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A Scandium-45 NMR Study of Ovotransferrin and Its Half-Molecules

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Abstract: The binding of Sc^{3+} to chicken ovotransferrin has been investigated by ^{45}Sc and ^{13}C NMR spectroscopy. In the presence of carbonate, one observes two ^{45}Sc and ^{13}C signals which can be assigned using the proteolytic half-molecules of ovotransferrin to bound Sc^{3+} and $^{13}CO_3^{2-}$ in both metal ion binding sites of the protein. When the synergistic anion is changed to oxalate, two overlapping ^{45}Sc resonances are again detected. Several properties of the transferrin-bound ^{45}Sc signals, such as their dependence on pulse length, magnetic field, protein size, and temperature, are consistent with the detection of only the central ($m = {}^1/_2 \rightarrow -{}^1/_2$) transition of a quadrupolar nucleus under far from extreme narrowing conditions. From ^{45}Sc chemical shift and line width data for the Sc^{3+}/carbonate form of ovotransferrin at four magnetic fields, we have calculated values for the quadrupole coupling constant (χ) and rotational correlation time (τ_c) for the bound metal ion in each site of the protein. In addition, from chemical shift information at two fields, we have obtained estimates of χ for the Sc^{3+}/oxalate form of ovotransferrin, as well as for the Sc^{3+}/carbonate derivative of human serotransferrin. The results in each case are comparable to the χ values we have determined for two octahedral Sc^{3+} organometallic complexes. From the χ data, we have calculated values for the electric field gradient ($|eq_{ionic}|$) at the metal nucleus for transferrin-bound Sc^{3+}, by taking into account the nuclear quadrupole moment for ^{45}Sc and the Sternheimer antishielding factor for Sc^{3+}. These results are compared to our previous ^{27}Al NMR data for the analogous Al^{3+} forms of the transferrins [Aramini, J. M.; Vogel, H. J. *J. Am. Chem. Soc.* **1993**, *115*, 245–252. Aramini, J. M.; Germann, M. W.; Vogel, H. J. *J. Am. Chem. Soc.* **1993**, *115*, 9750–9753]. This report represents the first ^{45}Sc NMR study of a metalloprotein and is another example of the applicability of quadrupolar metal ion NMR to the investigation of metal ion binding sites in large proteins.

Introduction

Recent ^{27}Al and ^{51}V nuclear magnetic resonance (NMR)[1] studies of the transferrins have demonstrated the feasibility of using quadrupolar metal ion NMR spectroscopy to probe the metal ion binding sites in large proteins.[2-6] The technique revolves around the detection of the central ($m = {}^1/_2 \rightarrow -{}^1/_2$) transition of a half-integer quadrupolar nucleus (i.e., $I = n/2$, $n = 3, 5, 7$), which is facilitated by increasing nuclear resonance frequency and protein size (i.e., $\omega_0\tau_c \gg 1$). It was also recently shown that important physical information about the metal ion binding site, namely the symmetry of the site (i.e., χ, the quadrupole coupling constant) and the motion of the bound metal ion (i.e., τ_c, the rotational correlation time), may be gleaned from the magnetic field dependence of the chemical shift and line width of the signal due to the bound metal ion.[2,4]

In this paper, we extend this methodology to another metal, scandium, by using ^{45}Sc NMR to monitor the binding of Sc^{3+} to the transferrins, a class of MW $\approx$ 80 000 proteins which contain two high-affinity Fe^{3+}-binding sites.[7] The solution chemistry of scandium is based entirely on its trivalent cation, Sc^{3+}, which almost exclusively forms six-coordinate complexes.[8] This makes Sc^{3+}, whose ionic radius is slightly larger than that of Fe^{3+} (0.75 *vs* 0.65 Å),[9] a suitable probe for the Fe^{3+}-binding sites of transferrins in which the metal ion is coordinated by six ligand atoms, four from the side chains of four protein residues and two from the synergistic anion (i.e., carbonate), in a distorted octahedral geometry.[10,11] Like the two other quadrupolar nuclei which have been successfully applied to the study of transferrins thus far (i.e., ^{27}Al and ^{51}V), ^{45}Sc ($I = {}^7/_2$) has both a high resonance frequency and a high receptivity, but it has a slightly larger quadrupole moment.[12] Despite these desirable qualities, ^{45}Sc NMR spectroscopy has scarcely been employed in studies of scandium complexes to date.[13]

Experimental Section

Materials. The apo-forms of chicken OTf and human sTf were purchased from Sigma Chemical Co. and used without further purification. The purification and characterization of the N- and C-terminal half-

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(1) Abbreviations used: NMR, nuclear magnetic resonance; OTf, ovotransferrin; sTf, serotransferrin; OTf/2N; N- (amino-) terminal half-molecule of ovotransferrin; OTf/2C, C- (carboxy-) terminal half-molecule of ovotransferrin; acac, 2,4-pentanedionato; hfaa, 1,1,1,5,5,5-hexafluoro-2,4-pentanedionato; Tris, tris(hydroxymethyl)aminomethane; TMS, tetramethylsilane; NOE, nuclear Overhauser enhancement; sTf/2N, N- (amino-) terminal half-molecule of serotransferrin.

