Synthesis of the P-Cluster Inorganic Core of Nitrogenases

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The nitrogenases catalyze the transformation of dinitrogen to ammonia under mild conditions.[1−3] A key to the function of nitrogenases is the efficient electron-transfer system, where the novel P-clusters serve as an electron transmitter from the Fe-protein, with ATP bound, to the FeMo-co.[4−11] The iron sulfide proteins, which are involved in electron-transfer processes, are represented by those consisting of [4Fe−4S], [3Fe−4S], and [2Fe−2S] clusters. The [8Fe−7S] core of P-clusters, either in the reduced form (P^N) or in the two-electron oxidized form (P^{2+}), is unique among the known biological [Fe−S] clusters and consists of two [4Fe−3S] incomplete cubanes linked by a hexa-coordinate sulfur atom (μ_6-S) and two bridging cysteinyl ligands.[12,13] Although the [4Fe−4S] and [2Fe−2S] clusters are stable and are readily prepared by spontaneous self-assembly reactions in polar aprotic solvents, the core structure of the P-clusters has been believed to be unstable. The extrusion of the [8Fe−7S] cluster from the FeMo-protein using a thiol-exchange method resulted in the conversion to conventional [4Fe−4S] cubanes in >90% yield.[14] The synthesis of the P-cluster has not been achieved in vitro, because the required gene products have yet to be identified.[15,16] The P-cluster geometry is so unusual that it has been thought to exist only in certain protein environments, and construction of the [8Fe−7S] core has posed a challenge for synthetic chemistry. The known model iron sulfide clusters, which may be close to the [8Fe−7S] structure, are limited to an S-bridged bis-cubane [8Fe−9S] cluster and a fused bis-cubane [8Fe−8S] cluster.[17,18] The M/Fe/S (M = V, Mo) clusters, $[(Tp)_2M_2Fe_6S_9(SH)_2]^{n-}$ (n = 3, 4), were shown to contain a $M_2Fe_6S_9$ framework topologically analogous to the [8Fe−7S] core.[19] Here we report that the structure of the inorganic core of P-cluster can be generated by a self-assembly reaction of Fe(II) bis-amide, tetramethylthiourea, 2,4,6-triisopropylbenzenethiol, and elemental sulfur (S_8).

Addition of tetramethylthiourea (tmtu), 2,4,6-triisopropylbenzenethiol (HStip), and elemental sulfur to a toluene solution of iron-(II) bis-amide, Fe{N(SiMe₃)₂}₂,[20] in the ratio of 8(Fe):3(tmtu): 12(HStip):7(S) leads to an immediate color change from green to dark purple and then to dark brown over the period of 2 days. Removal of the solvent under reduced pressure, washing the residue twice with 10 mL of hexane, followed by crystallization at −10 °C from toluene generated an [8Fe−7S] complex [{N(SiMe₃)₂}-{SC(NMe₂)₂}Fe₄S₃]₂(μ_6-S){μ-N(SiMe₃)₂}₂ (**1**) as analytically pure black crystals (28%) (Scheme 1). The neutral cluster complex **1** is highly air/moisture sensitive and degrades to an insoluble black material at room temperature in solution even under inert atmosphere. This reaction system is analogous to that reported for the high yield (92%) synthesis of a neutral [4Fe−4S] cubane cluster, Fe₄S₄{SC(NMe₂)₂}₂(Stip)₂ (**2**),[21] where the mole ratio of 8(Fe): 6(tmtu):16(HStip):8(S) was employed. We have confirmed that the reaction system with this mole ratio in fact produces **2** as a sole
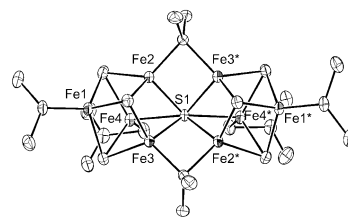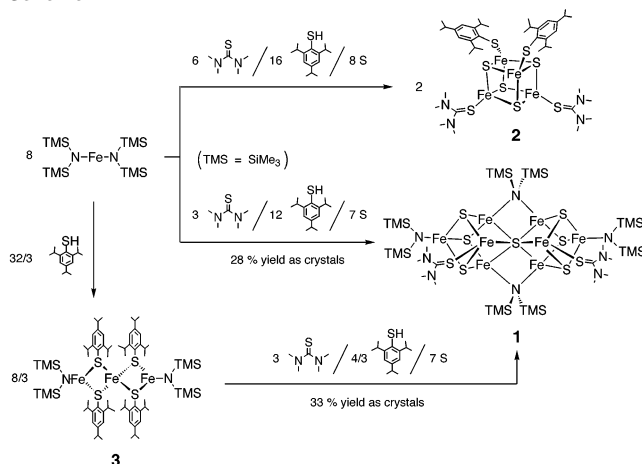
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Figure 1. A molecular view of **1**. The methyl groups of amide and tmtu are omitted for clarity.

Scheme 1

isolable product, in 96% yield as crystals, in our case. Thus, slight modification of the mole ratio gave rise to the entirely different iron sulfide cluster. Interestingly, formation of **2** was not discernible in the 8(Fe):3(tmtu):12(HStip):7(S) reaction system, although **2** is less soluble and more readily crystallized in toluene than **1**. The cluster **1** was alternatively synthesized in 33% yield as crystals from the reaction of the known trinuclear ferrous cluster Fe₃{N(SiMe₃)₂}₂-(μ-Stip)₄ (**3**)[22] with tmtu, HStip, and elemental sulfur in the mole ratio shown in Figure 1. Indeed, the initial color of the solution in the 8(Fe):3(tmtu):12(HStip):7(S) reaction system is very similar to that of **3**.

