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Preparation and Characterization of the L-prolino Co(III) Complexes with the Tetradentate N_2O_2-type Ligands

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Complexes of the type [Co(T)(L-pro)], where T is the quadridentate N_2O_2-type ligand, N,N'-dimethylethylenediamine-N, N'-diacetate or N,N'-dimethylethylenediamine-N,N'-di-α-butyrate, have been prepared. The complexes were separated into the two stereoisomers, *Δ-s-cis*-[Co(T)(L-pro)] and *Λ-s-cis*-[Co(T)(L-pro)]. They were characterized by their proton magnetic resonance, absorption and circular dichroism spectra, elemental analyses.

Introduction

In recent articles cobalt(III) complexes of the N,N'-dimethylethylenediamine-N,N'-diacetate (dmedda) have been obtained in the *s-cis* geometry only.[1-5] Jung *et al.* have synthesized the dmedba(N,N'-dimethylethylenediamine-N,N'-di-α-butyrate) ligand containing both the C-ethyl and N-methyl substitutions. The cobalt(III) complexes of the dmedba have been found also *s-cis* isomer only.[6] The stereochemistry of cobalt(III) complexes with optically active amino acids is an interesting problem, and many studies have been directed toward the stereoselectivity or stereospecificity of those complexes.[7-11]

Recently, the amino acid cobalt(III) complexes of dmedda ligand, [Co(dmedda)(aa)], (aa = S-methyl-L-cysteinate, L-glutamate, L-aspartate, L-serinate) have been prepared from the reaction between the *s-cis*-$[Co(dmedda)Cl_2]^-$ complex and the trifunctional amino acids. The trifunctional amino acids have shown remarkable stereospecificity in their coordination to the racemic *s-cis*-$[Co(dmedda)Cl_2]^-$ giving the *Δ-s-cis*-[Co(dmedda)(aa)] absolute racemic *s-cis*-[Co(dmedda)Cl_2]$^-$ giving the *Λ-s-cis*-[Co(dmedda)(aa)] absolute configuration only.[5]

In the present paper, some aspects of the stereochemistry of the [Co(T)(L-pro)] complexes are dealt with; T = dmedda, dmedba. It will be shown that the two stereoisomers, *Δ-s-cis*-[Co(T)(L-pro)] and *Λ-s-cis*-[Co(T)(L-pro)], are stereoselectively formed in the *s-cis*-cobalt(III) complexes of dmedda and dmedba.

Experimental

Physical Measurements. Electronic absorption spectra were obtained with a Shimadzu UV-240 Spectrophotometer. ^{1}H-NMR spectra were recorded on a varian EM 360L Spectrometer. Infrared spectra were taken with a Hitachi 270-30 Spectrophotometer. Elemental analyses were performed by Korea Advanced Institute of Science and Technology. Circular dichroism spectra were measured using a Jasco J-550 C Automatic Recording Spectropolarimeter.

Preparation of *s-cis*-Hydrogen(N,N'-dimethylethylenediamine-N,N'-di-a-butyrato) Dichloro Cobaltate (III), *s-cis*-H[Co(dmedba)Cl_2]. 2.0g of barium N,N'-dimethylethylenediamine-N,N'-di-α-butyrate[6] was dissolved in 20 m*l* of water. 1.4g of cobaltous sulfate heptahydrate dissolved in 20 m*l* of water was added to this solution and the

reaction mixture was maintained at 50 °C for 30 min. Barium sulfate was removed from the reaction mixture by filtration. 2.0 m*l* of 30% H_2O_2 was carefully added to the filtrate. 30 m*l* of concentrated hydrochloric acid was added to this solution and then the resulting solution was concentrated to a volume of 20 m*l*, which was added to an ion-exchange column filled with 200-400 mesh Dowex 1-8X anion exchange resin. The eluent was 0.1N HCl solution. The first band showed a purple color, which turned out to be the [Co(dmedba)Cl(H_2O)] complex, and the second band showed a green color which was the H[Co(dmedba)Cl_2] complex. The green complex was obtained by evaporating the solution to dryness on a water bath.

