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On the Cause of Fe_3S_4 Instability

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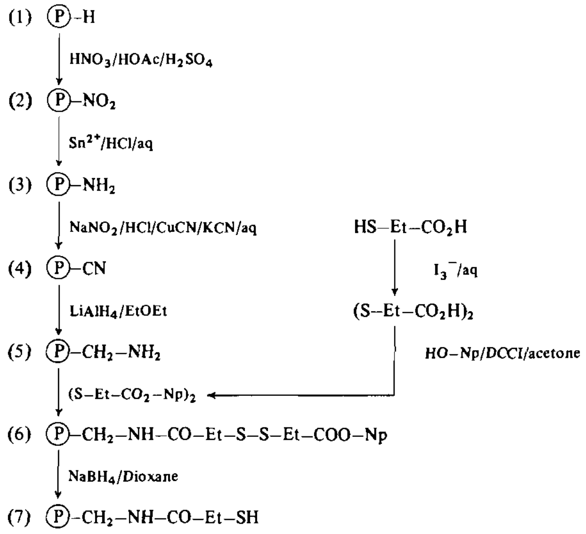
The class of three-iron proteins [1] have been known for about five years and the core present in the majority of them is believed to have the cubane-derived $[3Fe{-}4S]^{1+(1+,0)}$ structure [2]. In contrast with Fe_2S_2 and Fe_4S_4 clusters [3] this Fe_3S_4 core has not been prepared synthetically, although an analogue of the isomeric linear structure found in Aconitase has been reported recently [4]. We have obtained evidence that oxidation of a 4-Fe cluster generally produces the $[Fe_3S_4(SR)_3]^{2-}$ cluster and that the inherent stability of this 3-Fe cluster is limited [5]. In order to synthesize a stable model compound the origin of this instability has to be established so that an attempt can be made to reduce its effect as much as possible.

There are two types of reaction which could explain 3-Fe instability. The first type might be called rearrangement and it involves reaction of at least two 3-Fe clusters to yield iron–sulfur clusters of different nuclearity. The second type might be called degradation. First-order decay and higher-order reaction with for example the solvent are included in this type of reaction. The observation that 3-Fe core extrusion from proteins yields 2-Fe and 4-Fe clusters [6] and the recent isolation of metastable 6-Fe clusters [7] favour the rearrangement explanation. If rearrangement is the primary cause of 3-Fe lability then immobilization should yield a more stable species and extrusion should result in destruction. We here report the preparation of a suitable polymeric support and the immobilization and subsequent extrusion of a cubane cluster (Scheme 1). An immobilized cubane cluster was oxidized, yielding an immobilized 3-Fe cluster according to our hypothesis and its stability was studied by ESR.

Experimental

$(NMe_4)_2[Fe_4S_4(S\text{-}tBu)_4]$ was synthesized according to the literature [8]. Schlenk techniques were used to handle air-sensitive compounds. Macroporous



Scheme 1. Preparation of the polymeric support. Abbreviations Ⓟ, polymer; Ac, acyl; aq, aqueous medium; Et, ethyl or ethylene; Np, *para*-nitrophenyl; DCCI, *N,N'*-dicyclohexylcarbodiimide.

poly(divinylbenzene) was kindly supplied by Mr. P. Piet, Laboratory for Polymer Research, Eindhoven University of Technology. It was prepared from commercial divinylbenzene (which consisted of 55% *m*- and *p*-divinylbenzene and 44% *m*- and *p*-ethylvinylbenzene) and it showed a surface area of 450 m^2/g, about half of which was found in pores of diameter larger than 7 nm. Macroporous poly(styrene) (25 m^2/g, crosslinked by 3% divinylbenzene) and all other chemicals were purchased from Aldrich Chemical Company. Both polymers were modified using standard reactions according to the accompanying scheme. Chain lengthening was carried out because coupling of aromatic amines with the activated ester proceeds slowly.

$(S{-}Et{-}CO_2H)_2$

To a solution of 25.4 g (100 mmol) of I_2 and 20 g (120 mmol) of KI in 300 ml of water 20 ml (230 mmol) of 3-mercapto-propanoic acid was added. The precipitate was recrystallized twice from boiling water. The yield of the dry product was 18 g (91%); melting point (m.p.) 149–151 °C.

