Serinato-, Tyrosinato-, and Prolinato-derivatives of Fe_4S_4 Clusters

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Reaction of $[Fe_4S_4(SBu^t)_4]^{2-}$ with the methyl esters of L-serine, L-tyrosine, or L-proline yields the complexes $[Fe_4S_4(AA)_4]^{2-}$ (AA = anion of the amino acid methyl ester); all the complexes have been characterized in solution and the first two have been isolated as solids.

Thiolates constitute the major class of ligand for Fe_4S_4 clusters, and in nature the binding groups for such clusters are almost invariably cysteinyl residues, often in the sequence cys–X–X–cys, which spans a cluster face, and in which X may be a variety of amino acid residues.[1] However, examination of the amino acid sequences of the nitrogenase molybdenum–iron protein from a variety of sources[2] shows that there are not enough conserved cysteinyl residues to bind all the clusters present, and that there are no cys–X–X–cys sequences. Consequently, residues other than cysteinyl must be involved in binding these clusters. We have now shown that serine, tyrosine, and proline are all candidates for this function, but that under the same conditions neither tryptophan nor threonine are candidates.

A solution of $(Me_4N)_2[Fe_4S_4(SBu^t)_4]$ in MeCN was stirred with a ten-fold molar excess of solid L-serine or L-tyrosine methyl ester hydrochloride for three hours under slightly reduced pressure. The volatiles were removed from time to time by pumping. On work up (by removal of solvent, dissolution of the black residue in MeCN, precipitation of unreacted amino-acid ester with ethyl acetate, and removal of solvent from the filtrate), crude $(Me_4N)_2[Fe_4S_4$(methyl L-serinate$)_4]$ (**1**) or $(Me_4N)_2[Fe_4S_4$(methyl L-tyrosinate$)_4]$ (**2**) were obtained as black powders. Using a similar procedure other

Table 1. ^{1}H N.m.r. data.

		Chemical shift (δ)[a]		
	Q	β-CH_2	α-CH	CO_2CH_3
$Q_2[Fe_4S_4$(methyl L-serinate)$_4]$	Me_4N	24.5	10.0	4.13
	Et_4N	27.0	8.0	4.19
	$BnNMe_3$[b]	26.1	10.0	4.18
	$BnNEt_3$	30.0	13.0	4.13
	$BnNBu_3$	25.5	11.5	4.11
	PPh_4	20.4	10.1	4.02
$Q_2[Fe_4S_4$(methyl L-tyrosinate)$_4]$	Me_4N	20.7	12.5	3.80
	Et_4N	20.1	9.4	3.96
	$BnNMe_3$	21.0	14.0	4.06
	PPh_4	14.2	10.1	3.73

[a] In CD_3CN at ambient temperature, *ca.* 0.025 mol dm^{-3}. [b] Bn = benzyl.

salts of the butylthiolato clusters yielded salts of these clusters, but with different counter cations, equation (1).

$$[Fe_4S_4(SBu^t)_4]^{2-} + 4AAH \rightleftharpoons [Fe_4S_4(AA)_4]^{2-} + 4Bu^tSH \quad (1)$$
AA = methyl L-serinate or methyl L-tyrosinate†

