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^{13}C NMR Study of the Synergistic Anion in Transferrins

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^{13}C NMR spectra of Al(III), Ga(III), and Zn(II) derivatives of both human serum transferrin and ovotransferrin with ^{13}C-enriched bicarbonate or oxalate as synergistic anions have been measured at 75.4 MHz. The good quality of the spectra allows us to get detailed information on the state of the synergistic anion. The chemical shift values of the bound anion are interpreted on the basis of the effects arising from the interaction with the protein and the metal and through comparison with simple inorganic complexes. Our ^{13}C NMR spectral data support the idea that the synergistic anion bridges the metal to some positively charged residue of the protein in agreement with the interlocking-sites model proposed by Bates. In the case of the bicarbonate derivatives, chemical shift values suggest carbonate as the form of the bound anion; the arrangements of the anion in the N-terminal and C-terminal sites appear to be identical within the resolution of the technique. The spectral data of the tripositive metal ion derivatives, with oxalate as synergistic anion, show a strong inequivalence between the two carboxylate carbons and are indicative of oxalate monodentate binding to the metal. Oxalate arrangements in the two sites are very similar but not identical. Differences in ^{13}C NMR spectral data between tripositive and bipositive metal derivatives are also discussed.

Transferrins are a class of double-sited, single-chain proteins (M_r = 80 000) involved in iron transport in biological systems.[1-3] They can specifically and tightly bind 2 equiv of a large number of tripositive and bipositive metal ions besides iron. The metal binding sites have been extensively investigated. Both sites appear to possess a distorted octahedral geometry, the binding ligands probably being two tyrosines,[4-6] two histidines,[7-9] a water or hydroxide moiety,[6,10-12] and a synergistic anion.[13,14] The latter is the most striking feature of transferrin chemistry; indeed transferrins, in order to bind metals, require the presence of an anion (hence "synergistic") giving rise to ternary complexes at both sites. The native synergistic anion is (bi)carbonate, but also many other bifunctional anions can act as synergistic anions.[13,14] The synergistic anion modulates the affinity of transferrins for metals; therefore, it appears to play a central role in the reactivity of the metal centers, in particular in metal uptake and release processes.[15] A controversy is still open on whether it must be considered as carbonate or bicarbonate. The latest reports point toward an overcoming of the conflict by assuming that the above distinction is "only a matter of degree" owing to the presence of hydrogen bond interactions.[3,16]

On the other hand there is an almost general agreement on the steric and electronic requirements of the suitable anions; they must possess both a carboxylate group and a Lewis base function separated by not more than 7 Å.[13,14] The way by which the anion interacts with the protein and the metal has been described by Schlabach and Bates through the interlocking-sites model. In this model the Lewis base function is thought to bind directly to the metal whereas the carboxylate group interacts with a cationic group of the protein.[13,14] This hypothesis, suggested by electronic and EPR spectra of the iron derivative, has been subsequently supported by several experimental results obtained on different metal-substituted transferrins. Evidence of anion coordination to the metal has been obtained from electron spin-echo studies on the ^{13}C-enriched oxalate derivatives of copper(II)–ovotransferrin (Otf)[17] and copper(II)–transferrin (Tf)[18] through an analysis of the modulation of the echo decay by the ^{13}C nucleus. Other evidence has come from EPR spectra of oxovanadium(IV) derivatives,[19] from EXAFS studies on the Fe(III)–Otf–thioglycolate derivative,[20] and from resonance Raman data on the Fe(III)–Otf–dihydroxybenzoate complex.[20] The absence of a signal of ^{13}C-enriched bicarbonate bound to the protein in (bicarbonato)iron(III) transferrin indicates an upper limit of 9 or 4.9 Å for the iron–^{13}C distance according to different reports.[21,22]

The carboxylate function of the synergistic anion is thought to interact with some (one or more) positively charged groups of the protein;[7,22,23] this is consistent with recent biochemical studies, which indicate that both metal-binding domains are rich in positively charged residues such as Arg, Lys, and His.[24] This fact would also nicely account for the absolute requirement of the synergistic anion in order for metal binding to take place; the anion would allow complex formation by reducing the net positive charge in the binding cavity.

In order to obtain a deeper insight into the binding mode of the synergistic anion and further spectral evidence for the above outlined model, we prepared the Ga(III),[25] Al(III),[26] and Zn(II)[27] derivatives of Otf and Tf with ^{13}C-enriched bicarbonate and, for Otf, also the ^{13}C-enriched oxalate derivatives and investigated them through ^{13}C NMR spectroscopy.

