Natural Abundance ^{15}N Nuclear Magnetic Resonance Study of Amino Acids Coordinated to Cobalt(III)

N. JURANIĆ†*, R. L. LICHTER*

City University of New York, Hunter College, Department of Chemistry, 695 Park Avenue, New York, N.Y. 10021, U.S.A.

M. B. ĆELAP, M. J. MALINAR and P. N. RADIVOJŠA

Department of Chemistry and Physical Chemistry, University of Belgrade, P.O. Box 550, 11001 Belgrade, Yugoslavia

Received Feburary 12, 1982

^{15}N chemical shifts of cobalt(III) amino acid complexes of the type λ-cis*(NO_2)*-trans*(NH_2)-[Co(NO_2)$_2$(Am)$_2$]$^{(+/-)}$ AmH = amino acid) have been determined at the natural-abundance level. Coordination with cobalt induces displacements of the amino acid ^{15}N resonance positions by 24–42 ppm to higher shielding compared to those of the corresponding protonated α-amino acids. The chemical shifts of β-amino acids are affected less markedly, while those of uncoordinated nitrogens remain practically unchanged. These changes allow monitoring of coordination sites by cobalt(III).*

Introduction

Biological interest in metal complexes of amino acids is based on the awareness that metal–ligand interactions in such complexes exhibit some properties common to more complex biological systems. Therefore, ^{15}N nuclear magnetic resonance (NMR) spectroscopy of coordinated amino acids, where nitrogen is directly bonded to metal ions, is an area that attracts attention. The underlying biological importance of ^{15}N spectroscopy of diamagnetic coordination compounds already has been stressed and substantiated in the study of Zn(II) binding to the adenine ring of adenine triphosphate [1], and in the study of Zn(II) and Cd(II) bonding to imidazole complexes [2]. Only a few ^{15}N NMR studies of amino nitrogen directly bonded to transition metal ions exists [1, 3, 4]. Of special interest is the study of metal complexes in the low-spin d^6 electronic configuration, where large changes in ^{15}N chemical shifts of the amino nitrogen occur upon bonding to the ions [4]. Among those ions which readily form low-spin d^6 complexes with amino acids, only cobalt(III) is essential for living organisms. Thus examination of these complexes by ^{15}N NMR may be particularly informative. Previous ^{15}N studies of nitrogen directly bonded to cobalt(III) have used only ^{15}N-enriched samples [5, 6], so that the range of available compounds has been limited. The ability to observe resonances at the natural-abundance level of ^{15}N (0.4%) affords access to larger ranges of compounds. We report here the results of such a study on complexes of the type λ-*cis*(NO_2), *trans*(NH_2)-[Co(NO_2)$_2$(Am)$_2$]$^{(-/+)}$, (AmH = amino acid, see Table I).

Experimental

Preparation of the complexes, λ-*cis*(NO_2)-*trans*-(NH_2)-[Co(NO_2)$_2$(Am)$_2$]$^{(-/+)}$, has been reported previously [7] (see also Table I), except for complexes with S-lysine. This complex was prepared by the reaction of *trans*-NH_4[Co(NO_2)$_4$(NH_3)$_2$] (0.02 mol in 100 cm^3 of water) and S-lysine (0.04 mol of S-lysine hydrochloride in 40 cm^3 of 1 *M* sodium hydroxide) at 50 °C for three hours. The filtrate obtained from the cooled reaction mixture was eluted through a column of cationic resin (Dowex 50W-X8, 200–400 mesh), first by water, then by 0.1 *M* aqueous sodium perchlorate. The second band eluted with sodium perchlorate was found to contain the *cis*(NO_2)- *trans*(NH_2)-[Co(NO_2)$_2$(S-Lys)$_2$]ClO_4. Subsequent fractional crystallization afforded 0.5 g of the pure λ-diastereoisomer ($[\alpha]_D^{20°}$ = +232°). Its absolute configuration was assumed by comparing the sign of optical rotation with the signs of other members of the homologous series [7b].

For NMR measurements we were able to prepare about 1 *M* aqueous solutions of the very soluble

†Visiting Fulbright Scholar (1980–81), University of Belgrade, Department of Chemistry and Physical Chemistry.

*Author to whom correspondence should be addressed.

