TWO-COMPONENT INHIBITION AS A METHOD FOR STUDYING OF ENZYME ACTIVE CENTRE FLEXIBILITY. CO-INHIBITION OF TRYPTIC ACTIVITY BY Ag^+ AND Pb^{2+} IONS

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1. Introduction

The multicomponent inhibition of enzymatic reactions [1, 2] has proved to be a useful method for the study of the active centre topography of the enzyme, α-chymotrypsin [3, 4]. This method, it should be noted, may find a wider application, for example, in the study of conformational flexibility of the enzyme active centre. Some theoretical aspects of this problem have been recently pointed out by T. Keleti and Cs. Fajszi [5]. Investigations along this line are important as some theories of enzymatic catalysis proceed from the assumption of conformational flexibility of the protein globule.

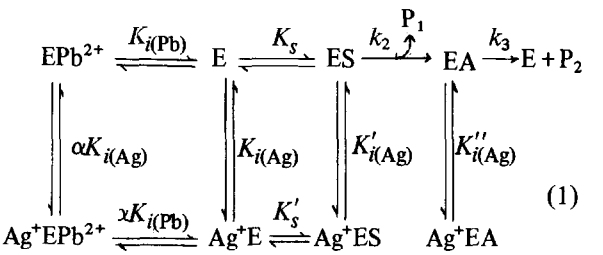
It has been established that the reversible inhibiting effect of Ag^+ ions on trypsin catalyzed reactions is due to the formation of the complex between the Ag^+ cation and the catalytically active imidazole group [6] which is likely to belong to the histidine no. 46. The formation of this complex results in the complete inhibition of trypsin both at the stage of its acylation and at the stage of deacylation of the intermediate acylenzyme.

Pb^{2+} ions are competitive inhibitors of trypsin [7]. The pH-dependent effect of Pb^{2+} on the specific rotation of trypsin suggested [7] the participation of the carboxyl group with pK_a 3.2 in the formation of the complex between Pb^{2+} and the active centre. This carboxyl group seems to belong to the residue of aspartic acid no. 182 [8] which is supposed [9] to take part in the maintenance of the catalytically active conformation of the enzyme.

The present paper reports the results of the co-inhibition of the trypsin catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester (BAEE) by Ag^+ and Pb^{2+} ions.

2. Methods

The effect of Ag^+ and Pb^{2+} ions on the tryptic hydrolysis of BAEE was studied using a Radiometer TTT1c pH-stat. On the ground of [6, 7] the reaction scheme involving both inhibitors may be proposed as follows:



(1)

When the equilibria have been achieved the ratio of stationary rates of enzymatic hydrolysis in absence and, respectively, in presence of inhibitors should be given as:

$$\frac{v}{v_i} = 1 + \frac{[Ag^+]}{K_{i(Ag)}} + K\frac{[Pb^{2+}]}{K_{i(Pb)}} + K\frac{[Ag^+]\,[Pb^{2+}]}{\alpha K_{i(Ag)} K_{i(Pb)}}, \qquad (2)$$

where

$$K = \frac{(K_m)_{app}}{(K_m)_{app} + [S]_0} \text{ and } (K_m)_{app} = \frac{K_s k_3}{k_2}.$$

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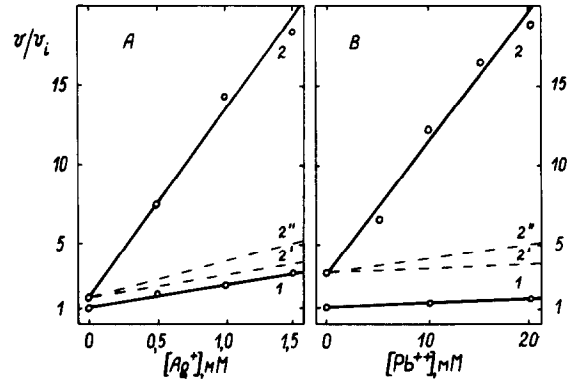

Fig. 1. Two-component inhibition of BAEE tryptic hydrolysis by Ag^+ and Pb^{2+} ions.

(A) v/v_i dependence upon Ag^+ ions concentration in absence of Pb^{2+} ions (line 1) and in presence of 20 mM $Pb(NO_3)_2$ (line 2).

(B) v/v_i dependence upon Pb^{2+} ions concentration in absence of Ag^+ ions (line 1) and in presence of 1.5 mM $AgNO_3$ (line 2).

