THE EFFECT OF pH ON THE ALLOSTERIC PROPERTIES OF SHEEP BRAIN 5'-NUCLEOTIDASE

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1. Introduction

The inhibition of 5'-nucleotidase by nucleoside triphosphates was first described by Ipata for the brain enzyme [1] and confirmed by Murray and Friedrichs for 5'-nucleotidase of Ehrlich ascites-tumour cells [2].

The inhibition exerted by ATP, UTP and CTP on the brain enzyme was found to be of the allosteric nature, as shown a) by the sigmoidal form of the inhibition curves obtained when the enzyme is assayed in the presence of increasing concentrations of nucleoside triphosphates, b) by the cooperative inhibition by pairs of nucleoside triphosphates, and c) by the desensitization to inhibition by ATP and UTP observed in the presence of p-chloromercuribenzoate [1, 3].

In this report, the effect of pH on the inhibition of sheep brain 5'-nucleotidase by nucleoside triphosphates is reported. The data show that the enzyme is desensitized to allosteric inhibition at alkaline pH, and that the values of the interaction coefficient, n', for the inhibitory nucleoside triphosphates are remarkably pH dependent.

2. Methods

Sheep brain 5'-nucleotidase was partially purified as described previously [3]. The activity was measured spectrophotometrically at 265 nm with 5'-AMP as substrate, by coupling the 5'-nucleotidase reaction to the deamination of adenosine formed, in the presence of an excess of adenosine [4]. The standard reaction mixture contained, in a final volume of 1 ml, 0.03 M tris acetate buffer at the desired pH, varying concentrations of substrate, and about 100 μg of enzyme preparation. 0.3 μg of commercial adenosine deaminase (obtained from Boehringer and Soehne, Mannheim, Germany), were added to the reaction mixture, and the decrease in absorbance at 265 nm was followed with a recording spectrophotometer at room temperature. AMP was omitted in the reference cuvette.

3. Results and discussion

Fig. 1 shows the effect of hydrogen ion concentration on the enzyme activity and on the inhibition exerted by ATP, UTP and CTP, at 0.05 mM AMP, a saturating substrate concentration. The present inhibition is referred to the initial velocity observed in the absence of inhibitors at each pH value tested. The binding of nucleoside triphosphates seems to occur at a site distinct from AMP, as in borne out by the fact that some treatments which preserve the catalytic activity bring about a desensitization of the enzyme to the inhibitory effect of ATP, UTP and CTP. Thus, at pH 6.5 the enzyme is inhibited about 90% by 20 μM ATP or 10 μM UTP, and this inhibition is reduced to about 20% at pH 8.0; at pH 7 the enzyme is inhibited 69% by 30 μM CTP, and the inhibition is reduced to zero at pH 8. At pH 8.5 a slight activation by 30 μM CTP was constantly observed.

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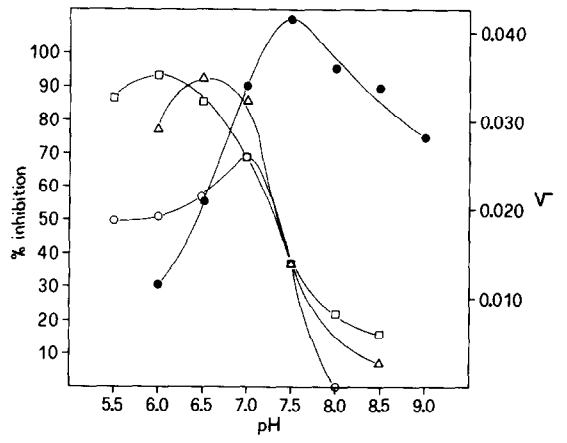

Fig. 1. The effect of pH on the activity of sheep brain 5′-nucleotidase in the absence and in the presence of nucleoside triphosphates. ●——●——●, velocity as a function of pH; △——△——△, percent inhibition of activity in the presence of 20 μM ATP; □——□——□, percent inhibition in the presence of 10 μM UTP; ○——○——○, percent inhibition in the presence of 30 μM CTP. In all assays 0.05 mM AMP was used. The velocity is expressed as Δ absorbance per minute at 265 nm.