$(S{-}Et{-}CO_2{-}Np)_2$

The above disulfide (8.4 g, 40 mmol) and HONp (11.4 g, 82 mmol) were dissolved in 600 ml of p.a. acetone. DCCI (16.1 g, 78 mmol) was dissolved in 65 ml of p.a. acetone. Both solutions were cooled to 0 °C and then mixed. After 30 min the mixture was brought to room temperature for several hours to complete the reaction. To precipitate as much

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N,N'-dicyclohexylurea as possible the temperature was lowered again to 0 ℃. The filtered solution was concentrated and treated with light petroleum. The crude product was recrystallized from 250 ml of acetone and 500 ml of n-hexane. Large needles were collected with a yield of 11.1 g (63%); m.p. 111–113 ℃. The purity was also checked by ^{1}H NMR (60 MHz; solvent $(CD_3)_2CO$; standard Me_4Si) 8.23 (2.1 H, d, Ar–H), 7.33 (2.4 H, d, Ar–H), 3.07 (4.0 H, m, Et–H).

Ⓟ–NO_2

A mixture of 25 ml of 95% nitric acid, 25 ml of sulfuric acid and 105 ml of acetic acid was cooled to 0 ℃ and 5 g of poly(divinylbenzene) were stirred into this medium for 20 min. After filtering, washing with water and drying, elemental analysis showed the introduction of 2.00% (1.4 mmol/g) of N. (In the case of poly(styrene) a mixture of 20 ml of 95% nitric acid, 20 ml of sulfuric acid and 20 ml acetic acid was used, resulting in 0.6 mmol N/g).

Ⓟ–NH_2

The nitrated polymer (5 g) was refluxed with 36 g of $SnCl_2$ and 30 ml of 36% aqueous hydrochloric acid for 6 h. The beads were filtered and thoroughly extracted with 3 N NaOH and ethanol. An IR spectrum of the dried polymer showed almost complete disappearance of the NO_2 absorptions at 1530, 1565 and 1640 cm^{-1} whereas elemental analysis showed the nitrogen content to be 1.91%.

Ⓟ–CN

The aminated polymer (4.9 g) and 20 ml of 36% aqueous hydrochloric acid were chilled on ice and 6 g of $NaNO_2$, dissolved in 12.5 ml of cold water, were added in small portions over a period of half an hour. CuCN [9] (12 g) and KCN (26 g) were dissolved in 60 ml of water and heated to 60 ℃. The diazotised polymer suspension was neutralized with Na_2CO_3 and the cyanide solution was added. This suspension was refluxed for an hour and then extracted with KCN solution, water and methanol. Elemental analysis showed 1.10% N and in the IR spectrum a CN absorption was observed at 2200 cm^{-1}.

Ⓟ–CH_2-NH_2

Reduction of 1.5 g of Ⓟ–CN was achieved by $LiAlH_4$ in dry ether. Excess $LiAlH_4$ was destroyed after six hours by the subsequent addition of ethyl acetate, ethanol, and water. The beads were extracted overnight with aqueous hydrochloric acid and then by 1 N NaOH, water and methanol. After drying the nitrogen content was 0.98% by elemental analysis and disappearance of the IR absorption at 2200 cm^{-1} was observed.

Ⓟ–CH_2–NH–CO–Et–S–S–Et–CO–Np

$(S-Et-CO_2-Np)_2$ (1.5 g) and 1.3 g of Ⓟ–CH_2-NH_2 were refluxed in 20 ml of ethyl acetate with 0.5 ml of triethylamine for 24 h. The resulting polymer was extracted with methanol/trimethylamine until colourless and then dried at 60 ℃. The IR spectrum of the modified poly(divinylbenzene) showed two different carbonyl absorptions: at 1680 cm^{-1} (amide) and at 1740 cm^{-1} (ester). (In the case of poly(styrene) only an amide absorption was seen. The absence of the ester absorption was interpreted as evidence that the disulfide had crosslinked. The modified poly(styrene), despite its better mechanical stability, was therefore put aside.) Evidently, no crosslinking by disulfide occurred in the modified poly(divinylbenzene). Elemental analysis of this polymer showed the presence of 1.55% sulfur.

