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Magnetic Circular Dichroic Spectra of Various Adducts of Co(II)–Bovine Carbonic Anhydrase

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On the basis of magnetic circular dichroic (MCD) spectra, the coordination geometries of various adducts of cobalt(II)–bovine carbonic anhydrase were determined. The 8-quinolinecarboxylate, acetate and oxalate adducts of cobalt(II)–bovine carbonic anhydrase, which had two clearly separated negative MCD bands at 17500 cm^{-1} and 21000—22000 cm^{-1}, were five-coordinate. The MCD spectra of the Cl^-, Br^-, benzoate, and 3-quinolinecarboxylate adducts of cobalt(II)–bovine carbonic anhydrase were very complex and were interpreted in terms of the overlapping of MCD spectra of tetrahedral and five-coordinate adducts. The coordination geometry of the alkaline form of cobalt(II)–bovine carbonic anhydrase is discussed on the basis of the above results.

Keywords——carbonic anhydrase; cobalt(II)–carbonic anhydrase; magnetic circular dichroic spectra; ligand adduct of cobalt-enzyme; coordination geometry; five-coordinate

Vallee and Holmquist[1] indicated that the magnetic circular dichroic (MCD) spectra of Co(II)–substituted metalloenzymes are very useful for the tentative estimation of the overall coordination of cobalt binding sites. The MCD spectra of Co(II)–enzymes with typical tetrahedral geometry have been widely investigated.[1-6]

Recently, Bertini *et al.*[7,8] proposed that adducts of some ligands (acetate, thiocyanate, and oxalate) with the cobalt(II)–enzyme are five-coordinate on the basis of a new characteristic band at 13000—15000 cm^{-1} (the highest F–F^* transition of the five-coordinate geometry) and low molar absorbance in the visible region of the absorption spectra.

MCD spectra of five-coordinate cobalt-substituted enzymes were not studied in detail by Vallee and Holmquist.[1] Therefore, we measured the MCD spectra of various adducts of Co(II)–bovine carbonic anhydrase to clarify the nature of the five-coordination.

Materials and Methods

Enzymes——Bovine carbonic anhydrase (carbonate hydrolyase) (BCA) was prepared from bovine erythrocytes by the method of Lindskog[9] and its purity was checked by gel electrophoresis. The metal content was checked by atomic absorption spectrophotometry. The zinc content of BCA was 1.0 ± 0.05 atom per molecule. Protein concentration was determined from the absorbance at 280 nm, by using a molar absorption coefficient of $5.7 \times 10^4\ M^{-1}\ cm^{-1}$.[10] Apo-carbonic anhydrase was prepared by successive dialysis of bovine carbonic anhydrase

against 10^{-2} M 2,6-pyridinedicarboxylate in 0.2 M acetate buffer (pH 5.0) and then water. The zinc content of apocarbonic anhydrase was less than 0.05 atom per molecule. Cobalt–bovine carbonic anhydrase (Co(II)–BCA) was prepared by dialysis of the apoenzyme against 10^{-3} M Co^{2+} solution in 0.2 M acetate buffer (pH 6.0) and then water. The cobalt content was 1.00 ± 0.05 atom of cobalt per molecule. Cobalt–enzyme concentration was determined from the absorbance at 280 and 550 nm.[10]

Reagents——8-Quinolinecarboxylic acid was synthesized by the method of Campbell *et al.*[11] and its melting point agreed with that reported in the literature. All other reagents used were of reagent grade and were used without further purification. HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid) and MES (2-(*N*-morphorino)ethanesulfonic acid) were used as buffer reagents.

Spectrophotometric Measurement——Visible and near-infrared spectra were recorded on a Shimadzu MPS-5000 spectrophotometer. The MCD spectra were recorded on a JASCO 40C spectropolarimeter in the magnetic field of an 11.4 kg magnet. The ligand concentrations at which most of Co(II)–BCA (>90%) forms the adduct were calculated from the formation constants[7,8,10] and added to Co(II)–enzyme solution. Absorption and MCD spectra of various adducts of Co(II)–BCA were unaffected by pH.[7,8,10]

Results and Discussion

8-Quinolinecarboxylate,[10] oxalate,[8] and acetate[7] form adducts with Co(II)–BCA. The binding ratios of these ligands to Co(II)–BCA are 1 : 1.[7,8,10] Figure 1 shows the MCD spectra of the 8-quinolinecarboxylate, oxalate and acetate adducts of Co(II)–BCA. The MCD spectra have two clearly separated negative main bands at about 17500 and 21000—22000 cm^{-1}.

