Chelation of the Fe_4S_4 Cluster by the Tripeptide N-Phenylacetylcysteinylglycylcysteinamide, Bzl-C(O)-Cys-Gly-Cys-NH_2

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The synthesis, spectroscopic characterization and study of the structure of low-molecular weight analogs of two-iron and four-iron ferredoxins have been the subjects of a variety of investigations [1]. Apart from simple thiolate ligands that coordinate to Fe_2S_2 or Fe_4S_4 clusters, bidentate thiolate type ligands such as diols and peptides with at least two cysteine residues, have been increasingly used as chelating ligands [2]. In studies using synthetic polypeptides as chelating ligands, up to now most investigators have used the so-called n, n + 3 rule [3], based on the observation that a spacing of two amino-acid residues appears to be present between two cysteines in the naturally-occurring proteins that chelate to a Fe_4S_4 cluster.

A first explanation of this phenomenon, based on the peptide conformation and the occurrence of N–H···S hydrogen bonds, was given by Adman *et al.* [4]. More recently, Burt *et al.* [2c] argued that the Fe_4S_4 core with a tetradentate peptide Cys-X_n-Cys-X_n-Cys-X_n-Cys can only be formed for $n \geq 2$. However, metal-binding Cys-X-Cys (n = 1) sequences do exist in nature, *e.g.* in metallothioneines, although the details of the structure and bonding are not known [5]. Up to the present a Fe_4S_4 chelating system containing Cys-X-Cys has not been reported. However, studying molecular models suggests that chelation of such a tripeptide system is not impossible [6].

In view of the ongoing discussion about the relationship between the structure of the cluster and its redox potential, it seemed interesting to study such a Cys-X-Cys unit, since possible steric constraints could give rise to interesting properties. In addition, variation of the group X would allow a systematic study of the electronic, steric and hydrogen-bonding effects.

At a first ligand system we looked at the tripeptide system Cys-Gly-Cys, and in this communication the first results, including UV and NMR spectra, are presented. The NMR results prove that chelation to a Fe_4S_4 cluster can indeed occur.

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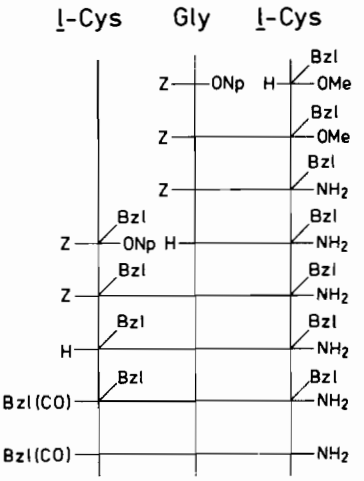
Experimental

Starting Materials

Solvents and amino acids were all used as commercially available products. The compound $(NMe_4)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$ was synthesized according to the literature [7].

Synthesis of the Tripeptide

N-Phenylacetylcysteinylglycylcysteinamide was prepared as depicted in Scheme 1. Standard literature



Scheme 1. Synthetic strategy used for Cys-Gly-Cys; Z = benzyloxycarbonyl; ONp = *p*-nitrophenyl ester.

procedures were used in all steps, *e.g. p*-nitrophenyl ester couplings [8], removal of the N-protecting Z group with hydrogen bromide in acetic acid [9] and removal of the S-protecting benzyl group with sodium in liquid NH_3 [10]. The route provided in Scheme 1 appeared to be convenient for the present tripeptide, because all intermediate products are sufficiently soluble in the used solvents and are obtained in high yield. They were purified by recrystallization and characterized by their ^1H NMR spectra.

Cluster Exchange Studies

All operations and manipulations were carried out in oxygen-free N_2, using Schlenk techniques. All solvents were purified by repeated distillation under nitrogen. Exchange reactions were performed with one and with two equivalents of the tripeptide added to the starting $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ cluster. DMSO was used as the solvent, allowing evaporation *in vacuo* of the liberated t-BuSH, thereby forcing a possible equilibrium to completion. NMR spectra of reaction mixtures were recorded using d_6-DMSO as solvent. For these reactions a 0.025 *M* solution

