Inorganica Chimica Acta, 158 (1989) 265–271

The Interaction of $Sc(OH)^{2+}\cdot aq$ with Serine and Small Peptides Investigated by ^{45}Sc NMR Spectroscopy

DIETER REHDER* and KIRSTEN HINK

Institut für Anorganische Chemie der Universität, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, F.R.G.

(Received August 8, 1988)

Abstract

Aqueous solutions of ScX_3 ($X = Cl^-$, NO_3^-) contain, in the pH/D range of *ca.* 2–4, the species $Sc(H_2O)_6^{3+}$ [$\delta(^{45}Sc) = 0$ ppm], $Sc(H_2O)_5OH^{2+}$ and $Sc_2(H_2O)_x(OH)_4^{4+}$ (all in relatively fast exchange) and, if $X = Cl^-$, an aqua–chloroscandium complex in slow exchange with the other species. At pH > 4, a slowly exchanging hydroxo complex $Sc_3(H_2O)_y(OH)_5^{4+}$ [$\delta(^{45}Sc) = +36$ ppm] becomes predominant. ^{45}Sc relaxation in the aqua/hydroxo system is exchange-dominated. For $X = HCO_2^-$ and an intrinsic pH of 5.75, the main species present is a formiato complex [$\delta(^{45}Sc) = +32$ ppm]. Complex formation has been observed in aqueous solutions containing scandium nitrate or scandium formiate [$c(Sc^{3+}) = 30$ mmol/l] and the dipeptides H-val-ala-OH and H-glu-val-OH, and the tripeptide H-ala-val-leu-OH, and also in the scandium nitrate/serine system. The $\delta(^{45}Sc)$ values for the complexes are +30 to +40 ppm [oxygen coordination: serine/$Sc(NO_3)_3$, dipeptides/ScX_3] and *ca.* +70 ppm [oxygen and nitrogen coordination: H-ala-val-leu-OH/$Sc(NO_3)_3$].

Introduction

We have been interested in studies of the interaction between metal ions such as HVO_4^{2-} [1a] and $Sc(OH)\cdot aq^{2+}$ [1b] with peptides in the context of developing and applying metal NMR to bioinorganic problems. Scandium certainly is not a biometal. However, since the radius of Sc^{3+} in an octahedral environment and in the aqueous system is about the same as that of Mg^{2+}, a fact which is reflected in certain similarities of the aqueous chemistry of these two ions, it may be justified to consider Sc^{3+} as a tool to mimic the biological functions of Mg^{2+}. As we have shown in a previous communication [1b], the ^{45}Sc nucleus is easily accessible to NMR experiments and hence much more suited than the nucleus ^{25}Mg. The nuclear properties of ^{45}Sc [nuclear spin = 7/2, quadrupole moment = -0.22×10^{-28} m^2, natural abundance = 100%, relative receptivity ($^1H = 1$) = 0.30, frequency at $B_0 = 2.3488$ T ($^1H = 100$ MHz) = 24.29 MHz] show that relative receptivity and measuring frequency are favourable for this nucleus. The quadrupole moment is a disadvantage with respect to spectral resolution. It provides, however, an additional source of information via the quadrupole relaxation mechanism which is effective in non-cubic environments and gives rise to broad lines mainly in compounds of low local symmetry and in large and/or bulky molecules.

Results and Discussion

The Aqueous Sc(3+) System

Figure 1 shows the ^{45}Sc NMR spectra of aqueous (D_2O) solutions containing 1 mol/l of $ScCl_3$ (which has been employed as the standard in this investigation) at pD 1.6 and 2.8, respectively. The systems $ScCl_3$–H_2O and $ScCl_3$–H_2O–HCl have been investigated earlier by several groups [2] and although a deshielding with increasing concentration of $ScCl_3$ and, more pronounced, with addition of HCl has

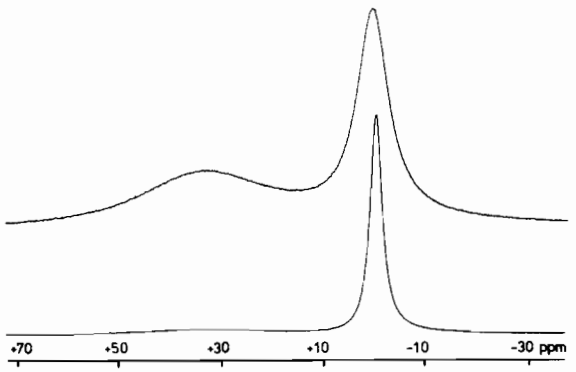


Fig. 1. Scandium chloride (1 mol/l) in D_2O at pH 1.6 (bottom) and 2.8. The broad signal at low field (high frequency) probably is a chloro complex $Sc(H_2O)_5Cl^{2+}$. The sharp signal at $\delta(^{45}Sc) = 0$ ppm, $Sc(H_2O_6)^{3+}$, has been taken as a reference throughout.

