A Raman study of the binding of Fe(III) to ATP and AMP

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ATP–Fe and AMP–Fe complexes in water (H_2O and $^2\text{H}_2\text{O}$) at pH 7.5 were studied using Raman spectroscopy. Parallel and perpendicular polarization spectra were recorded in the spectral range 200–1650 cm^{-1} , and the depolarization ratios for most of the bands were calculated. The changes in the frequencies, intensities and depolarization ratios of the ATP and AMP bands after the addition of FeCl_3 showed that the adenine moiety, in addition to the phosphate(s), was involved in the binding of Fe to both ATP and AMP. Direct interactions of Fe(III) with the phosphate chain and the N-7 nitrogen and indirect interaction (via water molecules) with the amide group were proposed for the ATP–Fe complex. In contrast, direct interaction with the phosphate group and indirect interaction with the amide group were observed for the AMP–Fe complex. The different interactions of the two complexes suggest an ‘anti’ conformation for the ATP–Fe complex and a ‘syn’ conformation for the AMP–Fe complex. The strong binding of Fe to ATP compared with AMP and the difference in the conformation of the ATP–Fe and the AMP–Fe complexes may be significant in the pathway of Fe release in mitochondria.

INTRODUCTION

Iron was found to bind with high affinity to a mitochondrial receptor incubated with the ATP–Fe complex, suggesting that phosphate-containing Fe compounds are important for mitochondrial uptake of Fe [1]. In addition, it was shown recently [2] that two kinds of mitochondrial receptors are responsible for the incorporation of Fe into haem. One of these receptors accepts Fe from the ATP–Fe complex, but not from the AMP–Fe complex. The other accepts Fe from the AMP–Fe complex but not from the ATP–Fe complex and is much more efficient in haem synthesis. It was argued that the main difficulty in transferring Fe from the ATP–Fe complex to the corresponding mitochondrial receptor is the tight binding of Fe to ATP, thus making the release of Fe less favourable. Therefore it was suggested that the major pathway for the delivery of Fe to haem is through the hydrolysis of cytosolic ATP–Fe to AMP–Fe. It is of interest therefore to understand better the binding of Fe to ATP and AMP. The main purpose of this study is to investigate the differences in these bindings using Raman spectroscopy, including polarization measurements.

The interactions of bivalent metal ions, Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , Cu^{2+} , Co^{2+} , Hg^{2+} , with adenine phosphates have been studied by various spectroscopic techniques, including resonance Raman spectroscopy [3–11]. Most Raman studies on metal–adenosine phosphate interaction have concentrated on the phosphate moiety which is known to interact strongly with all metal cations studied. Others have shown that some metals in addition interact with the adenine moiety [7,11]. These interactions are strongly influenced by changes in pH and are metal specific [7]. The changes in the Raman spectra due to these metal–adenine interactions (wavenumber shift and/or intensity change) are usually small, suggesting that the metals interact with the adenine moiety indirectly, probably through hydrogen-bonded water molecules. In contrast, Fe(II) and Fe(III) complexes with ATP or AMP have not been extensively examined spectroscopically [12,13] and have not been studied by Raman spectroscopy. Furthermore, it is well known that the

depolarization ratios of Raman bands can be used to detect symmetry changes due to changes in surroundings and interactions [14]. In this study, we have used the changes in the depolarization ratio, as well as intensity changes and wavenumber shifts, to gain qualitative structural information on the binding of Fe cations to ATP and to AMP.

EXPERIMENTAL

ATP (disodium) and AMP (disodium) were purchased from Sigma Chemical Co. and were used without further purification. A 1:1 to 4:1 stoichiometric amount of Fe^{3+} cation was introduced by adding ferric chloride (Sigma Chemical Co.) in a 0.1 M solution of HCl to a solution of AMP or ATP [2]. The pH of the samples was then adjusted to 7.5 with 0.1 M-NaOH and -HCl. When $^2\text{H}_2\text{O}$ was used, deuterated equivalents, NaO^2H and ^2HCl , were used. Concentrations of AMP or ATP and Fe^{3+} were between 40 and 300 mM. Some precipitation occurs at high concentrations.

