

site zinc of the enzyme, changed to yellow (Fig. 1) Excess zinc ions also inhibited the peptidase activity of [(Azo-CPD)Zn]. The interaction between

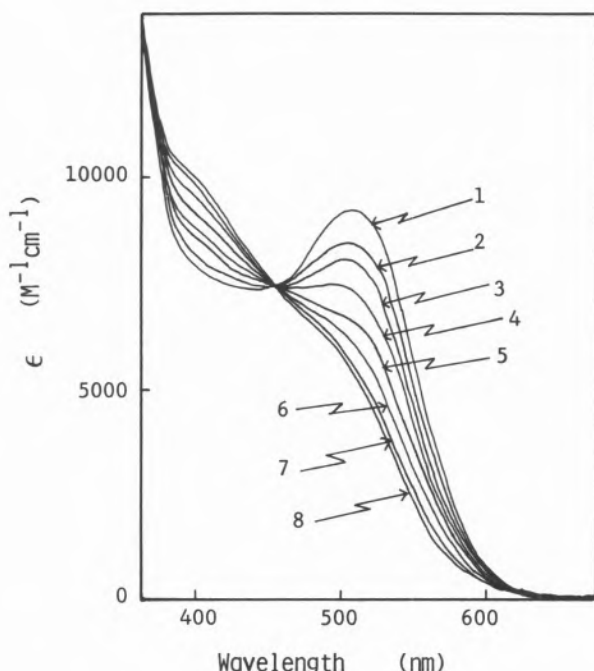
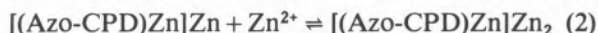
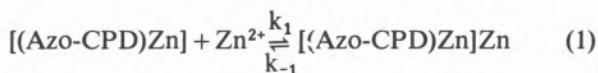


Fig. 1

Effect of excess zinc ions on the absorption spectrum of arsanilazotyrosine-248-carboxypeptidase A. [(Azo-CPD)Zn] = 3.4×10^{-5} M, Temp 25°C. pH 8.2, 0.05 M Tris-HCl buffer (0.5 M NaCl).

1, without zinc ions; 2, $Zn^{2+} = 10^{-5}$ M; 3, $Zn^{2+} = 2.0 \times 10^{-5}$ M; 4, $Zn^{2+} = 3.0 \times 10^{-5}$ M; 5, $Zn^{2+} = 5.0 \times 10^{-5}$ M; 6, $Zn^{2+} = 7.0 \times 10^{-5}$ M; 7, $Zn^{2+} = 1.5 \times 10^{-4}$ M; 8, $Zn^{2+} = 3.5 \times 10^{-4}$ M

excess zinc ions and [(Azo-CPD)Zn] has been studied by the stopped flow and spectrophotometric methods at pH 8.2, 7.7, I=0.5M (NaCl) and 25°C. [(Azo-CPD)Zn] has two binding sites for excess zinc ions and the binding constant of the first site (3.9×10^5 M⁻¹ at pH 8.2, 7.1×10^4 M⁻¹ at pH 7.7) is much larger than that of the second site (1.8×10^3 M⁻¹ at pH 8.2, 7×10^2 M⁻¹ at pH 7.7), as shown in the following equations.



The binding of excess zinc ions to the first site was completely correlated with both the inhibition of the peptidase activity and the color change of

the enzyme. The results can be explained in terms of the zinc ion reaction with only one of three conformational states of [(Azo-CPD)Zn] [2]. The second order rate constants (k_1) for binding of excess zinc ions to [(Azo-CPD)Zn] were 4.3×10^6 and 8.4×10^5 M⁻¹sec⁻¹, at pH 8.2 and 7.7, respectively, and the first order rate constants (k_{-1}) for the dissociation of zinc ions from [(Azo-CPD)Zn]Zn are 11 and 12 sec⁻¹, respectively. It has been proven that excess zinc ions promote the inhibition of the peptidase activity and the color change from red to yellow through specific binding of zinc ions to one conformational state of [(Azo-CPD)Zn].

