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'Insulation' to lattice effects on quadrupole splitting in the Mössbauer spectra of salts of $[Fe_4S_4(Cys)_4]^{2-}$, a ferredoxin model

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Abstract

The Mössbauer parameters for a series of tetraalkylammonium salts of $[Fe_4S_4(Cys)_4]^{2-}$ (HCys = L-cysteine ethyl ester hydrochloride) have been measured. The quadrupole splitting is found to be independent of cation size, the cluster core is 'insulated', and the expected lattice effect is not observed.

Key words: Mössbauer spectroscopy; Iron complexes; Sulfur complexes; Amino acid complexes

Iron–sulfur clusters are constituents of biological electron carriers such as ferredoxins and high potential iron proteins [1]. Generally the residues which bind the cluster to the protein backbone are all cysteines. In, for example, four-iron bacterial ferredoxins, three iron atoms of an Fe_4S_4 cluster are bound by a peptide with an amino acid sequence CXXCXXC (C = cysteine; X = another non-bonding amino acid) and the fourth iron atom by a cysteine further removed along the peptide chain. Mössbauer spectroscopy has been used extensively to characterise the Fe_4S_4 clusters, both in metalloproteins and complexes [2]. The ^{57}Fe Mössbauer spectra of such clusters, at the $Fe_4S_4^{2+}$ oxidation level, exhibit a simple quadrupole doublet, though the clusters formally contain two iron(II) and two iron(III) atoms. In biological systems [3] this doublet has a relatively invariant isomer shift (*IS*) of *c.* 0.43 mm s^{-1} at 77 K. However, the quadrupole splitting (*QS*) is sensitive to the whole protein (ranging from *c.* 0.9 to 1.2 mm s^{-1}) and varies in a way not easy to rationalise. It has been ascribed to some kind of protein–cluster interaction [3].

We have shown that the *QS* in a series of tetraalkylammonium salts of $[Fe_4S_4(SBu^t)_4]^{2-}$ clusters is cation-dependent [4]. Crystallographic data were used to show that increasing the bulk of the tetraalkylammonium cation increases separation between anionic cluster and cation. This in turn reduces the lattice contribution to the *QS* and is observed as an increase in *QS* with separation between iron and the cationic charge centres. These studies have since been extended to frozen solutions of the cluster salts in a number of solvents [5]. Over the concentration range studied there is little dependence of *QS* on solvent, concentration or cation. The cation and anion separation is too great for a lattice effect. We have also rationalised the Mössbauer spectra of a range of tetrachloroferrate(II) salts with symmetric and asymmetric cations by a consideration of both vibronic interactions and cationic charge effects [6]. Again, the influence of charges in the lattice on the *QS* appears to depend on cation size (the smaller the cation the closer it can approach the anion and the larger the effect, i.e. the smaller the *QS*). Here we report the unexpected 'insulation' of $Fe_4S_4^{2+}$ clusters by cysteinate ligation, to such lattice effects, as shown in their Mössbauer spectra.

Experimental

All manipulations were performed under dinitrogen. IR (Nujol mull, KBr plates) and ^1H NMR spectra (DMSO-d_6) were recorded, at ambient temperature, on a Perkin-Elmer 883 and a Jeol GSX270 spectrometer, respectively. Mössbauer spectra were determined at 77 K on an ES-Technology MS-105 spectrometer with a 925 MBq ^{57}Co source in a rhodium matrix at ambient temperature. Spectra were referenced to a 25 μm iron foil at 298 K.

Iron–sulfur clusters $[NR_4]_2[Fe_4S_4(Cys)_4]$ (R = Me, Et, Pr^n or Bu^n; HCys = L-cysteine ethyl ester hydrochloride) were prepared as solids by the literature method [7] and characterised by IR and NMR spectroscopy, by which criteria they were pure. Typically: IR, ν(CO) 1740 cm^{-1}; ^1H NMR, α-*CH c.* 4.5, β-CH_2 *c.* 16, COO*Et* 1.2 and 4.1 ppm.