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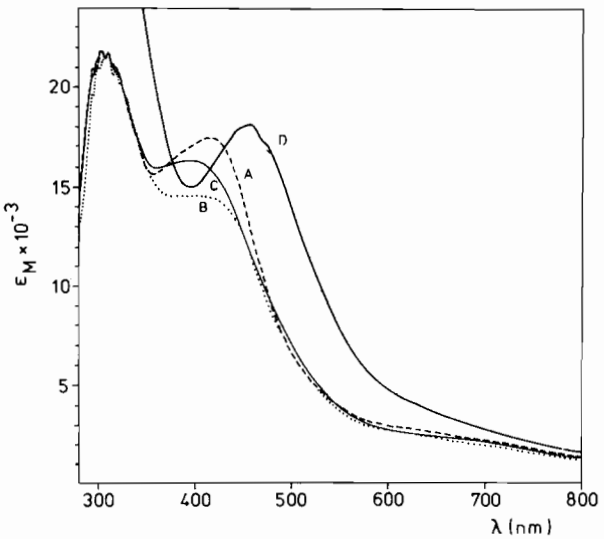

Fig. 1. Electronic spectra in DMSO solution, illustrating the ligand substitution reactions: (A) $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$; (B) as (A) plus 1 eq. of tripeptide; C) as (A) plus 2 eq. of tripeptide; (D) as (C) plus 10 eq. of PhSH. Concentration used 1.25×10^{-4} mol/l.

of the tripeptide was slowly added to a 0.025 *M* solution of the $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ cluster until the appropriate ratios were obtained.

Measurements

UV–vis spectra in the range 280–800 nm were recorded in DMSO on a Perkin-Elmer 330 spectrophotometer. 300-MHz ^{1}H NMR spectra in d_6-DMSO were obtained on a Bruker WM300 spectrometer.

Results and Discussion

UV–vis spectra (280–800 nm) of both the reaction mixtures shown in Fig. 1, together with the mixture obtained after subsequent treatment with excess of PhSH, indicate that the reactions are not accompanied by any significant degradation of the Fe_4S_4 core*. To prove that a substitution of the t-BuS$^-$ ligands has indeed occurred in both cases, ^{1}H NMR spectra were recorded. Figure 2a shows the 1:1 reaction product, with 50% of the t-BuS$^-$ groups still coordinated to iron. Figure 2b shows the 1:2 reaction product; in this case the signal of coordinated t-BuS$^-$ has disappeared (*N.B.* the freed thiol was removed by vacuum evaporation). NMR signals were assigned as indicated in Fig. 2 and data

* Calculations using the ε_M values given by DePamphilis *et al.* [1b] show that more than 98% of the Fe_4S_4 core is recovered in the PhSH reaction product $[Fe_4S_4(SPh)_4]^{2-}$.

