X-ray crystal structures of base-on CNCbl [23] and 5'-deoxyadenosylcobalamin [24] (the CH_3Cbl structure has not been determined) support a difference in phosphodiester geometry between CNCbl and an alkylcobalamin.

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Inhibition of Human Carbonic Anhydrase II by Anions and some 'Neutral' Compounds

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Anions inhibit carbonic anhydrase-catalyzed reactions by binding to the zinc ion in the active center. The pH dependence of the inhibition of the esterase activity shows that anions predominantly bind to enzymes having a protonated catalytic group. Formally, anion binding can be described as a competition with OH^- for a coordination site on the metal ion [1].

Pocker and Deits [2] recently showed that anions inhibit the CO_2 hydration catalyzed by bovine carbonic anhydrase at high pH values in an uncompetitive fashion, and they presented a kinetic scheme to explain this phenomenon.

We have studied the anion inhibition of human carbonic anhydrase II (or C). We have confirmed the

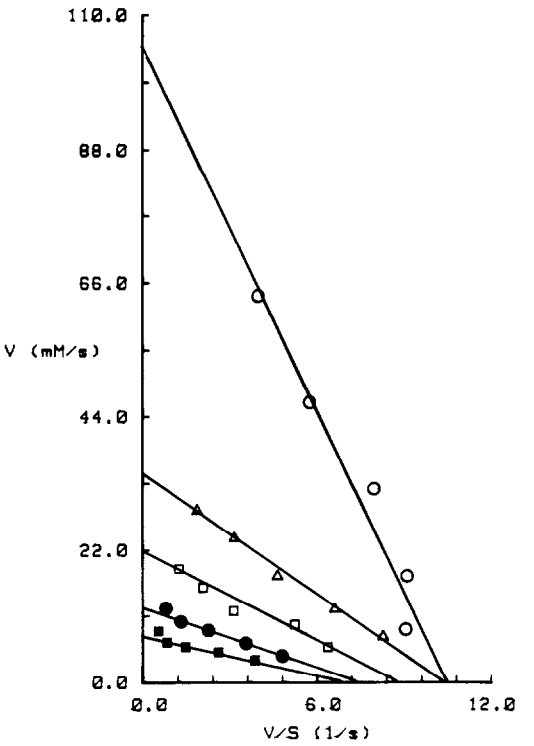

Fig. 1. Inhibition by N_3^- of CO_2 hydration catalyzed by human carbonic anhydrase II at pH 8.9, 25 °C. Buffer: 50 m*M* 1,2-dimethylimidazole-H_2SO_4 with Na_2SO_4 to yield an ionic strength of 0.2 *M*. Inhibitor concentrations: (○), 0 m*M*; (△), 1 m*M*; (□), 5 m*M*; (●), 10 m*M*; (■), 30 m*M*.

observations of Pocker and Deits that at least certain anions, such as SCN^- [3], OCN^-, and N_3^-, give rise to uncompetitive inhibition patterns of CO_2 hydration at high pH. This is illustrated for N_3^- in Fig. 1. However, we offer an alternative interpretation to that of Pocker and Deits. We will show that the uncompetitive pattern is a direct consequence of the kinetic mechanism which we have proposed previously [3, 4]. The crucial point is that a strongly anion binding enzyme species, $His–E–Zn^{2+}–OH_2$ (see ref. [5]), precedes the rate-limiting step in catalysis.

Recently, some organic compounds that bind at or near the metal ion have received attention. One of these is phenol which has been shown to act as a competitive inhibitor of CO_2 hydration and as a mixed noncompetitive inhibitor of HCO_3^- dehydration [6]. We have also studied *o*-nitrophenol, 1,2,4-triazole and tetrazole. However, these inhibitors behave kinetically like anions and give rise to mixed uncompetitive-noncompetitive patterns in the CO_2 hydration reaction at high pH.

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Spectroscopic Studies of Mercury Binding to Metallothionein

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Metallothioneins (MT) are a class of low-molecular weight, sulfur-rich metalloproteins that have been isolated from a wide variety of organisms [1]. Exposure to metal ions, such as Cd, Zn, Cu, Hg, Ag and Bi, result in tissues, especially the liver and kidneys, that contain much higher levels of these proteins. The most widely studied metallothioneins have been hepatic proteins isolated following induction with $CdCl_2$ [2–4]. In these proteins there is usually a mix of cadmium and zinc with a very little copper. Early reports have described some similar induction properties for mercury from which a metallothionein-like protein was obtained [5]. Despite this very wide interest in the metallothioneins there are very few papers describing mercury binding *in vitro* [6, 7].

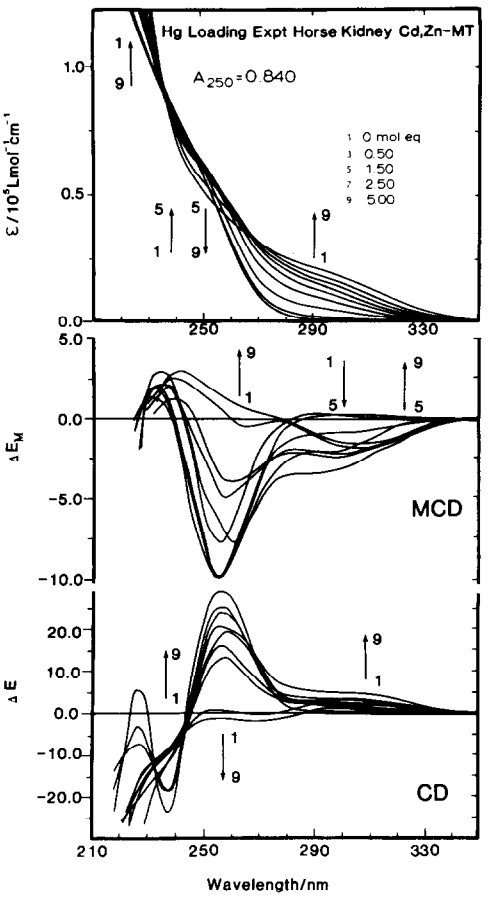

Fig. 1. Conversion of horse kidney Cd,Zn-MT 1 into Hg-MT.

Mercury has been shown to displace zinc and cadmium from metallothionein *in vitro* [6, 7]. Changes in the metal composition within Cd,Zn-MT are readily followed in the UV absorption, CD and MCD spectra as the prominent shoulder at 250 nm is a good indicator of the presence of cadmium [2, 3]. In titrations with mercury the 250 nm band is replaced by a broad band near 300 nm [6]. In this paper we present a detailed description of mercury binding to metallothionein. Titrations were carried out using horse kidney and rat liver Cd,Zn-MT and rat liver Bi,Zn-MT. Metal binding was followed by monitoring the absorption, CD and MCD spectra in the 210–350 nm region.

Rat liver Cd,Zn-MT and Bi,Zn-MT 2, and rat kidney Hg,Cu-MT 1 were induced in rat tissues by

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