraction indicates that the submicroscopic particles are oriented parallel to the fiber's longitudinal axis and have a rodlet form. The rodlet shape of the submicroscopic particles is compatible with the electron microscopic investigation of vitrosin, which showed that vitrosin is made up of long, thin filaments, measuring 200–300 A in width[3,4].

The positive crystalline double refraction indicates that vitrosin particles have a crystalline molecular structure, oriented parallel to their longitudinal axis. In fibrous proteins such a molecular structure usually corresponds to oriented polypeptide chains[5].

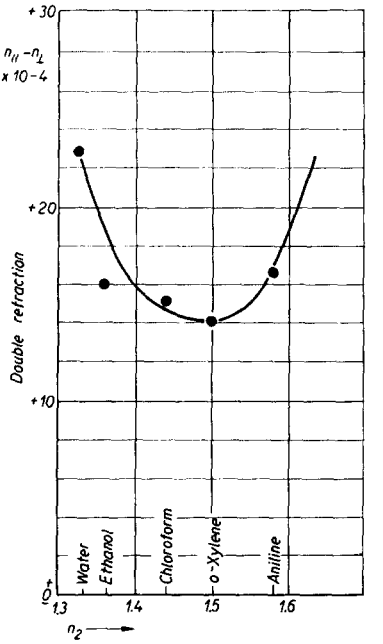

Fig. 1. Rodlet birefringence curve of vitrosin fibers. Abscissa: double refraction. Ordinate: refractive index of immersion fluids.

In biological objects the value of form double refraction is known to be always smaller than that of the crystalline double refraction[2]. The double refraction of vitrosin fibers, observed here, conforms to this rule.

SUMMARY

1. Fibers were prepared from a structural protein (vitrosin) of the vitreous body and their form and crystalline double refraction were determined.

2. The form double refraction was calculated as $9 \cdot 10^{-4}$, the crystalline double refraction as $14 \cdot 10^{-4}$.

3. On the basis of these data, and WIENER's theory, the conclusion was drawn that vitrosin fibers are composed of rodlet-shaped submicroscopic particles, which have a longitudinally oriented molecular structure.

RÉSUMÉ

1. Nous avons préparé des fibres à partir d'une protéine structurale (vitrosine) du corps vitré et nous avons déterminé la double réfraction de forme et la double réfraction cristalline.

2. Pour la double réfraction de forme, le calcul nous a donné une valeur de $9 \cdot 10^{-4}$, pour la double réfraction cristalline, $14 \cdot 10^{-4}$.

3. En nous basant sur ces données et sur la théorie de WIENER, nous concluons que les fibres de vitrosine se composent de particules submicroscopiques à forme de bâtonnets, ayant une structure moléculaire à orientation longitudinale.

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ZUSAMMENFASSUNG

1. Aus einem Strukturprotein (Vitrosin) des Glaskörpers wurden Fasern dargestellt und ihre Form- und Eigendoppelbrechung bestimmt.

2. Die Formdoppelbrechung wurde zu $9 \cdot 10^{-4}$, die Eigendoppelbrechung zu $14 \cdot 10^{-4}$ berechnet.

3. Auf grund dieser Daten und WIENER's Theorie wurde der Schluss gezogen, dass die Vitrosinfasern aus stäbchenförmigen, submikroskopischen Teilchen zusammengesetzt sind, die eine longitudinal orientierte Molekularstruktur haben.

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