Vallee and Holmquist[1] proposed that the MCD spectra of high-spin cobalt(II) complexes can be classified into three groups. The first group consists of complexes with octahedral geometry which exhibit a pronounced but weak negative band ($\Delta\varepsilon_M = 0.018$—$0.1\ M^{-1}\ cm^{-1}T^{-1}$), the second group consists of complexes with tetrahedral geometry which have a negative MCD band at low wave numbers and one or two smaller positive bands at higher wave numbers, and the third group consists of complexes with five-coordinate geometry which exhibit two distinct negative bands.

The MCD spectral patterns of the 8-quinolinecarboxylate, oxalate, and acetate adducts are very similar to those of the five-coordinate model complexes and the values of $\Delta\varepsilon_M$ (0.2—$0.6\ M^{-1}\ cm^{-1}T^{-1}$) of these adducts also correspond to those of five-coordinate model

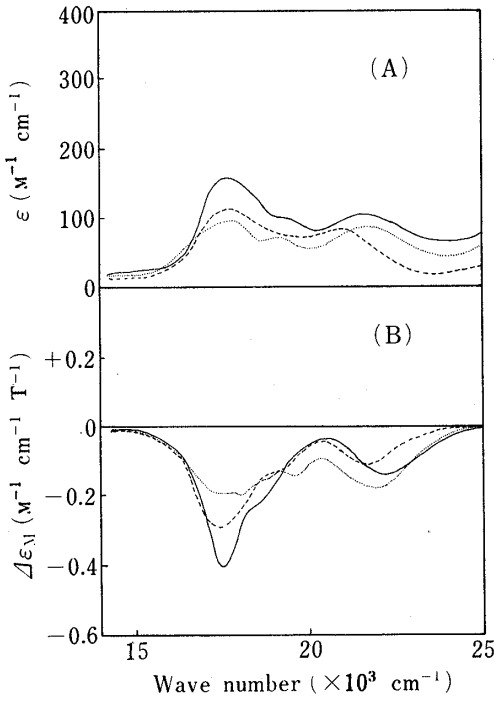

Fig. 1. Absorption (A) and MCD (B) Spectra of Co(II)–Bovine Carbonic Anhydrase Adduct

Co(II)–bovine carbonic anhydrase, 5.3×10^{-4} M. ---, 0.75 M acetate ion in 0.1 M HEPES buffer (pH 8.0); ——, 0.40 M oxalate ion in 0.1 M HEPES buffer (pH 8.0); ·····, 0.10 M 8-quinolinecarboxylate in 0.1 M HEPES buffer (pH 8.0).

complexes.[4,12] Therefore, the MCD spectra of 8-quinolinecarboxylate, oxalate, and acetate adducts indicate that the coordination geometry of these adducts is five-coordinate. This conclusion is consistent with that obtained from the absorption spectra (*F–F** transition band and low molar absorbance in the visible region).[7,10] On the basis of these properties, it is thought that 8-quinolinecarboxylate or oxalate coordinates as a bidentate ligand to the cobalt ion, the residual sites of which are occupied by three histidine residues of the enzyme.[7,8,10] In the case of the monodentate acetate adduct, a water molecule also coordinates to the cobalt ion in the enzyme,[7] because the acetate ion coordinates to Co(II)–BCA in a 1 : 1 ratio.[7]

Figure 2 shows the MCD spectra of various adducts (Cl^-, Br^-, benzoate, 3-quinolinecarboxylate, and bicarbonate) of Co(II)–BCA. The MCD spectrum of the bicarbonate adduct is very different from those of Fig. 1; it shows a large negative band at 17500 cm^{-1} and a small positive band at 21000 cm^{-1} (Fig. 2). The pattern of the MCD spectrum of the bicarbonate adduct is very similar to those of tetrahedral model complexes[1] and those of Co(II)–carboxypeptidase and Co(II)–thermolysin[4,5,13] which were assigned a distorted tetrahedral geometry. The visible spectral pattern of the bicarbonate adduct resembles that of the benzoate adduct, as shown in Fig. 2, so Bertini *et al.*[7] proposed that the bicarbonate adduct of Co(II)–BCA is in an equilibrium between tetrahedral and five coordinate species. However, the pattern of the MCD spectrum of the bicarbonate adduct indicates that the coordination geometry is tetrahedral. Therefore, it is considered that the predominant species of the bicarbonate adduct has tetrahedral geometry. The $\Delta\varepsilon_M$ values ($-0.5\,M^{-1}\,cm^{-1}\,T^{-1}$, $+0.1\,M^{-1}$

