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

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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

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