Raman measurements were done with the samples in melting-point capillaries. The 488.0 nm line from an argon ion laser (Spectra Physics model 164) was used as excitation. Typical power at the sample was 100 mW. Raman signals were collected via a 90° geometry by a triple monochromator (Spex model 1877 triplemate) and a photodiode array detector system consisting of a model IRY-1024 detector and a model ST-120 controller from Princeton Instruments (Trenton, NJ, U.S.A.) interfaced to an IBM-AT microcomputer. Calibrations for the Raman system were done for each measurement using known Raman lines of toluene. Peak positions are accurate to within $\pm 1 \text{ cm}^{-1}$ between measurements done on different days. Typical resolution was 6 cm^{-1} . In general, accurate frequency shifts are easier to observe. The accuracy of the frequency shifts due to complexation with iron was about 1 cm^{-1} for the low-intensity bands and better than 1 cm^{-1} for the high-intensity ones. Perpendicular and parallel spectra have their usual meanings [15], i.e. the polarization of the scattered light is selected to be perpendicular to, or parallel with, the polarization of the excitation light res-

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pectively. The depolarization ratio is calculated as the ratio of the intensity in the perpendicular to that in the parallel direction.

RESULTS AND DISCUSSION

The Raman spectra of AMP in water, with and without Fe(III), between 900 and 1650 cm^{-1} are shown in Fig. 1. Similarly, Fig. 2 shows typical spectra of ATP in water, with and without Fe(III), for the perpendicular and parallel polarizations between 900 and 1650 cm^{-1} . The corresponding unpolarized spectra for AMP and ATP in $^2\text{H}_2\text{O}$, with and without Fe(III), are shown in Fig. 3. Smooth backgrounds have been subtracted for these spectra. Depolarization ratios, ρ , of the Raman peaks of ATP, AMP and their respective Fe(III) complexes between 900 and 1650 cm^{-1} were obtained by dividing the perpendicular spectrum by the parallel spectrum point to point. The intensities of these spectra had been normalized (counts/unit integration time per mW of excitation power) before the division. The same procedures were attempted with spectra in the region from 200 cm^{-1} to 900 cm^{-1} (results not shown). Except for the band at approx. 720–730 cm^{-1} , the depolarization ratios of bands in this region could not be calculated with enough accuracy because of low intensity and/or strong overlapping. In general, two major factors affect the uncertainty. Bands with lower intensities have higher uncertainties, and the strongly polarized bands (i.e. small ρ values) have more uncertainties. The frequencies and depolarization ratios for the Raman bands of ATP, AMP and their Fe(III) complexes in H_2O and $^2\text{H}_2\text{O}$ between 700 and 1650 cm^{-1} are presented in Tables 1 and 2 respectively. Changes

Fig. 1. Raman spectra of AMP in water at pH 7.5 (a) and after addition of Fe(III) (b)

The sample was in a melting-point capillary tube at room temperature, and 100 mW of 488 nm excitation was used. Total accumulation time was 5 and 10 min respectively. A smooth background has been subtracted.

Fig. 2. Parallel and perpendicularly polarized Raman spectra of ATP in water at pH 7.5 (a) and after addition of Fe(III) (b)

Total accumulation time was 10, 15, 30 and 30 min respectively. Other conditions were the same as in Fig. 1.

in depolarization ratios and frequency shifts on addition of Fe are also included. The spectral region from approx. 800 cm^{-1} to approx. 920 cm^{-1} can be assigned to the sugar ring and is not included. These bands strongly overlap, which leads to a large uncertainty in the determination of small wavenumber shifts and of changes in the depolarization ratios on addition of metal.

The frequency shifts in the Raman spectra of ATP and AMP on complexation with Fe(III) are checked by varying the concentration of ATP and AMP and the ratio of adenine phosphate to Fe(III). Within the accuracy of the measurements, there is no dependence of the shifts in the ATP spectra on the concentration of ATP from 40 to 300 mM and the ATP to Fe(III) ratio of 1:1 to 1:4. Raman peaks of AMP show no shift within a similar range.

It is clear that the oscillators of the adenine moiety are strongly coupled and most modes involve more than one vibration. The assignments of the bands in Tables 1 and 2 are based primarily on those by Majoube [16] with modifications due to other researchers [17–19].