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molecules of OTf (OTf/2N and OTf/2C) were performed according to published methods.[2,14-16] Tris(2,4-pentanedionato)scandium ($Sc(acac)_3$) was prepared from scandium oxide (Sigma Chemical Co.) and 2,4-pentanedione (General Intermediates of Canada) by following published procedures.[17,18] Scandium chloride and tris(1,1,1,5,5,5-hexafluoro-2,4-pentanedionato)scandium ($Sc(hfaa)_3$) were obtained from Aldrich Chemical Co. and Strem Chemicals, respectively. The suppliers of all isotopically labeled compounds and solvents used in this study are listed elsewhere.[2,4]

NMR Spectroscopy. Procedures for the preparation and titration of protein samples for NMR are identical to those described in our earlier work with Al^{3+}.[2,4,19] ^{45}Sc NMR spectra were acquired at 5 and 25 °C on four instruments—Bruker ARX 300 (ν_0 = 72.9 MHz), AM 400 (ν_0 = 97.2 MHz), AMX 500 (ν_0 = 121.5 MHz), and AMX 600 (ν_0 = 145.8 MHz)—each equipped with a 10-mm broadband probe, with the following parameters: a 30–45° flip angle, a repetition time of 11–21 ms, a sweep width of 50–125 kHz, a 50-μs dead time, and 2–4 K data points. All data was zero-filled once and processed with a 100-Hz line broadening. For quantitative work with $Sc(H_2O)_6^{3+}$, a total time between pulses of 60 ms was used. The chemical shifts and line widths of overlapping ^{45}Sc NMR signals were obtained by fitting each spectrum a minimum of four times using the LINESIM routine (P. Barron, Bruker Australia). Because of the large observed ^{45}Sc line widths for the Sc^{3+} compounds used in this study, the ^{45}Sc chemical shifts reported are to the nearest ±1 ppm; the uncertainty in the ^{45}Sc line width data is ±5%. Proton-coupled ^{13}C NMR spectra of the proteins used in this study were acquired on the Bruker AM 400 (100.6 MHz) and AMX 500 instruments (125.7 MHz) and processed as described elsewhere.[2] $^{13}C\{^1H\}$ and ^{45}Sc NMR studies of 0.1–0.15 M solutions of $Sc(acac)_3$ and $Sc(hfaa)_3$ in benzene-d_6 were performed at 25 °C on the Bruker AM 400 spectrometer. ^{13}C longitudinal relaxation times (T_1) for these complexes were determined by the inversion–recovery method. All ^{45}Sc and ^{13}C NMR spectra are referenced to external 1.0 M $ScCl_3$ in D_2O and TMS, respectively.[20]

Theory

In general, the quadrupolar relaxation mechanism, which arises from the interaction of the nuclear quadrupole moment and fluctuating electric field gradients at the nucleus, is the most effective relaxation pathway for quadrupolar ($I > {}^1/_2$) nuclei.[21] For small molecules in solution under extreme narrowing conditions (i.e., $\omega_0\tau_c \ll 1$), the longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation rates for the single quantum transitions between the $2I + 1$ nuclear Zeeman levels are equivalent, resulting in a single Lorentzian peak with a line width ($\Delta\nu_{1/2}$) given by the following expression:[22]

$$\frac{1}{T_1} = \frac{1}{T_2} = \pi\Delta\nu_{1/2} = \frac{3\pi^2}{10}\frac{(2I+3)}{I^2(2I-1)}\chi^2\tau_c \qquad (1)$$

where χ is defined as the quadrupole coupling constant and τ_c is the rotational correlation time of the nucleus.

However, under far from extreme narrowing conditions (i.e., $\omega_0\tau_c \gg 1$), this degeneracy is lost and quadrupolar relaxation is multiexponential. Some time ago it was predicted that in this situation the central ($m = {}^1/_2 \rightarrow -{}^1/_2$) transition of a half-integer quadrupolar nucleus (i.e., $I = n/2$, n = 3, 5, 7) should produce a relatively narrow signal.[23,24] Westlund and Wennerström[25] derived analytical expressions for the transverse relaxation rate of this transition in $I = {}^5/_2$ and ${}^7/_2$ nuclei in the limit of slow isotropic molecular motion; for $I = {}^7/_2$ nuclei like ^{45}Sc, the line width of the central transition is[26]

$$\Delta\nu_{1/2} = 2.5 \times 10^{-3}\left(\frac{\chi^2}{\nu_0^2\tau_c}\right) \qquad (2)$$

Thus, in this window of molecular motion, the line width of the $m = {}^1/_2 \rightarrow -{}^1/_2$ transition is dependent on the nuclear resonance frequency, ν_0 (and hence the external magnetic field, B_0), and is inversely proportional to τ_c. Furthermore, the chemical shift of this transition when $\omega_0\tau_c \gg 1$ is also field dependent and for $I = {}^7/_2$ nuclei is[25,27]

$$\Delta\delta_d = -2.5 \times 10^3\left(\frac{\chi^2}{\nu_0^2}\right) \qquad (3)$$

This "second-order dynamic frequency shift" is an upfield (low frequency) shift, whose magnitude decreases with increasing B_0. Equations 2 and 3 are identical to analogous expressions for $I = {}^5/_2$ nuclei, like ^{27}Al, except that the leading coefficients in both equations are smaller for the $I = {}^7/_2$ case. Thus, if all the other parameters are equivalent, both the line width and the magnitude of the second-order dynamic frequency shift for the signal due to the central transition under far from extreme narrowing conditions will be smaller for $I = {}^7/_2$ nuclei compared to $I = {}^5/_2$ nuclei.

