fields up to 6.0 T we found that $A_z(1) > 0$, $A_z(2a) < 0$, and $A_z(2b) < 0$.

Cluster I has unique spectroscopic features. Formally, it contains two Fe^{2+} and one Fe^{3+}; thus the three iron sites accommodate one more electron than those of reduced Fd II. Since we have no spectroscopic data on the Zn, our spectra could indicate the formation of a superreduced Fe_3S_4 cluster, the reduction being achieved, however, only in the presence of Zn (but not with Fe, Co, Cu, or V); Zn^{2+} would bind to the protein in the vicinity of the cluster stabilizing the superreduced state. The oxidized/reduced Fd II couple has $E_m = -130$ mV (vs. NHE); in the absence of Zn^{2+} we have never observed, even at −600 mV, any EPR feature indicative of the $S = {}^5/_2$ state (3% $S = {}^5/_2$ clusters would have been detected). A superreduced state has never been indicated for any protein containing an Fe_3S_4 cluster. More plausibly, therefore, Zn has been incorporated into the vacant site of the Fe_3S_4 cluster to form $ZnFe_3S_4$. This interpretation is suggested by our earlier work which has demonstrated facile formation of $[Fe_4S_4]^{1+}$ and $[CoFe_3S_4]^{1+}$ under similar incubation conditions. Since I has half-integer spin, the incorporated Zn must be Zn^{2+}, suggesting that I is $[ZnFe_3S_4]^{1+}$ and thus the analogue of $[Fe_4S_4]^{1+}$. In order to prove incorporation of Zn directly, EXAFS and ENDOR experiments are in preparation.

$[Fe_4S_4]^{1+}$ cores seem to consist of two spin-coupled pairs; see ref 8. For the postulated $[ZnFe_3S_4]^{1+}$ the replacement of one Fe^{2+} by Zn^{2+} has disrupted one pair and created the trapped Fe^{2+} of site 1. In order to exploit the cluster for the study of spin coupling of cubanes, $A_x(i)$ and $A_y(i)$ need to be determined. Such efforts are in progress.

Acknowledgment. This work was supported by the National Science Foundation, the National Institutes of Health, the Instituto de Investigacão Cientifica Technologica, the Junta Nacional de Investigacão Cientifica Tecnologica, the Agency for International Development, and a NATO Collaborator Research Grant.

---

[8] (a) Aizman, A.; Case, D. A. *J. Am. Chem. Soc.* **1982**, *104*, 3269–3279. (b) Dickson, D. P. E.; Johnson, C. E.; Thompson, C. L.; Cammack, R.; Evans, M. C. W.; Hall, D. O.; Rao, K. K.; Weser, U. *J. Phys. (Paris)* **1974**, *35*, C6-343.

Synthesis, Structural Characterization, and Electronic Structures of the $[Fe_6S_6(X)_6(Mo(CO)_3)_2]^{3-}$ Clusters (X = Cl, Br)

D. Coucouvanis,* A. Salifoglou, M. G. Kanatzidis,
A. Simopoulos, and A. Kostikas

Department of Chemistry, University of Michigan
Ann Arbor, Michigan 48109
Nuclear Research Center, "Demokritos"
Aghia Paraskevi, Attiki, Greece

Received January 12, 1987

In the last few years, work in our laboratory has been aimed toward the design and synthesis of structural analogues for the Fe/Mo/S center in nitrogenase[1] or the nitrogenase cofactor.[2] Our synthetic efforts are directed mainly by the data available for the Fe/Mo/S center, from analytical,[3] Mössbauer,[4] EXAFS,[5] and ENDOR[6] studies. These studies broadly define the heterometallic center as an asymmetric, spin-coupled, $S = {}^3/_2$ aggregate with a Fe/Mo/S ratio of 6–7/1/6–8.

