SUBSTRATE ACTIVATION IN THE HYDROLYSIS OF L-ALANINE-4-NITROANILIDE CATALYZED BY AMINOPEPTIDASE M

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Received 30 August 1972

1. Materials

Aminopeptidase M, obtained from Röhm GmbH (Darmstadt) with a specific activity of 14.8 I.U. was further purified by repeated gel filtrations up to a specific activity of 60 I.U. L-alanine-4-nitroanilide was purchased from Merck (Darmstadt).

2. Methods

Protein concentrations were determined at 280 nm ($A^{1\%}_{1\,cm}$ = 17.0). The molecular weight of aminopeptidase M used in the calculations was 2.8×10^5. Kinetic measurements were performed as previously described [7].

3. Results and discussion

The effect of substrate concentration (S) on the activity (v) of aminopeptidase M does not comply with the Michaelis formulation of enzyme action over the whole range of substrate concentrations used in the present investigation (fig. 1). The deflection in the $[S]/v$ versus v plot shown in fig. 1 must be regarded as a case of activation by the substrate. Several different types of models have been invoked in the past to describe nonlinear kinetics including those involving interactions between catalytic sites [1, 2] and those with modifier sites separate from the catalytic sites [3–6].

Since there is obviously no apparent interaction of a catalytic site with another site (modifier or catalytic) in the aminopeptidase M catalyzed hydrolysis of L-alanine-4-nitroanilide (fig. 2) the observed phenomenon must be considered as a modification of the mechanism operative at the active site.

Our model (fig. 3) assumes that the free enzyme may exist in two conformations, E and E*.

Both conformations are considered to be present in equilibrium but E is thermodynamically more stable and the dominating conformation in solution. The interconversion process $E \rightleftarrows E^*$ is assumed to be slow. There is no greater affinity of the substrate for one conformational state, and the binding of the substrate will be per se without any effect on conformation.

It is further assumed that the occurrence of the reaction induces a rate limiting conformational change at the active site of the enzyme substrate complex, $ES \rightarrow E^*S$ and that the enzyme is regenerated in the thermodynamically unfavorable conformation E*.

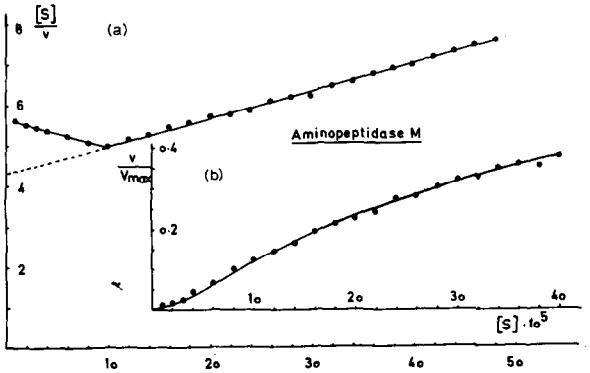

Fig. 1. Nonlinear kinetics in the hydrolysis of L-alanine-4-nitroanilide catalyzed by aminopeptidase M. a) Michaelis plot; b) Hanes plot. Enzyme concentration: 10^{-8} M; pH 8.0, T = 25°.

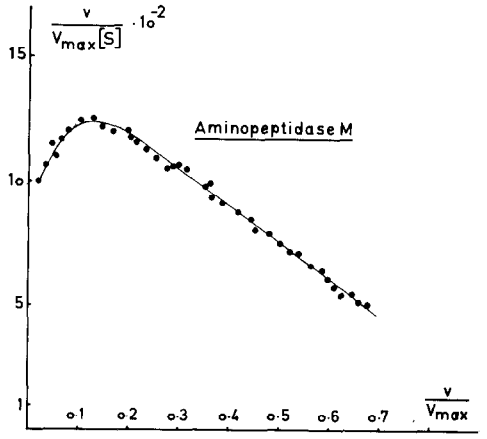

Fig. 2. Scatchard-plot of the kinetic data from fig. 1 indicating a positive cooperativity of 1.18 "interacting sites".

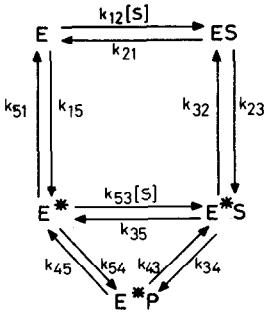

Fig. 3. Schematic illustration of the proposed change in mechanism of reactions catalyzed by aminopeptidase M with different substrate concentrations.

If the time required for the enzyme to relax to the state E is smaller than the average time interval before another substrate molecule binds, $k_{51} > k_{53}$, the reaction path will be a function of substrate concentration: as long as $k_{51} > k_{53}[S]$ the original cycle will be valid unless by raising the substrate concentration $k_{53}[S]$ will become greater than k_{51} and the reaction proceeds via the short cut $E^* + S \rightarrow E^*S \rightarrow E^* + P$, thereby avoiding the time consuming, rate limiting isomerisation step $ES \rightarrow E^*S$. The complex kinetics shown in fig. 1 will be the consequence of two separate reaction pathways:

$$\text{i) } E + S \rightarrow ES \rightarrow E^*S \rightarrow E + P$$

$$\text{ii) } E^* + S \rightarrow E^*S \rightarrow E^* + P$$

and the overall process will be described by the equation:

$$v = \frac{V_a\,[S]}{K_a + [S]} + \frac{V_b\,[S]}{K_b + [S]} =$$

$$= \frac{(V_aK_b + V_bK_a)\,[S] + (V_a + V_b)\,[S]^2}{[S]^2 + (K_a + K_b)\,[S] + K_aK_b},$$

$$\frac{[S]}{v} = \frac{[S]^2 + (K_a + K_b)\,[S] + K_aK_b}{[S]\,(V_a + V_b) + V_aK_b + V_bK_a}$$

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