The quadrupole coupling constant, χ, in eqs 1–3 is generally given by the expression

$$\chi = \frac{e^2Qq_{obs}}{h} \qquad (4)$$

where eQ is the quadrupole moment of the nucleus and eq_{obs} is the observed electric field gradient at the nucleus. This key parameter provides information about the relative symmetry of the electronic environment surrounding the nucleus, where a decrease in χ reflects an increase in symmetry. The larger (absolute) value of Q for ^{45}Sc compared to ^{27}Al (0.22 *vs* 0.14 b)[28]

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(20) The chemical shift of aqueous Sc^{3+} is dependent on a number of factors, such as concentration and the deuterium isotope effect (Haid, E.; Köhnlein, D.; Kössler, G.; Lutz, O.; Messner, W.; Mohn, K. R.; Nothaft, G.; van Rickelen, B.; Schich, W.; Steinhauser, N. *Z. Naturforsch.* **1983**, *38A*, 317–321. Melson, G. A.; Olszanski, D. J.; Rahimi, A. K. *Spectrochim. Acta* **1977**, *33A*, 301–309). The shift of the standard employed in this study is δ = −5.5 ppm with respect to 0.1 M $Sc(H_2O)_6^{3+}$, the standard most commonly used in ^{45}Sc NMR spectroscopy.
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(26) Equation 2 was derived from the transverse relaxation matrix for $I = {}^7/_2$ nuclei in the slow motion limit:

$$R_2(m = {}^1/_2 \rightarrow -{}^1/_2) = [10J(\omega) + (70 - 20\sqrt{6})J(2\omega)]K$$

where

$$K = \frac{1}{588}\left(\frac{eQ}{\hbar}\right)^2 \quad \text{and} \quad J(\omega) = \frac{3(eq)^2}{10\omega^2\tau_c}$$

Higher-order contributions to $R_2(m = {}^1/_2 \rightarrow -{}^1/_2)$ have been neglected; see ref 25 herein.
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is somewhat of a disadvantage since an increase in Q translates into an increase in signal line width under either extreme or nonextreme narrowing conditions (i.e., see eqs 1 and 2). In order to meaningfully compare χ data for *different* quadrupolar nuclei in analogous compounds, one must consider that, aside from the distribution of the juxtaposed ligands, the eq_{obs} at the nucleus is also a function of deviations from spherical symmetry of the inner electron orbitals induced by the valence electrons, an effect termed Sternheimer antishielding.[29,30] In general, Sternheimer antishielding factors ($1 - \gamma_\infty$) markedly increase with atomic size, though the uncertainties in these factors make the calculation of electric field gradients quite problematic. However, $1-\gamma_\infty$ values have been calculated for free closed shell ions and for closed shell ions in ionic crystals,[31] and under these circumstances χ is[30]

$$\chi = \frac{e^2 Q q_{ionic}(1 - \gamma_\infty)}{h} \quad (5)$$

where eq_{ionic} is the field gradient due to the ionic charges about the nucleus. There are examples in the literature of the use of Sternheimer antishielding factors in calculations of electric field gradients and quadrupole coupling constants for complexes of quadrupolar metal ions in both the liquid and solid states.[32,33]

Results

Sc^{3+} and Carbonate Binding to OTf and Its Half-Molecules. In general, two approaches have been used to monitor the binding of diamagnetic metal ions to transferrins by NMR spectroscopy: (1) direct detection of the metal nucleus[2-6,34-36] and (2) detection of the bound isotopically enriched anion by ^{13}C NMR.[2,3,37-39] ^{13}C (125.7 MHz) and ^{45}Sc (121.5 MHz) NMR spectra of OTf and its half-molecules in the presence of Sc^{3+} and a molar excess of ^{13}C-labeled carbonate are shown in Figure 1. In the case of OTf, one observes two partially overlapping signals in both the ^{13}C and ^{45}Sc NMR spectra corresponding to bound $^{13}CO_3^{2-}$ and Sc^{3+} in both metal ion binding sites of the protein. From titration experiments, we have found that both sets of signals increase simultaneously, indicative of a lack of site preference for this metal ion in the presence of carbonate (data not shown); such behavior may also be indicative of cooperativity. The ^{13}C and ^{45}Sc NMR signals for the $Sc^{3+}/^{13}CO_3^{2-}$ derivative of OTf have been assigned by studying the proteolytic half-molecules of this protein. The near perfect agreement between the ^{45}Sc and ^{13}C signals due to the bound metal ion and anion in the two sites of OTf and the corresponding resonances in the half-molecules suggests that proteolytic digestion of the intact protein does not perturb the metal ion binding sites in each lobe of the protein. Notice that the ^{45}Sc NMR signals for both OTf/2N and OTf/2C are significantly broader than the corresponding resonances in the intact protein (see Table 1).

In the analogous $Sc^{3+}/^{13}CO_3^{2-}$ form of sTf, we also observe two distinct ^{13}C signals due to the bound anion in both sites of the protein, but only one ^{45}Sc signal (spectra not shown; see Table 2).