Preparation and seperation of isomers of *s-cis*-(L-prolinato)(N,N′-dimethylethylenediamine-N,N′-diacetato)cobalt(III), *s-cis*-[Co(dmedda)(L-pro)]. A solution containing 0.67g (2 mmol) of *s-cis*-H[Co(dmedda)Cl_2][4] in 40 m*l* of water was heated at 60 °C for 20 min. To this solution was added a solution containing 0.23 g (2 mmol) of L-proline 10 m*l* of water. The pH of the solution was adjusted to 8.0 by addition of 1N NaOH aqueous solution. After 0.15g of activated charcoal have been added to the solution, the mixture was mechanically stirred at 60 °C for 3 hr. The charcoal and insoluble material were removed by filtration and washed with hot water. The combined filtrate and washings were concentrated to *ca.* 5 m*l* with rotary evaporator. The resulting violet solution was poured into a column containing cation-exchange resin (Dowex 50 W-X4, 200-400 mesh, H^+ form). The mixture of products absorved at the top of the column and pink impurities were eluted with water. The adsorbed band was separated into two bands. From their absorption and CD spectra, the first band turned out to be Δ-*s-cis* isomer and the second band Λ-*s-cis* isomer, respectively. The two stereoisomers were concentrated to a small volume, and to the solution was added ethanol and aceton. The resulting crystals were collected washed with ethanol, and dried. Anal. Calcd. for $CoC_{13}H_{22}O_6N_3 \cdot H_2O$ (Δ-*s-cis*). C, 40.6; H, 5.8; N, 10.9. Found C, 40.5; H, 5.8; N, 10.7.

Preparation and seperation of isomers of *s-cis*-(L-prolinato)(N,N′-dimethylethylenediamine-N,N′-di-α-butyrato)cobalt(III), *s-cis*-[Co(dmedba)(L-pro)]. The complex was prepared and separated into two isomers in the same procedure as that used for [Co(dmedda)(L-pro)] using dmedba inplace of dmedda. The reaction solution was concentrated and chromatographed on the cation exchange column. The complex was eluted with distilled water. From their absorption and CD spectra, the first band turned out to be Δ-*s-cis* isomer and the second band Λ-*s-cis* isomer, respectively. The two stereoisomers were concentrated to a small volume, and to the solution was added ethanol and aceton. The resulting crystals were collected, washed with ethanol, and dried. Anal. Calcd. for $CoC_{17}H_{30}O_6N_3 \cdot H_2O$ (Δ-*s-cis*). C, 45.4; H, 7.2; N, 9.4. Found C, 45.2; H, 7.3; N, 9.3.

Results and Discussion

The dichloro cobalt(III) complex of dmedda has yielded only one isomer of *s-cis* geometry,[4] and then the dichloro cobalt(III) complex of dmedba has been prepared only one isomer, the *s-cis* isomer, in this work. Therefore, the optically active L-proline cobalt(III) complexes of dmedda and dmedba have been prepared from the reaction between the *s-cis*-[Co(T)Cl_2]$^-$ complex and the L-proline amino acid.

Table 1. The COO Stretching Frequencies of the Co(III) Complexes

Compounds	(C=O)*	(C-O)*
H_2dmedda	1735	1440
dmedba	1600	1410
Δ-*s-cis*-[Co(dmedda)(L-pro)]	1650	1360
Λ-*s-cis*-[Co(dmedda)(L-pro)]	1645	1360
Δ-*s-cis*-[Co(dmedba)(L-pro)]	1650	1360
Λ-*s-cis*-[Co(dmedba)(L-pro)]	1660	1380

*These correspond to ν_a(COO^-) and ν_s(COO^-) of the symmetrical COO^- group. (in cm^{-1})

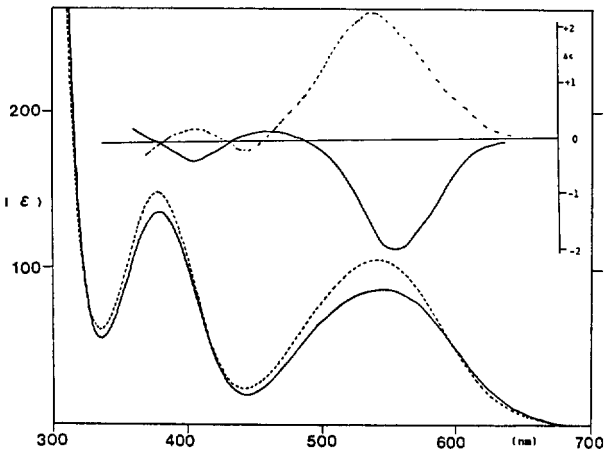

Figure 1. CD spectrum and electronic absorption spectrum of Δ-*s-cis*-[Co (dmedda)(L-pro)](—) and Λ-*s-cis*-[Co(dmedda) (L-pro)](····) complexes.

