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 corrins [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, $k_{obsd} = 1.1 \times 10^{-4}\ s^{-1}$): $< 10^{-4}$ (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 S_N1 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

entropy for groups bulkier than the methyl group is consistent with S_N2 character since nucleophilic attack is an important feature of the mechanism. One substituent about which we report herein for the first time is the ethylacetato group. The *N*-ethylacetatotetraphenylporphine ligand has been reported previously [10] and the Cu(II) complex (formed *in situ* from $Cu(CF_3SO_3)_2$ and *N*-$CH_2CO_2C_2H_5$ HTPP in CH_3CN) shows a visible spectrum typical of a Cu(II) *N*-substituted porphyrin complex [8]. Although we expected that the carbonyl functionality might inhibit carbocation formation and thereby increase the activation enthalpy, the reactivity of this complex is very similar to the corresponding *N*-ethyl TPP complex. The acetato group is a versatile functionality and we might expect to be able to synthesize a variety of *N*-substituted porphyrins that could have similar dealkylation reactivity.

The contributions of factors other than the *N*-substituent are consistent with S_N1 characteristics in some cases and with S_N2 reactions in other cases. The dependence of the rate of dealkylation on the metal ion is characteristic of S_N1 reactions. The rate is highly dependent on the metal ion. Under comparable conditions, the rate order is [5-9] $Pd(II) > Cu(II) > Ni(II) > Co(II) \gg Zn(II) > Mn(II)$ which parallels the stability order of the product (non-*N*-substituted) metalloporphyrin. This result and the dependence on carbocationic stabilization noted above infer that the activated complex resembles the product and that bond-breaking is important in determining the rate. The dependence of the rate on the nucleophile and the solvent are characteristic of an S_N2 mechanism. Although these factors have not been studied in detail, it is quite clear that the dealkylation rates are highly dependent on solvent [7]. Highly polar solvents appear to stabilize the nucleophile and retard reaction, as expected. It must be noted here that we are discussing the dependence of the dealkylation step itself. The solvent can also have a profound effect by changing the rate determining step of the mechanism, as illustrated by the reactions of Pd(*N*-CH_3TPP)$^+$ in CH_3CN (in which complexation is fast and dealkylation slow) and in DMF (in which complexation is slow relative to dealkylation) [9]. In reactions of Cu(*N*-CH_3TPP)$^+$, ion-pairing of the complex with a nucleophilic anion may be the origin of the observed first order rather than second order rate law [7,11]. The nucleophilicity of amines is much greater than pyridine which is, in turn, much more nucleophilic than water or alcohols [4], but this aspect has not been studied extensively.

The rate of dealkylation is not affected greatly by the nature of the porphyrin ring [7], so results deduced from synthetic porphyrins can be transferred to naturally-derived porphyrins (*e.g.* under comparable conditions, the rate constants for dealkylation of Cu(*N*-CH_3TPP)$^+$, Cu(*N*-CH_3TPPS_4)$^{3-}$ and Cu(*N*-CH_3 deuteroporphyrin IX dimethylester) are 2.9×10^{-3}, 3.7×10^{-3}, 1.4×10^{-3} M^{-1} sec^{-1} (25°C, CH_3CN as solvent, di-n-butylamine as nucleophile) [7]. The results of kinetic studies of dealkylation of *N*-substituted metalloporphyrins to date suggest that the rate can vary over wide limits which are controllable in synthetic schemes by choice of *N*-substituent, metal ion, solvent and/or nucleophile. In biological reactions of *N*-substituted hemes or hemins it might be expected that dealkylation by nucleophilic attack would be different except in cases of very favorable carbocationic stabilization. In the presence of strong reductants *in vivo*, migration to form σ-iron(III) species could result in removal of the *N*-substituent. It is not surprising that *N*-aryl and *N*-alkyl porphyrins are not dealkylated after their formation from myoglobin or hemoglobin [12,13] in light of the relative stability of such complexes against dealkylation and the relative ease of hydrolysis of iron complexes of these *N*-substituted species [14].

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PS2.32 — TU

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INHIBITION OF HUMAN CARBONIC ANHYDRASE II BY SOME ORGANIC COMPOUNDS

In addition to monovalent inorganic anions a variety of organic compounds inhibit carbonic anhydrase-catalyzed reactions by binding at or near the zinc ion in the active center. The best known of these are certain aromatic and heterocyclic sulfonamides [1].

We have studied the inhibition of human carbonic anhydrase II by four organic compounds, tetrazole, 1,2,4-triazole, 2-nitrophenol and trichloroacetaldehyde hydrate (chloral hydrate). These inhibitors can be classified in two categories depending on their effects on CO_2 hydration at pH near 9. The first category is represented by tetrazole and 2-nitrophenol giving rise to predominantly uncompetitive inhibition patterns. At this pH these compounds are anions and their behaviour is completely analogous to the behaviour of simple inorganic anions [2,3]. We also show that tetrazole is a competitive inhibitor of HCO_3^- dehydration in analogy with the behaviour of anions [9]. The second category is represented by 1,2,4-triazole and chloral hydrate yielding noncompetitive inhibition patterns at high pH. 1,2,4-triazole was also studied at pH 7.2 and found to be a noncompetitive inhibitor of both CO_2 hydration and HCO_3^- dehydration. However, at chemical equilibrium 1,2,4-triazole and CO_2/HCO_3^- bind to the enzyme in a mutually competitive fashion.

A third category of organic inhibitors is represented by phenol which has been shown to be a competitive inhibitor of CO_2 hydration and a non-competitive inhibitor of HCO_3^- dehydration by Simonsson *et al.* [4]. The diverse kinetic patterns of these organic inhibitors can be explained by the mechanism model of Fig. 1, an extended version

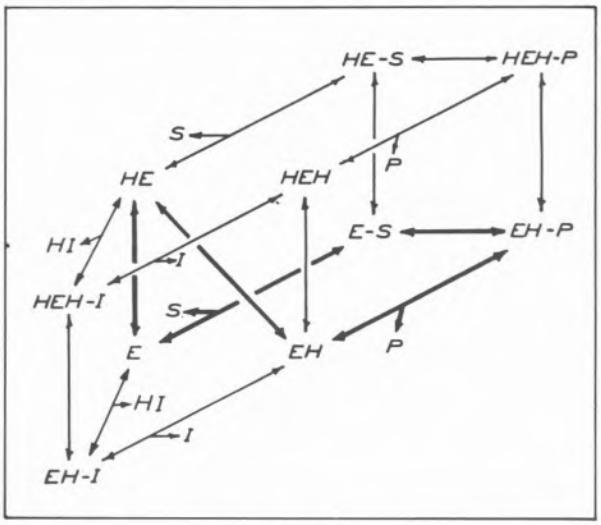

Fig. 1

*Kinetic mechanism scheme for carbonic anhydrase. **H** to the right of **E** represents a protonated catalytic group and **H** to the left of **E** represents a protonated proton-transfer group. **S** represents CO_2, **P** represents HCO_3^-, **I** represents the anionic form of the inhibitor, and **HI** represents a neutral inhibitor. Reaction steps indicated by thick lines represent the catalytic pathway that is thought to dominate at high pH. The diagonal line corresponds to the intramolecular H^+ transfer step. Vertical lines correspond to buffer-facilitated H^+ transfer steps*

of the scheme originally proposed by Steiner *et al.* [5]. In this scheme **H** to the right of **E** represents a protonated catalytic group believed to be a zinc-bound H_2O molecule ionizing to OH^-. **H** to the left of **E** represents a protonated proton trans-