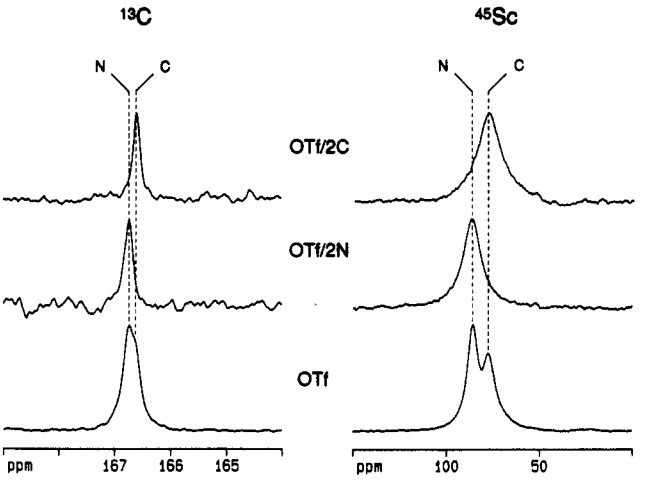

Figure 1. ^{13}C (125.7 MHz) and ^{45}Sc (121.5 MHz) NMR spectra of OTf (1.05 mM, 1.9 equiv of Sc^{3+}, pH 7.6), OTf/2N (^{13}C, 0.22 mM; ^{45}Sc, 0.42 mM; 0.8 equiv of Sc^{3+}, pH 7.6), and OTf/2C (^{13}C, 0.28 mM; ^{45}Sc, 0.46 mM; 0.8 equiv of Sc^{3+}, pH 7.6) in the presence of excess $^{13}CO_3^{2-}$ (10–20 mM) at 25 °C: ^{13}C, 2–3 × 10^4 scans; ^{45}Sc, 1–3 × 10^6 scans.

Table 1. ^{13}C and ^{45}Sc NMR Data for the $Sc^{3+}/^{13}CO_3^{2-}$ Forms of OTf, OTf/2N, and OTf/2C at 11.7 T and 25 °C

protein	site[a]	δ ^{13}C (ppm)	δ ^{45}Sc (ppm)	$\Delta\nu_{1/2}$ (Hz)
OTf	N	166.74	85	740
OTf	C	166.61	77	940
OTf/2N		166.74	86	1300
OTf/2C		166.61	78	1700

[a] ^{13}C and ^{45}Sc signals for intact OTf were assigned using the N- and C-terminal half-molecules of OTf.

Table 2. ^{13}C and ^{45}Sc NMR Data for the $Sc^{3+}/^{13}C_2O_4^{2-}$ and $Sc^{3+}/^{13}CO_3^{2-}$ Derivatives of OTf and sTf at 9.4 and 11.7 T and 25 °C[a]

protein	anion	site[b]	δ ^{13}C (ppm)	δ ^{45}Sc (ppm)	$\Delta\nu_{1/2}$ (Hz)
OTf	$^{13}C_2O_4^{2-}$	N	170.60[c]	70	1160
			167.78	(54)[d]	(1900)
OTf	$^{13}C_2O_4^{2-}$	C	170.52[e]	65	1030
			167.00	(54)[d]	(1900)
OTf/2N	$^{13}C_2O_4^{2-}$		170.60[c]	70	1900
			167.78		
OTf/2C	$^{13}C_2O_4^{2-}$		170.52[e]	65	1900
			167.00		
sTf	$^{13}CO_3^{2-}$	N and C	167.19	76	1020
			166.76	(64)	(1320)

[a] ^{45}Sc data at 9.4 T for the $Sc^{3+}/^{13}C_2O_4^{2-}$ and $Sc^{3+}/^{13}CO_3^{2-}$ derivatives of OTf and sTf, respectively, are shown in parentheses. [b] ^{13}C and ^{45}Sc signals for intact OTf were assigned using the N- and C-terminal half-molecules of OTf; the assignment of the two ^{13}C signals for the $Sc^{3+}/^{13}CO_3^{2-}$ adduct of sTf has not been determined. [c] $^1J_{C-C}$ = 74 Hz. [d] ^{45}Sc signals at this field are not resolvable but can be fit to two resonances: δ = 58 ppm, $\Delta\nu_{1/2}$ = 1600 Hz; δ = 51 ppm, $\Delta\nu_{1/2}$ = 1650 Hz. [e] $^1J_{C-C}$ = 73 Hz.

Experiments with Oxalate as the Synergistic Anion. ^{13}C (100.6 MHz) and ^{45}Sc (121.5 MHz) NMR spectra of OTf and its half-molecules in the presence of Sc^{3+} and a molar excess of ^{13}C-labeled oxalate are shown in Figure 2. In the carbonyl region of the ^{13}C spectrum, one observes two pairs of doublets (i.e., two AB spin systems) assigned to the carboxyl carbons of the bound anion in both metal ion binding sites of the protein.[2,38] The ^{45}Sc signals from the bound metal ion in each site are nearly degenerate and are slightly upfield of those for the $Sc^{3+}/^{13}CO_3^{2-}$ form of OTf. Again, the ^{45}Sc and ^{13}C signals due to the bound metal ion and anion in the two sites of OTf line up exactly with the signals for the half-molecules of OTf, and the ^{45}Sc line widths for OTf/2N

