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SELECTION OF BINDING SITES IN COENZYME A BY NICKEL(II) AND COPPER(II)

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The sites of metal binding in coenzyme A(CoA) have been investigated in an aqueous solution bv visible absorption, proton magnetic resonance and electron spin resonance spectra, and compared with those in the related ligands. Spectroscopic results indicate that Ni(II) binding occurs at cysteamine-β-alanyl portion of CoA and that Cu(II) binding occurs at adenosine portion of CoA.

One of the most functional of the coenzymes, coenzyme A(CoA) is a complex of adenine, ribose, phosphoric acid, pantothenic acid and cysteamine. The multiplicity of potential metal binding sites present in CoA, especially selective binding affinity of cysteamine-β-alanine and adenosine portions, has attracted much interest. Herein, Ni(II) and Cu(II) bindings of CoA have been clarified by spectroscopic investigations. Recently, the preferred conformation of CoA in an aqueous solution has been studied by proton magnetic resonance(PMR), and the coupling constants and mole fractions of trans rotamers of cysteamine and β-alanine moieties have been reported.[1)]

CoA was purchased from P-L Biochemicals and was used without further purification.[2)] Adenosine-5'-diphosphate(ADP), pantethine and glutathione were obtained from Sigma Chemical Co., and β-mercaptopropionylglycine(β-MPG) was a gift of Santen Seiyaku Co. Pantetheine was obtained by the reduction of pantethine with sodium borohydride. PMR and electron spin resonance(ESR) spectra were recorded at 100 MHz on a Varian HA-100 NMR and at 100 KHz on a Joco ME-3X ESR spectrometers.

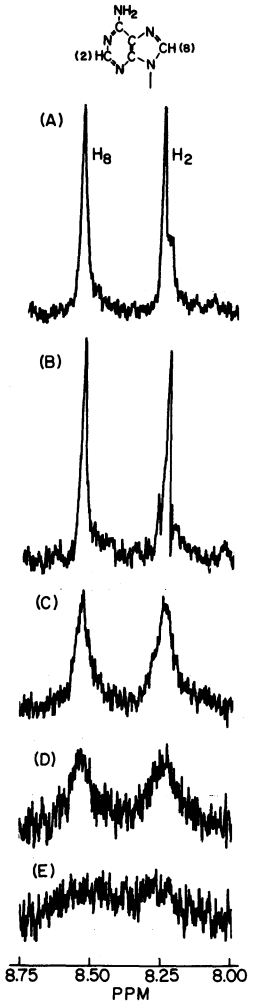
Visible absorption data of Ni(II) and Cu(II) complexes formed from CoA and its related ligands are summarized in Table I. The spectral feature of CoA-Ni(II) complex is characteristic of sulfur coordination, and is very similar to those of pantetheine-, β-MPG- and glutathione-Ni(II) complexes which coordinate through thiolate sulfur and deprotonated amide nitrogen atoms.[3] On the contrary, CoA-Cu(II) complex exhibits an absorption maximum at 700 nm, and its spectral characteristics resemble those of the ADP-Cu(II) complex, but not those of pantetheine-, β-MPG- and glutathione-Cu(II) complexes. This fact suggests that CoA interacts predominantly with Ni(II) through the cysteamine-β-alanyl moiety and with Cu(II) through the adenosine moiety.

Table I Spectral Data of Ni(II) and Cu(II) Complexes with Coenzyme A and Its Related Ligands

Ligand	Absorption Maxima, nm (ε)	
	Ni(II) Complex	Cu(II) Complex
Coenzyme A[a]	410(600) 505(270)	700(20)
Pantetheine[a]	407(700) 510(250)	625(60)
β-Mercaptopropionylglycine[a]	400(1200) 510(290)	630(100)
Glutathione[a]	420(680) 520(200)	620(70)
N,N-Dimethylcysteamine[b]	495(90)	not determined
Pantothenic Acid[b]	not determined	660(70)
Adenosine-5'-diphosphate[a]	not determined	725(25)
Pantethine[b]	not determined	not determined

a) These data were obtained by mixing ligand(1.0 mM) and $NiCl_2$(1.0 mM), or ligand(10.0 mM) and $CuCl_2$(10.0 mM) in borate buffer solution of pH 9.2.

b) The conditions are the same as in a), except for the concentration of ligand[10 mM for Ni(II) and 100 mM for Cu(II)].