The electronic spectrum of each cluster in MeCN exhibits an absorption at 680 nm corresponding to a transition within the $[Fe_4S_4]^{2+}$ core,[3] as well as unresolved bands to lower wavelength. Compound (**1**) also shows a shoulder at 450 nm tentatively assigned to a terminal ligand-to-metal charge-transfer transition. The ^{1}H n.m.r. spectra of the novel clusters were recorded in CD_3CN (0.025 mol dm^{-3}, ambient temperature) (Table 1). In both (**1**) and (**2**) the α-CH and β-CH_2 resonances are isotropically shifted downfield to about δ 10 and 25, respectively. These shifts are approximately twice as large as those observed for the α-CH (δ 5.34) and β-CH_2 (δ 13.6 and 12.4) of the cysteinyl complex $[Fe_4S_4$(Ac–CyS–NHMe)$_4]^{2-}$,[4] an observation consistent with the reported doubling of isotropic shifts on comparison of $[Fe_4S_4$(arenethiolate)$_4]^{2-}$ and $[Fe_4S_4$(phenolate)$_4]^{2-}$.[3] The increase in magnitude of the shift is explained by a larger hyperfine interaction in the phenolate complexes, possibly arising from the higher covalency of the Fe–O bond. The isotropic shifts exhibit a temperature dependence. The shift is to higher field with increase in temperature. This is opposite to that observed for a range of $[Fe_4S_4(SR)_4]^{2-}$,[5] oxidized ferredoxins,[6] and reduced 'high potential' iron proteins.[7] Addition of >5 equivalents of PhSH to a CD_3CN solution of (**1**) or (**2**) generates $[Fe_4S_4(SPh)_4]^{2-}$, showing that the Fe_4S_4 core has remained intact. Mössbauer spectroscopy shows a simple quadrupole doublet in (**1**), isomer shift (I.S.) 0.45, ΔE_Q 1.02 mm s^{-1}, and (**2**), I.S. 0.39, ΔE_Q 0.92 mm s^{-1} (77 K) plus a high-spin iron(III) impurity.

Addition of a six-fold molar excess of L-proline methyl ester hydrochloride to a CD_3CN solution of $[Fe_4S_4(SBu^t)_4]^{2-}$ produces $[Fe_4S_4$(methyl L-prolinate)$_4]^{2-}$ (**3**) *in situ*, as shown by ^{1}H n.m.r. spectroscopy (ambient temperature, conc. *ca.* 0.025 mol dm^{-3}). Four isotropically shifted resonances are observed in the region δ 6—11, all of which shift to higher field with increase in temperature. The magnitude of the isotropic shifts is much smaller than that observed in the serinato-, tyrosinato-, and cysteinyl[4] Fe_4S_4 clusters.

† The more accurate IUPAC names for the amino acid derived ligands are L-2-amino-2-methylcarboxylato-ethanolato- and L-4-[(2-amino-2-methylcarboxylato)ethyl]phenolato-.

Addition of an excess of PhSH to (**3**) in CD_3CN generates resonances characteristic of $[Fe_4S_4(SPh)_4]^{2-}$, showing the Fe_4S_4 core to be intact. The u.v.–visible spectrum of a solution of (**3**) prepared in MeCN exhibits a peak at 485 nm and unresolved bands to lower wavelength. Attempts to isolate (**3**) failed.

The methyl ester hydrochlorides of tryptophan and threonine do not react with $[Fe_4S_4(SBu^t)_4]^{2-}$ under our conditions.

Co-ordination of the amino acids to the Fe_4S_4 core through the carboxylate oxygen can be discounted by i.r. and ^{1}H n.m.r. spectroscopy. The carboxylate stretches in (**1**), 1748 cm^{-1}, and in (**2**), 1742 cm^{-1}, are not shifted relative to those of the unco-ordinated amino acid esters. Also, liberated methanol is not observed in ^{1}H n.m.r. spectra of solutions of (**1**), (**2**), and (**3**) generated *in situ*, consistent with the methyl ester remaining intact. Binding is proposed, therefore, through the anionic oxygen in (**1**) and (**2**) and the anionic amino nitrogen in (**3**).

Whereas there is ample precedent for the reaction of tyrosine methyl ester described above in phenolato clusters such as $[Fe_4S_4(OPh)_4]^{2-}$,[3] there are no alcoholato analogues of the serinato complex, nor aminato analogues of the prolinato complex. Thus (**1**) and (**3**) are unique examples of two new classes of cluster, and the existence of all three complexes demonstrates as yet unrecognized ways in which Fe_4S_4 clusters might be incorporated into proteins.

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