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Spectroscopic Investigation on a New Class of Inhibitors which Bind either the Acidic or Basic Form of Cobalt Substituted Carbonic Anhydrase

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Imidazole has been shown to bind the native and cobalt substituted human carbonic anhydrase B (CoHCAB) with a dependence of the affinity constant versus pH substantially different from that shown by the other usual inhibitors [1, 2]. Indeed, whereas the affinity constant of inhibitors increases with a sigmoidal pattern as pH decreases [3, 4], the affinity constant of imidazole towards the enzyme is almost constant in the range of pH 7.3–9.5 [1]. This means that imidazole, contrarily to the other inhibitors, is able to bind also the alkaline form of the enzyme [1, 5].

In order to understand the mechanism of the imidazole binding, ^{1}H NMR studies on the CoHCAB–imidazole adduct have been performed, together with an analysis of the inhibiting properties of several substances, structurally related to the imidazole. In particular we found that 1,2,3- and 1,2,4-triazole are also able to bind either the acidic or alkaline forms of carbonic anhydrase. The latter two compounds show a larger affinity for CoHCAB than imidazole itself and are able to bind also the cobalt substituted bovine enzyme. The apparent affinity constants for the two inhibitors as a function of pH are reported in Fig. 1. The large decrease observed in the two curves at high pH values is due to the dissociation of the N–H group with a pK_a = 9.4 and 10.2 for 1,2,3- and 1,2,4-triazole, respectively. This demonstrates that only the neutral inhibitor species is interacting at the active site. The limit electronic spectra of the two inhibitors are not pH dependent.

The inhibitor properties of the N-methyl imidazole ligand have also been investigated. This ligand shows an affinity constant for CoHCAB of 4 M^{-1} at pH 7.2 which decreases at alkaline pH in the usual way shown by anionic inhibitors. ^{1}H NMR investigation on the CoHCAB–imidazole and –N-methyl imidazole

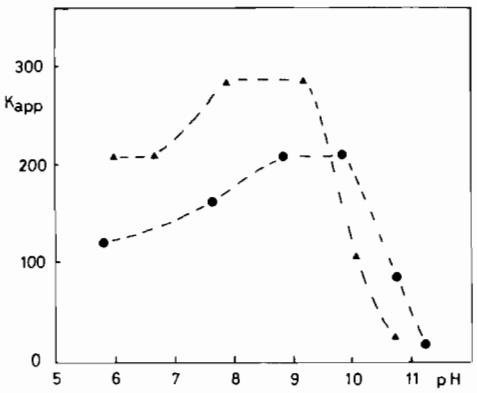

Fig. 1. pH dependence of the apparent affinity constants for cobalt substituted bovine carbonic anhydrase of 1,2,3-triazole (▲) and 1,2,4–triazole (●).

adducts have shown that in both cases the inhibitors, in spite of their different behaviour versus pH, interact at the metal.

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Crystal and Molecular Structure of the Ternary Complex Zn(II)–ATP–2,2′-Bipyridyl

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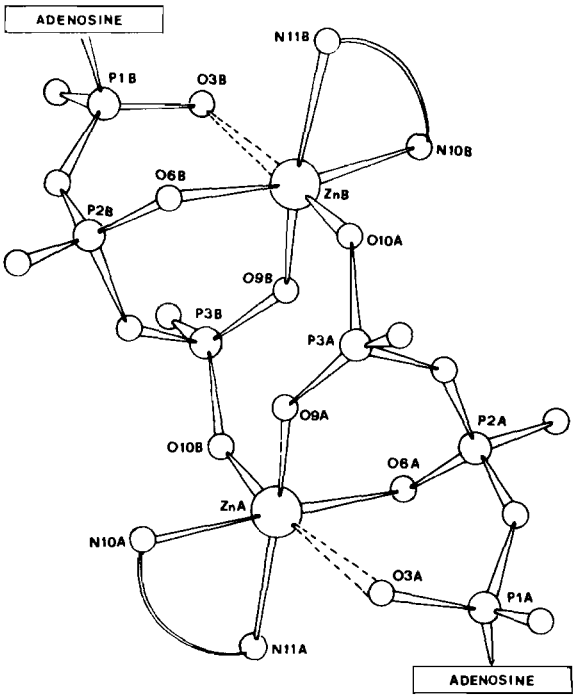
A series of microcrystalline compounds between adenosine 5′-triphosphoric acid (ATP), 2,2′-bipyridyl and some 3d metal ions, such as Mn(II), Co(II), Cu(II) and Zn(II), have been obtained in 1:1:1 ratio [1]. Crystals suitable for X-ray analysis were obtained for the zinc compound. Diffractometer data, collected on one of these crystals, gave the following results: a = 11.105(3), b = 25.223(7), c = 10.539(3) Å, β = 91.34(4)°, monoclinic, space group $P2_1$. 1617 reflections with intensity greater then twice their



standard deviation were used for the structure determination and refinement ($R = 0.098$).

The structure of the compound consists of dimeric molecules in which two zinc atoms are held together by two –OPO– bridges from the γ-phosphate groups of two ATP molecules (Fig. 1). Both zinc atoms show a distorted octahedral coordination, formed by two oxygen atoms from different γ-phosphate groups, one oxygen atom from the β-phosphate group and the two nitrogen atoms of the bipyridyl ligand. The sixth position is completed by an α-phosphate oxygen atom which is only weakly bound. Zn–N and Zn–O distances average respectively 2.15(4) and 2.02(3) Å. The structure is held together by strong intermolecular bipyridyl–purine and bipyridyl–bipyridyl stacking interactions. Weaker bipyridyl–purine intramolecular stacking is also observed.

The molecule provides a possible model for ATP transport and phosphate group transfer mechanism.

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