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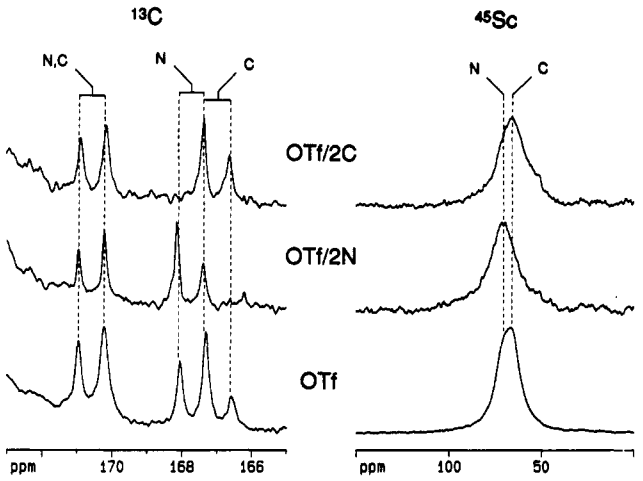

Figure 2. ^{13}C (100.6 MHz) and ^{45}Sc (121.5 MHz) NMR spectra of OTf (1.05 mM, 1.9 equiv of Sc^{3+}, pH 7.6), OTf/2N (0.42 mM, 0.8 equiv of Sc^{3+}, pH 7.5), and OTf/2C (0.50 mM, 0.8 equiv of Sc^{3+}, pH 7.8) in the presence of excess $^{13}C_2O_4^{2-}$ (5–10 mM) at 25 °C: ^{13}C, 2–6 × 10^4 scans; ^{45}Sc, 1–2.5 × 10^6 scans.

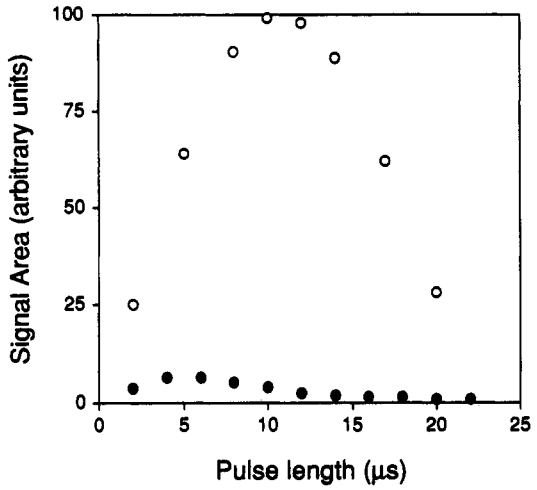

Figure 3. Pulse length dependence of the ^{45}Sc signal areas of $Sc(H_2O)_6^{3+}$ (○) and the $Sc^{3+}/^{13}CO_3^{2-}$ derivative of OTf (●) containing equimolar amounts of Sc^{3+} at a radio frequency pulse strength (ω_1) of 21.7 kHz; 1 × 10^5 scans per experiment.

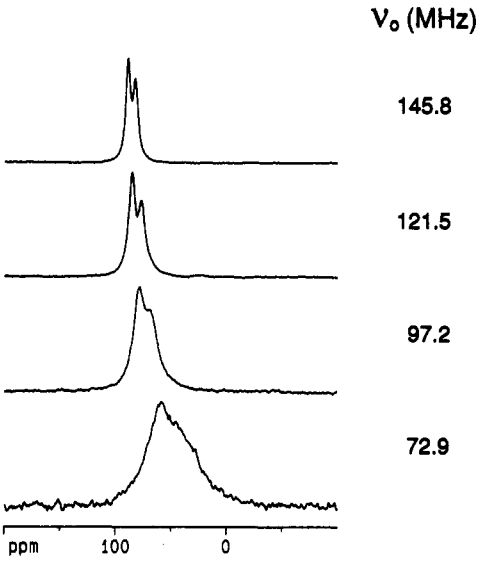

Figure 4. ^{45}Sc NMR spectra of OTf (1.05 mM, 1.9 equiv of Sc^{3+}, pH 7.6) at four magnetic fields (25 °C): 7.0 T (ν_0 = 72.9 MHz), 9.4 T (ν_0 = 97.2 MHz), 11.7 T (ν_0 = 121.5 MHz), and 14.1 T (ν_0 = 145.8 MHz); 1 × 10^6 scans each.

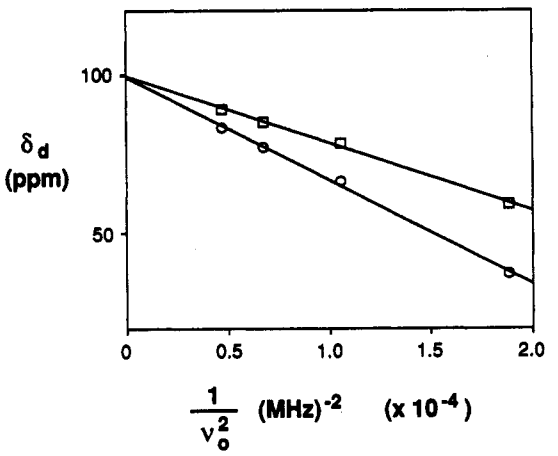

Figure 5. Field dependence of the chemical shifts of OTf-bound ^{45}Sc signals: (□) OTf N-site; (○) OTf C-site.

and OTf/2C are appreciably larger than in the intact protein (see Table 2). We again found no evidence for any difference in the affinities of the sites for Sc^{3+} when the anion is changed to oxalate (data not shown).