The Raman spectra of adenine and analogues are well known. The main features of the Raman spectra of ATP and AMP in Figs. 1–3 and in the low-frequency regions are in general agreement with previous reports [7,11]. In $^2\text{H}_2\text{O}$ solutions, the amide protons exchange quickly. Under our experimental conditions, the proton at the C-8 position (see Fig. 4) is also

Fig. 3. Raman spectra of AMP and ATP in $^2\text{H}_2\text{O}$ (a and c) and after addition of Fe(III) (b and d) respectively

Total accumulation time was 5, 5, 10 and 15 min respectively. Other conditions were the same as in Fig. 1.

exchanged even though its H– ^2H exchange rate is very slow [20]. The C-2 proton, however, is not exchanged. The changes in the Raman spectrum of AMP or ATP from H_2O to $^2\text{H}_2\text{O}$ can be understood by the deuterations of these three protons. At least two normal mode calculations on the adenine moiety have been

reported [16,17]. Most of the observed bands can be assigned to vibrations of the phosphate and adenine moiety, which are predominantly involved in the interaction with the metal ions [7]. Most assignments are in good general agreement with published reports. There are, however, some differences in the detailed assignments of the vibrations to specific parts of the molecules.

As can be seen in Figs. 1–3, the addition of FeCl_3 leads to a decrease in the intensities of the bands at approx. 974 cm^{-1} and 996 cm^{-1} in the AMP spectra and at approx. 1088 cm^{-1} and 1116 cm^{-1} in the ATP spectra. These bands are due to the in-phase and out-of-phase modes of the symmetric (PO_2^-) vibration and the stretching vibration of the terminal phosphate group in ATP [10,21]. Similar changes have been observed for most other metal cations binding to adenosine phosphate [7]. Note also the huge changes in depolarization ratios of these two bands on addition of Fe(III) ions (Tables 1 and 2). Sizable frequency shifts also occur for the approx. 996 cm^{-1} band of AMP in H_2O and the 1114 cm^{-1} band of ATP in $^2\text{H}_2\text{O}$ with the formation of the Fe complexes (Tables 1 and 2). Furthermore, the band at 1116 cm^{-1} (and the corresponding peak in $^2\text{H}_2\text{O}$) for ATP seems to be broadened with complexation (Figs. 2 and 3). This shows unambiguously that the phosphate moiety is directly involved in the complexation of Fe(III) cations to both AMP and ATP.

The peak of the Raman spectra of ATP and AMP at approx. 729 cm^{-1} in H_2O has been assigned to a ring-breathing mode with strong coupling to the deformation of the attachment of the entire adenine to the sugar [7,16]. Its frequency is clearly sensitive to the solvent, shifting down by 9 cm^{-1} in $^2\text{H}_2\text{O}$. Although the frequency does not change on addition of Fe(III) to either ATP or AMP, there is always a trend for a positive change in the depolarization ratio of this band, with a pronounced change for ATP in $^2\text{H}_2\text{O}$ (Tables 1 and 2). A strain in the attachment of adenine to the sugar can cause such a change (see discussion below).

Almost every band between 900 and 1650 cm^{-1} in the Raman spectrum of ATP is shifted on binding to Fe(III) (Tables 1 and 2). Except for the two phosphate-related bands, these bands have been assigned to modes involving the adenine moiety. In contrast, the adenine bands of AMP show no significant change in frequency. This is consistent with a much stronger binding of

Table 1. Tentative assignments, Raman shifts and depolarization ratios of the Raman bands of AMP and ATP in H_2O between 700 cm^{-1} and 1650 cm^{-1} and their absolute changes (in parentheses) after addition of FeCl_3