The theoretical lines 2′ and 2″ are based on equation (2) for incompatible ($\alpha \to \infty$) and mutually independent ($\alpha = 1$) mechanisms of inhibition. Conditions: 25°, pH 5.5, ionic strength 0.1 (inhibiting salts and $Ca(NO_3)_2$), $[E_0] = 2.5 \times 10^{-7}$ to 5×10^{-6} M, $[S]_0 = 0.1$ mM. Under these conditions the $(K_m)_{app}$ value is equal to 0.02 mM.

The expression (2) is valid with $[Ag^+]$, $[Pb^{2+}]$, $[S]_0 \gg [E]_0$ and $K_{i(Ag)} = K''_{i(Ag)}$. The latter equation was proved for pH 5.5 in [6]. As it was shown that for the given substrate, BAEE, $k_2 \gg k_3$ [10, 11], the expression (2) does not contain the $K'_{i(Ag)}$ value.

3. Results

According to scheme (1) at least three cases should be distinguished:

(a) $\alpha = 1$, binding of one inhibitor does not affect the binding of the second one (mutually independent inhibitors);

(b) $\alpha \neq 1$, binding of one inhibitor facilitates ($0 < \alpha < 1$) or hinders ($\alpha > 1$) the binding of the second one (mutually dependent inhibitors);

(c) $\alpha \to \infty$, inhibitors are incompatible and no formation of the triple Ag^+EPb^{2+} complex occurs.

Our experimental data on co-inhibition of tryptic activity by Ag^+ and Pb^{2+} are presented in coordinates of equation (2) in fig. 1. The points of the lines 1 are

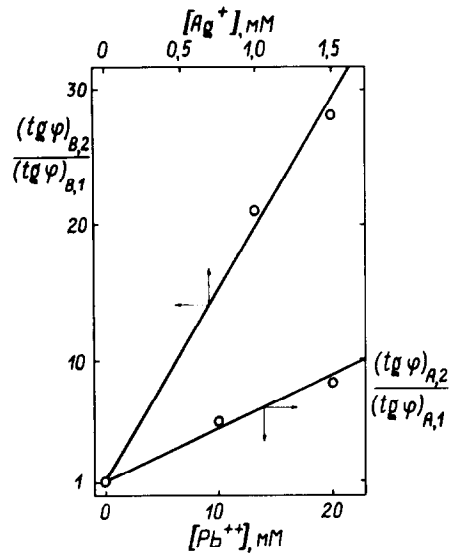

Fig. 2. Kinetic data on two-component inhibition of BAEE tryptic hydrolysis by Ag^+ and Pb^{2+} ions, presented in coordinates of equations (3) and (4).

obtained in presence of one inhibitor only. The constants $K_{i(Ag)}$ (0.68 mM) and $K_{i(Pb)}$ (5.5 mM) are calculated from the slopes of lines 1. Their values are in agreement with those obtained earlier [6, 7]. The theoretical lines 2′ and 2″ are based on equation (2) for incompatible ($\alpha \to \infty$) and mutually independent ($\alpha = 1$) mechanism of two-component inhibition. As is evident from fig. 1, the co-inhibition of tryptic activity by Ag^+ and Pb^{2+} ions (lines 2) results in a high degree of the reversible enzyme inactivation. The comparison of the experimental data (lines 2) with theoretical lines 2′ and 2″ permits one to conclude that the inhibiting effect of one of the cations becomes stronger in presence of the second one, i.e. $\alpha < 1$. The α value may be found in accordance with (2) from equations (3) or (4). The tgφ values in equations

$$\frac{(\mathrm{tg}\varphi)_{A,2}}{(\mathrm{tg}\varphi)_{A,1}} = 1 + \frac{K\,[Pb^{2+}]}{\alpha\, K_{i(Pb)}} \qquad (3)$$

$$\frac{(\mathrm{tg}\varphi)_{B,2}}{(\mathrm{tg}\varphi)_{B,1}} = 1 + \frac{[Ag^+]}{\alpha\, K_{i(Ag)}} \qquad (4)$$

(3) and (4) are the slopes of straight lines 1 and 2 in fig. 1A and 1B, respectively. The belonging of the tangent to the respective curve is shown by the index. Fig. 2 represents the experimental results given in the coordinates of equations (3) and (4). The α value calculated from the straight lines slopes in fig. 2 is equal to ~ 0.08 in both cases.

The obtained value of α shows that although the Ag^{+} and Pb^{2+} ions form complexes with different sites of the active centre (see Introduction) which are not close to each other [9], the binding of one of the inhibitors facilitates the binding of the second one more than 10 times, possibly, by some mechanism of allosteric nature. This is the indication of a conformational flexibility (mobility) of the active centre of trypsin.

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