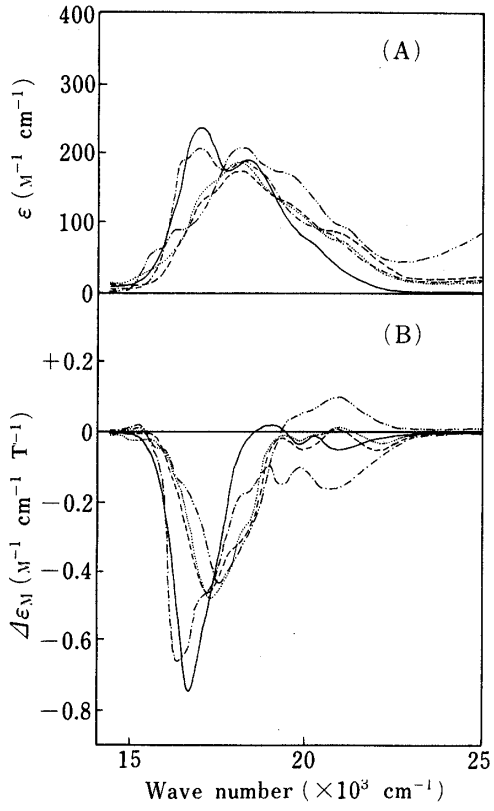

Fig. 2. Absorption (A) and MCD (B) Spectra of Co(II)–Bovine Carbonic Anhydrase Adduct

Co(II)–bovine carbonic anhydrase, 5.3×10^{-4} M. –·–·–, 0.90 M $NaHCO_3$ in 0.1 M HEPES buffer (pH 8.2); ––––, 0.44 M 3-quinolinecarboxylate in 0.1 M MES buffer (pH 6.5); ·····, 0.40 M benzoate in 0.1 M MES buffer (pH 6.5); ——, 0.50 M NaCl in 0.1 M MES buffer (pH 6.5); –·–·–, 0.50 M KBr in MES buffer (pH 6.5).

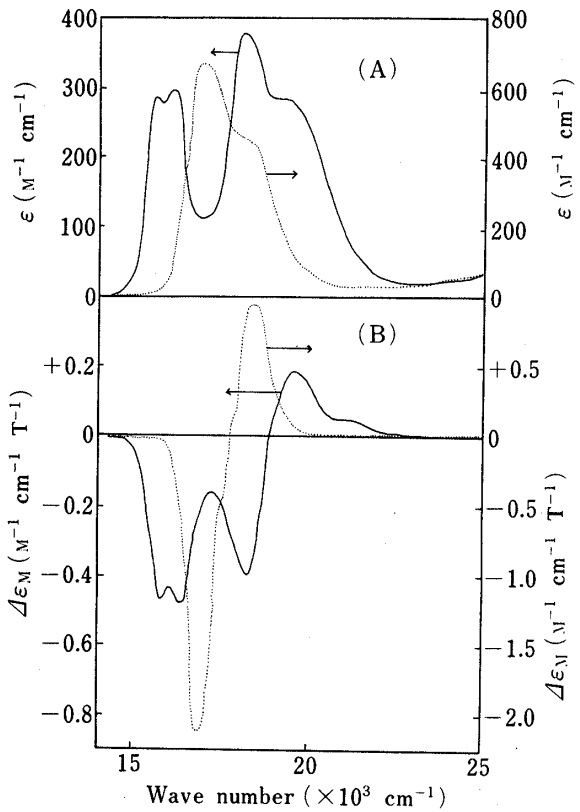

Fig. 3. Absorption (A) and MCD (B) Spectra of Co(II)–Bovine Carbonic Anhydrase and Its Cyanide Adduct

Co(II)–bovine carbonic anhydrase, 5.3×10^{-4} M. ——, Co(II)–bovine carbonic anhydrase in 0.1 M HEPES buffer (pH 8.0); ·····, 3.3×10^{-3} M cyanide ion in 0.1 M HEPES buffer (pH 8.0).