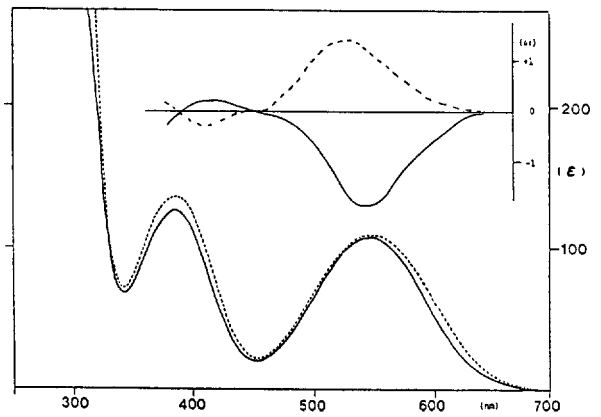

Figure 2. CD spectrum and electronic absorption spectrum of Δ-*s-cis*-[Co (dmedba)(L-pro)](—) and Λ-*s-cis*-[Co(dmedba)(L-pro)](····) complexes.

Table 1 shown the IR spectral data of the ligands as well as for the cobalt(III) complexes. The electronic absorption spectra of the complexes prepared in this work are shown in Figures 1 and 2. In the Table 2 the *d-d* transitions occure at 375-550 nm. In the *s-cis*-meridional isomer, the holohydrized symmetry is rhombic, with the average contribution being different along all three axes. This loss of symmetry of the *s-*

Table 2. Electronic Absorption Spectral Data for Aqueous Solutions of the Cobalt(III) Complexes

Compounds	Absorption maxima nm(ε, $M^{-1}cm^{-1}$)
Δ -*s*-*cis*-[Co(dmedda)(L-pro)]	542(85) 500 sh 380(135)
Λ -*s*-*cis*-[Co(dmedda)(L-pro)]	540(105) 500 sh 380(149)
Δ -*s*-*cis*-[Co(dmedba)(L-pro)]	548(107) 500 sh 385(125)
Λ -*s*-*cis*-[Co(dmedba)(L-pro)]	550(109) 500 sh 385(135)

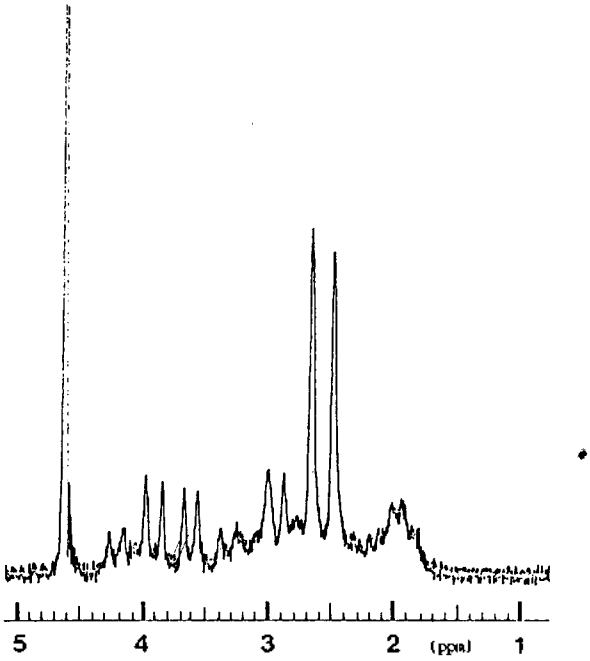

Figure 3. The ^{1}H-NMR spectrum of Δ-*s*-*cis*-[Co(dmedda)(L-pro)] complex.

cis-mer-[CoN_3O_3] system is expected to cause a splitting or at least a broadening of the lowest energy absorption.[12,13] Such phenomena are also observed in Figures 1 and 2.

