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THE INFLUENCE OF METAL IONS AND SOME INHIBITORS ON THE ACTIVITY OF THE PROTEINASE ISOLATED FROM THE HATCHING LIQUID OF *COREGONUS PELED* * *

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A specific proteinase (chorionase) was isolated from the hatching liquid, obtained by electric stimulation of the hatching glands in *Coregonus peled* embryos.

The enzymatic preparation was electrophoretically homogeneous, and showed proteolytic activity towards the egg membranes. Ions of Na^+ at concentration from 10^{-5} to 10^{-4} M activated the chorionase of *C. peled*, whereas ions of K^+, Na^+, Ca^{2+} and Mg^{2+} at concentrations above 10^{-4} M inhibited the enzyme.

Addition of EDTA to the incubation mixture significantly reduced the enzyme activity. The chorionase was not inhibited by the natural soybean trypsin inhibitor.

Chorionase is a specific protease produced by the hatching gland cells of fish embryos. The enzyme enables digestion of the inner layer of egg membrane (chorion), facilitating hatching of embryos. As the mechanism of the enzymatic reaction has not been elucidated, the investigations on the isolated preparations of chorionase from different fish species are of special interest [1-4].

The aim of the present study was a preliminary investigation on the effect of metal ions on the activity of purified hatching enzyme of *C. peled*, and determination of the sensitivity of the enzyme's active sites to the action of some inhibitors.

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MATERIALS AND METHODS

Isolation of the enzyme. Chorionase was isolated from the hatching liquid obtained from embryonic hatching glands of *C. peled* by electric stimulation of developing eggs [5]. The enzyme was purified by successive fractionation with ammonium sulfate (60% saturation). Sephadex G-75 gel filtration, and CM-cellulose column chromatography [6].

Effects of some ions and inhibitors. Tests were made using the water solution of the enzyme. In tests with the inhibitors, the enzyme was preincubated at 20°C for 15 min with EDTA, or for 60 min with soybean trypsin inhibitor. Proteolytic activity was assayed with casein by the method of Kunitz [7].

RESULTS

Electrophoretically homogeneous chorionase showed biological activity towards its natural substrate (Fig. 1). Ions of Na^+ activated the enzyme when

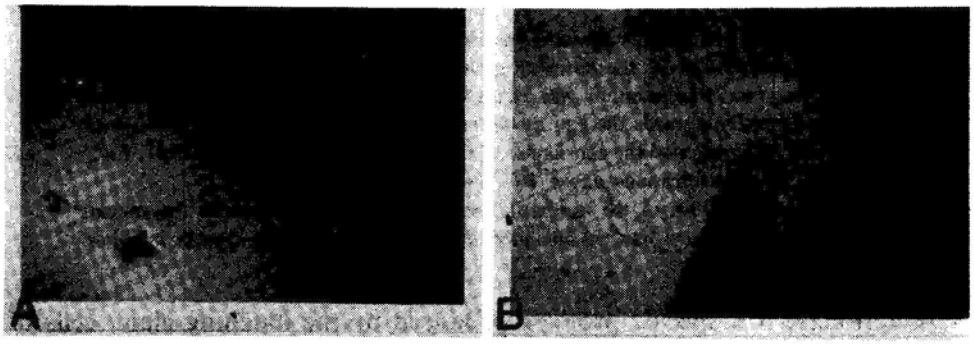

Fig. 1. Digestion of native egg chorions by the isolated hatching enzyme of *C. peled.* Digestion was performed in a medium consisting of 0.17 M Tris/HCl (pH 7.1), 0.2 M NaCl and purified enzyme (0.5 mg/cm^{-3}) for 40 min at 28°C. A, control without enzyme; B, experimental (× 100)

applied at concentrations ranging from 10^{-5} to 10^{-4} M. Both univalent (Na^+, K^+) and divalent ions (Ca^{2+}, Mg^{2+}) at concentrations exceeding 10^{-4} M (Fig. 2) inhibited the hatching proteinase.

Addition to the reaction mixture of EDTA at concentrations higher than 10^{-7} M inhibited the activity of the hatching enzyme. Complete inhibition of the examined proteinase was observed at EDTA concentration of 10^{-3} M (Fig. 3).

DISCUSSION

Fish species differing in their environmental requirements possess chorionases of similar properties, such as pH optima and thermal stability,

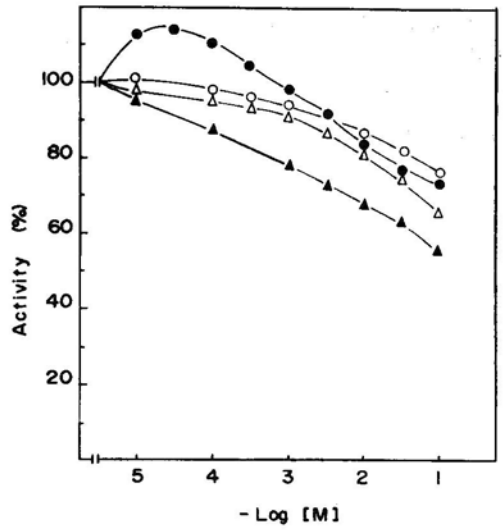

Fig. 2

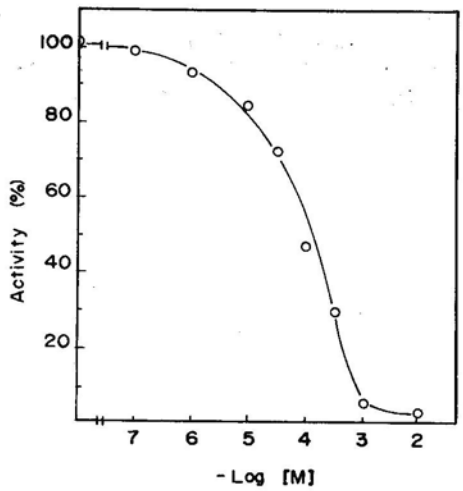

Fig. 3

Fig. 2. Effect of some ions on the proteolytic activity of the isolated hatching enzyme of *C. peled*. The amount of the enzyme was 12 μg per tube. KCl, (○); NaCl, (●); $CaCl_2$ (Δ); $MgCl_2$, (▲)
Fig. 3. Effect of EDTA on the proteolytic activity of the isolated hatching enzyme of *C. peled*. The enzyme (25 $\mu g/cm^{-3}$) was preincubated at 20°C for 15 min with various concentrations of EDTA

which, however, differ in their response to proteinase inhibitors. The chorionase of *C. peled* was inhibited by EDTA (a metalloprotease inhibitor), similarly as was the hatching enzyme of *Salmo gairdneri* [8] and *Oryzias latipes* [4]. On the other hand, the activity of the *C. peled* hatching enzyme was not influenced by soybean trypsin inhibitor. DiMichele & Taylor [1] demonstrated that chorionase of *Fundulus heteroclitus* could be inhibited by phenylmethanesulphonyl fluoride (a serine protease inhibitor) and EDTA. Thus, both a metal and a serine-active site may be involved in the catalysis in the hatching enzyme of *F. heteroclitus* [1]. These differences between the hatching enzymes presumably originate from the varying substrates in which they are encased [8].

Our results concerning the influence of some ions on the proteolytic activity of *C. peled* chorionase correspond to the data of other authors [2, 4]. Thus it may be concluded that the hatching enzyme of teleost fishes is inhibited by higher concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+}.

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