*Author to whom correspondence should be addressed.

0020-1693/89/$3.50

been reported and attributed to the presence of aqua–chloro complexes, distinct signals corresponding to different species present in solution have not been detected. Of the two signals observed with the experimental conditions maintained in our experiment, the sharper signal at high field corresponds to $Sc(H_2O)_6^{3+}$, which is the species predominating in aqueous solution at low pH [$\delta(^{45}Sc)$ = 0 ppm]. Since, at pH = 2.8, stable hydroxo complexes of Sc are not yet present (*vide infra*), the broad signal to low field can be attributed to a complex $Sc(H_2O)_{6-n}Cl_n^{(3-n)+}$ (with n probably attaining the value of 1), exchanging with the hexaqua complex at a rate where the position and linewidth of the signal of the latter are influenced. The NMR parameters of the two species are as follows:

pH = 1.6: $Sc(H_2O_6^{3+}$; δ = 0 ppm, $W_{1/2}$ = 250 Hz. $Sc(H_2O)_5Cl^{2+}$; δ = +33.8 ppm, $W_{1/2}$ = 1700 Hz.

pH = 2.8: $Sc(H_2O)_6^{3+}$; δ = 1.7 ppm, $W_{1/2}$ = 670 Hz. $Sc(H_2O)_5Cl^{2+}$; δ = 34.4 ppm; $W_{1/2}$ = 2000 Hz

with $W_{1/2}$ the width of the resonance signal at half-height. The broadening of the resonance signal for the chloro complex is in accord with the quadrupole relaxation mechanism, which is effective in this species of local C_{4v} symmetry. However, there is an additional contribution arising from exchange at medium exchange rates, as evidenced by the broadening of the resonance for the hexaqua cation, for which quadrupole relaxation should be ineffective due to its cubic symmetry. The downfield shift of the resonance of the chloro complex is in accord with both a decrease in symmetry and the replacement of an oxygen ligand by the less electronegative Cl^-, an effect commonly observed in d^0 complexes [3b, 3c, 4, 5] and usually termed 'inverse electronegativity dependence of metal shielding'.

We have carried out a more detailed analysis of the aqueous chemistry of the Sc^{3+} ion with dilute (30 mmol/l) scandium nitrate solutions in H_2O/D_2O 1/1 at varying temperatures and pH values. At these low concentrations, nitrate does not coordinate to the scandium ion, and hence the equilibria to be considered involve hexaquascandium, mono-, di- and trinuclear hydroxo complexes, eqn. (1) [6]

$$n Sc^{3+} + m H_2O \rightleftharpoons Sc_n(OH)_m^{(3n-m)+} + m H^+ \qquad (1)$$

with the following species present in solution (in parenthesis: log K*, K = formation constant for the hydroxo complex):

$Sc(H_2O)_6^{3+} \equiv [Sc]$

*The equilibrium constants have been taken from ref. 6 (25 °C, 0.1 mol/l KNO_3). Constants from other sources (*cf.* ref. 6) are close to those quoted.

TABLE 1. ^{45}Sc NMR Data of Aqueous Scandium Nitrate Solutions (30 mmol/l; H_2O/D_2O 1/1

	$\delta(^{45}Sc)$ (ppm)[a]		$W_{1/2}$[b] (Hz)	
Temperature (K) (pH = 3.8)				
285	+0.9		115	
300	+2.7		110	
315	+4.8		105	
322	+5.8		165	
330	+7.2		205	
345	+11.2		280	
360	+15.5		370	
pH (temperature = 298 K)				
4.1	+3.5	+36.2	195	2000[150]
4.5	+9.7	+36.8	650	2150[20]
5.0	+37.3[c]		3150[200][c]	
5.5	+29.4[d]		3150[150][d]	
6.5	+36.4[c]		3550[750][c]	

[a]Relative 1 M $ScCl_3$ in D_2O at pD 1.6. [b]Estimated error in brackets; *ca.* ±2% where not indicated. [c]Only a single broad signal is observed. [d]Unresolved low-field (high-frequency) shoulder.