The ^{1}H-NMR spectra are particularly helpful in distinguishing the coordinating mode. In the ^{1}H-NMR spectra of [Co(dmedda)(L-pro)] (Figure 3) the methyl protons attached to the nitrogen atoms show two singlets at 2.47 ppm and 2.67 ppm. The occurrence of two bands is probably due to the steric interaction between the N-methyl group and the proline ring. The ^{1}H-NMR spectrum of [Co(dmedda)(L-pro)] shows the same pattern as observed for *s*-*cis*-[Co(dmedda)(aa)], aa = S-methyl-L-cysteinate, L-glutamate, L-aspartate.[5] Particulary, the ^{1}H-NMR spectrum of [Co(dmedda)(L-pro)] complexes (Figure 3) is shown two AB quartet pattern due to the glycinate protons. The chemical shifts of the glycinate protons are 4.28, 3.98, 3.68, 3.38 ppm and 4.16, 3.86, 3.53, 3.23 ppm, respectively, and the coupling constant, J_{AB}, is 18 Hz. This values are in agreement with those which have been previously with the analogous complexes.[11,14,15] This results indicate that the [Co(dmedda)(L-pro)] complex is the *s*-*cis* isomer. In the previous report, *s*-*cis*-[$Co(N_2O_2)$AA] complexes. AA = two monodentate ligands or bidentate ligand with same donor atom, observed only one AB quartet pattern with the J_{AB} = 18Hz (J_{AB} = coupling constant) due to the glycinate ring protons. The *s*-*cis*-[$Co(N_2O_2)$AB] complexes, AB = two monodentate ligands with different donor atom or bidentate ligand with different donor atom, observed two AB quartet pattern due to the different chemical environment. In contrast to the relatively simple pattern of the *s*-*cis* isomer, the *uns*-*cis* isomer exhibits two AB quartet with different coupling constant or one AB quartet and a single intense absorption peak. In the *s*-*cis* configuration, however, the two glycinate rings are not equivalent in these complexes because of the coordination of the two different molecules as the additional ligands. Thus, as may be seen in Figure 3, two AB quartet patterns are observable. The *s*-*cis* configuration is, therefore, assigned to this complex. The optically active L-proline amino acid have shown remarkable stereoselectivity to give the stereoisomer in their coordination to racemic *s*-*cis*-$[Co(dmedda)Cl_2]^-$ out of two possible stereoisomers of Δ-*s*-*cis* and Λ-*s*-*cis* (Figure 5).

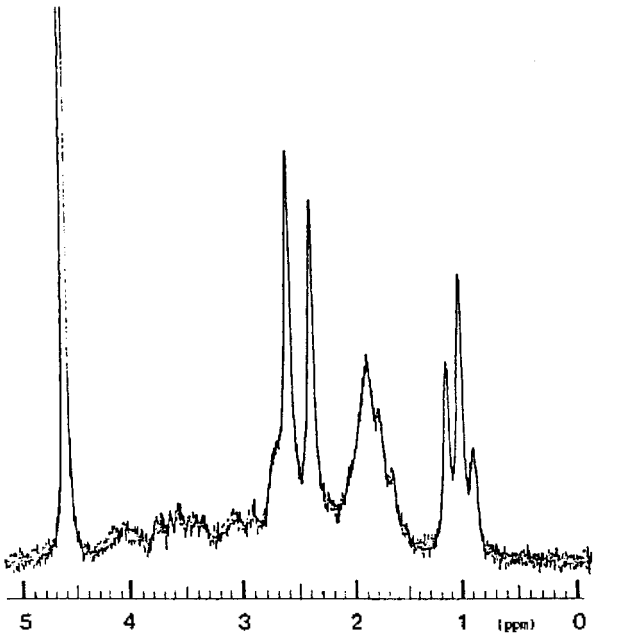

Figure 4. The ^{1}H-NMR spectrum of Δ-*s*-*cis*-[Co(dmedba)(L-pro)] complex.