Ⓟ–CH_2–NH–CO–Et–SH

The above poly(divinylbenzene) (0.9 g) was immersed in 30 ml of 1,4-dioxane and treated for two days with $NaBH_4$. Excess $NaBH_4$ was then destroyed by the addition of acetone and water. The IR spectrum showed disappearance of the ester group. The sulfur content was found to be 0.97%. From the difference, 0.58% S, it was inferred that 0.58% of the S remaining in the polymer is found in an SH group, *i.e.* the final concentration of SH groups is 0.18 ± 0.04 mmol/g.

Cluster Chemisorption

Ten ml of a 15 mM solution of $(NMe_4)_2[Fe_4S_4(S\text{-}tBu)_4]$ in acetonitrile was added anaerobically to 90 mg of Ⓟ–CH_2–NH–CO–Et–SH. After 24 h the polymer was filtered and extracted several times with 10 ml of acetonitrile until no more chromofores were liberated. The iron content was determined by atomic absorption spectroscopy and showed that 0.13 mmol of the cluster had chemisorbed per gram of cubane-saturated polymer. If the polymer was treated overnight with a $K_3[Fe(CN)_6]$ solution prior to chemisorption 0.09 mmol/g was chemisorbed, indicating that only a minority of the SH groups is able to crosslink.

Cluster Extrusion

To 50 mg of the polymer containing chemisorbed cubanes a mixture of 15 ml of acetonitrile and 1 ml of thiophenol was added. Judged by the colours of the polymer and of the solution the extrusion must have been complete within 5 min; standing for 4 h did not produce any visible changes. The polymer was filtered and extracted with 30 ml of acetonitrile. Analysis of the iron content by AAS showed 85% of the iron to be extruded. In another run extrusion was carried out with 20 μl of thiophenol. The UV–Vis spectrum of the extruded clusters showed an optimum at 448 nm (*cf.* $[Fe_4S_4(SPh)_4]^{2-}$: 457 nm,

$[Fe_4S_4(S{-}tBu)_4]^{2-}$: 417 nm and $[Fe_2S_2(SPh)_2]^{2-}$: 490 nm) [10, 11].

Results and Discussion

In the range tBuSH < EtSH < Cysteine < PhSH the electron withdrawing capability of the RS group increases. Two important consequences of this property have been applied. The first, a parallel supersession series [10], is used in coupling $[Fe_4S_4(S{-}tBu)_4]^{2-}$ to the $-CH_2-NH-CO-Et-SH$ group and subsequently in extrusion by PhSH. The second is that $tBuS^-$ is more easily oxidized than the anchoring thiolate, which can be considered as cysteine lacking the amino function.

The concentration of thiol groups (0.18 mmol/g) in the polymer can be expressed as 1 SH group per 4 nm^2. The length of the $-CH_2-NH-CO-Et-SH$ spacer is less than 1 nm. A significant number of clusters will therefore be isolated. Coupling of the support with the $[Fe_4S_4(S{-}tBu)4]^{2-}$ cluster yielded a brown polymer and iron analysis showed that more than 70% of the spacers had bound a cluster. An extrusion experiment was also successful. It showed that the 4-Fe clusters in this polymer are accessible within minutes. Oxidation of cubanes chemisorbed on this polymer is therefore expected to yield 3-Fe clusters which are still immobilized and well separated.

A sample of the cubane-containing polymer was treated for 5 min with one equivalent of ferricyanide in tris•HCl buffer pH 7.5. The grey polymer suspension was then frozen in liquid nitrogen and the X-band ESR spectrum was recorded at 4.2 K. Under the conditions used, a quantitative formation of 3-Fe clusters would result in a strong absorption at $g = 2.01$. However, no signal of this kind was observed. A signal, three orders of magnitude weaker than would be expected if 100% of the cubanes had been converted to 3-Fe clusters, had to be assigned to impurities of the polymer. Therefore we conclude that degradation instead of rearrangement reactions play an important role in the destruction of the 3-Fe clusters.

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