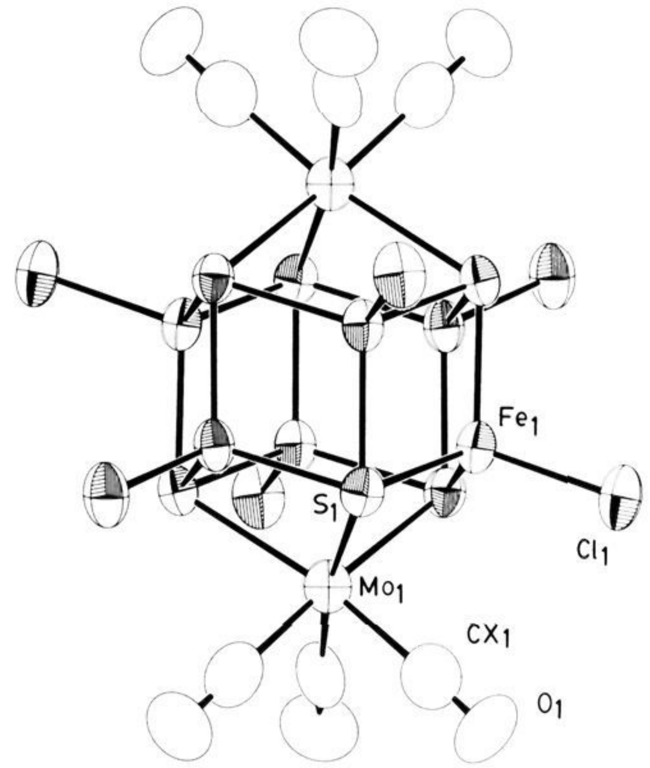

Figure 1. Structure and labeling of the anion in I. Thermal ellipsoids as drawn by ORTEP (Johnson, C. K. ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, TN, 1965) represent the 40% probability surfaces.

Recently, we reported on the synthesis spectroscopic properties and molecular structures of the $[Fe_6S_6(OR)_6(W(CO)_3)_2]^{3-}$ [7] and $[Fe_6S_6(Cl)_6(Mo(CO)_3)_2]^{4-}$ [8] complex anions and of the synthesis and spectroscopic properties of $[Fe_6S_6(OR)_6(Mo(CO)_3)_2]^{3-}$.[9] These clusters are obtained by the addition of two $M(CO)_3$ fragments (M = Mo, W) to the $[Fe_6S_6(L)_6]^{3-}$ prismanes[10] and adopt a heterometallic pentlandite type of structure with $[Fe_6M_2S_6]^{3+,2+}$ cubic cores. In this paper we report on the synthesis, crystal structures, and Mössbauer spectra of the trianionic $[Fe_6S_6(L)_6(Mo(CO)_3)_2]^{3-}$ clusters (L = Cl, Br).

The $[Fe_6S_6(L)_6(M(CO)_3)_2]^{n-}$ clusters display two reversible waves in cyclic voltammetry that correspond to the 3−/4− and 4−/5− couples. The low potentials of the 3−/4− couples, at +0.05 and +0.08 V ($E_{1/2}$ values in CH_2Cl_2 on a Pt electrode vs. SCE) for L = Cl and Br, respectively, suggest that the trianions would be susceptible to reduction under mildly reducing conditions. Indeed the adduct-forming reactions of the $[Fe_6S_6(L)_6]^{3-}$ prismanes with an excess of the mildly reducing $Mo(CO)_3(CH_3CN)_3$ reagent, in CH_3CN solution, produce primarily the $[Fe_6S_6(L)_6(Mo(CO)_3)_2]^{4-}$ tetraanions when L = Cl or Br. The $Mo(CO)_3(CH_3CN)_3$ complex ($E_{1/2}$ for the 0/+1 couple, +0.30 V

---

* Address correspondence to this author at The University of Michigan.

[1] (a) Orme-Johnson, W. H.; Davis, L. C. In *Iron Sulfur Proteins*; Lovenberg, W., Ed.; Academic: New York, 1976; Vol. III, Chapter 2. (b) Burgess, B. K. In *Advances in Nitrogen Fixation Research*; Veeger, C., Newton, W. E., Eds.; Nijhoff-Junk-Pudoc: Wageningen, 1983.

[2] Shah, V. K.; Brill, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3249.

[3] Nelson, M. J.; Levy, M. A.; Orme-Johnson, W. E. *Proc. Nat. Acad. Sci. U.S.A.* **1983**, *80*, 147.