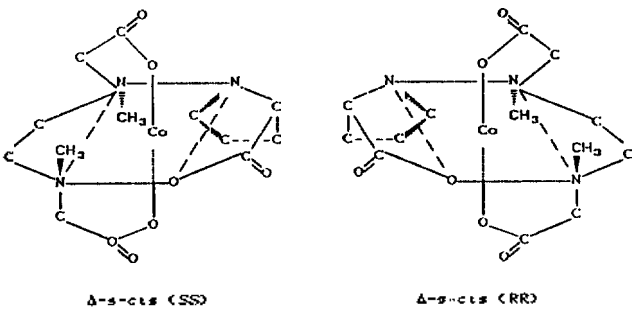

Figure 5. *s*-*cis* configurational isomers of [Co(dmedda)(L-pro)].

The CD spectra of the complexes prepared in this work (Figures 1 and 2) show the dominant peak in the T_{1g} region, indicating the fact that the negative dominant peak in the T_{1g} region have the Δ configuration and the positive dominant peak in the T_{1g} have the A configuration.[16-18] The optically active L-proline amino acid have shown remarkable stereospecificity to give the stereoisomer in their coordination to racemic *s*-*cis*-[Co(T)(L-pro)].

In Figure 4, which shows the ^{1}H-NMR spectrum of [Co(dmedba)(L-pro)], the methyl protons at the α-carbon atom are shown 1.0 ppm as a triplet and the methyl protons at the N-methyl protons are shown as a two singlet at 2.4, 2.6 ppm. If the complex has the *uns-cis* configuration, the methyl protons at the α-carbon atom would have shown two butyrato arms are no longer equivalent in the *uns-cis* geometry. We also confirm the stereoisomer of the *s-cis*-[Co(dmedba)(L-pro)] complexes from the CD spectra in Figure 2.

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NMR Studies of Lipid–Protein Interaction in Apolipoprotein B / Phosphatidylcholine Recombinants

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^{31}P{^{1}H} nuclear Overhauser effects (NOEs) have been obtained for complexes formed between apolipoprotein B (apo B) and dipalmytoylphosphatidylcholine (DPPC) vesicles. NOE measurements have been conducted with broad-band irradiation of the entire ^{1}H spectrum in order to identify the proton source of the NOE. In a unilamellar vesicle formed spontaneously upon mixing aqueous suspensions of long-chain phospholipid with small amount of short-chain lecithin, the maximum NOE occurs at the N-methyl proton resonance position of the choline moiety. With addition of cholesterol to vesicles, the position of the NOE maximum shifts further away from the choline methyl frequency. For the ternary apo B-vesicle-cholesterol complex, the position of the maximum NOE lies halfway between those in vesicles with and without cholesterol.

Introduction

The low-density lipoprotein (LDL) of human plasma plays a central role in the control of cholesterol transport and in the pathogenesis of atherosclerosis[1,2]. The overall structure of the LDL is that of a spherical particle with a neutral lipid core surrounded by phospholipids and apolipoprotein B (apo B)[3,4]. Phospholipid polar head-group behavior of the LDL has only recently been explored in any detail due to the lack of adequate probing method. NMR has proven to be a useful nonperturbing probe of the head-group region[5-9]. For Phosphatidylcholine, sphingomyelin bilayer, and LDL Yeagle *et al.*[5,6] reported a ^{31}P{^{1}H} nuclear Overhauser effect (NOE) profile with a maximum at the choline methyl ^{1}H frequency. Addition of cholesterol apparently disrupted the interactions, causing the NOE maximum to shift toward ^{1}H frequencies of methylene groups on either side of the phosphate. This study reports ^{1}H frequency dependence of ^{31}P NOEs and ^{1}H NMR chemical shift data for complexes formed between apolipoprotein B and small unilamellar vesicles with cholesterol. The object of our NMR studies is to provide deeper understanding of lipid-protein interactions as they occur in this model system.

Experimental

Materials. Egg phosphatidylcholine and phosphatidylethanolammine were obtained from Calbiochem; Dipalmitoylphosphatidylcholine and diheptanoylphosphatidylcholine was purchased from Sigma. Cholesterol was purchased from General Biochemical and the phospholipid purity was monitored by thin-layer chromatography.