

$M < c_{Me^{++}} < 10^{-4}$ 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

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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-

-Hückel Limiting Law and the transition state theory, leads to the assumption that the active site of urease carries a negative charge.

In order to confirm the hypothesis set up, Michaelis constants have been determined for three different metal concentrations (3×10^{-6} , 1.5×10^{-5} , and 9×10^{-5} M) in a urea concentration range from 0.1 M to 1.6×10^{-3} M. The data are compiled in Table 1 and have to be compared

-values are reduced up to 50% and more compared with those measured below 30°C. These results also indicate that the added alkaline earth halides directly influence the reaction between urea and the active site of the enzyme urease.

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Table 1

c_{MeCl_2} (mole/l)	Michaelis constant $K_{M(\text{app})} \cdot 10^3$ (mole/l)			
	MgCl ₂	CaCl ₂	SrCl ₂	BaCl ₂
$3 \cdot 10^{-6}$	3.22 ± 0.20	3.18 ± 0.16	2.90 ± 0.30	2.89 ± 0.09
$1.5 \cdot 10^{-5}$	3.13 ± 0.15	2.77 ± 0.07	2.70 ± 0.11	2.58 ± 0.07
$9 \cdot 10^{-5}$	2.85 ± 0.13	2.60 ± 0.10	2.52 ± 0.07	2.07 ± 0.09

with K_M for pure urease preparations 0.00328 ± 0.00011 M. The data show, that K_M decreases with increasing concentration as well as with increasing ionic radius of the inhibiting metal ion. The inhibitory effect caused by a 3×10^{-6} M BaCl₂ solution is of the same magnitude as that of a MgCl₂ solution with a 30 fold metal ion concentration. The corresponding Lineweaver Burk plots show that the kind of inhibition changes from a predominantly noncompetitive inhibition for low MgCl₂ concentrations to completely uncompetitive inhibition in the presence of BaCl₂. From this it can be deduced that the active site of urease reacts with metal ion — urea complexes.

The temperature dependence of the urea hydrolysis catalyzed by urease is quite complex. If the reaction rate is determined in small temperature intervals, the measuring points no longer fall close to a straight $\ln v/T^{-1}$ — Arrhenius plot, but fit a wave like curve due to subsequent conformational changes with increasing temperature [1]. This anomalous temperature behaviour of urease is distinctly intensified in the presence of alkaline earth chlorides. Measurements, that have been performed in ΔT intervals of 1 K between 3°C and 80°C, further show that the temperature optimum of urease is raised from 60°C up to 73°C if the reaction solution contains 5×10^{-4} M SrCl₂. The mean activation energies decrease with increasing temperature, especially above 30°C where the E_A -



PS2.30 — TH

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PROTEIN INTERACTION WITH THE COPPER(II)/ASCORBIC ACID/OXYGEN SYSTEM: EVIDENCE FOR A NON-SITE SPECIFIC REACTION

The ability of ascorbic acid, in the presence of low levels of copper(II), to deactivate enzymes such as catalase [1], alkaline phosphatase [2], Na,KATPase [3] and acetylcholinesterase [4] has been well documented. Recent investigations [4] have used the catalytic effect of copper(II) on enzyme deactivation as evidence for the "site specific" mechanism of biomolecule deactivation. In