$Sc(H_2O)_5OH^{2+} \equiv [Sc(OH)]$ (4.8)

$Sc_2(H_2O)_8(OH)_2^{4+} \equiv [Sc_2(OH)_2]$ (6.1)

$Sc_3(H_2O)_x(OH)_5^{4+} \equiv [Sc_3(OH)_5]$ (17.6)

The exchange between [Sc], [Sc(OH)] and [$Sc_2(OH)_2$] is fast, exchange involving the trinuclear species [$Sc_3(OH)_5$] is slow, and this is evidenced by the ^{45}Sc NMR results which are summarized in Table 1 and Figs. 2a and b.

With increasing temperature, there is a downfield shift and, above 320 K, an increase in linewidth. Although a decrease of shielding with increasing temperature is in accord with theory, the effect is too large to be accounted for simply in terms of the temperature-dependent increase of the paramagnetic deshielding contribution. Further, the broadening of the resonance signal indicates that chemical exchange takes place (quadrupole relaxation becomes less effective as the temperature goes up and hence, if mere quadrupole relaxation prevails, the signals should sharpen). At pH 3.8 and room temperature, about 10% of the species present are the hydroxo complexes [Sc(OH)] and [$Sc_2(OH)_2$]. Increasing temperature leads to higher concentrations of hydroxo complexes, and hence the NMR parameters are increasingly influenced by these species of relatively low local symmetry. At 360 K, about 60% of the overall scandium concentration is represented by rapidly exchanging hydroxo species, for which the extrapolated shift value $\delta(^{45}Sc)$ is +27 ppm.

