

## SHORT COMMUNICATIONS

STUDY OF THE PROPERTIES OF ALKALINE PHOSPHATASE  
IN *TAENIA CRASSICEPS* (ZEDER, 1800) *CYSTICERCI*

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**Abstract.** The properties of alkaline phosphatase present in *Taenia crassiceps* cysticerci were studied. Approximately a half of the total activity was free and the remaining part was bound to membranes. Kinetic studies did not show any differences between the free and bound alkaline phosphatases. It was found that high substrate concentrations produced an inhibitory effect on the enzyme. This effect was much greater at lower pH. pH optimum changed with the concentration of the substrate.

The activity of alkaline phosphatase (AP) has been studied in many parasitic worms. Sharma (1977) detected histochemically the AP activity in the gut of 12 trematode species. Nizami et al. (1975) studied pH optimum of this enzyme in 8 trematode species. Parshad and Guraya (1978) demonstrated the effect of pH and various anthelmintics on the AP activity in different helminth species. Probert and Lwin (1974) studied kinetic parameters of AP from *Fasciola hepatica*. The location of AP in *Cysticercus bovis* was detected by histochemical methods by Žďárská (1973, 1975, 1976) and Žďárská and Machnicka (1978). As to the significance of AP, it is assumed that it participates in the transport of nutrients, particularly of saccharides, through the cell membrane, since it was found that this enzyme is localized at the sites of active transport (Sharma 1977, Žďárská 1973). Dike and Read (1971) demonstrated in *Hymenolepis diminuta* how the membrane-bound AP participates in the glucose-6-P transport and found that AP catalyzes splitting off of the phosphate which is followed by glucose transport through an independent system. The membrane-bound AP from isolated plasmatic membranes of brush border of *H. diminuta* was studied by Pappas (1982) who compared the properties of this enzyme before and after solubilization.

The distribution and kinetic properties of AP in *Taenia crassiceps* were studied in the present work.

## MATERIAL AND METHODS

Cysticerci of *Taenia crassiceps* were passaged on C 57 BLACK 6 mice at regular 3-month intervals. Six ml of these cysticerci were washed in physiological saline and homogenized in 60 ml of physiological saline for  $2 \times 2$  min using MSE knife homogenizer (8,000 — 10,000 RPM). The homogenate was centrifuged (30 min, 19,200 g, 4 °C) and the supernatant was used for the study of free AP. The sediment was suspended in physiological saline and then centrifuged (19,200, 10 min, 4 °C). The sediment was resuspended in physiological saline and centrifuged (19,200 g, 10 min, 4 °C). Finally the sediment was suspended in physiological saline and the saline was added to obtain the same volume as was that of the original homogenate. This suspension was used for the study of bound AP.

Detection of AP activity: 0.1—0.2 ml of enzyme preparation was added to 1.6—1.7 ml of 0.05 M carbonate buffer, pH 9.6, containing  $1 \cdot 10^{-3}$  M  $MgCl_2$ . The mixture was tempered to 30 °C for 5 min and the reaction was triggered by addition of 0.2 ml of 0.1 M p-nitrophenylphosphate (resulting concentration  $1 \cdot 10^{-2}$  M). After 10-min incubation (it was verified that the reaction speed does not change during this time) at 30 °C the reaction was stopped by the addition of 4 ml of 0.1 M NaOH containing 0.015 M EDTA.  $A_{405}$  was determined at 1 cm optical path against blanks obtained by gradual addition of enzyme preparation and stopping mixture (NaOH + EDTA) in a reverse order. The following modifications were used for the determination of kinetic parameters: For the determina-

tion of pH optimum at  $1 \cdot 10^{-2}$  M substrate concentration, pH of carbonate buffer was changed from 9 to 11. The range of  $1 \cdot 10^{-2}$  M— $5 \cdot 10^{-4}$  M of substrate concentration was used for the determination of  $K_M$ . The effect of substrate concentration on AP activity at pH 7.0 was studied using 0.05 M Tris and the same buffer was also used for the determination of pH optimum at  $2 \cdot 10^{-4}$  M substrate concentration. The effect of cysteine on AP activity was studied using 0.05 M carbonate buffer at pH 9.6. In all experiments the reaction mixture contained  $MgCl_2$  at the concentration of  $1 \cdot 10^{-3}$  M.  $K_M$  was determined by plotting after Lineweaver-Burke. The straight was made by the method of smallest squares using TI-58c computer. Quantitative data for the determination of specific activity were read from the calibration curve obtained from p-nitrophenol solutions in a medium suitable for AP determination.