Assignment		Raman shift (cm^{-1})		Depolarization ratio	
		AMP	ATP	AMP	ATP
D.N ₉ R, N ₃ C ₄	(5/6)*	729 (0)†§	730 (–1)†§	0.08 (+0.02)‡§	0.07 (+0.02)‡§
Phosphate		977 (0)§	1088	0.01 (+0.04)§	0.10 (+0.03)
Phosphate		996 (+3)	1116 (+2)	0.07 (–0.01)§	0.02 (+0.11)
C ₆ N ₁₀ , N ₃ C ₄	(6?)	1174 (0)§	1175 (0)§	0.12 (+0.21)	0.12 (+0.07)
D.C ₈ H	(5)	1218 (0)§	1220 (–2)	0.20 (+0.02)§	0.26 (+0.01)§
D.CNH ₂ , C ₆ N ₃ , N ₉ R	(6)	1253 (–1)§	1253 (+1)§	0.28 (+0.01)§	0.27 (–0.12)
D.C ₂ H, N ₁ C ₂	(6)	1307 (0)§	1307 (–2)	0.30 (+0.01)§	0.28 (+0.03)
C ₈ N ₉ , C ₆ N ₃	(5/6)	1338 (0)§	1338 (–2)	0.26 (+0.03)	0.27 (–0.03)
C ₅ N ₇ , C ₈ N ₉	(5/6)	1378 (–1)§	1378 (–2)	0.18 (+0.03)	0.20 (–0.01)§
N ₇ C ₈ , C ₆ N ₁₀ , D.C ₉ H	(5/6)	1423 (0)§	1423 (–2)	0.25 (+0.03)	0.27 (–0.07)
N ₉ C ₄ , D.C ₂ H, C ₂ N ₃	(6)	1483 (0)§	1483 (–2)	0.10 (+0.02)§	0.10 (+0.02)§
N ₇ C ₈ , C ₆ N ₉ , D.C ₈ H	(5)	1508 (0)§	1508 (–3)	0.28 (+0.02)§	0.28 (–0.04)
N ₃ C ₄ , C ₄ C ₅	(5/6)	1579 (0)§	1579 (–1)§	0.59 (+0.05)	0.61 (–0.05)
C ₄ C ₅ , D.N ₃ C ₄		Not observed			

* Major involvement: five- or six-membered ring.

† Frequency shifts on addition of Fe(III) are given in parentheses.

‡ The absolute changes in ρ on addition of Fe(III) are given in parentheses.

§ Within experimental uncertainty of no change.

|| Shoulder or strongly overlapped component.

Table 2. Tentative assignments, Raman shifts and depolarization ratios of the Raman bands of AMP and ATP in $^2\text{H}_2\text{O}$ between 700 cm^{-1} and 1650 cm^{-1} and their absolute changes (in parentheses) after addition of FeCl_3

Assignment		Raman shift (cm^{-1})		Depolarization ratio	
		AMP	ATP	AMP	ATP
D.N ₉ R, N ₃ C ₄	(5/6)*	720(+1)†§	721(-1)†§	0.08(+0.02)‡§	0.07(+0.04)
Phosphate		974(-1)§	1089	0.01(+0.02)§	0.03(+0.08)
Phosphate		997	1114(+4)	0.14(+0.18)	0.02(+0.11)
N ₁ C ₂ , N ₉ R, D.ND ₂	(6)	1183	1182(-1)§	0.14(+0.20)	0.15(+0.06)§
D.C ₈ D	(5)	1198	1198	0.27(+0.16)	0.33(+0.01)§
C ₄ N ₃ , N ₉ R			Not observed?		
D.C ₂ H, N ₁ C ₂	(6)	1306(-1)§	1305(-2)	0.29(+0.04)	0.29(-0.01)§
C ₈ N ₉ , C ₂ N ₃	(5/6)	1342(-1)§	1342(-2)	0.27(+0.02)§	0.27(-0.02)§
C ₅ N ₇ , C ₈ N ₉	(5)	1383(-1)§	1383(-3)	0.21(-0.02)§	0.20(+0.01)§
N ₇ C ₈ , C ₄ N ₁₀ , D.C ₈ D	(5/6)	1426(0)§	1426(-3)	0.32(+0.07)	0.32(-0.06)
D.C ₂ H, C ₂ N ₃ , N ₉ C ₄	(6)	1484(0)§	1483(-2)	0.11(+0.03)	0.11(+0.02)§
N ₇ C ₈ , C ₈ N ₉ , D.C ₈ D	(5)	1520(-1)§	1520(-4)	0.26(+0.01)§	0.29(-0.02)§
N ₃ C ₄ , N ₉ C ₄ C ₅ , C ₄ C ₅	(5/6)	1576(+1)§	1576(-1)§	0.61(+0.09)	0.62(-0.06)
C ₄ C ₅ , D.N ₃ C ₄	(5/6)	1622(0)§	1622(-3)	0.67(+0.11)	0.71(-0.13)

* Major involvement: five- or six-membered ring.

† Frequency shifts on addition of Fe(III) are given in parentheses.‡ The absolute changes in ρ on addition of Fe(III) are given in parentheses.

§ Within experimental uncertainty of no change.

|| Shoulder or strongly overlapped component.

