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*of the 9th International Conference on Fundamental
and Applied Aspects of Physical Chemistry*

Volume I

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NON-ESSENTIAL ACTIVATION OF PEPSIN BY Al^{3+} “*IN VITRO*”

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Abstract

The *in vitro* effect of Al^{3+} ions on pepsin activity at pH 2, *via* kinetic parameters was evaluated. Kinetic study showed that Al^{3+} ions increase the maximal velocity (V_{max}) rather than apparent affinity for substrate (K_s) implying the non-competitive nature of activation which indicated that aluminium was a non-essential activator of partial non-competitive type.

Introduction

Porcine pepsin A (EC 3.4.23.1), a prominent member of the aspartic protease, is the principal proteolytic enzyme of gastric juice and as the best understood of this family of proteases it was used as a model enzyme [1]. As the mechanism of Al^{3+} ions (known as a moderate toxic element) on pepsin activity is not still clear, the objective of this study is to investigate the *in vitro* influence of different concentrations of Al^{3+} ions, physiological and toxic ones, on pepsin activity.

Experimental

Pepsin activity was determined in an incubation medium containing 1 mL of pepsin solution (20 μ g/mL in 0.01M HCl, pH 2), 5 mL haemoglobin solution (2% solution of haemoglobin in 0.01M HCl). The working solutions were incubated for 10 minutes at 37°C. The reaction was stopped by addition of 10 mL 5% TCA. Kinetic analysis was carried out according to a slightly modified method of Anson [2], by following the initial velocity of the enzymatic reaction in the absence and presence of Al^{3+} ($1.7 \cdot 10^{-6}$ – $8.7 \cdot 10^{-3}$ M) and rising concentrations of hemoglobin (from $0.025 \cdot 10^{-3}$ M to $4 \cdot 10^{-3}$ M) that used as a specific substrate. All the assays were performed at pH 2.

Results and Discussion

The dependence of the initial reaction rate *vs.* substrate concentration in the presence and the absence of aluminium exhibited typical Michaelis – Menten kinetics (data not shown). The Lineweaver-Burk plots (Figure 1) show that increase of reaction velocity in a presence of Al^{3+} ions resulted in a family of linear plots intersecting at $1/[Hb]$ axis. Aluminum was found to stimulate the enzyme activity in dose-dependent manner.

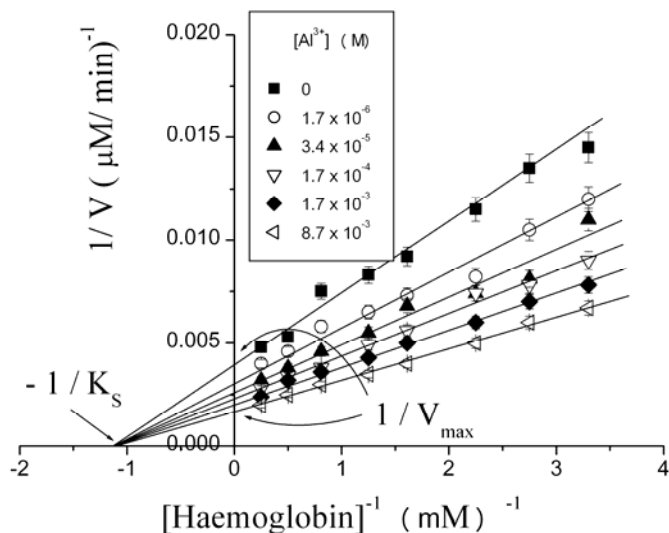
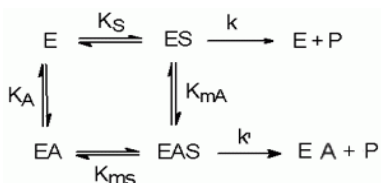


Fig. 1. Lineweaver-Burk plot of a series of kinetic measurements in a presence of different Al^{3+} ions concentrations at pH 2.

As can be seen, increasing of aluminium concentration increased V_{\max} values from $254 \pm 7 \mu\text{M min}^{-1}$ to $599 \pm 17 \mu\text{M min}^{-1}$, without producing a significant change in the value of apparent enzyme affinity for the substrate K_s , of $0.907 \pm 0.072 \text{ mM L}^{-1}$, according to the proposed reaction scheme (Scheme 1).



Scheme 1. Reaction scheme for non-essential activation; Abbreviations are E-enzyme, S-substrate, A-activator, and P-product.

At equilibrium conditions partially non-competitive activation exists if the following conditions are satisfied, i.e. $K_S = K_{mS}$, $K_A = K_{mA}$ and $k < k'$ (Scheme 1) [3, 4]. To evaluate the nature of activation secondary Lineweaver-Burk plots were considered. As the secondary plots of the slopes and intercepts vs. activator concentrations are not linear (data not shown), the reciprocal of the change in slope and intercept (Δ slope and Δ intercept) vs. $1/[\text{Al}^{3+}]$, that are linear [3, 5], were used for calculating equilibrium constants K_{mS} and K_{mA} (Figure 2). The calculated values for constants, K_{mS} , K_{mA} and K_A are $0.904 \pm 0.083 \text{ mM}$, $8.56 \pm 0.51 \mu\text{M}$ and $8.82 \pm 0.90 \mu\text{M}$ respectively, which is consistent with a non-essential partial non-competitive activation system (Scheme 1). Activation constant, K_A , was determined graphically and by direct measurement of Al^{3+} binding (data not shown).

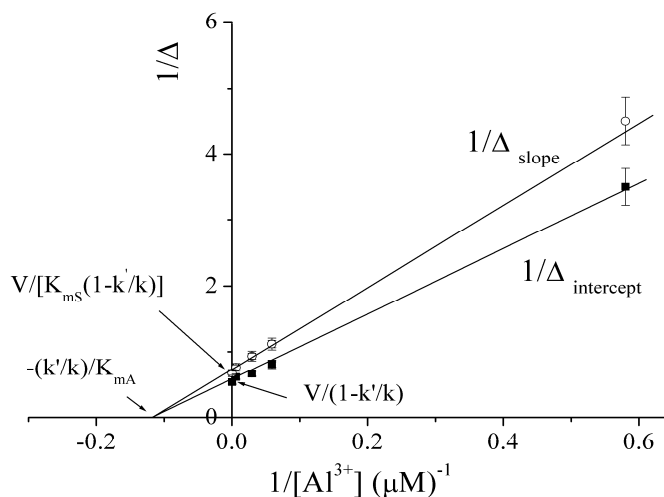


Fig. 2. The secondary plots of $1 / \Delta$ against $1 / [\text{Al}^{3+}]$, where Δ is defined as slope or intercept in the absence of activator (Al^{3+} ions) minus that in its presence.

Conclusion

In summary, the present kinetic analyses, which classify this phenomenon as a case of non-essential activation, indicate that the activation by Al^{3+} ions was of partial non-competitive type.

Acknowledgement

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