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PROTEOLYTIC ACTIVITY OF VITREOUS-HUMOUR

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It has been found that the bovine vitreous-humour did not digest in vitro collagen
at physiological and acidic pH. A proteolytic activity against haemoglobin in acid pH
was found both in bovine and human vitreous-humours. The activity of human
vitreous-humour increased significantly in endophthalmitis and glaucoma. In all
pathological conditions studied the pH optima were at more acidic values than in
control.

Vitreous-humour of the eye contains various proteins at the total
concentration of about 1.5 mg/ml. Both collagens (types II, IX and XI) and
non-collagenous proteins (pre-albumins, albumins, transferrin and immuno-
globulin G) were identified in this fluid [1-3].

The mechanism of enzymatic degradation of vitreous-humour proteins is
not known. Under physiological conditions the collagens are digested only by
specific proteases: collagenses, and collagenolytic cathepsins [4, 5].

Our aim was to study whether vitreous-humour contains a collagenase or
collagenolytic cathepsin and a protease(s) which digest non-collagenous
proteins.

MATERIALS AND METHODS

Bovine vitreous-humour (v.h.) was obtained as described previously [3]
and samples of human v.h. were taken from the material removed during
surgical operations of patients with various eye diseases: tumours (5 cases),
cataract (7), glaucoma (6) and endophthalmitis with retinal detachment (4). For
obvious reasons it was not possible to obtain control material from normal
human eye therefore vitreous-humour from an eye removed because of an
extensive accidental injury served as a control (it was further referred to as
“normal”). The investigated samples were free of pathological material like
blood, pus, etc.

Type I collagen was isolated from rat tail tendons as described by Chung
& Miller [6]. Types II, IX and XI collagens were kindly supplied by Dr. Shirley
Ayad from the University of Manchester (Great Britain). The collagens were dissolved (1 mg/ml) in 0.02 M acetate buffer, 0.05 M CaCl₂, pH 4.8, or in 0.05 M Tris/HCl buffer, 0.05 M CaCl₂, pH 7.4. The vitreous-humours were dialysed against proper buffers. The incubation mixtures containing 0.5 ml of each component were as follows: a, collagen solution and a buffer; b, collagen solution and vitreous-humour; c, a buffer and vitreous-humour. All samples were incubated at 37°C for 4 h and dialysed against 0.01 M phosphate buffer, pH 7.0 at room temperature for 24 h. Then the samples were supplemented with sodium dodecyl sulphate (SDS) and 2-mercaptoethanol to 1% concn. and submitted to electrophoresis on SDS-5% polyacrylamide gel [7].

Proteolytic activity was estimated by the method of Worowski & Roszkowska [8], at pH range 2.5-8.5 with denatured haemoglobin as a substrate. The 4% haemoglobin solution was diluted (1:1) with Britton-Robinson buffer of the desired pH. To 0.4 ml of the substrate 0.1 ml of vitreous-humour was added and the mixture incubated at 37°C for 4 h. The reaction was terminated by the addition of 1.25 ml of 5% trichloroacetic acid. The control sample contained the same constituents but trichloroacetic acid was added at “zero time”. The samples were filtered and tyrosine content in the acid-soluble fraction was determined by the Folin-Ciocalteau method [9].

RESULTS AND DISCUSSION

It was found that bovine vitreous-humour at pH 4.8 and 7.4 did not induce degradation of type I, II, IX and XI collagens. The electrophoretic mobilities of collagens treated with the humour were found to be the same as those of intact substrates. A typical electrophoreogram is presented in Fig. 1.

On the other hand, normal bovine vitreous-humour digested haemoglobin at acidic pH with the optimum at pH 4.0. Similar proteolytic activity was found in “normal” human vitreous-humour (Fig. 2).

Both the proteolytic activity and pH optimum of human vitreous-humour differed largely in various pathological conditions. In the cases of eye tumours and cataract this activity was almost the same as in “normal” material but in glaucoma and endophthalmitis it was, respectively, 2-4 times higher (Fig. 2).

These results allow to conclude that vitreous-humour does not contain any collagenolytic activity either at acidic or at neutral pH, therefore it can not degrade its own collagen. It seems that collagen fibrils present in vitreous-humour undergo phagocytosis by hyalocytes or other cells and are digested in lysosomes.

On the other hand, the vitreous-humour is able to digest other proteins. It seems that the injury of various cells of the diseased eye may result in release of some cathepsins into vitreous-humour, and that these enzymes may participate in further damage of eye tissues.
Fig. 1. The effect of incubation of type I collagen with vitreous humour at pH 7.4. a, Type I collagen and buffer; b, type I collagen and vitreous humour; c, vitreous humour and buffer
Fig. 2. Proteolytic activity of bovine and human vitreous-humours measured at different pH values with denatured haemoglobin as a substrate. Mean values ± SD are presented.

REFERENCES