Owing to the similarity of ionic radius and charge density, Ga(III) and Al(III) appear to be good probes for iron(III) substitution. Indeed, transferrins are known to form very stable complexes with tripositive metal ions like Ga(III) and Al(III).[25,26] In particular, in the case of the gallium(III) derivative, accurate thermodynamic data for complex formation have been reported,[25] which are very similar to those found for the iron derivative. This chemical analogy allows us to be rather confident in transferring

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(1) Chasteen, N. D. *Coord. Chem. Rev.* **1977**, *22*, 1.
(2) Aisen, P.; Listowski, I. *Annu. Rev. Biochem.* **1980**, *49*, 357.
(3) Chasteen, N. D. *Adv. Inorg. Biochem.* **1983**, *5*, 201–233.
(4) Gaber, B. P.; Miskowski, V.; Spiro, T. G. *J. Am. Chem. Soc.* **1974**, *96*, 6868.
(5) Williams, J. *Biochem. J.* **1982**, *201*, 647.
(6) Pecoraro, V. L.; Harris, W. R.; Carrano, C. J.; Raymond, K. N. *Biochemistry* **1981**, *20*, 7033.
(7) Alsaadi, B. M.; Williams, R. J. P.; Woodworth, R. C. *J. Inorg. Biochem.* **1981**, *15*, 1.
(8) Rogers, T. B.; Gold, R. A.; Feeney, R. E. *Biochemistry* **1977**, *16*, 2299.
(9) Bertini, I.; Luchinat, C.; Messori, L.; Scozzafava, A. *Eur. J. Biochem.* **1984**, *141*, 373.
(10) Koenig, S. H.; Schillinger, W. E. *J. Biol. Chem.* **1969**, *244*, 6520.
(11) Zweier, J. L.; Aisen, P. *J. Biol. Chem.* **1977**, *252*, 6090.
(12) Chasteen, N. D.; White, L. K.; Campbell, R. C. *Biochemistry* **1977**, *16*, 3631.
(13) Schlabach, M. R.; Bates, G. W. *J. Biol. Chem.* **1975**, *250*, 2177.
(14) Bates, G. W.; Schlabach, M. R. *J. Biol. Chem.* **1975**, *250*, 2182.
(15) Aisen, P.; Brown, E. B. *Prog. Hematol.* **1975**, *9*, 25.
(16) Carrano, C. J.; Spartalian, K.; Appa Rao, G. V. N.; Pecoraro, V. L.; Sundaralingam, M. *J. Am. Chem. Soc.* **1985**, *107*, 1651.
(17) Zweier, J. L.; Peisach, J.; Mims, W. B. *J. Biol. Chem.* **1982**, *257*, 10314.
(18) Zweier, J. L.; Aisen, P.; Peisach, J.; Mims, W. B. *J. Biol. Chem.* **1979**, *254*, 3512.
(19) Campbell, R. F.; Chasteen, N. D. *J. Biol. Chem.* **1977**, *252*, 5996.
(20) Schneider, O. J.; Roe, A.; Mayer, R. J.; Que, L. Jr. *J. Biol. Chem.* **1984**, *259*, 9699.
(21) Harris, D. C.; Gray, G. A.; Aisen, P. *J. Biol. Chem.* **1974**, *249*, 5261.
(22) Zweier, J. L.; Wooten, J. B.; Cohen, J. S. *Biochemistry* **1981**, *20*, 3505.
(23) Rogers, T. B.; Boerresen, T.; Feeney, R. E. *Biochemistry* **1978**, *17*, 1105.
(24) Williams, J.; Elleman, T. C.; Kingston, I. B.; Wilkins, A. G.; Kuhn, K. A. *Eur. J. Biochem.* **1982**, *122*, 297.
(25) Harris, W. R.; Pecoraro, V. L. *Biochemistry* **1983**, *22*, 292.
(26) Cochran, M.; Coates, J.; Neoh, S. *FEBS Lett.* **1984**, *176*, 129.
(27) Harris, W. R. *Biochemistry* **1983**, *22*, 3920.

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Table I. ^{13}C NMR Chemical Shifts[a] for ^{13}C-Enriched Synergistic Anions in Transferrins

Bicarbonate Derivatives

	chem shift, ppm		chem shift, ppm
HCO_3^-	161.4		
CO_3^{2-}	169.4		
Al_2–Otf(H)CO_3	165.9	Al_2–Tf(H)CO_3	165.4
Ga_2–Otf(H)CO_3	166.3	Ga_2–Tf(H)CO_3	166.5
Zn_2–Otf(H)CO_3	168.2	Zn_2–Tf(H)CO_3	168.5
		Co_2–Tf(H)CO_3	169.4[b]

Oxalate Derivatives

		chem shift, ppm		
		C1[c]	C2[c]	J(C–C), Hz
$C_2O_4^{2-}$		174.4		
Al_2–Otf(ox)	group 1	169.2	165.8	71
	group 2	169.2	166.2	68
Ga_2–Otf(ox)	group 1	168.4	165.7	71
	group 2	168.4	166.2	66
Zn_2–OTf(ox)		169.6[d]		

[a] Chemical shifts are given in ppm from Me_4Si. [b] See ref 22; the reported value is that of the nonexchangeable resonance. [c] C1 is assigned to protein-bound carbon and C2 to metal bound carbon. [d] Only one signal detectable.

chemical information obtained via ^{13}C NMR spectroscopy on these diamagnetic complexes to the native paramagnetic iron(III) system.

Substitution of bicarbonate with oxalate, although modifying the system, gives us the opportunity to analyze separately (i.e. on two different carbon nuclei) the interaction of the synergistic anion with both the protein chain and the metal. The investigation of the zinc(II) derivative allows us to compare the anion binding in the tripositive and bipositive series.