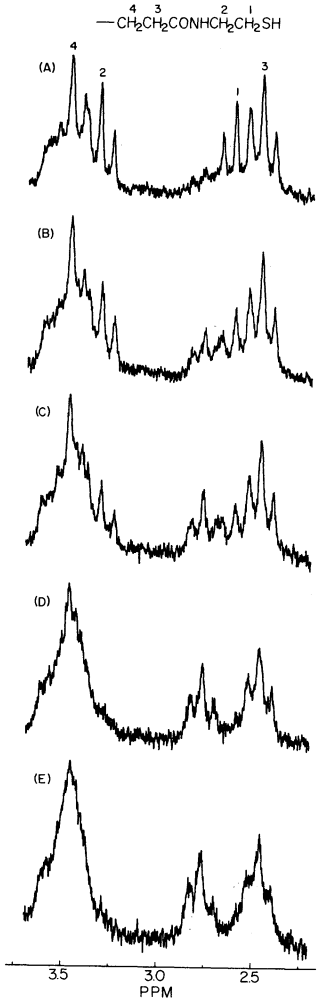


Figure 1 (left) PMR(100 MHz) spectra of adenine portion in CoA in D_2O(pD=9.6) at various $[Cu^{2+}]$: [CoA] values : A, no Cu^{2+}; B, 1:1000; C, 1:100; D, 1:10; E, 1:1. The concentration of CoA is 0.05 M. Chemical shifts were measured from internal TSP.

Figure 2 (right) PMR(100 MHz) spectra of cysteamine-β-alanyl portion in CoA in D_2O(pD=9.6) at various $[Ni^{2+}]$: [CoA] values : A, no Ni^{2+}; B, 1:1000; C, 1:100; D, 1:10; E, 1:1. The concentration of CoA is 0.05 M.

Cu(II) has often been used as a paramagnetic ion probe of binding sites in molecules of biological interest,[4] because of its selective broadening function with little or no accompanying isotropic shifts. Addition of $CuCl_2$ solution(D_2O) to D_2O solution of CoA results in selective broadening of H-2 and H-8 signals of the adenine ring(see Figure 1). Since no effects on other PMR signals of CoA in the presence of Cu(II) were evident, it is concluded that Cu(II) binding occurs through pyridine and amino nitrogen atoms of the adenine ring and presumably through the phosphate group as found in ADP- and ATP-Cu(II) complexes.[5] On the other hand, Ni(II)-induced shifts and broading of CoA proton signals are clearly different from those of CoA-Cu(II) complex. Figure 2 shows that significant PMR shifts and broading are found only for methylene signals of the cysteamine-β-alanyl moiety in the presence of Ni(II). In CoA-Ni(II) complex, the methylene resonances of the cysteamine-β-alanyl portion are observed as triplets at 2.77 (CH_2S, J_{av}=6.0 Hz) and 2.46(CH_2CO, J_{av}=6.5 Hz) ppm, and the methylene signals of two $NHCH_2$(2 and 4 positions) residues are broadened.[6] The result supports the binding of Ni(II) at thiolate sulfur and amide nitrogen groups of the cysteamine-β-alanyl moiety, consistent with the visible absorption data. Binding of Ni(II) at the adenosine portion is ruled out since H-2 and H-8 signals of the adenine ring are not perturbed in the presence of Ni(II), in contrast to the evidence in the presence of Cu(II).