0020-1693/82/0000-0000/$02.75

TABLE I. ^{15}N Chemical Shifts of Aminocarboxylato Ligands Coordinated in λ-*cis*(NO_2)- *trans*(NH_2)-[$Co(NO_2)_2(Am)_2$]$^{(-/+)}$ Complexes and Corresponding Protonated Amino Acids.[a]

Amino Acid	Coordinated	Protonated	Δ_c[b]	Ref.[c]
Glycine[d]	−10.8	30.9[e]	−41.7	7a, 7b
S-Aminobutyric acid	−4.0	30.0	−34.0	7c, 7d
S-Valine	−1.5	37.3[e]	−38.8	7c, 7d
S-Leucine	1.8	42.9[e]	−41.1	7d
S-Isoleucine	0.0	38.9[f]	−38.9	7c, 7d
S-Phenylalanine	3.6	40.8	−37.2	7e
S-Proline	18.6	54.6[e]	36.0	7e
S-Methionine	4.7	39.1	−34.4	7e
S-Lysine	5.0	42.6[e] (α-NH_3^+)	−37.6	
	33.4	35.6[e] (ε-NH_3^+)	−2.2	
S-Arginine	8.2	42.8[e] (α-NH_3^+)	−34.6	7e
	75.9	74.1[e] ($NH_2 \rightleftharpoons NH_3^+$)	1.8	
	89.2	86.6[e] (−NH−)	2.6	
β-Alanine	9.8	34.2[f]	−24.4	7f, 7b

[a]In ppm from anhydrous liquid ammonia [8]. Positive sign indicates a shift to lower shielding. [b]Defined as the difference in ^{15}N chemical shifts between coordinated aminocarboxylato ligand and protonated amino acid. [c]References for the preparation and/or determination of absolute configuration of the complexes. [d]Racemic mixture. [e]According to reference 8 and references given therein. [f]Reference 12 (calculated by means of conversion constant = 378 ppm, obtained from the value for glycine).

sodium salts of the anionic complexes. The cationic complex with lysine was readily soluble, while the same concentration for the argininato complex was achieved only by dissolving it in a concentrated aqueous solution of LiBr. All solutions contained 5% D_2O for NMR field locking. Proton- noise-decoupled Fourier transform ^{15}N spectra were recorded in a 10-mm tube at 10.09 MHz on a JEOL PS/PFT-100 NMR spectrometer. With a pulse angle of about 60° and a repetition time of 3 s, 10–15 hours were required to achieve a 3:1 signal-to-noise ratio. Chemical shifts are reported relative to anhydrous liquid ammonia at 25 °C using nitromethane as the external standard (capillary) and a conversion constant of 380.2 ppm [8].

Results and Discussion

A ^{15}N NMR signal of nitrogen bonded to ^{59}Co (I = 7/2) may be expected to be split into an octet because of spin–spin coupling. Although the octet structure has been observed for a very symmetrical [$Co(NH_3)_5(^{15}NH_3)$]Cl_3 complex [6], fast quadrupolar relaxation of ^{59}Co in less symmetrical complexes effectively decouples cobalt from nitrogen and a single resonance line is observed [5]. However, the line generally is broadened by relaxation through modulation of the scalar spin–spin coupling. Under these conditions the ^{15}N resonance linewidth at half-height, $\nu_{1/2}$ (^{15}N), is given by:

$$\nu_{1/2}(^{15}N) = 21J^2/\nu_{1/2}(^{59}Co) \quad (1)$$

where J is the ^{15}N–^{59}Co coupling constant and $\nu_{1/2}$-(^{59}Co) the linewidth at half-height of the corresponding ^{59}Co resonance. Low signal-to-noise ratios have prevented determination of precise linewidths in the ^{15}N spectra of the complexes. Nevertheless, observed linewidths of the amino nitrogen directly bonded to cobalt(III) vary over the range 5–10 Hz, and there is a distinct inverse proportionality with linewidths in the ^{59}Co NMR spectrum of the same complexes [9], as required by eqn. (1). We have estimated the coupling constant thus to be $J(^{15}N–^{59}Co) = 60 \pm 10$ Hz. This value is comparable to the amino nitrogen coupling constant $J(^{15}N–^{59}Co) = 62.5$ Hz in [$Co(NH_3)_5(^{15}NH_3)$]Cl_3 [6].

^{15}N NMR chemical shifts of amino acids coordinated as the aminocarboxylato chelate ligands to cobalt(III) in λ-*cis*(NO_2)- *trans*(NH_2)-[$Co(NO_2)_2$-$(Am)_2$]$^{(-/+)}$ complexes are given in Table I. Since all complexes studied have C_2 symmetry, two aminocarboxylato ligands exhibit the same ^{15}N chemical shifts. The chemical shift of the amino nitrogen directly bonded to cobalt(III) lies considerably to higher shielding compared with those of the free aminocarboxylato ligand [8], the zwitterion [8],

or the protonated amino acid. In order to eliminate amino group lone pair effects, we have compared in Table I the chemical shifts of coordinated and doubly protonated aminocarboxylato ligands. With the exception of (S)-phenylalanine, (S)-methionine, and (S)-aminobutyric acid, whose chemical shifts were determined in this work, values for protonated amino acids have been taken from the literature [8, 12]. The comparison shows that an additional shielding, Δ_c, of 24–42 ppm arises when a proton is replaced by cobalt(III). Very similar shifts to higher shielding have been observed for protonated diamine ligands when protons are replaced by rhodium(III) [4]. Table I also shows substantial differences in Δ_c among amino acids. In particular, an especially low value of Δ_c is observed for β-amino acids. It is well established that α-amino acids, which form five-membered aminocarboxylato chelate rings with cobalt(III), exhibit a stronger metal–ligand bond than β-amino acids, which form six-membered ones [9]. Therefore, the low Δ_c value observed for β-alanine may indicate a weaker nitrogen–cobalt bond. If such a relationship is assumed, it is difficult to explain the observed variations in Δ_c among the α-amino acids, because there is no clear correlation between their composition and the coordination shifts. However, a correlation may be obscured by other factors, *e.g.*, formation of second-sphere complexes, none of which is dominant enough to display clearly systematic trends. In any case, sensitivity of the coordination shift to the nature of amino acid is likely to be useful in further study of their bonding to the metal ions.