$cm^{-1}\ T^{-1}$) of the bands of the bicarbonate adduct are much smaller than those of cyanide adduct (Fig. 3) of Co(II)–BCA, which has typical tetrahedral geometry.[3] This difference may indicate that the degree of distortion from tetrahedral geometry is large in the bicarbonate adduct.[13]

The MCD spectra of the benzoate, 3-quinolinecarboxylate, bromide and chloride adducts resemble one another and are more complicated than those of five-coordinate adducts. The MCD spectra of the former adducts have large negative bands at 16000—18000 cm^{-1} and two very small negative bands at 19000—22000 cm^{-1}.

In general, tetrahedral coordination species have a small positive MCD band in the region from 19000 to 22000 cm^{-1} and have a large negative band in the region from 16000 to 19000 cm^{-1}.[1] If the five-coordinate and tetrahedral species coexist for a given adduct, the observed spectrum would be a superimposed form of the two types of spectra. Such spectra are observed for the 3-quinolinecarboxylate, chloride, benzoate, and bromide adducts, as shown in Fig. 2. The splitting of the bands in the wave number region of 19000 to 22000 cm^{-1} may be due to the overlapping of the MCD spectra of the tetrahedral and five-coordinate species, and the absolute intensity of the negative bands may depend on the population of the five-coordinate species. This conclusion is consistent with that reached in the case of the visible and near-infrared spectra by Bertini *et al.*[7] and Hirose *et al.*[10] The MCD spectra of the 3-quinolinecarboxylate, benzoate, bromide, and chloride adducts can be easily interpreted in this way. For example, the MCD spectrum of the bromide adduct has a large negative band and two small negative bands at the corresponding wave number region. The pattern of the MCD spectrum of the chloride adduct resembles that of the bromide adduct except that the intensity of the negative bands at 19000—22000 cm^{-1} of the former is much smaller than that of the latter. The larger absolute intensity of the negative bands at 19000—22000 cm^{-1} in the bromide adduct as compared with the chrolide adduct can be also interpreted in terms of a higher population of five-coordinate species in the former than the latter.[7] Therefore, the negative MCD bands in the region from 16000 to 19000 cm^{-1} are less valuable for identification of the coordination geometry, because tetrahedral and five-coordinate species have similar negative MCD bands. However, the MCD bands in the region from 19000 to 22000 cm^{-1} are very useful, because tetrahedral species have a positive band but five-coordinate species have a negative band in this region.

The alkaline form of Co(II)–BCA is highly active but its coordination geometry has remained unsettled (trigonal, distorted tetrahedral or five-coordinate).[1,4,7,14] It was proposed that the alkaline form (Fig. 3) might be five-coordinate on the basis that its MCD spectrum has two negative bands in the 16000—19000 cm^{-1} wave number region and is very similar to that of [Co(tris(2-methylaminoethyl)amine)Br]Br, and that its visible spectrum also greatly resembles that of [Co(tris(2-methylamino ethyl)amine)Br]Br complex.[1,14]

Previously, Bertini *et al.*[7] proposed that the alkaline form has severely distorted tetrahedral geometry because its visible and near-infrared spectrum lacks the highest *F–F** transition band in the region from 13000 to 15000 cm^{-1} which is characteristic of five-coordinate geometry. The MCD spectra of the 8-quinolinecarboxylate, oxalate and acetate adducts of Co(II)–BCA clearly indicate that five-coordinate species have two main negative bands in the visible region (16000—19000 and 20000—22000 cm^{-1}) which are almost independent of the nature of the coordinating atoms or coordination number of ligands (monodentate or bidentate ligand). These band positions are almost identical with those in the MCD spectra of the chloride, bromide, benzoate and 3-quinolinecarboxylate adducts, which are in an equilibrium between four- and five-coordinate species. However, the two negative band positions (16000 and 18000 cm^{-1}) in the MCD spectrum of the alkaline form are very different from those of the 8-quinolinecarboxylate, oxalate, and acetate adducts. These results suggest that the two negative MCD bands of the alkaline form are not due to the five-

coordinate species. Recently, it was shown that the absorption and MCD spectra of Co(II)–lactamase[15] are remarkably similar, both in the positions of absorption bands and their intensities, to those of the alkaline form of Co(II)–BCA. However, the coordination geometry postulated for Co(II)–lactamase II is distorted tetrahedral, and the ligands bound to the cobalt(II) ion in the enzyme are postulated to be three histidine residues and the S^- group of the sole cysteine. Therefore, it be reasonable to consider that the two negative bands of the MCD spectrum of the alkaline form of Co(II)–BCA may originate from highly distorted tetrahedral geometry rather than a five-coordinate form.

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