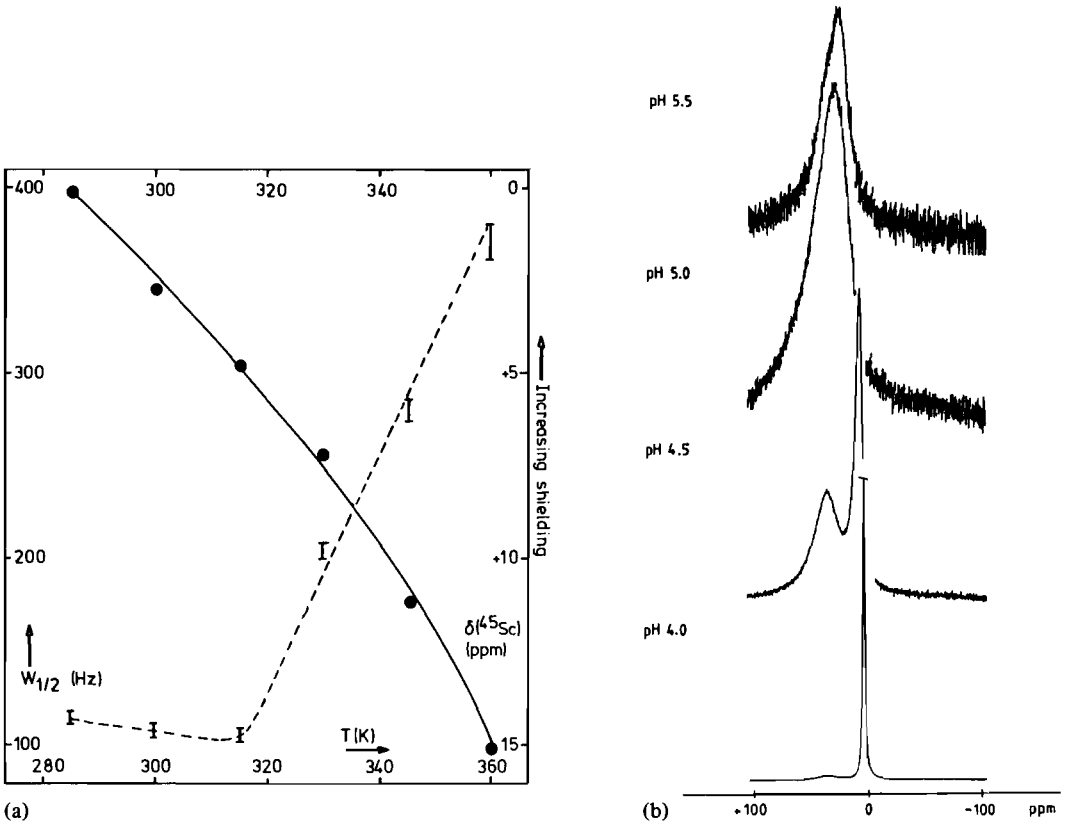


Fig. 2. (a) Temperature (T) *vs.* the ^{45}Sc chemical shift, $\delta(^{45}Sc)$ (right-hand ordinate, full circles and full line) and the half-widths of the resonance signals, $W_{1/2}$ (left-hand ordinate, vertical bars and broken line) for scandium nitrate (30 mmol/ l) in H_2O/D_2O 1/1, pH/D (room temperature) = 3.8. (b) ^{45}Sc NMR spectra of scandium nitrate solutions (30 mmol/l, H_2O/D_2O 1/1, 298 K) at varying pH values as indicated. The broader low-field signal corresponds to a trinuclear pentahydroxoscandium species, the sharper signal close to δ = 0 ppm reflects the equilibrium position for the species $Sc(H_2O)_6^{3+}$, $Sc(H_2O)_5OH^{2+}$ and $Sc_2(H_2O)_8(OH)_2^{4+}$.

At pH > 4, substantial amounts of $[Sc_3(OH)_5]$ are formed which give rise to a broad signal at 36 ppm (Fig. 2b). At pH = 5, practically all of the scandium is incorporated in the trinuclear complex. The signal parameters remain substantially unchanged up to the point where precipitation of $Sc(OH)_3$ begins (pH 6 to 6.5).

Sc(3+), Serine and Peptides

The signal for $[Sc_3(OH)_5]$ is also present in a 1/1 mixture of scandium nitrate (30 mmol/l) and serine (Fig. 3a; Table 2) at the intrinsic pH = 3.9. The formation constant of the serine/scandium(3+) complex [eqn. (2)], determined by pH-metric titration is 160, which is two orders of magnitude less than with other hydroxycarboxylic acids such as α-hydroxyisobutyric acid and glycolic acid [7]. Nonetheless, the introduction of the serine ligand into the coordination sphere of Sc^{3+} is evident from the low-field shift and the broadening of the resonance line for the exchanging [Sc]/[Sc(OH)]/ $[Sc_2(OH)_2]$ (compare Fig. 3 and the data in Tables 1 and 2). These effects are more pronounced as a four-fold molar excess of serine is employed (Fig. 3 b; Table 2). Ongoing from medium exchange rates, increasing temperature leads to further line broadening, and there is also an apparent involvement of $[Sc_3(OH)_5]$ in the exchange process, which gives an additional contribution to $W_{1/2}$. Close to the boiling point, the signal narrows, and this can be explained by rapid exchange owing to the labilization of the complexes. Here, quadrupole-dominated relaxation again prevails.

$$[Sc(H_2O)_5(OH)]^{2+} + HL \rightleftharpoons [Sc(H_2O)_4L(OH)]^{+} + H^{+} \quad (2)$$

with HL = $^{+}H_3N{-}CH(R){-}CO_2^{-}$ and equilibria of the kind [Sc] ⇌ [Sc(OH)] ⇌ $[Sc_2(OH)_2]$, $[Sc(H_2O)_4L(OH)]^{+} + H^{+} \rightleftharpoons [Sc(H_2O)_5L]^{2+}$, and $[Sc(H_2O)_5L]^{2+} + H^{+} = [Sc(H_2O)_5HL]^{3+}$ probably coupled to eqn. (4).

We have earlier reported on the interaction between Sc^{3+} and α-alanine, where a signal around

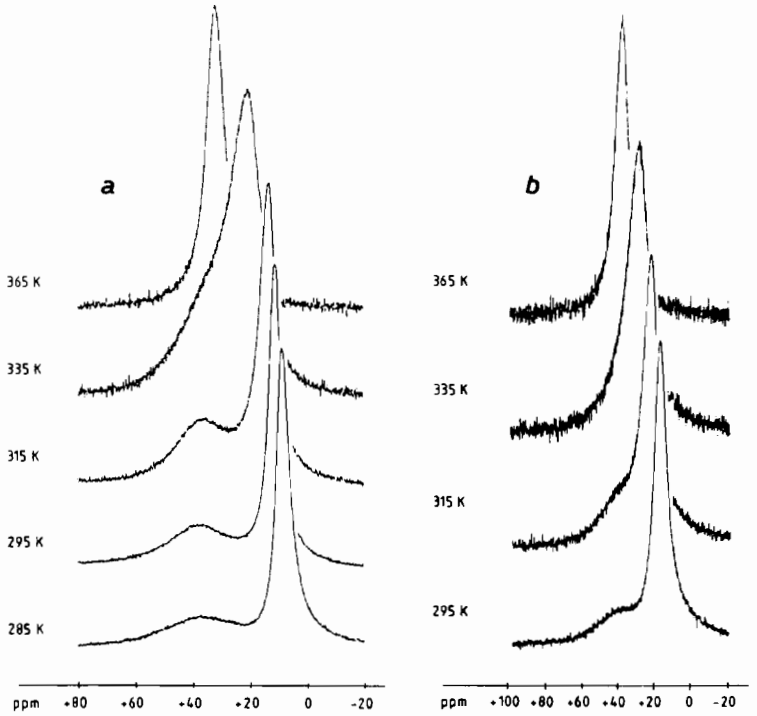


Fig. 3. ^{45}Sc NMR spectra of scandium nitrate/serine 1/1 (a) and 1/4 (b) at variable temperatures. c(Sc) = 30 mmol/l.