Fig. 4. Models of ATP-Fe (a) and AMP-Fe (b) complexes

Fe(III) to ATP. Interaction of Fe(III) with the adenine moiety of AMP is likely to be indirect, through the solvent. Direct interaction usually leads to greater changes in the Raman spectrum (greater wavenumber shifts and/or intensity changes [7]. On the other hand, frequency changes in all of the adenine bands of ATP suggest a global involvement of the entire adenine moiety with Fe(III) , with direct interactions being more likely.

Direct interactions with ATP are also consistent with the fact that these frequency changes in H_2O and $^2\text{H}_2\text{O}$ on the addition of Fe(III) are very similar.

The involvement of the adenine moiety of ATP and AMP in the binding of Fe(III) can also be seen by the change in depolarization ratio of most of the adenine-related bands. From Tables 1 and 2, it is clear that the involvement with ATP is very different from that with AMP. Complexation of AMP with Fe(III) leads to an increase in the depolarization ratio for all of the bands associated with the adenine moiety, in H_2O and in $^2\text{H}_2\text{O}$. The only possible exception is the band at approx. 1383 cm^{-1} in $^2\text{H}_2\text{O}$ (see below). In contrast, some of the adenine-related vibrations of ATP (in H_2O and in $^2\text{H}_2\text{O}$) show a decrease in the depolarization ratio on addition of Fe(III) .

According to the theory of the Raman scattering [15], the mean polarizability invariant a , the anisotropy invariant γ and off-resonance depolarization ratio ρ are defined by:

$$a = (1/3)(\alpha_{xx} + \alpha_{yy} + \alpha_{zz}) \quad (1)$$

$$\gamma^2 = (1/2)[(\alpha_{xx} - \alpha_{yy})^2 + (\alpha_{yy} - \alpha_{zz})^2 + (\alpha_{zz} - \alpha_{xx})^2 + 6(\alpha_{xy}^2 + \alpha_{yz}^2 + \alpha_{zx}^2)] \quad (2)$$

$$\rho = (3\gamma^2)/(45a^2 + 4\gamma^2) \quad (3)$$

It is known that all the symmetry properties of the molecule are retained by the derived polarizability ellipsoid as described by the derived polarizability tensor [15,22]. The elements of the derived polarizability tensor depend on the properties of 'loose electrons' which are free to move or to be polarized [23], i.e. they are determined by the shape and density of the electron cloud of the molecule. A change in the electron cloud can change the derived polarizability ellipsoid and thus the depolarization ratio. Complexation of Fe(III) to the adenine moiety can lead to a redistribution of the electron cloud. If this complexation occurs at the N-7 position (see Fig. 4), the electron cloud at the N-7 nitrogen atom can be partially removed, thus balancing in part the strong covalent bonding of the sugar moiety at the N-9 position. As a result, the polarizability ellipsoid for the five-membered ring may become more isotropic, i.e. the absolute

difference between the diagonal elements of the polarizability tensor, α_{xx} , α_{yy} and α_{zz} , should decrease. According to eqn. (3), the depolarization ratio should also decrease, as ρ is a monotonously increasing function of γ^2 for constant a . On the other hand, the value of a is a measure of the 'average' polarizability [15] and will probably change much less and remain essentially unchanged since no additional electron is introduced or removed from the adenine moiety. Thus complexation at the N-7 position can lead to a decrease in depolarization ratio of modes that are due to the five-membered ring. In contrast, if the complexation of Fe(III) occurs at the N-10 position through the amide group, additional anisotropy in the polarizability ellipsoid is introduced by further withdrawing electrons to the amide's protons. Furthermore, any decrease in the motion of the amide's protons due to complexation is likely to strain the six-membered ring and increase the anisotropy of the electron cloud of the six-membered ring. Using similar arguments as described above, an increase in the depolarization ratio of the affected modes can be expected.

As mentioned above, the detailed assignments of some bands are not unambiguous. Most modes involve highly coupled vibrations where most of the atoms of the adenine moiety participate. The assignments in Tables 1 and 2 are our best estimates. Modes involving mainly the six-membered ring are labelled by the numeral 6. Similarly, numeral 5 designates modes associated primarily with the five-membered ring. Modes that involve the entire adenine moiety are labelled as 5/6. Interpretation of the changes of ρ should be viewed as tentative for some modes.