Experimental Section

Human serum apo-transferrin and chicken egg apo-ovotransferrin were purchased from Sigma Chemical Co. and further purified according to the standard procedure.[13] The concentration of the protein samples was determined through UV absorption spectroscopy (ϵ_{280} = 92 300 M^{-1} cm^{-1} for Tf; ϵ_{280} = 91 200 M^{-1} cm^{-1} for Otf).

Preparation of M–Otf and M–Tf Adducts. The bicarbonate derivatives of Otf and Tf with Al(III), Ga(III), and Zn(II) were prepared by adding stoichiometric amounts of metal chloride solutions to millimolar protein solutions, buffered at pH 8 with Tris–HCl 5 × 10^{-2} M in presence of a twofold excess of 91.2% ^{13}C-enriched bicarbonate. In order to avoid carbon dioxide contamination from the air, apoprotein solutions were degassed at low pH (pH 4.5) by bubbling nitrogen; then the pH was adjusted to 8 with solid Tris and ^{13}C-enriched bicarbonate was added together with the required amount of metal salt upon stirring; all sample handling was performed under nitrogen atmosphere. The analogous oxalate (90% ^{13}C enriched on both carbons) derivatives were prepared in the same way. Complex formation was followed through UV difference spectroscopy on 10^{-5} M protein samples by monitoring the development of the characteristic tyrosinate bands upon metal addition, according to the reported procedure.[6]

^{13}C NMR Measurements. Proton-decoupled ^{13}C NMR spectra were recorded on a Bruker CXP 300 spectrometer operating at 75.46 MHz. Typically 30 000–40 000 scans were collected for each spectrum, by using a 50° flip angle, a pulse delay of 1.6 s and a spectral window of 10 000 Hz. Quadrature detection was employed, and the spectra were recorded with line broadening of 3–5 Hz. The chemical shift values are referenced to Me_4Si (Table I). The NMR experiments were performed at 35 °C, using 1.5 mM protein samples (1.5 mL in 10 mm tubes).

Results

^{13}C NMR Spectra of Otf–Bicarbonate–Metal Complexes. The ^{13}C NMR spectra in the carbonyl region of Al_2–Otf, Ga_2–Otf and Zn_2–Otf complexes with ^{13}C-enriched bicarbonate as synergistic anion are reported in Figure 1; the spectrum of apo–Otf at the same pH in the presence of ^{13}C bicarbonate is also reported. All the spectra of the metal derivatives show the typical pattern of natural-abundance protein carbonyl signals (see the spectrum of apo–Otf as reference),[28] the signal of free bicarbonate (161.4 ppm) and a further rather broad signal in the 166–168 ppm region. The latter signal is lacking in the apoprotein spectrum and can be assigned to bicarbonate specifically bound to the two metal sites. This assignment is consistent with previous observations on transferrin derivatives;[29] the fact that only one signal is detectable for bound bicarbonate in all the derivatives suggests that the anion environment in both sites is very similar. The chemical shift value of the above signal changes through the three derivatives; however, these differences are small and can be accounted for by the different contribution of each metal to the carbon nucleus shielding constant.

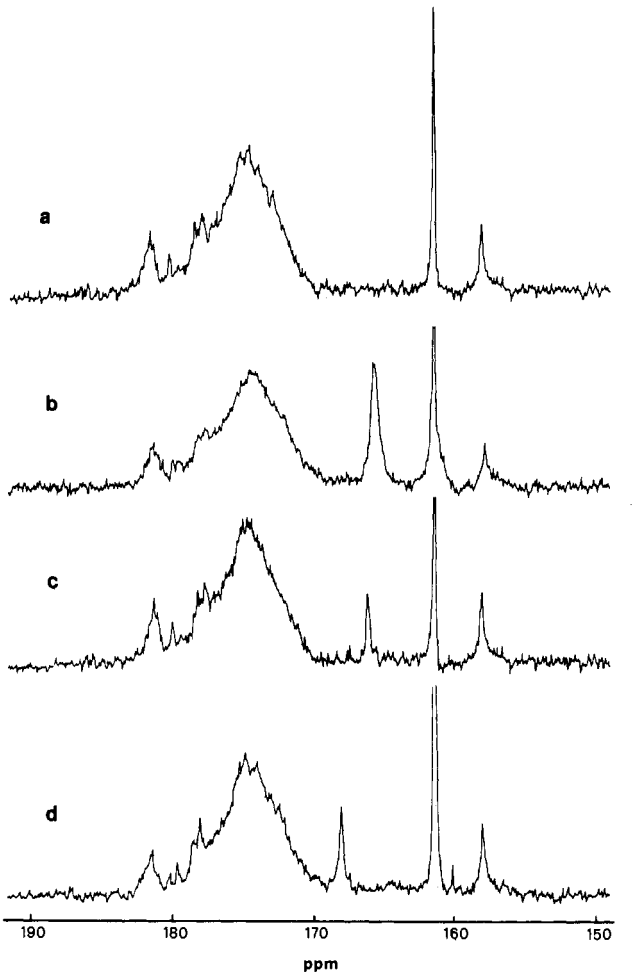


Figure 1. 75.4-MHz ^{13}C NMR spectra of the carbonyl region of Otf–bicarbonate–metal complexes in presence of a twofold excess of ^{13}C-enriched bicarbonate, pH 8 (protein concentration 1.5 mM): (a) apo–Otf; (b) Al_2–Otf; (c) Ga_2–Otf; (d) Zn_2–Otf.

