Interaction between Cobalt(II) Bovine Carbonic Anhydrase B and Cyanometallates

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The binding affinities of various cyanometallates towards cobalt(II) bovine carbonic anhydrase B have been investigated by means of optical and nuclear magnetic resonance spectroscopies.

In order to establish whether the analyzed cyano-complexes bind the cobalt enzyme or not, the addition of increasing amounts of complexes to CoBCA, both in buffered solutions, was followed by electronic spectroscopy in the visible region. Of all the cyanoderivatives investigated only $Au(CN)_2^-$ and $Ag(CN)_2^-$ cause a marked variation in the electronic spectrum of the cobalt enzyme, while $Hg(CN)_2$, $Au(CN)_4^-$, and $Fe(CN)_6^{4-}$ do not cause any appreciable change, indicating that they do not interact with the enzyme. In the case of $Ni(CN)_4^{2-}$ and $Pd(CN)_4^{2-}$ the formation of the CoBCA·CN adduct is obtained; this may be expected since the K_4 value for the above complexes is lower than the stability constant of the enzyme–cyanide derivative.

The importance of the bulkiness of the molecules or ions with respect to the binding capabilities of cyanometallates for CoBCA is evidenced by the consideration that only the small linear mononegative $Au(CN)_2^-$ and $Ag(CN)_2^-$ ions show remarkable affinity for the metalloenzyme [1]. The apparent affinity constants at pH 8 are 6.2×10^2 and 1.6×10^3 for $Au(CN)_2^-$ and $Ag(CN)_2^-$, respectively; they decrease with increasing pH with an apparent pK_a around 7.

The electronic spectrum of the $Au(CN)_2^-$–CoBCA adduct, which shows absorption maxima at 12.5×10^3 cm^{-1} ($\epsilon \simeq 5$ M^{-1} cm^{-1}), 18×10^3 cm^{-1}(90), and 21.5×10^3 cm^{-1}(60), is interpreted as indicative of pentacoordinate species [2] present in solution: this is in agreement with X-ray studies performed on the native enzyme showing that $Au(CN)_2^-$ is located in the active cavity but does not remove the water molecule bound to the zinc(II) ion [3]. The electronic spectrum of the $Ag(CN)_2^-$ derivative is attributed to an equilibrium between tetrahedral and pentacoordinate species.

^{13}C NMR studies of $Au(^{13}CN)_2^-$ and $Ag(^{13}CN)_2^-$ in the presence of CoBCA show that both longitudinal and transverse relaxation times of carbon nuclei strongly decrease in the presence of the paramagnetic cobalt(II) ion of the cobalt enzyme with respect to the pure cyanometallate solutions. The analysis of the relaxation times confirms that direct bonding occurs between the above dicyanometallates and the metallo-enzyme. The effectiveness of the coupling between the unpaired electrons and the resonating nuclei is consistent with the proposed geometries.

References

1 J. E. Coleman, 'Inorganic Biochemistry', G. L. Eicchorn, Ed., Vol. 1, Elsevier, New York, N.Y. (1973), p. 488.
2 I. Bertini, G. Canti, C. Luchinat and A. Scozzafava, *J. Am. Chem. Soc., 100,* 4873 (1978).
3 P. C. Bergstén, I. Waara, S. Lövgren, A. Liljas, K. K. Kannan and U. Bengtsson, *Proceedings of the Alfred Belzon Symp. IV,* M. Rorth and P. Astrup, Ed., Munksgaard, Copenhagen, p. 363 (1972).

Vibronic Studies of Daunorubicin and Its Complex with DNA

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Daunorubicin belongs to an important class of antitumor antibiotics which are able to bind DNA and to inhibit its enzymatic synthesis. Several physical methods [1, 2] have been employed to investigate the nature of the bonds between the drug and the nucleic acids. It has been so possible to suggest the existence of at least two binding sites involving the chromophore and the amino sugar moiety of daunorubicin [3].

In order to obtain information on the electronic and vibrational excited states of the drug as well as on the interaction mechanism, we have studied the resonance Raman scattering and the fluorescence spectra and excitation profiles of the pure compound and the complex. The results obtained from the combined analysis of the data allowed to interpret the complex absorption feature of the pure compound at about 500 nm as due to a single electronic state with its vibrational structure. In addition evidence for the existence of other electronic excited states has been obtained especially from the fluorescence excitation profile.

The remarkable spectral changes by DNA addition furnish further evidence for the formation of the complex and show the specific interaction of the chromophore.

References

1 R. J. Sturgeon and S. G. Shulman, *J. Pharm. Sci., 66,* 958 (1977).

2 E. Calendi, A. Di Marco, M. Reggiani, B. Scarpinato and L. Valentini, *Biochem. Biophys. Acta, 103,* 25 (1965).
3 W. A. Remers, 'The Chemistry of Antitumor Antibiotics', Vol. 1, Ch. 2, John Wiley and Sons (1979).

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

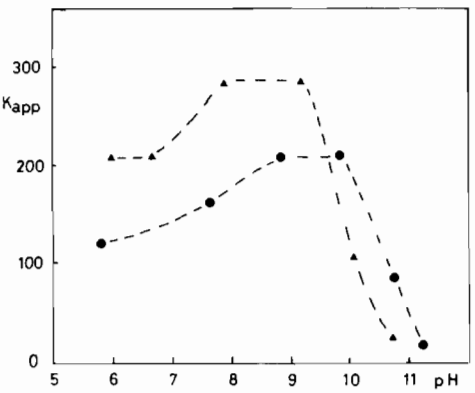

Fig. 1. pH dependence of the apparent affinity constants for cobalt substituted bovine carbonic anhydrase of 1,2,3-triazole (▲) and 1,2,4–triazole (●).

References

1 R. Bauer, P. Limkilde and J. T. Johansen, *Carlsberg. Res. Commun., 42,* 325 (1977).
2 K. K. Kannan, M. Petef, K. Fridborg, H. Cid-Dresdner and S. Lövgren, *FEBS Lett., 73,* 115 (1977).
3 I. Bertini, G. Canti, C. Luchinat and A. Scozzafava, *J. Am. Chem. Soc., 100,* 4873 (1978).
4 I. Bertini, C. Luchinat and A. Scozzafava, *Bioinorg. Chem., 9,* 93 (1978).
5 D. W. Appleton and B. Sarkar, *Bioinorg. Chem., 4,* 309 (1975).

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