TABLE 2. ^{45}Sc NMR Data of Scandium Complexes[a]

System/temperature (K)	δ(^{45}Sc) (ppm)		$W_{1/2}$[b] (Hz)	
$Sc(O_2CH)_3$, pH 5.8	+32.2[c]		690	
$Sc(NO_3)_3$/ser 1/1, pH 3.9				
285	+8.1	+37.1	550	2880[180]
295	+10.5	+37.9	485	2205[135]
315	+13.6	+35.6	630	2115[45]
335	+20.3	[d]	1495[45]	
365	+30.9		730	
$Sc(NO_3)_3$/ser 1/4, pH 4.0				
295	+20.0	+40	785	[d]
315	+24.5	+42	950	[d]
335	+30.3		1295[35]	
365	+38.3		825	
$Sc(NO_3)_3$/val-ala 1/1, pH 4.0				
285	+13.1	+34	640	3375[225]
295	+14.8	+38	585	2680[290]
335	+24.6	[d]	1530[45]	
365	+33.9		750	
$Sc(NO_3)_3$/val-ala 1/4, pH 4.6				
285	+23.5	+71	1565	[d]
335	+29.7	[d]	2025[200]	
365	+39.0	[d]	3035[450]	
$Sc(O_2CH)_3$/val-ala 1/4, pH 3.8				
295	+24.6		960	
315	+25.6		725	
335	+27.6		735	
365	+32.7		720	

(continued)

TABLE 2. *(continued)*

System/temperature (K)	δ(^{45}Sc) (ppm)			$W_{1/2}$[b] (Hz)	
$Sc(O_2CH)_3$/glu-val 1/4, pH 4.1					
297	+19.6			1125	
315	+19.7			930	
330	+20.9			950	
360	+24.6			1050	
$Sc(NO_3)_3$/ala[e] 1/6, pH 4.0					
283	+23.0	+26	+77	1470[f]	
313	+31.1	+45	+71	1400[f]	
343	+42.5	+68		1680[f]	
$Sc(NO_3)_3$/ala-val-leu 1/1					
295	+18.5	+40	+77	980[f]	
305	+19.0	+37	+74	1050[f]	
315	+21.3	+34	+67	1540[f]	
$Sc(NO_3)_3$/ala-val-leu 1/4					
297	+23		+73	2300[230]	4200[350]
305	+24		+72	2100	4200[280]
315	+25		+74	1960	3640[320]

[a]In D_2O/H_2O 1/1, c(Sc) = 30 mmol/l. [b]Estimated error in brackets; *ca.* ±2% if not indicated. [c]Plus a very weak shoulder at low field. [d]Unresolved or poorly resolved low-field shoulder. [e]Selected values from ref. 1b. [f]For the high-field resonance (main component).

70 ppm indicates the formation of an alanine–scandium complex [1b]. This is clearly a greater deshielding of the ^{45}Sc nucleus than the limiting 30 ppm for the scandium/serine system, and there are two possible explanations for this observation, *viz.* (i) a preferred coordination of the hydroxy group of serine over the amino group and/or (ii) a stronger complex formation in the case of alanine, *i.e.* a shift of the equilibrium position (eqn. (2)) to the right-hand side. With these explanations, it is assumed that the amino acid is coordinated as a bidentate ligand, employing the carboxylato group and an additional ligand function (NH_2 or O^-). A scandium–alanine complex has in fact been isolated in substance by ethanol precipitation from an aqueous medium containing scandium sulfate and the amino acid [8]. We have not succeeded in isolating a corresponding serine compound, probably as a consequence of insufficiently strong interaction between Sc^{3+} and serine.