According to the X-ray structure analysis (Figure 1), cluster **1** comprises two [4Fe−3S] units connected by a μ_6-sulfur (S1) through interactions with six irons, and by two amide ligands each bridging two iron atoms. There are two terminal amides and two tmtu molecules completing the coordination sphere of irons. Being crystallized in the $P2/c$ space group, a crystallographic C_2 axis runs through μ_6-S1. The [8Fe−7S] cluster core of **1** reproduces very well the basic core geometry of the P^N-cluster,[12,13] except that the iron atoms of P^N carry six cysteinyl sulfurs, two at the bridging positions and four at the terminal positions.

Table 1 compares the important bond lengths of **1** and P^N. The mean Fe−S bond distance of μ_6-S1 in **1** (2.373 Å) is somewhat

Table 1. Comparison of the Selected Bond Distances (Å) of **1** and the P^N-Cluster in Kp1[12]

	Fe2	Fe3	Fe4		Fe2	Fe3	Fe4		Fe6	Fe7	Fe8
S1	2.3719(7)	2.3969(7)	2.3489(5)	S1	2.52	2.40	2.42	S1	2.41	2.49	2.47
Fe1	2.8150(6)	2.8263(7)	2.7346(6)	Fe1	2.75	2.67	2.79	Fe5	2.82	2.79	2.85
Fe2		2.7020(6)	2.6433(6)	Fe2		2.58	2.52	Fe6		2.52	2.52
Fe3			2.6507(6)	Fe3			2.53	Fe7			2.58
Fe2−Fe3*		2.7903(6)		Fe2−Fe7		2.94		Fe3−Fe6		2.92	

shorter than that in P^N (2.45 Å), while the other Fe−S distances are comparable between **1** (av. 2.285 Å) and P^N (av. 2.31 Å). The S1 sulfur is surrounded by six iron atoms in a severely distorted octahedral arrangement: 143.64(5)° (Fe4−S1−Fe4*) and 71.62-(2)° (Fe2−S1−Fe3*) for **1**, 157° (Fe4−S1−Fe8) and 72° (Fe2−S1−Fe7) for P^N. The Fe−Fe distances within each [4Fe−3S] unit can be classified into two groups. The longer Fe−Fe bonds are those associated with the iron atoms at both ends of the cluster, that is, Fe1 in **1** (av. 2.792 Å) and Fe1 and Fe5 in P^N (av. 2.77 Å). The other Fe−Fe distances between the two [4Fe−3S] units are shorter for **1** (Fe2−Fe3*, 2.7903(6) Å), as compared with the corresponding Fe2−Fe7 and Fe3−Fe6 distances of P^N (av. 2.93 Å). This is probably due to the difference in the bridging ligands, where the short Fe−N (amide) bond of 2.059(2) Å relative to the Fe−S (μ-Cys) bond of 2.40 Å brings two iron atoms closer.

The oxidation state of all of the iron atoms in P^N has been assigned as Fe(II), and this is supported by the diamagnetic character suggested by the EPR spectrum and the Mössbauer spectrum which shows two types of iron sites, all with isomer shifts consistent with the Fe(II) oxidation state.[23−26] Oxidation to the P^{2+} state has been reported to produce a cluster with Fe(II)$_6$ and Fe(III)$_2$,[25,27] which corresponds to the oxidation state of the model cluster we report here. The cluster **1** was found to be EPR silent at 4−100 K. Temperature-dependent magnetic susceptibility of **1** in the solid state was measured from 2 to 300 K, and minor impurity and diamagnetic corrections were applied to the data. The magnetic behavior is qualitatively consistent with an antiferromagnetic exchange coupling among the eight iron atoms. The magnetic moment μ_{Fe} decreases with a lowering of the temperature from 0.575 μ_B/Fe at 300 K and approaches 0.023 μ_B/Fe (0.06 μ_B/Fe without impurity correction) at 2 K, indicating a singlet ground state. The Mössbauer spectrum of our model compound **1** is consistent with both the structure and the formal Fe(II)$_6$Fe(III)$_2$ oxidation state, showing two quadrupole doublets approximately in a 3:1 ratio, where the IS and QS values of the former doublet are 0.61 and 0.54 mm/s, and those of the latter doublet are 0.37 and 1.28 mm/s. The environment of Fe2 and Fe3 closely resembles that of Fe4, and presumably these three sites contribute to the more intense doublet and are thus assigned as Fe(II). The Fe(III) site is therefore Fe1 and occupies the peripheral position. In the reported Mössbauer spectrum of P^N, the larger doublet presumably corresponds to the central iron positions, while the other doublet with a larger quadrupole splitting corresponds to the peripheral iron position.

While the Mössbauer spectrum of our model resembles that of P^N, it should not because the oxidation state of our model is that assigned to P^{2+}. Indeed the Mössbauer spectrum of P^{2+} is quite different from our model, consisting of complicated subspectra corresponding to a paramagnetic state, indicating that the two Fe-(III) sites in the [8Fe−7S] core are not antiferromagnetically coupled. There are two possible explanations for this difference. One is that the bridging amides in our model allow antiferromagnetic coupling between Fe(III) ions resulting in a nearly diamagnetic cluster, even though the Fe(III) sites are on opposite ends of the cluster, while the bridging thiolates of P^{2+} do not allow such coupling. The second is that the highly distorted structure suggested for the P^{2+}-cluster[13] inhibits antiferromagnetic coupling between Fe(III) ions. Further work will be required to shed light on this question.

Supporting Information Available: Details of synthesis and characterization, and crystallographic data for **1** (PDF and CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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