Pulse Length Dependence of OTf-Bound ^{45}Sc NMR Signals. The dependence of ^{45}Sc signal area on pulse length for $Sc(H_2O)_6^{3+}$ and the Sc^{3+}/carbonate form of OTf containing equimolar amounts of Sc^{3+} is shown in Figure 3. The total area of the OTf-bound ^{45}Sc signals is appreciably less than that for the free metal ion and reaches a maximum at a flip angle (30–35°) that is significantly shorter than the 90° pulse length for $Sc(H_2O)_6^{3+}$. In addition, the maximum area of the protein-bound signals is only 12–15% of that for free Sc^{3+} at that pulse length; this is somewhat less than the theoretically predicted value of the contribution of the central component of a $I = {}^7/_2$ nucleus (19%).[24] Similar results have been obtained when oxalate acts as the synergistic anion and for sTf (data not shown). As in the previous ^{27}Al and ^{51}V NMR studies of transferrins,[2,6] these effects are attributable to the near selective excitation of the central (m = ${}^1/_2 \rightarrow -{}^1/_2$) transition of the quadrupolar nucleus bound to a large molecule (i.e., $\omega_0\tau_c \gg 1$).

Field Dependence of OTf-Bound ^{45}Sc NMR Signals. The chemical shifts and line widths of ^{45}Sc NMR signals due to bound Sc^{3+} in the transferrins show a marked dependence on the strength of the external magnetic field. ^{45}Sc NMR spectra of the Sc^{3+}/carbonate form of OTf acquired at four different magnetic fields at 25 °C are shown in Figure 4. The resonances for both sites of this protein are shifted downfield and become appreciably sharper with increasing magnetic field. Such trends have also been observed for the $Sc^{3+}/^{13}C_2O_4^{2-}$ and $Sc^{3+}/^{13}CO_3^{2-}$ adducts of OTf and sTf, respectively, at magnetic fields of 9.4 and 11.7 T (Table 2). In addition, decreasing the temperature to 5 °C causes a substantial decrease in the line widths of both signals at each field, while their respective resonance positions are unaffected (spectra not shown). From the field dependence of the chemical shift (Figure 5) and the line width (Figure 6) of the OTf-bound ^{45}Sc NMR signals, one can obtain values for the quadrupole coupling constant, χ, and the rotational correlation time, τ_c, for the bound metal ion in both sites of OTf in the presence of carbonate using eqs 2 and 3 (see Table 3).

Experiments with $Sc(acac)_3$ and $Sc(hfaa)_3$. Using ^{45}Sc and ^{13}C NMR spectroscopy, we have obtained χ values for two scandium complexes, $Sc(acac)_3$ and $Sc(hfaa)_3$. For these small, six-coordinate molecules, extreme narrowing conditions ($\omega_0\tau_c \ll 1$) apply. To calculate χ using eq 1, we have obtained τ_c values for these compounds from the T_1 relaxation times of their methine

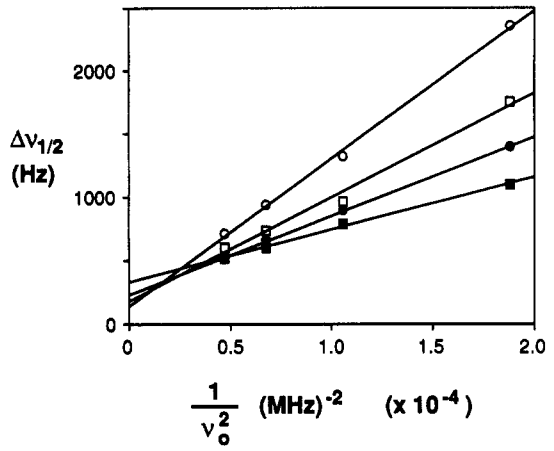

Figure 6. Field dependence of the line widths of OTf-bound ^{45}Sc signals at 5 and 25 °C: (□) OTf N-site, 25 °C; (○) OTf C-site, 25 °C; (■) OTf N-site, 5 °C; (●) OTf C-site, 5 °C.

Table 3. χ and τ_c Data for the Sc^{3+}/Carbonate Derivative of Ovotransferrin at 5 and 25 °C

site	temperature (°C)	χ (MHz)	τ_c (ns)
N	25	9.2	26
	5	9.2	51
C	25	11.4	28
	5	11.4	52

Table 4. ^{13}C and ^{45}Sc NMR Data for $Sc(acac)_3$ and $Sc(hfaa)_3$ at 25 °C

a.^{13}C NMR[a]

complex	δ (ppm)	η^b	T_1^{DD} (s)	τ_c (s)
$Sc(acac)_3$	103.5	2.0	1.05	4.4×10^{-11}
$Sc(hfaa)_3$	93.8	2.0	1.05	4.4×10^{-11}

b.^{45}Sc NMR

complex	δ(ppm)	$\Delta\nu_{1/2}$ (Hz)	T_2 (ms)	χ (MHz)
$Sc(acac)_3$	87	935	0.34	12.9
$Sc(hfaa)_3$	32	485	0.66	9.3

[a] The ^{13}C data shown are for the methine carbons in each complex. [b] NOE enhancement.