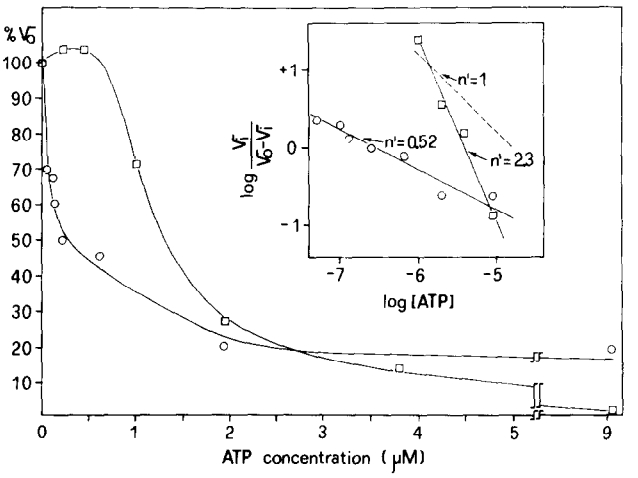

Fig. 2. Effect of varying concentration of ATP on the initial velocity of 5′-nucleotidase, at pH 7 □——□——□, and at pH 5.5 ○——○——○. The final AMP concentration was 0.03 mM. The reaction was started by addition of the enzyme. Velocities are expressed as % of the velocity in the absence of inhibitor. The inset shows the Hill plots for the determination of interaction coefficients.

Table 1
The effect of pH on the interaction coefficient for ATP.

pH→	5.5	5.8	6.0	6.5	7.0	7.5	8.0
n'→	0.52	0.96	1.24	1.50	2.30	0.90	1.00

n' Values were determined as shown in the inset of fig. 2.

In tris acetate buffer the K_m values for AMP were 12 μM at pH 5.5, 7 and 8 respectively. At all pH values the inhibition exerted by the nucleoside triphosphates was of the mixed competitive and non-competitive type with respect to AMP.

The pH effect was further investigated by applying the Hill system of coordinates to kinetic measurements of 5′-nucleotidase at different pH values. The

$\log \frac{v_i}{v_o - v_i}$ (where v_i is the initial reaction velocity in the presence of ATP, and v_o is the initial reaction velocity in the absence of the inhibitor) was plotted against log of ATP concentration, to obtain the values of the interaction coefficient, n', from the negative slopes. Table 1 shows that these values are markedly dependent on the hydrogen ion concentration, ranging from 0.52 at pH 5.5 to 2.3 at pH 7. Fig. 2 shows the shapes of the inhibition curves obtained at these pH values. Similar results were obtained with CTP and UTP as inhibitors.

Interaction coefficients lower than 1 probably indicate that at certain hydrogen ion concentrations a "negative cooperative effect" for the binding of ATP to 5′-nucleotidase occurs, as first described by Conway and Koshland for the binding of NAD molecules to phosphoglyceraldehyde dehydrogenase from rabbit muscle [5].

The effect of pH on the allosteric properties of 5′-nucleotidase could be a consequence of either a conformational alteration of the enzyme protein, or a change in the protonation of the inhibitory nucleoside triphosphate. However, in the alkaline pH region, where nucleoside triphosphates exist as tetraanions, the marked decrease of the inhibition might be mainly a consequence of a change in the protein structure, causing a variation in the accessibility of the binding sites to the inhibitors. On the other hand, the variation of the interaction coefficient for ATP as a function of pH cannot be explained on the basis of an electrostatic repulsion leading to decreasing affinities for the binding of inhibitor

molecules, all carrying negative charges. In fact the data obtained clearly show that the fall-off of the interaction coefficient is found both at acidic and alkaline pH, while maximal cooperativity is observed at hydrogen ion concentrations around the pK value for the conversion of ATP trianions to ATP tetraanion [6]. This further supports the idea that structural alteration of the enzyme protein is the main factor affecting the interaction between ligands.

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References

[1] P.L.Ipata, Biochem. Biophys. Res. Commun. 27 (1967) 337.
[2] A.W.Murray and B.Friedrichs, Biochem. J. 111 (1969) 83.
[3] P.L.Ipata, Biochemistry, 7 (1968) 507.
[4] P.L.Ipata, Anal. Biochem. 20 (1967) 30.
[5] A.Conway and D.E.Koshland Jr., Biochemistry 7 (1968) 4011.
[6] H.R.Mahler and E.H.Cordes, Biological Chemistry, (Harper and Row, New York, 1967) p. 203.