When more than one nitrogen of an amino acid may be involved in bonding to cobalt(III), the large change in the ^{15}N resonance position of the nitrogen bonded to cobalt(III) gives direct insight into the type of bonding that has taken place. This is well illustrated by the chemical shifts of complexes with lysine and arginine (Table I), where only the α-amino group nitrogen is shielded, while chemical shifts of other potentially coordinating nitrogens remain practically unaffected. This feature of the d^6 metal-ion influence on nitrogen chemical shifts is likely to make cobalt(III) very useful for determining bonding sites in more complicated ligands by ^{15}N NMR spectroscopy.

The large shielding of the ^{15}N nucleus of the Co(III)-bound amino nitrogen seems to arise from the spin-paired d^6 electronic configuration of the metal ion. In this configuration, readily excitable d-electrons give rise to the second-order paramagnetism of the cobalt(III). In a molecular orbital description, the corresponding d–d electronic transition occurs between antibonding molecular (metal–ligand) orbitals, the consequence of which may be described in terms of the Cornwell-Santry effect [10, 11]. Essentially, this means that a large paramagnetic circulation on the metal ion is accompanied by a diamagnetic circulation on the directly bonded nucleus. Calculations [11] show that this mechanism may induce an additional nitrogen shielding of up to a few hundred ppm, the extent of which is sensitive to metal–ligand bond strength. A more detailed study of the influence of composition of complexes and of ligand field symmetry and strength of ^{15}N chemical shifts is in progress.

Acknowledgements

This work was supported by funds from NIH Grant No. GM-21148 and by PSC-CUNY Grant Nos. 13118 and 13344 to R. L. Lichter, and by the Serbian Republic Research Fund. R. L. Lichter is grateful to Sandoz, Inc. for its hospitality during part of the preparation of this manuscript.

References

1 J. A. Happe and M. J. Morales, *J. Am. Chem. Soc., 88,* 2077 (1976).
2 M. Alei, Jr., L. O. Morgan and W. E. Wageman, *Inorg. Chem., 17,* 2288 (1978); *17,* 3314 (1978); *20,* 940 (1981).
3 R. Hagen, J. P. Warren, D. M. Hunter and J. D. Roberts, *J. Am. Chem. Soc., 95,* 5712 (1973).
4 K. S. Bose and E. H. Abbott, *Inorg. Chem., 16,* 3190 (1977).
5 Y. Nakashima, M. Muto, J. Takagi and K. Kuwano, *Chem. Letters,* 1075 (1975).
6 Y. Akiro, M. Yoshie, Y. Yuzo and Y. Hideo, *Denki Tsushin Digaku Gakuho, 28,* 107 (1977).
7 a) M. B. Ćelap, T. J. Janjić and D. J. Radanović, *Inorg. Synth., 9,* 173 (1967);
b) R. G. Denning, M. B. Ćelap and D. J. Radanović, *Inorg. Chim. Acta, 2,* 58 (1968);
c) M. B. Ćelap, M. B. Dimitrijević, D. J. Radanović, F. A. Coha, T. J. Nikolić and T. J. Janjić, *Glasnik Hem. drustva Beograd, 35,* 449 (1970);
d) M. B. Ćelap, R. G. Denning, D. J. Radanović and T. J. Janjić, *Inorg. Chim. Acta, 5,* 9 (1971);
e) B. A. Kamberi, M. B. Ćelap and T. J. Janijić, *Glasnik Hem. drustva Beograd, 43,* 149 (1978);
f) M. B. Ćelap, D. J. Radanović, T. J. Nikolić and T. J. Janjić, *Inorg. Chim. Acta, 2,* 52 (1968).
8 G. C. Levy and R. L. Lichter, 'Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy', Wiley–Interscience, New York (1979).
9 N. Juranić, M. B. Ćelap, D. Vucelić, M. J. Malinar and P. N. Radivojša, *J. Coord. Chem., 9,* 117 (1978).
10 a) C. D. Cornwell, *J. Chem. Phys., 44,* 874 (1966);
b) D. P. Santry, 'Theoretical Chemical Research at Carnegie Institute of Technology', April 1965.
11 N. Juranić and R. L. Lichter, *J. Am. Chem. Soc.,* in press.
12 H. R. Kricheldorf, *Org. Magn. Reson., 12,* 414 (1979).