[4] Orme-Johnson, W. H.; Munck, E. In *Molybdenum and Molybdenum Containing Enzymes*; Coughlin, M. P., Ed.; Pergamon: Oxford, 1980; pp 427–438.

[5] (a) Cramer, S. P.; Gillum, W. D.; Hodgson, K. O.; Mortenson, L. E., Stiefel, E. I.; Chisnell, J. R.; Brill, W. J.; Shah, V. K. *J. Am. Chem. Soc.* **1978**, *100*, 4630. (b) Cramer, S. P.; Hodgson, K. O.; Gillum, W. O.; Mortenson, L. E. *J. Am. Chem. Soc.* **1978**, *100*, 3398. (c) Newton, W. E.; Burgess, B. K.; Cummings, S. C.; Lough, S.; McDonald, J. W.; Rubinson, J. F.; Conradson, S. D.; Hodgson, K. O. In *Advances in Nitrogen Fixation Research*; Veeger, C., Newton, W. E., Eds.; Nijhoff-Junk-Pudoc: Wageningen, 1983; p 160. (d) Antonio, M. R.; Teo, B. K.; Orme-Johnson, W. H.; Nelson, M. J.; Groh, S. E.; Lindahl, P. A.; Kauzlarich, S. M.; Averill, B. A. *J. Am. Chem. Soc.* **1982**, *104*, 4703.

[6] Hoffman, B. M.; Venters, R. A.; Roberts, J. E.; Nelson, M.; Orme-Johnson, W. H. *J. Am. Chem. Soc.* **1982**, *104*, 4711–4712.

[7] Salifoglou, A.; Kanatzidis, M. G.; Coucouvanis, D. *J. Chem. Soc.,, Chem. Commun.* **1986**, 559.

[8] Kanatzidis, M. G.; Coucouvanis, D. *J. Am. Chem. Soc.* **1986**, *108*, 337.

[9] Coucouvanis, D.; Kanatzidis, M. G. *J. Am. Chem. Soc.* **1985**, *107*, 5005.

[10] (a) Kanatzidis, M. G.; Dunham, W. R.; Hagen, W. R.; Coucouvanis, D. *J. Chem. Soc., Chem. Commun.* **1984**, 356. (b) Coucouvanis, D.; Kanatzidis, M. G.; Dunham, W. R.; Hagen, W. R. *J. Am. Chem. Soc.* **1984**, *106*, 2978. (c) Kanatzidis, M. G.; Hagen, W. R.; Dunham, W. R.; Lester, R. K.; Coucouvanis, D. *J. Am. Chem. Soc.* **1985**, *107*, 953. (d) Kanatzidis, M. G.; Salifoglou, A.; Coucouvanis, D. *J. Am. Chem. Soc.* **1985**, *107*, 3358. (e) Kanatzidis, M. G.; Salifoglou, A.; Coucouvanis, D. *Inorg. Chem.* **1986**, *25*, 2460.

0002-7863/87/1509-3807$01.50/0

Table I. Selected Distances[a] (Å) and Angles (deg) in the $(Et_4N)^+$ Salts of the $[(Fe_6S_6X_6)(Mo(CO)_3)_2]^{n-}$ Ions (A, $n = 4$, X = Cl^-; B, $n = 3$, X = Cl^-; C, $n = 3$, X = Br^-)

	A[b]	B[c]	C[c]
		Distances	
Fe–Mo	3.005 (3, 11)	2.929 (2,2)	2.946 (3, 17)[d]
Fe–Fe[e]	3.785 (3, 10)	3.761 (2, 3)	3.755 (3, 6)
Fe–Fe[f]	2.761 (3, 10)	2.744 (2, 3)	2.733 (3, 10)
Mo–S	2.619 (3, 3)	2.579 (2, 3)	2.572 (3, 7)
Fe–S[e]	2.333 (3, 3)	2.314 (2, 4)	2.318 (3, 4)
Fe–S[f]	2.286 (6 5)	2.282 (4, 4)	2.277 (6, 5)
Fe–X	2.245 (3 3)	2.225 (2, 3)	2.355 (3, 4)
Mo–C	1.958 (3, 11)	2.01 (2, 2)	1.98 (3, 2)
		Angles	
S–Mo–S	93.4 (3, 2)	95.8 (2, 1)	95.2 (3, 7)
Fe–Mo–Fe	78.07 (3, 2)	79.9 (2, 1)	79.2 (3, 2)
Fe–S–Fe[e]	111.8 (3, 5)	111.0 (2, 3)	111.1 (3, 7)
Fe–S–Fe[f]	73.4 (6, 2)	73.3 (4, 3)	73.0 (6, 2)
S–Fe–S[e]	113.0 (3, 4)	114.1 (2, 3)	113.1 (3, 5)