When the pH is lowered to pH 5, the metal protein complexes break down and the signal relative to the bound anion disappears.

^{13}C NMR Spectra of Tf–Bicarbonate–Metal Complexes. The results for the Ga_2–Tf, Al_2–Tf, and Zn_2–Tf bicarbonate complexes (Figure 2) are similar to those of the corresponding Otf derivatives. Again, only one signal for bound (bi)carbonate was detected with a chemical shift value almost identical with that of the homologous Otf derivative. These results are consistent with an observation on the digallium(III)–transferrin–(bi)carbonate system[29] and partly with a report on the dicobalt(III)–transferrin–(bi)carbonate system;[22] in the latter, further signals of fractional intensities were also observed and attributed to specifically bound bicarbonate.

^{13}C NMR Spectra of Otf–Oxalate–Metal Complexes. The ^{13}C NMR spectra of the dimetal derivatives of Otf with Al(III),

(28) Wütrich, K. *NMR in Biological Research: Peptides and Proteins*; North-Holland: Amsterdam, 1976; pp 157–209.
(29) Harris, D. C.; Aisen, P. In *Proteins of Iron Storage and Transport in Biology and Medicine*; Elsevier: Amsterdam, 1975; pp 157–209.

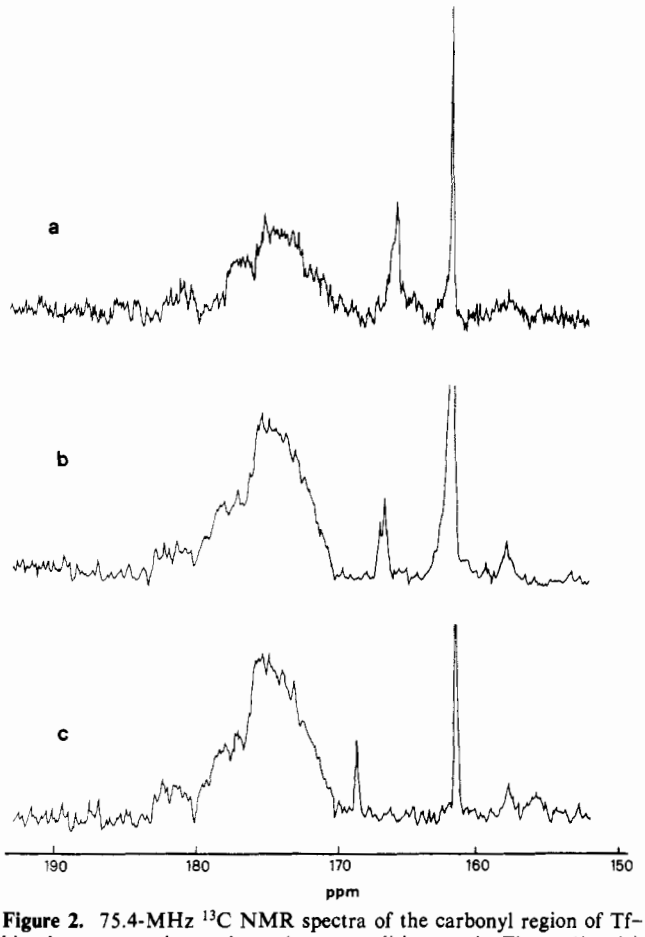


Figure 2. 75.4-MHz ^{13}C NMR spectra of the carbonyl region of Tf–bicarbonate–metal complexes (same conditions as in Figure 1): (a) Al_2–Tf; (b) Ga_2–Tf; (c) Zn_2–Tf.

Ga(III), and Zn(II) in presence of a twofold excess of ^{13}C-enriched oxalate at pH 8 and the spectrum of apo–Otf under the same conditions are reported in Figure 3.

If we analyze the spectrum of the Al_2–Otf derivative, we observe, besides natural-abundance protein signals and a free oxalate signal, a complex envelope of resonances in the spectral region between 170 and 165 ppm. This group of resonances is interpreted as arising from the overlapping of two AB groups (see Table I for the NMR parameters);[30] each AB group would correspond to an oxalate anion specifically bound to one of the two protein metal sites. Also the observed J(C–C) value is in agreement with that expected for the coupling of two sp^2 carbons according to the Karplus formula.[31] As previously shown, oxalate enhances the structural differences between the two sites.[32]

The two sites can be discriminated by lowering the pH to 6. It is generally known that at this pH value transferrins can bind just one metal equivalent, since only the C-terminal site appears to be acid resistant. Indeed, UV difference spectra show evidence that at pH 6 only one aluminum ion is bound to the protein. Performing the NMR spectrum at this pH, we observe that the signals of one of the two AB systems (group 2 resonances) selectively disappear as shown in Figure 4. On this basis, we can suggest that the group 1 resonances are assigned to oxalate bound to the C-terminal site, whereas group 2 resonances are assigned to oxalate in the N-terminal site. However, owing to the slight differences in the chemical shift values between the two groups of resonances, the anion should be arranged in a quasi-equivalent way in both sites.