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Results and discussion

Salts of the cluster $[Fe_4S_4(Cys)_4]^{2-}$ were prepared as solids by the metathetic reaction, eqn. (1) [7].

$$[Fe_4S_4(SBu^t)_4]^{2-} + 4HCys \rightleftharpoons [Fe_4S_4(Cys)_4]^{2-} + 4HSBu^t \quad (1)$$

The equilibrium can be drawn to the right by removal of the liberated volatile thiol. Characterisation of the clusters by IR and ^1H NMR spectroscopies showed the materials to be pure. However, in all Mössbauer spectra some high-spin iron(II) impurity was observed. Attempts to crystallise the clusters failed. As previously demonstrated, the cysteinato group is bound to cluster iron atoms through sulfur, and not carboxylate or amine. The amine remains as the hydrochloride salt.

The Mössbauer parameters for the series of salts $[NR_4]_2[Fe_4S_4(Cys)_4]$ (R = Me, Et, Pr^n, Bu^n) are collected in Table 1. The *IS* is invariant, as expected for a common cluster oxidation level and ligand environment about iron. The *IS* of *c.* 0.45 mm s^{-1} is fully consistent with ligation of the iron atoms by thiolato groups. To our surprise there is no significant variation of *QS* with cation. A comparison of *QS* with cation for the butylthiolato– and cysteinato–cluster series is shown in Fig. 1.

The observed 'insulation' of the cluster core in $[Fe_4S_4(Cys)_4]^{2-}$ to the effect of cation on *QS* may arise by a combination of at least two ways. (i) A cation cannot approach the cluster core nearer than a limiting distance as defined by the size of the ligand. For $[Fe_4S_4(Cys)_4]^{2-}$ this distance is greater than that over which the lattice effect can operate, even for the smallest cation, so there is no observed dependence of *QS* on cation size. However, for $[Fe_4S_4(SBu^t)_4]^{2-}$ the ligands are smaller, atoms can approach closer to the core and a lattice effect is manifest. In the absence of crystallographic data to define the relative ligand/cluster dimensions, the volume of the van der Waals' surface of each cluster was calculated from molecular models [9]. Values of 463 and 621 $Å^3$ were found for

TABLE 1. Mössbauer parameters for $[NR_4]_2[Fe_4S_4(Cys)_4]$ at 77 K

R	Isomer shift (mm s^{-1})	Quadrupole splitting (mm s^{-1})	HWHM[a] (mm s^{-1})
Me[b]	0.46(1)	1.06(2)	0.28(2)
Et	0.44(1)	1.11(2)	0.27(2)
Pr^n	0.46(1)	1.08(2)	0.25(2)
Bu^n	0.46(1)	1.08(2)	0.44(2)

[a]HWHM = half-width at half maxima. [b]Ref. 7.

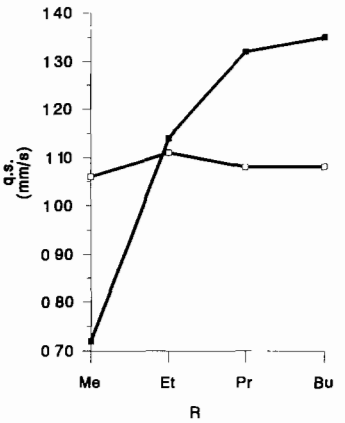

Fig. 1. Dependence of quadrupole splitting (*QS*) on cation for $[NR_4]_2[Fe_4S_4(SBu^t)_4]$ [4, 7, 8] (■) and $[NR_4]_2[Fe_4S_4(Cys)_4]$ (□) at 77 K.

$[Fe_4S_4(SBu^t)_4]^{2-}$ and $[Fe_4S_4(Cys)_4]^{2-}$, respectively, consistent with the above proposal. (ii) The positive charge of the quaternised amine must always be closer to the irons than the positive charge of a lattice cation. There is, in effect, a positive charge cloud shielding the cluster core from the positive charge on the cations. Also, the effect of the lattice cation will be modulated by the lattice anion (Cl^-). The shielding and modulation overrides any lattice effects. Attempts to prepare a series of cysteinato–iron–sulfur cluster salts with non-protonated amine groups in order to investigate this effect have, as yet, been unsuccessful.

Our programme on the nature and extent of charge effects in iron–sulfur clusters and simpler iron complexes, as reflected in their Mössbauer spectra, continues.

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