The formation constants of dipeptides are larger by about one order of magnitude than those of serine and other amino acids, *viz.* 1200 (H-val-ala-OH), 3600 (H-glu-val-OH) and 5400 (H-gly-gly-OH) and, consequently, the resonance for [Sc]/[Sc(OH)]/[$Sc_2(OH)_2$] in the scandium nitrate/dipeptide systems is still more effectively shifted to low field (Table 2; Fig. 4a) than in the case of Sc^{3+}/serine. Further, the signals are broader. Although in sufficiently slow exchange with [$Sc_3(OH)_5$] to allow for their separate observation, the signal parameters show that there is close communication between all of the species involved. This close communication is also exhibited by the spectral pattern for the tripeptide H-ala-val-leu-OH (Fig. 5). Here, an additional low-field resonance at *ca.* +70 ppm, reminiscent of the situation encountered in the scandium/alanine system [1b], is observed. There is a significant increase in the signal intensity of this resonance as the molar ratio peptide/Sc increases. Persuing the

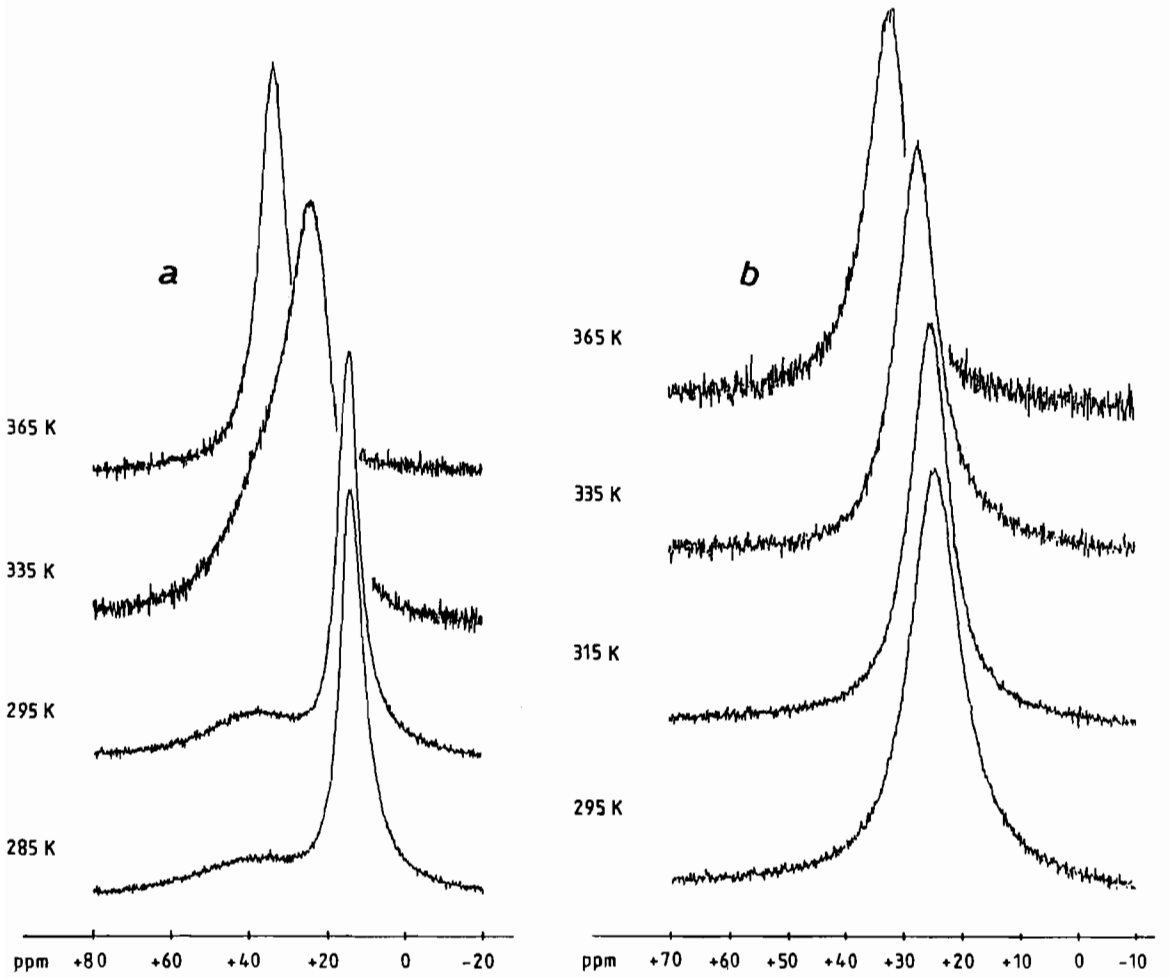


Fig. 4. ^{45}Sc NMR spectra of mixtures of the dipeptide H-val-ala-OH with scandium nitrate (1/1; a) and scandium formiate (4/1; b), respectively. $c(Sc) = c(peptide) = 30$ mmol/l. pH values are 4.0 (a) and 3.2 (b).