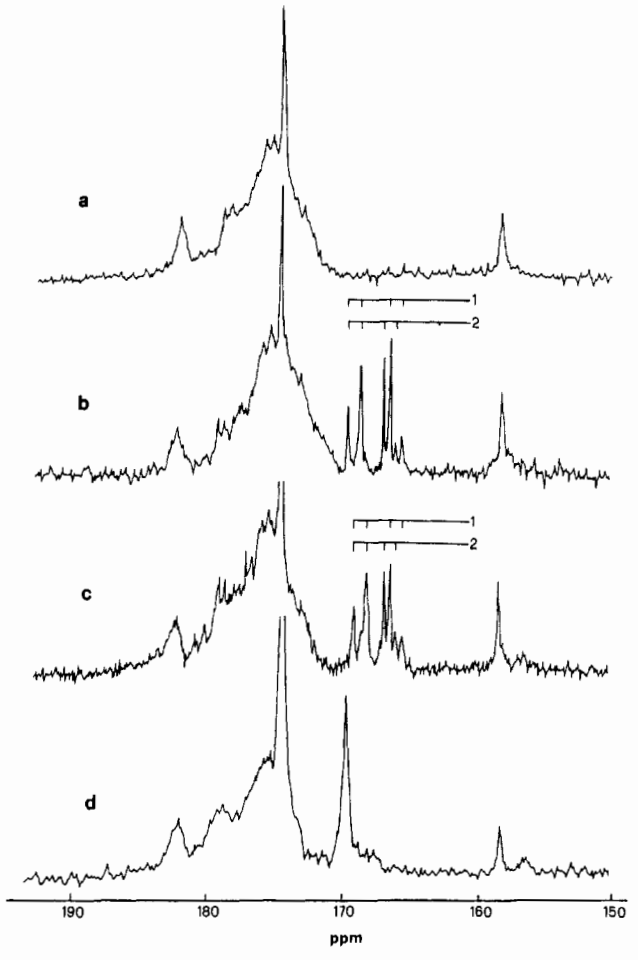


Figure 3. 75.4-MHz ^{13}C NMR spectra of the carbonyl region of Otf–oxalate–metal complexes in presence of a twofold excess of ^{13}C-enriched sodium oxalate: (a) apo–Otf; (b) Al_2–Otf; (c) Ga_2–Otf; (d) Zn_2–Otf. Except that for the synergistic anion, the conditions are the same as in Figure 1.

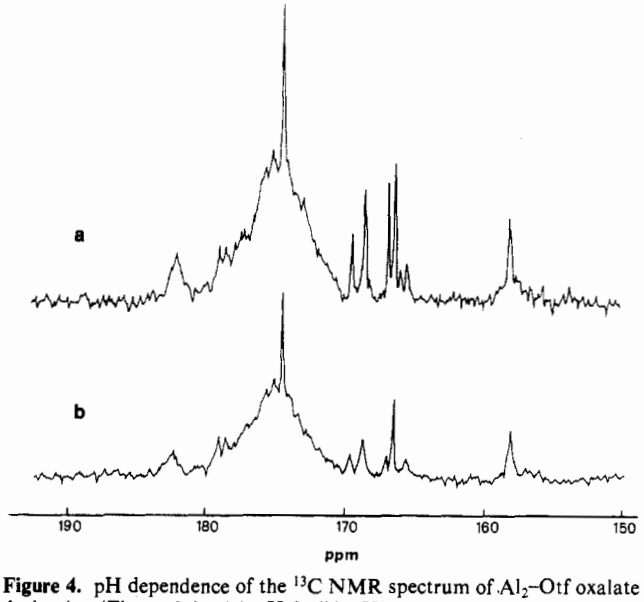


Figure 4. pH dependence of the ^{13}C NMR spectrum of Al_2–Otf oxalate derivative (Figure 3c): (a) pH 8; (b) pH 6.

The spectrum of the Ga_2–Otf oxalate derivative exhibits features almost superimposable on those of the above aluminum(III) complex (see Table I). Again, there is a group of resonances in the 170–165 ppm region that can be interpreted as the sum of two AB systems. Only very slight differences with respect to aluminum data are present in chemical shift and J(C-C) values, which can be ascribed to metal ion effects.

(30) Bertini, I.; Borghi, E.; Luchinat, C.; Scozzafava, A. *J. Mol. Struct.* **1984**, *113*, 191.
(31) Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon 13 NMR Spectra*; Heyden: London, 1978.
(32) Bertini, I.; Luchinat, C.; Messori, L.; Monnanni, R.; Scozzafava, A. *J. Biol. Chem.* **1986**, *261*, 1139.