carbons using eq 6.[40] On the basis of $^{13}C\{^1H\}$ NOE data and the

$$\frac{1}{T_1^{DD}} = \left(\frac{\mu_0}{4\pi}\right)^2\left(\frac{N_H\gamma_H^2\gamma_C^2\hbar^2\tau_c}{r_{CH}^6}\right) \quad (6)$$

fact that identical χ values for the Sc^{3+} ion in these complexes were obtained at different temperatures (i.e., eliminating spin rotation as a possible relaxation pathway for the carbons of interest[41]), the methine carbons relax exclusively by dipole–dipole relaxation. The ^{45}Sc and ^{13}C NMR data for $Sc(acac)_3$ and $Sc(hfaa)_3$ are presented in Table 4.[42]

Discussion

In this report, we have used high-field quadrupolar NMR spectroscopy to monitor the binding of Sc^{3+} to the metal ion binding sites in chicken OTf. From pulse length and field dependence experiments, we have shown that the ^{45}Sc signals resulting from the bound metal ion arise almost exclusively from the central ($m = {}^1/_2 \rightarrow -{}^1/_2$) transition of this quadrupolar nucleus, a result that is predicted for quadrupolar nuclei under far from extreme narrowing conditions by quadrupolar relaxation theory.[2,6,23-25,27] Using quadrupolar NMR under these conditions offers some important advantages over working with small molecules. For example, in the limit of slow molecular motion, values for both χ and τ_c can be derived from the field dependence of the chemical shift and line width of the bound quadrupolar nucleus, whereas under extreme narrowing conditions, quadrupolar relaxation data only can give the product of these two physical parameters. In the latter situation, χ may only be calculated if a value for τ_c can be determined by some other method. Moreover, detection of a quadrupolar nucleus in a macromolecule is dramatically enhanced by increasing the magnetic field (ν_0) and/or molecular size (τ_c), in contrast to the extreme narrowing case (i.e., compare eqs 1 and 2).

The values of χ obtained for the Sc^{3+}/carbonate form of OTf, $Sc(acac)_3$ and $Sc(hfaa)_3$, fall in a range ($\chi \approx$ 5–15 MHz) established by solution and solid-state ^{45}Sc NMR studies of a handful of Sc^{3+} salts.[43,44] From the chemical shift data for the Sc^{3+}/oxalate derivative of OTf and the Sc^{3+}/carbonate adduct of sTf at 9.4 and 11.7 T, one can also obtain estimates of χ for bound Sc^{3+} in these molecules using eq 3 (OTf N/oxalate, 11.2; OTf C/oxalate, 12.1 MHz; sTf N,C/carbonate, 11.2 MHz). This gives the following series for the symmetry of the ligand environment about the bound Sc^{3+} ion (in order of decreasing symmetry):

$$Sc(hfaa)_3 \approx \text{OTf N} > \text{sTfN,C} \approx \text{OTf C} \geq \text{OTf N,C (oxalate)} > Sc(acac)_3$$

Interestingly, a similar sequence was found for Al^{3+} bound to the same proteins by ^{27}Al NMR, except that the value of χ for $Al(acac)_3$ (χ = 3.1 MHz) is less than that for the bound metal ion in any transferrin studied (χ = 3.3–4.5 MHz).[2,4] By using the quadrupole moments of ^{27}Al and ^{45}Sc[28] and the Sternheimer antishielding factors for free Al^{3+} and Sc^{3+},[31] we have calculated values for $|eq_{obs}|$ and $|eq_{ionic}|$ for Al^{3+} and Sc^{3+} in $M(acac)_3$ and the various M^{3+} forms of OTf and sTf (M = Al, Sc) from our χ data using eqs 4 and 5 (Table 5). From X-ray crystal data for the acac complexes of several trivalent metal ions, it was proposed that the bonds between the metal ion and the ligands are largely ionic in character;[45] because of the hard ligands which participate in metal ion binding, this notion can be extended to the Fe^{3+}-binding sites in the transferrins. Thus, it seems reasonable to invoke the Sternheimer antishielding factors for the free closed shell metal ions in calculations of $|eq_{ionic}|$ for the bound metal nuclei in these compounds. When the Sternheimer antishielding effect is taken into account, the values of $|eq_{ionic}|$ for the central metal ion in the acac complexes are very comparable. This is in agreement with X-ray crystal data for $Sc(acac)_3$ and $Al(acac)_3$,[46,47] in which six oxygen atoms from the ligands are arranged in a slightly distorted octahedral geometry around the metal ion. However, from inspection of Table 5, it is obvious that the electric field gradients ($|eq_{ionic}|$) for all protein-bound Sc^{3+} ions are appreciably smaller than those for the analogous Al^{3+} adducts. Hence, our data suggest that the symmetry of the ligand environment in the transferrin metal ion binding sites is much higher for the larger Sc^{3+} ion compared to Al^{3+} (r = 0.75 *vs* 0.54 Å).[9]

In addition to a means of finding χ, the field dependence of the chemical shift of a protein-bound ^{45}Sc NMR signal can also

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(42) Our ^{45}Sc NMR results for $Sc(acac)_3$ and $Sc(hfaa)_3$, in particular the line width data, are at variance with previous work on these complexes (Bougeard, P.; Mancini, M.; Sayer, B. G.; McGlinchey, M. J. *Inorg. Chem.* **1985**, *24*, 93–95).