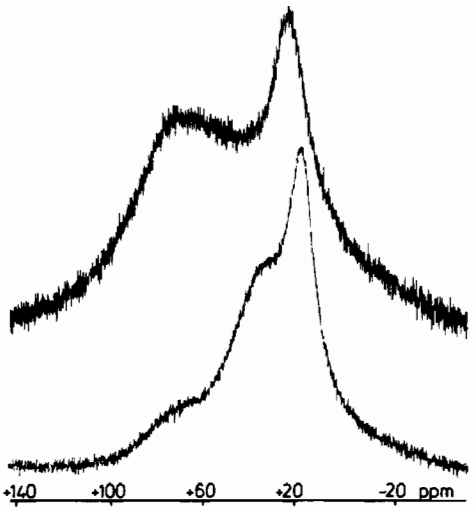


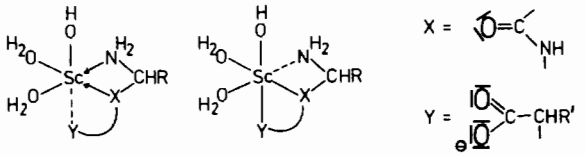
Fig. 5. ^{45}Sc NMR spectra of scandium nitrate solutions (30 mmol/l, H_2O/D_2O 1/1) containing equimolar amounts (bottom spectrum) and a four-fold molar excess of the tripeptide H-ala-val-leu-OH. The spectra have been obtained at 315 K; the signal at *ca.* +70 ppm is typical of an involvement of a nitrogen function in coordination to scandium.

arguments used in the preceding section, the low-field shift of this signal for a slowly exchanging scandium–peptide complex indicates participation of a nitrogen function in coordination to the scandium centre.

Following the preparative route to carboxylato complexes of Sc^{3+} described in the literature [e.g. $Sc(O_2CiPr)_3$] [9], we have also reacted scandium formiate in the presence of small amounts of acetic anhydride with the dipeptides H-val-ala-OH, H-gly-gly-OH and H-glu-val-OH. While H-gly-gly-OH does not replace the formiato ligand, complexes of varying composition are formed with the two other dipeptides. The IR spectra of these compounds give evidence for the coordination of the carboxylato groups but not for a participation of nitrogen. This is also evident from the ^{45}Sc NMR spectra of aqueous solutions containing $Sc(O_2CH)_3$ and the dipeptides (Fig. 4b; for data see Table 2), where only the resonance characteristic of exclusive oxygen coordination appears.

Conclusions

In aqueous solution, and at the pH values employed (*ca.* 4), the dominating species present are $Sc(H_2O)_6^{3+}$, $Sc(H_2O)_5OH^{2+}$ and $Sc_2(H_2O)_8(OH)_2^{4+}$ in fast exchange with each other. At pH > 4, a slowly exchanging trinuclear complex, $Sc_3(OH)_5(H_2O)_n^{4+}$, is also present. Sc^{3+} directly coordinates to negatively charged carboxylate functions, with concomittant removal of H_2O or OH^- from the coordination sphere.



Scheme 1.

Sc^{3+} has been shown to form rather stable complexes with hydroxy acids [7], dicarboxylic acids [10] and the tricarboxylic aconitic acid [11]. The complexes with amino acids and small peptides are labile (formation constants are 1.5×10^2 to 5×10^3; by potentiometry). Depending on the ligand, the complexation, as revealed by ^{45}Sc NMR, involves oxygen functions only, or chelate formation with the inclusion of nitrogen-containing groups. The signals are shifted to low field (high frequency) by 30 to 40 ppm with respect to $Sc(H_2O)_6^{3+}$, and these deshielding contributions are due to a decrease of symmetry ($O_h \rightarrow C_{4v}$ or less) and, in the case of N coordination, a decrease of ligand electronegativity. Possible structures are shown in Scheme 1.

Experimental

Physical Measurements

NMR spectra were obtained on a Bruker AM 360 spectrometer at 298 K in rotating 10 mm diameter vials containing the samples in D_2O or D_2O/H_2O solution under the following typical conditions: 87.48 MHz, sweep width 35 kHz (acquisition time 0.115 s), 8 K data set, digital resolution 9 Hz/point, pulse angle 30°, line broadening factor 0; standard 1 M $ScCl_3/D_2O$ (acidified to pD = 1.5); number of scans 2000 to 8000.