A completely different spectrum is shown by the zinc(II)–Otf–oxalate derivative (Figure 3); in this case only one rather broad signal, attributable to specifically bound oxalate is present. This behavior could be interpreted as an evidence of a different anion arrangement in the bipositive metal series with respect to the tripositive series or to a minor difference between the two carbon atoms of each oxalate as the result of a smaller shielding effect exerted by the metal on the carbon atom.

Discussion

In principle, nuclear shielding of a carbon atom can be considered as the result of three terms

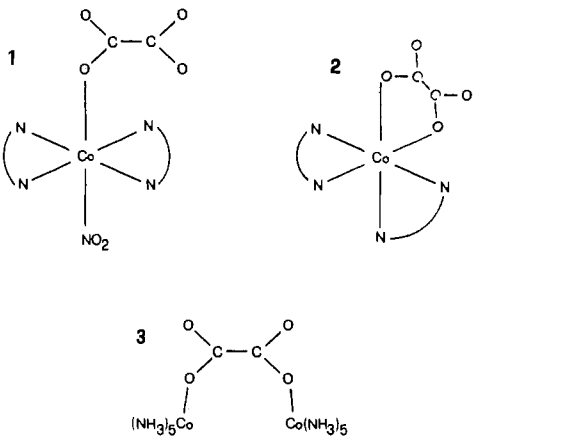
$$\sigma = \sigma_d + \sigma_p + \sigma'$$

where σ_d is the diamagnetic term, σ_p is the paramagnetic term, and σ' is a term including contributions due to electron circulation in distant parts of the molecule.[33,34] Extensive literature exists on ^{13}C NMR spectroscopy of coordination compounds.[35,36] Many attempts have been made in order to theoretically predict the pattern of chemical shifts in diamagnetic inorganic complexes through separate calculation of the changes in the three contributions to the total shielding constant; however, also in simple cases, the agreement with the experimental data is not fully satisfactory.[37,38] Therefore, to date, ^{13}C NMR data have been mainly treated by using semiempirical criteria, with the help of model systems.

Much work has been performed to estimate in a qualitative way the influence of various effects such as metal complexation, local electric fields, hydrogen bond interactions, protonation, etc., on the chemical shift values of carbonyl and carboxylic carbons. They can provide information on the environment experienced by the investigated nucleus. Namely, protonation of a carboxylate anion causes an upfield shift of the carboxylic carbon as large as 4–8 ppm,[39–41] whereas the effect of hydrogen bond interaction on a given nucleus is to shift its resonance about 2–6 ppm downfield.[42] The latter effect however can only be observed on passing from a nonprotic to a protic solvent; in water, solute–solvent and solute–solute hydrogen-bonding interactions are expected to be of the same size.

Local electric fields, extensively studied by Batchelor,[43] induce a more complicated pattern of chemical shift variations, owing to the presence of both uniform and gradient field contributions of comparable size and opposite sign as well as to orientation dependence of the contributions. However in most cases the final effect of "through-space" interaction of a positive charge with a carbonyl carbon nucleus is an upfield shift of the resonance.

Finally, the effect of complexation has to be taken into account. Again, the influence on the chemical shift of the ligand carbon nuclei is not easily predictable owing to the high number of factors that are important in determining the total effect (such as metal charge, filling of the *n*d shell, electron density distributions, energies of the electronic states, π bond order, etc.).[37] Literature data show that downfield shifts are observed in coordinated RCH_2COO^- moieties;[44–49] however oxalate and carbonate ions



Complex	Chemical shift	
1) Monodentate oxalate in $[Co(en)_2(NO_2)(Ox)]$	170[a], 178[b]	(ref.52)
2) Bidentate oxalate in $[Co(en)_2(Ox)]^+$	169	(ref.52)
3) Bridging oxalate in $[Co(NH_3)_5(Ox)Co(NH_3)_5]^{4+}$	165.8	(ref.50)

Figure 5. Schematic drawing of cobalt(III) oxalate model complexes. The ^{13}C NMR chemical shifts of oxalate carbons are reported (ppm from Me_4Si): a, metal-bound carboxylate; b, free carboxylate.

are usually reported to experience upfield shifts upon coordination, with larger effects on the former.[50–52]

Bicarbonate Derivatives. Our ^{13}C NMR data on Otf and Tf bicarbonate derivatives show a rather homogeneous behavior. The chemical shift of bound (bi)carbonate is in the range 168–166 ppm. Such a value is quite downfield with respect to bicarbonate (161.4 ppm) and slightly upfield with respect to carbonate (169.4 ppm). This value is analogous to that found for carbonate in the model complex $Co(NH_3)_4(CO_3)^+$ (168.5 ppm, ref 50). It seems therefore that the description of the natural synergistic anion is close to that of a carbonate. Indeed this is consistent with the interpretation given by Zweier for the downfield signal observed in the dicobalt(III)–Tf–(bi)carbonate system.[22] Finally, spectroscopic studies on the (bi)carbonate–Otf–cobalt(II) derivative have shown no pH dependence attributable to deprotonation of bicarbonate.[31] Only one resonance is found for bound carbonate; this means that the anion environments are identical in the two sites, within the resolution of the technique.