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PS2.28 — TH

P. GRUNWALD

Institut für Physikalische Chemie der Universität
Laufgraben 24, 2000 Hamburg 13
F.R.G.

KINETIC SALT EFFECT OF ALKALINE EARTH CHLORIDES ON THE UREASE CATALYZED UREA HYDROLYSIS

The kinetics of the Ni-containing [1] enzyme urease have turned out to be very complex. In order to get some informations about the reaction mechanism of the urease catalyzed urea hydrolysis we investigated the reaction in presence of the alkaline earth chlorides MgCl₂, CaCl₂, SrCl₂, and BaCl₂ in a concentration range of 10⁻⁶

$M < c_{Me^{++}} < 10^{-4}$ M where the Debye-Hückel Limiting Law is valid and where the alkaline earth carbonates do not precipitate. The activities were determined by conductivity measurements [2] in buffer free solutions [3].

The result of all measurements is that the activities A as a function of ionic concentration c fit the equation

$$\ln A = \ln A_0 - B \sqrt{c} \quad (1)$$

The sign of the slope being negative expresses that the urea hydrolysis is inhibited by the added salts. The value for B changes only slightly if $BaCl_2$ ($B = -38.05 \pm 1.73$) instead of $MgCl_2$ ($B = -37.01 \pm 1.33$) is present, although the radii of the metal ions differ from each other by about 0.7 Å and the degree of hydration is 13.9 (Mg^{++}) and 8.4 (Ba^{++}). Further the B -value is only insignificantly affected by the course of the reaction: $B = -38.17 \pm 1.76$ (Mg^{++}) and -40.92 ± 1.73 (Ba^{++}) for a conversion of 1.3×10^{-3} mole urea. This points out that the reaction is mainly influenced by the concentration of the added ions. Therefore, an interpretation of the results on the basis of the Debye-Hückel Limiting Law and the activated complex theory seemed to be meaningful. Applying the theory of transition state to reactions in solution, the equation for the rate constant k is

$$k = \frac{k_B \cdot T}{h} \cdot K^\ddagger \cdot \frac{f_A \cdot f_B}{f_{AB^\ddagger}} \quad (2)$$

At infinite dilution the activity coefficients f for the reactants, A, B and for the activated complex AB^\ddagger are unity and $k = k_0$. Inserting the Debye-Hückel Limiting Law for ionic activity coefficients into the logarithmic form of equation (2) gives

$$\lg(k/k_0) = 1.02 z_A z_B \sqrt{I} \quad (T = 298 \text{ K}) \quad (3)$$

This expression, that has been derived by Brønsted and Bjerrum, shows that if the charges z of the reactants are both positive or both negative $\lg(k/k_0)$ will increase with increasing ionic strength I , whereas the plot of the $\lg(k/k_0)$ versus $I^{1/2}$ has a negative slope if ions of opposite charge are reacting.

An evaluation of the data by use of equation (3) again leads to linear relationships. In all cases the

extrapolation of $\lg(k/k_0)$ to zero ionic strength yields values close to zero and the gradient $1.02 \cdot z_A \cdot z_B$ of every line is about -10 .

From that, it follows that one of the reactants must have a negative charge. No conclusion can be drawn from the experiments concerning the size of z_A or z_B , but we assume that this kind of kinetic salt effect results from the reaction of urease with positively charged metal-urea complexes and that the active site of the enzyme carries a negative charge. This is contrary to the reaction model that has been published by ZERNER [4].

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PS2.29 — TH

P. GRUNWALD

Institut für Physikalische Chemie der Universität
Laufgraben 24, 2000 Hamburg 13
F.R.G.

DEPENDENCE OF THE UREASE CATALYZED UREA HYDROLYSIS ON SUBSTRATE CONCENTRATION AND TEMPERATURE IN THE PRESENCE OF ALKALINE EARTH HALIDES

The alkaline earth halides $MgCl_2$, $CaCl_2$, $SrCl_2$, and $BaCl_2$ inhibit the urease catalyzed urea hydrolysis. The interpretation of the inhibition by a model, that has first been developed by Brønsted and Bjerrum on the basis of the Debye-