Formation constants of the scandium complexes were determined by potentiometric titration in thermostatted (298 K) solutions containing 0.2 mol/l KNO_3, 3.8 mmol/l $Sc(NO_3)_3$ and *ca.* 5 mmol/l of the ligand. The pH measuring system consisted of a micro glass electrode (Ingold, LoT-405-M3) and a Knick model 646 digital pH meter. The titration curves were evaluated by the tangent method [12]; formation constants were calculated following the Bjerrum method [13], assuming the formation of 1:1 complexes. Acid constants were taken from the literature.

Materials and Sample Preparation

Amino acids and peptides (L forms have been employed throughout) have been obtained from commercial sources (Serva) and used without further purification. Scandium nitrate was prepared by dissolving Sc_2O_3 (0.43 g = 3.1 mmol; obtained by thermal decomposition of $Sc_2(C_2O_4)_3 \cdot 6H_2O$ (Ventron)) in hot, concentrated HNO_3 (7 ml), and

evaporation and drying at 80 °C. These samples contained 16 to 17.5% Sc, corresponding to crystal water contents between 2.5 and 1.5 H_2O per Sc^{3+}. A solution of the concentration $c(Sc)$ = 3.8 mmol/l consumed between 5.1 and 7.1 ml of 0.1 M NaOH. Scandium formiate $Sc(O_2CH)_3$ was prepared from scandium nitrate and formic acid as described in the literature [14].

Scandium nitrate and scandium formiate solutions in D_2O/H_2O containing 30 to 35 mmol/l of Sc were treated with the ligand (serine or peptide) and measured at their intrinsic pH/D values. The pH values for measurements with ligand-free scandium nitrate and scandium chloride (Ventron) were adjusted by addition of KOH and HCl, respectively.

References

1 (a) D. Rehder, *Inorg. Chem.*, in press; (b) D. Rehder and M. Speh, *Inorg. Chim. Acta, 135* (1987) 73.

2 (a) G. A. Melson, D. J. Olzanski and A. Z. Rahimi, *Spectrochim. Acta, Part A, 33* (1977) 301; (b) O. Lutz, *Phys. Lett., 29A* (1969) 58; (c) E. Haid, D. Köhnlein, G. Kössler, O. Lutz, W. Messner, K. R. Mohn, G. Nothaft, B. van Rickelen, W. Schich and N. Steinhauser, *Z. Naturforsch., Teil A, 38* (1983) 317; (d) G. A. Kirakosyan and V. P. Tarasov, *Koord. Khim., 8* (1982) 261; for reviews of ^{45}Sc NMR see also refs. 3b and c.

3 (a) D. Rehder, *Bull. Magn. Reson., 4* (1982) 33; (b) D. Rehder, *Magn. Reson. Rev., 9* (1984) 125; (c) D. Rehder, in J. Mason (ed.), *Multinuclear NMR*, Plenum, New York, 1987, Ch. 19.

4 D. Rehder, *Chimia, 40* (1986) 186.

5 J. Mason, *Chem. Rev., 87* (1987) 1299.

6 P. L. Brown, J. Ellis and R. N. Sylva, *J. Chem. Soc., Dalton Trans.*, (1983) 35.

7 H. Itoh, N. Itoh and Y. Suzuki, *Bull. Chem. Soc. Jpn., 57* (1984) 716.

8 I. A. Fedorov, T. A. Balakaeva and A. N. Kuchumova, *Russ. J. Inorg. Chem., 11* (1986) 906.

9 A. K. Rai and G. K. Parashar, *Synth. React. Inorg. Met.-Org. Chem., 9* (1979) 301.

10 H. Itoh, Y. Ikegami and Y. Suzuki, *Bull. Chem. Soc. Jpn., 57* (1984) 3426.

11 N. A. Skorik, A. S. Kochmanyuk and O. Yu. Voronkova, *Russ. J. Inorg. Chem., 31* (1986) 646.

12 (a) C. F. Tubbs, *Anal. Chem., 26* (1954) 1670; (b) S. Ebel and W. Parzefall, *Experimentelle Einführung in die Potentiometrie*, Verlag Chemie, Weinheim, 1975, p. 80.

13 (a) J. Bjerrum, *Metal Ammine Formation in Aqueous Solution*, P. Haase and Son, Copenhagen, 1957; (b) H. L. Schläfer, *Komplexbildung in Lösung*, Springer, Berlin, 1961.

14 J. S. Sterba-Böhm and M. Melichar, *Coll. Czech. Chem. Commun., 7* (1935) 57.