ATP + Fe(III) complex

In addition to the small but significant frequency changes in most of the bands of ATP between 900 and 1650 cm^{-1} on complexation, there are also increases and decreases in ρ (Tables 1 and 2). Although these frequency shifts show clearly that the adenine moiety is directly involved in binding, it is difficult to get information about specific interactions from these small shifts. In contrast, changes in ρ can give more specific insight. On the basis of our considerations above, modes labelled by 5 should have a smaller ρ if complexation involves the N-7 nitrogen and modes labelled by 6 should have a larger ρ if interaction with the $-\text{NH}_2$ group is present. Changes in ρ for modes that involve the entire adenine cannot be predicted if complexation involves both N-7 and $-\text{NH}_2$. Such changes are generally observed from Tables 1 and 2. All modes due to the six-membered ring alone have ρ increased on complexation with the clear exception for the band at approx. 1253 cm^{-1} in H_2O . This mode is due mainly to the bending of the $-\text{NH}_2$ group. It becomes highly coupled in $^2\text{H}_2\text{O}$ and cannot be clearly identified. The observed negative change of ρ in H_2O may be due to the change of N_6R stretching vibration from the N-7-Fe(III) interaction. The largest increase in ρ occurs for the band at approx. 1174 cm^{-1} . The origin of this mode, however, is the least certain of all the assignments. Nevertheless, interaction with the five-membered ring is clear from the overall pattern of changes in ρ . We propose that Fe(III) binds to ATP through interactions with the phosphate and the N-7 and $-\text{NH}_2$ of the adenine as shown in Fig. 4(a). Furthermore, the ATP molecule has to be in the 'anti' configuration to accommodate interaction with the N-7 position. It is interesting to note that the bands due to the five-membered ring alone have the largest frequency shifts (e.g. the band at approx. 1508 cm^{-1} in H_2O and approx. 1383, 1520 cm^{-1} in $^2\text{H}_2\text{O}$). This agrees well with the proposed interactions in our model.

Very similar changes occur with the addition of Fe(III) to ATP in $^2\text{H}_2\text{O}$ (Fig. 3 and Table 2). The spectral change of ATP from H_2O to $^2\text{H}_2\text{O}$ can be understood as the deuteration of the amide

and C-8 protons. For example, there are large changes in the spectral features in the 1100–1300 cm^{-1} region between H_2O and $^2\text{H}_2\text{O}$ (compare Figs. 2 and 3). The mode at approx. 1253 cm^{-1} in H_2O disappears in $^2\text{H}_2\text{O}$ and is probably coupled with one or both of the two bands at approx. 1182 and 1198 cm^{-1} . The band at approx. 1175 cm^{-1} in H_2O is probably upshifted to 1182 cm^{-1} in $^2\text{H}_2\text{O}$. The two bands have similar changes in ρ on complexation. The changes in the phosphate bands on complexation seem to be more pronounced in $^2\text{H}_2\text{O}$ with large changes in ρ for both phosphate bands (Table 2).

AMP-Fe(III) complex

In contrast with Fe binding to ATP, there is essentially no change in the frequency of any bands associated with adenine in AMP on complexation with Fe. However, the depolarization ratios of nearly all bands between 900 and 1650 cm^{-1} increase (Tables 1 and 2). The only exception is the band at approx. 1383 cm^{-1} in $^2\text{H}_2\text{O}$. If the interaction of Fe(III) with the adenine moiety is through the six-membered ring, only increases in ρ are expected. Interaction through the N-7 position would have decreased ρ for some bands as in ATP, which is not observed. Therefore we propose that Fe(III) binding involves indirect interactions with adenine through the six-membered ring only. The unexpected decrease in ρ of the approx. 1383 cm^{-1} band in $^2\text{H}_2\text{O}$ is small and is within the experimental uncertainty. Since the phosphate group is clearly involved in the binding and there is only one PO_4^- group in the phosphate chain, we propose a closed 'syn' configuration for AMP when chelated with Fe(III). An 'anti' configuration is not possible, since it requires an interaction of the Fe(III) with the N-7 nitrogen from the close proximity of the two atoms and would lead to changes in the depolarization ratio of the adenine bands similar to those observed for the ATP-Fe(III) complex. Fig. 4(b) shows schematically a possible 'syn' configuration of the Fe(III)-AMP complex.