## RESULTS

The basic properties of AP are shown in Table 1. The behaviour of AP was studied at physiological pH 7.0, i.e. outside pH optimum. The dependence of AP activity on substrate concentration was measured. By contrast with pH 9.6, when the course of the

Table 1. Properties of free and bound AP from *T. crassiceps cysticerci*

| Free AP   | Bound AP                                 |
|---|--|
| Specific activity   |  |
| 0.512 $\mu$ mol substrate/min/mg protein                  | 0.613 $\mu$ mol substrate/min/mg protein |
| pH optimum<br>(0.05 M carbonate buffer, 0.01 M substrate) |  |
| 9.6   | 9.6                                      |
| $K_M$ for p-nitrophenylphosphate, pH 9.6                  |  |
| $3.08 \times 10^{-3}$ M                                   | $3.60 \times 10^{-3}$ M                  |

Table 2. Effect of cysteine on AP activity

| Resulting cysteine concentration<br>in reaction mixture | % inhibition |          |
|---|--------------|----------|
|   | Free AP      | Bound AP |
| $10^{-2}$ M   | 99.1         | 97.8     |
| $10^{-3}$ M   | 98.4         | 97.8     |
| $10^{-4}$ M   | 82.6         | 82.8     |
| $10^{-5}$ M   | 8.65         | 2.0      |
| $10^{-6}$ M   | 0            | 2.0      |
| $10^{-7}$ M   | 0            | 1.3      |
| $10^{-8}$ M   | 0            | 0.3      |

curve of enzyme saturation with substrate was almost normal, at pH 7.0 the activity increased with decreasing substrate concentration and reached the maximum at  $2.10^{-4}$  M concentration of the substrate (inhibition by substrate—Fig. 1). A further decrease of concentration resulted in the decrease in AP activity. The obtained rela-

tionship had a similar course for both bound and free AP. At  $2.10^{-4}$  M substrate concentration, pH optimum was found to be 8.8 for both bound and free AP; consequently, a shift of pH optimum occurred.

The results of the studies of cysteine effect on AP activity are shown in Table 2. The cysteine produced almost the same inhibitory effect on both bound and free AP.

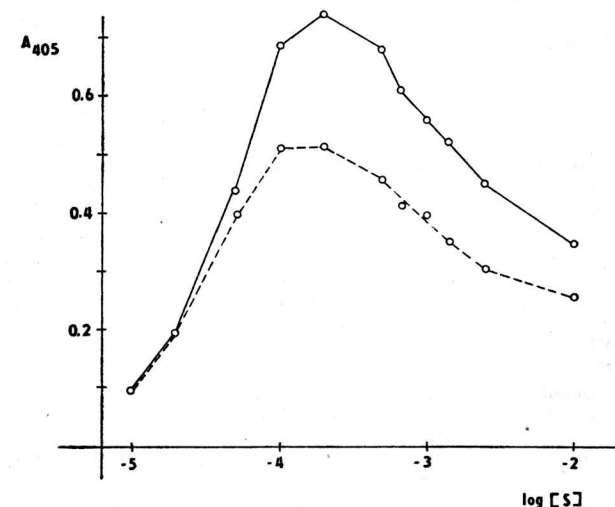


Fig. 1. Dependence of activity AP on concentration of substrate in pH 7.0. Full curve — free AP, dashed curve — fixed AP.

## DISCUSSION

The results indicate that approximately a half of AP activity is free and the remaining part is tightly bound in *Taenia crassiceps cysticerci*. The two AP could not be distinguished on the basis of kinetic and inhibitory studies. Pappas (1982), who studied the AP properties in *H. diminuta*, found a shift of pH optimum associated with the solubilization of AP, but other kinetic parameters remained unchanged after the solubilization.

The observed shift of pH optimum caused by the change of substrate concentration and inhibition by substrate are the phenomena described in AP from higher organisms (Frenley 1971). Both phenomena seem to be interrelated in such a way that the inhibition by substrate is more marked at lower pH values. The mechanism of these phenomena has not been studied in detail and particularly the problem of their physiological significance remains unsolved. It is evident that at lower substrate concentrations, which are awaited in the organism, pH optimum shifts to lower, "more physiological" values and therefore AP can affect these substrates (at these low concentrations the inhibition is small). At the same time, the affinity of AP to the substrates is increased due to the decrease in  $K_M$  (Frenley 1971). The inhibition by the substrate and shift of pH optimum with the change of substrate concentration may play a role in some regulatory mechanisms, but it remains unclear in which of them.

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**Резюме.** Изучали свойства щелочной фосфатазы у цистицерков *Taenia crassiceps*. Приблизительно половина общей активности этого фермента свободна, а другая часть крепко связана с мембранами. При изучении кинетики не нашлись различия между свободной и связанной фосфатазами. Было обнаружено, что высокие концентрации субстрата оказывают ингибирующее действие, которое больше при низких pH, и что pH оптимум, меняется в зависимости от концентрации субстрата.

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