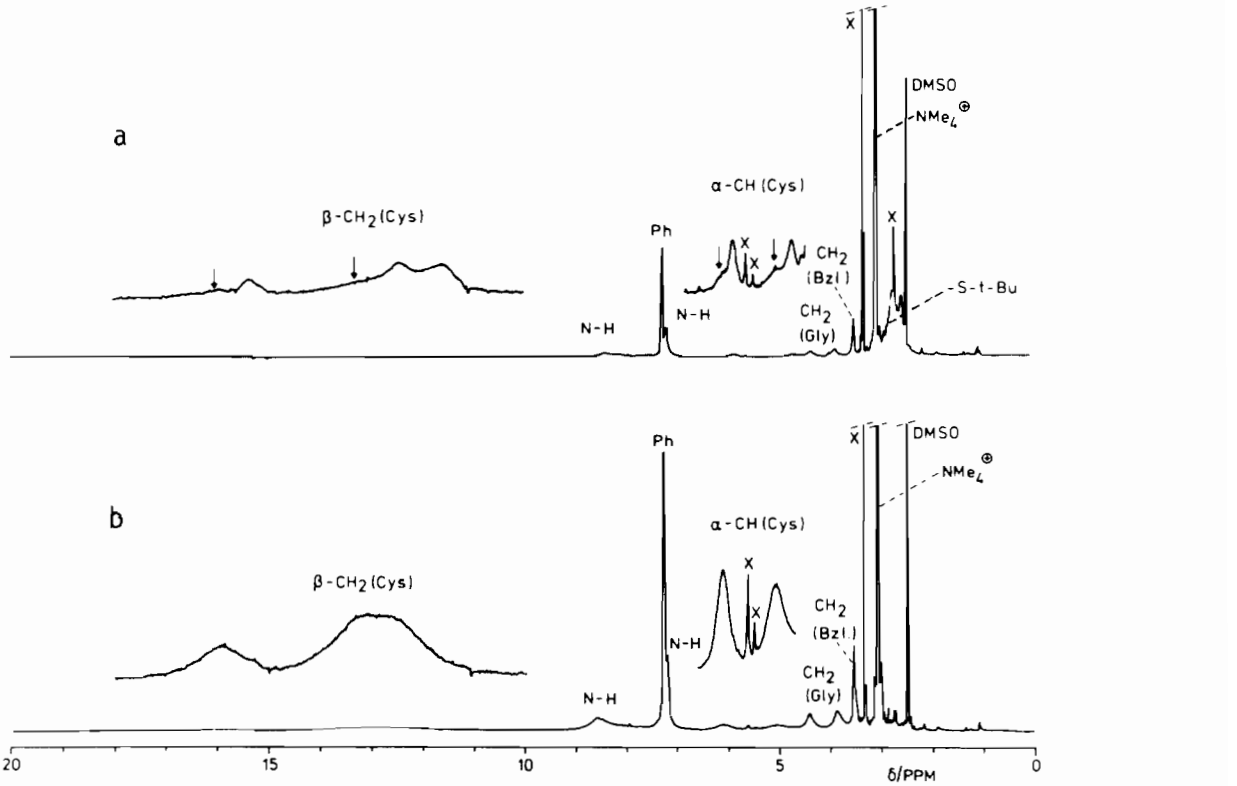

Fig. 2. 300-MHz ^{1}H NMR spectra in d_6-DMSO at 20 °C of (a) $(NMe_4)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$ plus 1 eq. of tripeptide, and (b) plus 2 eq. of tripeptide; x is impurity.

TABLE I. 300-MHz ^{1}H NMR Spectral Data (δ/ppm) in d_6-DMSO at 20 °C, with Tentative Assignments.

	Free peptide[b]	1:1	1:2
α-CH(Cys)	4.35 m	4.75	5.09
		5.92	6.12
β-CH_2(Cys)	2.70 m	11.6	12.5
	2.76 m	12.4	13.2
		15.4	15.9
CH_2(Gly)	3.78 d	3.93	3.89
		4.39	4.43
CH_2(Bzl.)	3.52 d	3.54	3.55
Ph(Bzl.)	7.27 s	7.28	7.27
	7.29 s		
N–H	7.23 s	7.21	7.21
	7.42 s	[a]	[a]
	7.98 d	[a]	[a]
	8.42 m	8.43	8.59
S–H	2.38 s	–	–

[a]Not clearly observed. [b]s = singlet, d = doublet, m = multiplet.

are given in Table I. Concerning the assignments the following comments are made:

1) It is noted that no resonances of the free peptide are observed.

2) The isotropically shifted α-CH(Cys) and β-CH_2-(Cys) are observed in the usual range for coordination to the $Fe_4S_4^{2+}$ core [2a], *i.e.* in the range δ 4.5–6.5 and 10–20 ppm respectively.

3) A remarkable feature of the spectra is the simultaneous disappearance of the α-CH(Cys) and β-CH_2-(Cys) resonances of the 1:1 product, accompanied by the appearance of new resonances for the 1:2 product, upon addition of the second equivalent of peptide. It is clearly seen that in Fig. 2a traces of the 1:2 product are already present, as indicated by the arrows, due to a slight excess of the tripeptide.

These NMR spectra were carefully compared with the NMR spectra of reaction mixtures obtained in reactions with the dipeptide N-acetylcysteinylcysteinamide (Ac-Cys-Cys-NH_2). Severe sterical constraints upon chelation of this dipeptide to two iron atoms of the Fe_4S_4 core were already evident from molecular models. Therefore, it is expected that this dipeptide does mainly or perhaps exclusively form bridges between different Fe_4S_4 clusters, forming oligomeric species. The observed behaviour in the NMR spectra of the dipeptide products differs from the behaviour of the tripeptide complexes. Substitution of t-BuS^- is also observed with the dipeptide, but the isotropically shifted resonances of α-CH(Cys) and β-CH_2(Cys) do not change upon addition of the second equivalent of the dipeptide. In the 1:2 adduct these signals are only more intense than in the 1:1 adduct (relative to the NMe_4^+ signal). This phenomenon would also be expected for the tripeptide if only bridged species were formed. Therefore, the observation of a different spectrum for the 1:1 and 1:2 products in the tripeptide reaction obviously indicates no formation of bridged species and hence chelation of the tripeptide to one Fe_4S_4 core should occur. With this investigation it is shown for the first time that Cys-X-Cys can indeed chelate to a Fe_4S_4 cluster. Current work is dealing with the redox properties of this and related systems, as well as with the synthesis of Cys-X-Cys chelating peptides with other central amino acids, such as Ala or His.

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