As mentioned above, the mode at approx. 730 cm^{-1} is mainly due to $\text{D.N}_6\text{R}$ deformation. A trend for an increase in ρ for this mode was observed for ATP and AMP on complexation with Fe (the effect is pronounced for Fe(III)-ATP complex in $^2\text{H}_2\text{O}$, Table 2), indicating a possible asymmetric strain on this bond (see the discussion about the relationship between the change of ρ and the isotropy of the polarizability ellipsoid). The proposed chelate complexes of Fe-ATP and Fe-AMP can exert such a strain and cause the observed changes in ρ .

A possible explanation for the observed differences in the complexation of AMP and ATP with Fe(III) in solution might be differences in oligomerization between the two adenine phosphate-Fe(III) complexes. Adenine is known to oligomerize at high concentrations, most probably through base-stacking interactions. Generally speaking, the stacking interactions are mainly due to the electronic properties of the base and one should not expect the two adenine phosphates to differ. It was recently shown by Raman spectroscopic measurements that two characteristic bands of the adenine moiety can be used to monitor the amount of base stacking [24]. A linear relationship between the log of the concentration of AMP, ATP and adenine nucleoside (in the range 1–1000 mM) and the intensity ratio of the two Raman bands was established. Furthermore, this linear relationship is not significantly different for AMP, ATP and adenine nucleoside, suggesting similar stacking behaviour for AMP and ATP. In addition, we find no dependence of the observed changes on the concentration of the adenosine phosphates within our measured range of concentrations, supporting a lack of interference from oligomerization. Unfortunately, the concentration for reliable Raman measurements is too high for the monitoring of the u.v./visible

absorption with the same sample. Our model of chelate complexes of AMP and ATP does not easily allow oligomerization of the complexes and is consistent with the observed lack of concentration-dependence.

Nevertheless, the stoichiometry of the complexes cannot be determined from our data alone. From the lack of dependence on concentration and the ratio of Fe to ATP (AMP), the mononuclear complex is quite probable [12]. Measurements on samples with 1 Fe to 2 ATP show clear evidence of uncoupled (unchanged) ATP, suggesting a stoichiometry of at least 1:1 (Fe:ATP). Certainly, specific configurations of dimers or polynuclear complexes are possible. Water molecules are known to be additional ligands for the metals in this type of complex [8]. It is likely that water molecules make up the missing ligands for the proposed mononuclear complexes. In our opinion, the observed differences in the Raman spectra of ATP and AMP complexes cannot be accounted for by a possible difference in stoichiometry, since measurements on complexes of Fe(III) with ATP and AMP with initial ratios from 1:1 to 4:1 and concentrations from 40 to 300 mM show no change in their respective spectra. Similarly, we think that the differences in the ionic strength of the solutions are not the reason for the observed spectral differences between ATP and AMP complexes since the samples with different ratios and concentrations also have different amounts of NaCl.

Our results show clearly that ATP binds more tightly to Fe(III) than AMP does. The longer phosphate chain of ATP gives rise to the possibility of a closer structure of the ATP-Fe complex. In addition to the extra phosphate bonds, the interaction with the N-7 nitrogen of adenine is also important. A tight complex is favourable for the uptake of iron but such a complex cannot facilitate the eventual release of iron. The proposed pathway of the conversion of ATP-Fe into AMP-Fe through ATPase seems likely [2]. The existence of two types of receptor, one specific to ATP-Fe and the other to AMP-Fe, is also interesting [2]. We proposed above that the ATP-Fe and AMP-Fe complexes involve two different conformations of the adenine/sugar. Such a difference in conformation could offer a possible explanation for the specificity of the two types of receptor. N.m.r. studies could possibly verify the proposed interaction.

In summary, AMP and ATP form chelate complexes with Fe(III), i.e. not only phosphate but also the adenine moiety are involved in the binding of iron. Frequency changes in adenine-related modes can only be observed with ATP, and not with AMP, on complex-formation. Pronounced differences in the changes in the depolarization ratios of the adenine moiety bands are observed for both AMP and ATP. The changes in the depolarization ratios are qualitatively and quantitatively very different between ATP and AMP. These data strongly suggest

that the chelate complex of AMP-Fe is mainly an outer-sphere structure [25], i.e. Fe(III) interacts with the adenine moiety not directly but through hydrogen-bonded water molecules. In contrast, it is likely that Fe(III) interacts directly with the adenine moiety of ATP at the N-7 position. We propose an 'anti' conformation for the ATP-Fe complex and a 'syn' conformation for the AMP-Fe complex.

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