Short Paper

Effect of Substrate Concentration on the Inactivation Velocity of Carp Muscle 5'-Nucleotidase by EDTA^{*1}

In the previous paper,¹⁾ it was found that, in the presence of 5'-AMP and EDTA, the inactivation of carp 5'-nucleotidase proceeded time-dependently according to the following equation;

$$\log V_t/V_o = -k[\text{EDTA}]t \quad (1)$$

where V_o or V_t was enzymatic reaction velocity at an incubation time o or t . It was also found that, with increasing concentration of 5'-AMP, a rate constant k in eq. (1) converged to a smaller value than that observed in the absence of 5'-AMP. In order to account for these experimental results, the present report deals with a kinetic consideration on the inactivation of 5'-nucleotidase by EDTA in the presence of various concentration of 5'-AMP.

The experimental result reported in the previous paper is assumed to be caused by the following mechanisms. i) When the mixture of carp muscle 5'-nucleotidase (E), substrate (S), and EDTA is incubated, the removal of bound metal from E and that from 5'-nucleotidase-substrate complex (ES) should occur time-dependently and respectively. ii) The rate constant (k) of the removal of bound metal by EDTA from E should be different from the rate constant (k_s) of that from ES. iii) The metal-free 5'-nucleotidase thus formed should have no enzymatic activity or be incapable of binding with substrate.

Rates of the removal of bound metal, v_o and v_s , in this reaction mixture, can be written as follows;

$$v_o = k_o[E][\text{EDTA}] \quad (2)$$

$$v_s = k_s[ES][\text{EDTA}] \quad (3)$$

It is reasonable to suppose from the previous experimental result that k_o is larger than k_s . Then if the removal of bound metal proceeds followed only by eq. (2) and (3), $[E]/[ES]$ may gradually decrease. In this reaction mixture, however, following equation must simultaneously be satisfied;

$$[E][S]/[ES] = K_m \quad (4)$$

where K_m is the Michaelis constant. The decrease of $[E]/[ES]$ should be compensated by the dissociation of ES to E and S. The change of $[E]$ and $[ES]$ in a minute time, dt , should be expressed as follows;

$$d[E] = -k_o[\text{EDTA}][E]dt + \alpha \quad (5)$$

$$d[ES] = -k_s[\text{EDTA}][ES]dt - \alpha \quad (6)$$

where α is the change of $[E]$ in dt by the dissociation of ES.

By rearranging eqs. (4), (5), and (6) for $[ES]$, the following equation is obtained;

$$\begin{aligned} d[ES]/[ES] &= -\{k_o + (k_s - k_o)/(1 + K_m/[S])\}[\text{EDTA}]dt \\ \text{and the integrated form of this equation is} \\ \log [ES_t]/[ES_o] &= -\{k_o + (k_s - k_o)/(1 + K_m/[S])\}[\text{EDTA}]t \quad (7) \end{aligned}$$

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^{*1} EDTA によるコイ 筋肉 5'-ヌクレオチダーゼ不活性化に及ぼす基質濃度の影響.

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Fig. 1. Effect of 5'-AMP concentration on the rate constant k in the inactivation of carp muscle 5'-nucleotidase by EDTA.

The change in absorbance at 260 nm was continuously recorded during the incubation at 20°C of reaction mixture which contained various concentration of 5'-AMP, 1 mM EDTA, 5'-nucleotidase,²⁾ and an excess of adenosine deaminase. From the slopes on the recorded curve at various incubation times or enzymatic reaction velocities, k in eq. (1) was calculated. A K_m value of 9.3×10^{-5} M³⁾ was used.

where $[ES_o]$ or $[ES_t]$ is concentration of ES at an incubation time o or t . As the 5'-nucleotidase reaction velocity is proportional to $[ES]$, eq. (7) is rewritten as follow;

$$\begin{aligned} \log V_t/V_o &= -\{k_o + (k_s - k_o)/(1 + K_m/[S])\}[\text{EDTA}]t \quad (8) \end{aligned}$$

On referring to eqs. (1) and (8), the following equation is obtained;

$$k = k_o + (k_s - k_o)/(1 + K_m/[S]) \quad (9)$$

In order to verify this equation, k values obtained in the previous paper were plotted against $(1 + K_m/[S])^{-1}$. As shown in Fig. 1, a straight line was obtained, and values of k_o and k_s were read to be 41 and 21 $\text{min}^{-1} \cdot \text{M}^{-1}$ respectively.

This figure shows that the inactivation of 5'-nucleotidase proceeds according to eq. (8) in the presence of EDTA and 5'-AMP, and that k_o is larger than k_s . This indicates that the stability of metal-apoenzyme binding is increased by the formation of enzyme-substrate complex. It is also reasonable to suppose that the metal which is removed by EDTA may bind to apoenzyme at a position, where a conformational change is caused by the formation of enzyme-substrate complex, that is, at a position closely related to enzyme-substrate binding site.

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References

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