

-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

this mechanism a metal-ion bound to the biomolecule acts as a centre for the production of hydroxyl radicals (via a modified Haber-Weiss cycle) which react rapidly with the surrounding biomolecule producing "site specific" deactivation [5].

In this presentation we aim to show that this type of experiment does not allow one to state with certainty that the "site specific" mechanism is operating. We show that a heterogeneous copper(II) catalyst (which does not form biomolecule/metal-ion complexes) is as effective as a homogeneous catalyst at deactivating the enzyme acetylcholinesterase in sharp contrast to the predictions of the "site specific" mechanism.

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PS2.31 — MO

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REACTIVITY PATTERNS IN THE NUCLEOPHILIC DEALKYLATION OF *N*-SUBSTITUTED METALLOPORPHYRINS

The reactions of *N*-substituted porphyrins are of interest in two quite different respects: for explanations of the variable efficiency in the formation

of *N*-alkylporphyrins by drugs that interact with cytochrome P-450 and for applications to synthesis of radiolabelled porphyrins for diagnostic imaging or therapy.

There are two types of mechanism for dealkylation of *N*-substituted metalloporphyrin that have precedence in the literature. One possible process involves reduction of a Co(II) or Fe(II) *N*-substituted metalloporphyrin to form a σ -alkyl Co(III) or Fe(III) complex [1-3]. The complexes could then lose the σ -alkyl group by several different types of reaction mechanisms, *i.e.*, through carbocationic, carboanionic or radical intermediates as have been documented for the Co(III) σ -alkyl corins [4]. A second type of reaction involves nucleophilic removal of the *N*-substituent through a carbocationic intermediate. This type of reaction has been a focus of our work [5-9]. In this article, we will present an overview of our previous results and present some new findings which are relevant.

Features of the nucleophilic dealkylation reaction which could affect the overall rate include the nature of the *N*-substituent, the nucleophile, the solvent, the porphyrin and the metal ion. We have found that the *N*-substituent has a profound effect on the dealkylation rate. The types of substituent which we have investigated are acyl (ethylacetato), alkyl (benzyl, methyl, and ethyl) and aryl (phenyl). For the sake of comparison, we used the same solvent (CH_3CN), metal ion (copper (II)) and nucleophile (di-*n*-butylamine). At 25°C, the relative rates for dealkylation of the *N*-substituted tetraphenylporphinatocopper(II) complex to form CuTPP are 100 (benzyl) [8]: 1 (methyl) [8]: 0.1 (ethyl) [8]: 0.15 (ethylacetato, $k_{\text{obsd}} = 1.1 \times 10^{-4} \text{ s}^{-1}$): $< 10^{-4}$ (phenyl) [8]. The activation parameters for this series are too few to be definitive but the trends they suggest are reasonable: the activation enthalpy is least for the *N*-benzyl complex and the activation entropy of the *N*-methyl complex is the least unfavorable. The first of the two features and the extremely slow loss of the *N*-phenyl group are consistent with $\text{S}_{\text{N}}1$ character. For this type of mechanism, the ability of the benzyl group to delocalize positive charge would stabilize a carbocationic activated complex relative to the other *N*-substituents and the phenyl group would be disfavored. The more unfavorable