Zweier et al. had found a complex behavior for bound bicarbonate signals in cobalt(III) transferrin, which pointed toward a remarkably inequivalent arrangement of the synergistic anion in the two sites.[22] No evidence of this is found in the present systems.

Oxalate Derivatives. The complex pattern of resonances of oxalate–tripositive metal derivatives can be satisfactorily interpreted as due to the superposition of two AB systems. A further comment is necessary on the chemical shift values. There are some interesting literature data on ^{13}C chemical shift values of simple inorganic complexes of cobalt(III) with oxalate. We have found three different situations that are helpful in elucidating our data (see Figure 5). It should be recalled that in homologous series the chemical shift values of cobalt(III) carboxylate and carbonyl ligands are always slightly less upfield than in the corresponding gallium and aluminum complexes.[44–46]

System **3** is the most suitable for our comparison purposes; it exhibits only one resonance at 165.8 ppm for the two equivalent oxalate carbons. Upon comparison of the latter value with that

(33) Saika, A.; Slichter, C. P. *J. Chem. Phys.* **1954**, *22*, 26.
(34) Karplus, M.; Pople, J. A. *J. Chem. Phys.* **1963**, *38*, 2803.
(35) Chisolm, M. H.; Godleski, S. *Prog. Inorg. Chem.* **1976**, *20*, 299.
(36) Pregosin, S. P. *Annu. Rep. NMR Spectrosc.* **1981**, *11a*.
(37) Pesek, J. J.; Mason, W. R. *Inorg. Chem.* **1979**, *18*, 924.
(38) Evans, J.; Norton, J. R. *Inorg. Chem.* **1974**, *13*, 3042.
(39) Hagen, R.; Roberts, J. D. *J. Am. Chem. Soc.* **1969**, *91*, 4504.
(40) Keim, P.; Vigna, R. A.; Morrow, J. S.; Marshall, R. C. Gurd, F. R. N. *J. Biol. Chem.* **1973**, *248*, 7811.
(41) Hagen, R.; Warren, J. P.; Hunter, D. H.; Roberts, J. D. *J. Am. Chem. Soc.* **1973**, *95*, 5712.
(42) Maciel, G. E.; Natterstad, J. J. *J. Chem. Phys.* **1965**, *42*, 2752.
(43) Batchelor, J. G. *J. Am. Chem. Soc.* **1975**, *97*, 3410.
(44) Hammel, J. C.; Smith, J. A. S. *J. Chem. Soc. A* **1969**, 2883.
(45) Howarth, O. W.; Moore, P.; Winterton, N, *J. Chem. Soc., Dalton Trans.* **1975**, 360.
(46) Howarth, O. W.; Moore, P.; Winterton, N. *J. Chem. Soc., Dalton Trans.* **1974**, 2271.
(47) Meiske, L. A.; Angelici, R. J. *Inorg. Chem.* **1982**, *21*, 738.
(48) Sherry, A. D.; Yang, P. P.; Morgan, L. O. *J. Am. Chem. Soc.* **1980**, *102*, 5755.
(49) Juranic, N.; Celap, M. B.; Vucelic, D.; Malinar, M. J.; Radivojsa, P. N. *Inorg. Chem.* **1980**, *19*, 802.
(50) Angelici, R. J. *Synthesis and Technique in Inorganic Chemistry*; Saunders: Philadelphia, 1969; p 16.
(51) Ogino, H.; Tsukahara, K.; Morioka, J.; Tanaka, M. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1736.
(52) Cooper, J. N.; Pennell, C. A.; Johnson, B. C. *Inorg. Chem.* **1983**, *22*, 1956.

of the monodentate complex (169 ppm), we see that the binding of the free carboxylate to a second $Co^{III}(NH_3)_5$ group determines a further upfield shift on the former carbon atom. If in the present system the second carboxylate of oxalate is bound to a positively charged protein residue whose effect is similar to the binding to a metal ion but smaller, the observed ^{13}C NMR spectra can be accounted for. The signal at 169 pp. would correspond to the protein-bound carbon whereas that at 166 to the metal-bound carbon. In the case of bipositive metal ions the effect of the metal ion is more similar to that of the protein, and a single signal (although broad) is observed.

The spectrum of the monometal derivative is very helpful in discriminating between the two groups of resonances and allows us a comparison between anion arrangements in the two sites. Group 1 resonances refer to oxalate in the C-terminal site and group 2 to oxalate in the N-terminal site. On the basis of the chemical shift values, anion binding at the two sites appears very similar. Namely, the protein-bound carbon exhibits the same chemical shift value at both sites (so pointing toward an identity of the anion binding ligand(s)) whereas the chemical shift value of the metal-bound carbon is just slightly different.

Concluding Remarks. Our ^{13}C data support the idea that the synergistic anion is bridging the metal to some positively charged group of the protein in agreement with the interlocking-sites model. The structure of the two carbonate binding sites appears identical within the resolution of the technique. Chemical shift values point toward carbonate as the form of the bound anion.