The ESR spectrum of CoA-Cu(II) complex at 77°K is typical of pseudo square-planar Cu(II) environment with C_{2v} or D_{4h} symmetry(see Figure 3). The line-shape and the ESR parameters of CoA-Cu(II) complex are very similar to those of ADP-Cu(II) complex, rather than to those of β-MPG-Cu(II) complex in which thiolate sulfur, deprotonated peptide nitrogen and carboxylate oxygen atoms coordinate to Cu(II) atom(see Table II). In addition, the evaluated spin-orbital coupling constants for the Cu(II) complexes of CoA, ADP and β-MPG are 522, 530 and 500 cm^{-1}, respectively.[7] Again, the ESR evidence favors Cu(II) binding at the adenosine moiety of CoA. Of interest is the selection of binding metal ion by cysteamine and adenosine portions in CoA. The information will perhaps lead to a better understanding of the binding and specificity in multienzymes requiring CoA as a cofactor.

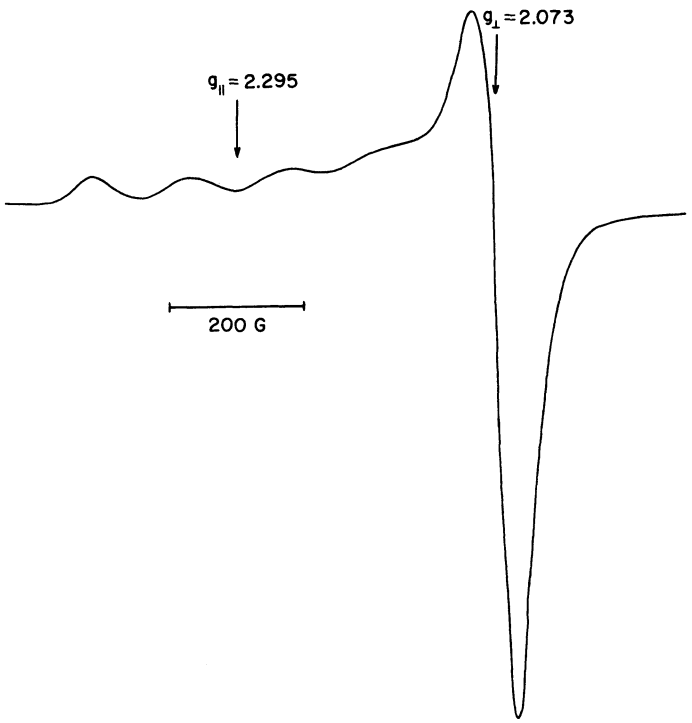


Figure 3 ESR spectrum of CoA-Cu(II) complex

The spectrum was measured by mixing CoA(10.0 mM) and $CuCl_2$(10.0 mM) in borate buffer solution(pH 9.2). The conditions of ESR spectroscopy : microwave power, 5 mW; frequency, 9.27 GHz; modulation amplitude, 5 G; temperature, 77°K.

Table II ESR Parameters for Cu(II) Complexes of Coenzyme A and Its Related Compounds

Ligand	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$ (G)	$A_{\perp}$ (G)
Coenzyme A	2.295	2.073	150	not resolved
β-Mercaptopropionylglycine	2.292	2.063	145	24
Adenosine Diphosphate	2.304	2.077	150	not resolved

References and Notes

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5) G.L.Eichhorn, in "Inorganic Biochemistry" Vol. 2, Ed. by G.L.Eichhorn, Elsevier Scientific Publishing, Amsterdam, 1973, p.1191.

6) On the basis of 220-MHz PMR measurements, methylene resonances of free CoA give rise to deformed triplet resonances at 2.58(CH_2S) and 3.29 ppm for the cysteamine portion, and at 2.43(CH_2CO) and 3.43 ppm for the β-alanine portion [see reference 1].

7) For many tetragonal Cu(II) complexes where an unpaired electron is in the $d_{x^2-y^2}$ orbital, the following relations are given approximately.

$$g_{\parallel} = 2(1 - \frac{4\lambda}{\Delta_1}) \qquad g_{\perp} = 2(1 - \frac{\lambda}{\Delta_2})$$

Δ_1 and Δ_2 are the ligand field splitting of d_{xy}-$d_{x^2-y^2}$ and $d_{xz,yz}$-$d_{x^2-y^2}$, respectively. The spin-orbital coupling constant(λ) was calculated from the experimental g-values and Δ_2 obtained from the visible spectrum. The λ-value of free Cu(II) ion is 828 cm^{-1}.

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