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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  $\text{CO}_2$  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 comple-

tely analogous to the behaviour of simple inorganic anions [2,3]. We also show that tetrazole is a competitive inhibitor of  $\text{HCO}_3^-$  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  $\text{CO}_2$  hydration and  $\text{HCO}_3^-$  dehydration. However, at chemical equilibrium 1,2,4-triazole and  $\text{CO}_2/\text{HCO}_3^-$  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  $\text{CO}_2$  hydration and a non-competitive inhibitor of  $\text{HCO}_3^-$  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

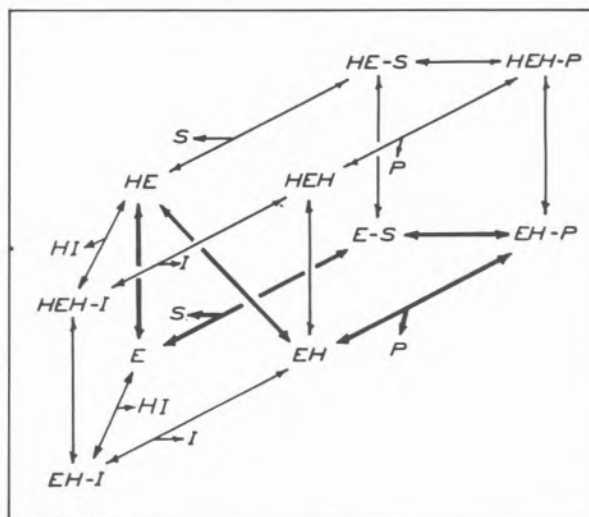


Fig. 1

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  $\text{CO}_2$ , **P** represents  $\text{HCO}_3^-$ , **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  $\text{H}^+$  transfer step. Vertical lines correspond to buffer-facilitated  $\text{H}^+$  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  $\text{H}_2\text{O}$  molecule ionizing to  $\text{OH}^-$ . **H** to the left of **E** represents a protonated proton trans-

fer group, thought to be His-64. At high substrate and buffer concentrations the intramolecular proton transfer ( $\text{EH} \rightleftharpoons \text{HE}$ ) is probably rate limiting.  $\text{pK}_i/\text{pH}$  profiles for tetrazole, 1,2,4-triazole and chloral hydrate suggest that enzyme-inhibitor complexes with the stoichiometric composition  $(\text{H})\text{EHI}$  are formed.  $(\text{H})$  denotes that binding occurs regardless of the ionization state of the proton transfer group. The complex between enzyme and tetrazole or 2-nitrophenol is formed mainly by a combination of  $(\text{H})\text{EH}$  and the anionic form of the inhibitor,  $\text{I}$ . The competitive inhibition of  $\text{CO}_2$  hydration by phenol suggested that  $(\text{H})\text{EHI}$  is formed mainly in a reaction between  $(\text{H})\text{E}$  and neutral inhibitor  $\text{HI}$ . The noncompetitive patterns obtained for 1,2,4-triazole and chloral hydrate could then be explained by assuming that both of these pathways for the formation of  $(\text{H})\text{EHI}$  are kinetically significant.

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PS2.33 — TH

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#### INHIBITORY ACTIVITY OF $\text{Cu(II)}$ , $\text{Co(II)}$ AND $\text{Ni(II)}$ COMPLEXES WITH 1,4-BIS(3-AMINOPROPYL)PIPERAZINE AND 3,3'-DIAMINO-N-METHYLDIPROPYLAMINE TOWARD LENTIL SEEDLINGS DIAMINEOXIDASE

Diamine oxidases (amine: oxygen oxidoreductase, deaminating E.C. 1.4.3.6) are enzymes evenly distributed among living organisms. They catalyse the oxidation of one primary amino group of the substrate according to the following reaction  $\text{R-CH}_2\text{-NH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{R-CHO} + \text{NH}_3 + \text{H}_2\text{O}_2$ . An important tool in studying the function of an enzyme is to find out specific inhibitors and to follow their effects.

Recently a DAO from Lentil seedlings (LSAO) has been purified to homogeneity [1]. This enzyme is similar to other plant amine oxidases.

LSAO is inhibited *in vitro* by various complexes of type  $\text{M(L)}_2\text{X}_2$  where  $\text{M} = \text{Cu(II)}$ ,  $\text{Co(II)}$  and  $\text{Ni(II)}$ ;  $\text{L} = 1,4\text{-Bis(3-Aminopropyl)piperazine (APP)}$ ,  $3,3'\text{-Diamino-N-methyldipropylamine (AMPA)}$ ;  $\text{X} = \text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{SO}_4^{2-}$ .

The inhibition constants were determined by Dixon's and Lineweaver Burk plots.

All inhibitors tested are non competitive and their inhibition constant change with the nature of the metal and anion of the complex. No inhibition is revealed with the  $\text{Co(II)}$  complexes.  $\text{Cu(AMPA)}_2\text{Br}_2$  in aqueous solution ( $3.7 \times 10^{-5} \text{ M}$ ) gives the most high inhibitor value [2].

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