In the case of oxalate, a close analysis of the spectral data allows us to differentiate between the two sites and to state that the anion arrangement is very similar but not identical in the two protein domains. The results appear relevant as far as the structure–function relations of transferrins are concerned. Oxalate seems to act as a monodentate with the second carboxylate group interacting with positively charged residues of the protein.

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Magnetochemistry of Copper(II): Exchange Interactions in Catenated $[Cu(NH_3)_2(CH_3COO)Br]$

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The specific heat and susceptibility of polycrystalline samples of $[Cu(NH_3)_2(CH_3COO)Br]$ have been measured over the respective temperature intervals of 1.5–50 and 1.2–60 K. Long-range antiferromagnetic order is found at $T_c = 4.146$ K. The specific heat data have been analyzed in terms of the Bonner–Fisher model for linear chains with $J/k = -4.3 \pm 0.4$ K; the susceptibility data above 10 K may be fit by the same model and exchange constant, with a large molecular field correction, $zJ'/k = -1.9$ K. With the critical entropy $S_c = 42\%$ and with a zigzag linear-chain structure, the substance exhibits unexpectedly large interchain interactions. The mechanism of intra- and interchain interactions is discussed. This latter phenomenon is attributed to the hydrogen-bonding networks linking the chains together.

Introduction

The acetate ion can provide a strong superexchange path in copper compounds. This is illustrated by the early studies[2,3] on hydrated copper acetate, $[Cu(CH_3COO)_2(H_2O)]_2$, in which the copper ions are bridged symmetrically by four acetate groups. Given the Bleaney–Bowers formulation for the exchange coupling in this molecule, the best value[4] of the exchange constant is $2J/k = -429$ K. A variety of related molecules are known in which the exchange is also strong.[5,6] The ability of the carboxylate group to transmit the interaction between two copper(II) ions separated by more than 5 Å was also demonstrated in the case of (μ-oxalato)copper(II) compounds.[7,8] For instance, in $[tmen(H_2O)Cu(C_2O_4)Cu(H_2O)tmen](ClO_4)_2$, $2J/k$ was found as large as −558 K with a Cu···Cu separation of 5.14 Å.[7]

Copper ions are bridged differently, in a zigzag fashion, by nitrate ions in $Cu(NO_3)_2 \cdot 2^1/_2H_2O$.[9] This leads to an exchange interaction which is much weaker than that in the case described above. The best-fit analysis[10] of a variety of data on copper nitrate is in terms of an alternating-linear-chain antiferromagnet, with α (the degree of alternation) = 0.27 and $J/k = -2.58$ K. This substance does not order spontaneously.

In light of the above, we were drawn to $[Cu(NH_3)_2(CH_3COO)Br]$, which has a polymeric structure.[11] The acetate ion is in a bridging situation between two copper atoms, so that the structure consists of zigzag chains of coordination polyhedra running parallel to [100]. The Cu–O bond distances of the acetate that bridges two metal ions are the same (1.995 (5), 2.001 (6) Å), but the Cu–O–C–O–Cu superexchange path is not a symmetric one. A sketch of the structure is illustrated in Figure 1. The question asked is as follows: How strong would the exchange be, and what is the nature of its dimensionality?

Experimental Section

The compound was prepared as described by Tomlinson and Hathaway.[12] Slow cooling and evaporation of a warm solution of $CuBr_2$ (5 g) in ammonia solution (10 mL, $d = 0.88$ g/cm^3), acetic acid (7.3 mL), and ethanol (70 mL) result in small needle-shaped blue single crystals within a few hours. The compound was washed with ethanol and ether

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(2) Bleaney, B.; Bowers, K. D. *Proc. R. Soc. London, Ser. A* **1952**, *214*, 451.
(3) Figgis, B. N.; Martin, R. L. *J. Chem. Soc.* **1956**, 3837.
(4) Güdel, H. U.; Stebler, A.; Furrer, A. *Inorg. Chem.* **1979**, *18*, 1021.
(5) Carlin, R. L.; van Duyneveldt, A. J. *Magnetic Properties of Transition Metal Compounds*; Springer-Verlag: New York, 1977.
(6) Carlin, R. L. *Magnetochemistry*; Springer-Verlag: West Berlin, Heidelberg, New York, Tokyo, 1986.
(7) Julve, M.; Verdaguer, M.; Gleizes, A.; Philoche-Levisalle, M.; Kahn, O. *Inorg. Chem.* **1984**, *23*, 3808.
(8) Michalowicz, A.; Girerd, J. J.; Goulon, J. *Inorg. Chem.* **1979**, *11*, 3004.
(9) Morosin, B. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1970**, *B26*, 1203.
(10) Bonner, J. C.; Friedberg, S. A.; Kobayashi, H.; Meier, D.; Blöte, H. W. J. *Phys. Rev. B: Condens. Matter* **1983**, *27*, 248.
(11) Ferrari, M. B.; Capacchi, L. C.; Fava, G. G.; Nardelli, M. *J. Cryst. Mol. Struct.* **1972**, *2*, 291.
(12) Tomlinson, A. A. G.; Hathaway, B. J. *J. Chem. Soc. A* **1968**, 2578.

0020-1669/86/1325-1786$01.50/0