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Table 5. Electric Field Gradients for $M(acac)_3$, the $M^{3+}/^{13}CO_3^{2-}$ Forms of OTf and sTf, and the $M^{3+}/^{13}C_2O_4^{2-}$ Form of OTf (M = Al, Sc)

complex	χ (MHz)[a]	Q (10^{-28} m^{-2})[b]	$\lvert eq_{obs}\rvert$ (10^{20} V/m^2)[c]	$1-\gamma_\infty$ (M^{3+})[d]	$\lvert eq_{ionic}\rvert$ (10^{20} V/m^2)[c]
$Al(acac)_3$	3.1	0.14	9.2	3.6	2.5
Al^{3+}–OTf N	3.3	0.14	9.7	3.6	2.7
Al^{3+}–OTf C	3.8	0.14	11.2	3.6	3.1
Al^{3+}–sTf N,C	3.6	0.14	10.6	3.6	3.0
Al^{3+}–OTf N (ox)	4.1	0.14	12.1	3.6	3.4
Al^{3+}–OTf C (ox)	4.5	0.14	13.3	3.6	3.7
$Sc(acac)_3$	12.9	–0.22	24.3	12.4	2.0
Sc^{3+}–OTf N	9.2	–0.22	17.3	12.4	1.4
Sc^{3+}–OTf C	11.4	–0.22	21.4	12.4	1.7
Sc^{3+}–sTf N,C	11.2	–0.22	21.1	12.4	1.7
Sc^{3+}–OTf N (ox)	11.2	–0.22	21.1	12.4	1.7
Sc^{3+}–OTf C (ox)	12.1	–0.22	22.7	12.4	1.8

[a] ^{27}Al χ data for $Al(acac)_3$ and the Al^{3+} adducts of OTf and sTf are taken from refs 2 and 4. [b] Taken from ref 28. [c] To convert the values for $\lvert eq_{obs}\rvert$ and $\lvert eq_{ionic}\rvert$ from SI units into atomic units (au), divide by 9.717×10^{21}. [d] Values of $1-\gamma_\infty$ given are for the free closed shell ions; taken from ref 31.

be used to obtain its true isotropic chemical shift (δ_i) by extrapolating the curve (i.e., Figure 5) to infinite field. In the case of Sc^{3+} bound to the N- and C-sites of intact OTf in the presence of carbonate, the values of δ_i are virtually identical ($\delta_i \approx 100$ ppm). This value is toward the high-frequency end of the ca. 340-ppm chemical shift window obtained from compilations of the known ^{45}Sc NMR data to this point.[13a,48] The isotropic ^{27}Al chemical shifts for the Al^{3+}/carbonate forms of OTf, sTf, and lTf are also practically the same.[4] X-ray crystal structures of the Fe^{3+}/carbonate adducts of human lTf[10] and rabbit sTf/2N[11] plus amino acid sequence information[49] have established that in all transferrins the same four highly conserved residues (one Asp, two Tyr, and one His) directly participate in metal ion binding. Thus, our results suggest that discrepancies in the chemical shifts of transferrin-bound ^{45}Sc (and ^{27}Al) signals are completely due to subtle differences in the symmetry of the metal ion binding sites in these proteins (i.e., the second-order dynamic frequency shift, eq 3) rather than to differences in the chemical nature of the ligands involved in chelating the metal ion. Although the atoms in the immediate binding sites of human lTf are highly superimposable, X-ray data for human sTf and chicken OTf which could further support our data, though imminent,[7a] have not been presented to date.

The τ_c values for bound Sc^{3+} in the two metal ion binding sites of OTf are slightly lower than those obtained by ^{27}Al NMR but are still in good agreement with recent perturbed angular correlation (PAC) data for OTf ($\tau_c \approx 33$ ns).[50] In addition, the vast increase in the ^{45}Sc line widths observed for the half-molecules of OTf compared to the corresponding sites in the intact protein plus the sharp decrease in line width with decreasing temperature for the ^{45}Sc signals from intact OTf are consistent with quadrupolar relaxation under far from extreme narrowing conditions (i.e., see eq 2; increasing τ_c causes a decrease in line width).

In conclusion, this ^{45}Sc NMR report represents another example of the potential of quadrupolar metal ion NMR for the study of large metalloproteins. We have shown in this study that χ data from field dependent NMR measurements of different quadrupolar metal ions bound to the same protein may be compared by calculation of the electric field gradient at the nucleus ($\lvert eq_{ionic}\rvert$) from χ using the Sternheimer antishielding factor ($1-\gamma_\infty$) for the metal ion. Taken together with our earlier ^{27}Al NMR studies on the transferrins,[2-4] the ^{45}Sc NMR results presented here suggest that in many respects the interaction of transferrins with Sc^{3+} mirrors that with Al^{3+}. However, in addition to the differences in the calculated electric field gradients ($\lvert eq_{ionic}\rvert$) at the metal nucleus for OTf-bound Sc^{3+} and Al^{3+}, we note two other exceptions to this. First, from titration experiments, we have found no site preference for Sc^{3+} binding to OTf when either carbonate or oxalate serves as the synergistic anion, in contrast to our ^{27}Al NMR work on OTf[2] and recent ^1H NMR studies of Al^{3+} and Ga^{3+} binding to human sTf and its recombinant N-terminal lobe.[51,52] Second, from competition experiments,[53] we have found that, in the presence of carbonate, OTf binds Sc^{3+} with a higher affinity than Al^{3+} and even Ga^{3+}. Quadrupolar metal ion NMR plays an integral role in such experiments, which broaden our understanding of the metal ion binding behavior of the transferrins.

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