[a] The mean values of chemically equivalent bonds are given. In parentheses the first entry represents the number of independent distances or angles averaged out and the second entry represents the larger of the standard deviations for an individual value estimated from the inverse matrix or of the standard deviation, $\sigma = [\sum_{i=1}^{N} (x_i - x)^2/N(N-1)]^{1/2}$. [b] From ref 8. [c] This work. [d] Range: 2.913 (2)–2.954 (2) Å. [e] Distances or angles within the Fe_3S_3 structural units. [f] Distances or angles within the Fe_2S_2 rhombic units.

vs. SCE) in CH_3CN solution, however, is inadequate for the reduction of the $[Fe_6S_6(OR)_6(Mo(CO)_3)_2]^{3-}$ adduct. The latter shows the potential for the 3–/4– couple at –0.35 V and is the only product isolated in the reaction of $[Fe_6S_6(OR)_6]^{3-}$ with excess $Mo(CO)_3(CH_3CN)_3$. The $[Fe_6S_6(Cl)_6(Mo(CO)_3)_2]^{3-}$ adduct, (I) can be obtained in ~60% yield by the reaction of $[Fe_6S_6(Cl)_6]^{3-}$ with $Mo(CO)_3(CH_3CN)_3$ in a 4:1 molar ratio, in dichloroethane[11] at 70–80 °C for ~50 min. The synthesis of I and the corresponding Br^- analogue II, also can be accomplished in similar yields by the reactions between the $[Fe_6S_6(X)_6(Mo(CO)_3)_2]^{4-}$ complexes and $[Fe(C_5H_5)_2][PF_6]$ in a 1:1 molar ratio in CH_3CN solution. The crystalline Et_4N^+ salts of I and II obtained in this fashion are X-ray isomorphous. A different crystalline modification of II is obtained when the oxidation is carried out with the oxidant in a 2:1 molar excess. The structure of this monoclinic modification is reported herein (vide infra).

The purity of I and II can be monitored conveniently by infrared spectroscopy. The C–O stretching vibrations in I and II occur as sharp doublets in the infrared spectra[12] at 1918, 1945 cm^{-1} and 1912, 1948 cm^{-1}, respectively, and reflect the C_{3v} microsymmetry of the coordinated $Mo(CO)_3$ units. These values are higher than those found in the corresponding tetraanions at 1847, 1908 cm^{-1} and 1852, 1912 cm^{-1} respectively for the chloro and bromo homologues and suggest that the oxidation of the tetraanions involves a molecular orbital with considerable Mo character. The electronic spectrum of I shows an absorption band at 460 nm ($e = 11\,500$), while that of II shows bands at 470 (sh) and 314 nm ($e = 22\,150$).

Single crystals of I and II were obtained by the slow diffusion of ether to CH_3CN solutions of these compounds. The crystal structures[13] of the trianions in both I and II are very similar and contain the $[Fe_6Mo_2S_6]^{3+}$ cores (Figure 1). The two crystallographically independent anions in the structure of I are situated on special positions ($\bar{3}$-site symmetry) and possess nearly exact D_{3d} symmetry. In the structure of II the anions are located on crystallographic centers of symmetry. Selected structural parameters for these anions are shown in Table I. As observed previously,[8] coordination of the $Mo(CO)_3$ to the Fe_6S_6 central cages results in an elongation of the latter along the $\bar{3}$-axis in I and the idealized $\bar{3}$-axis in II. A structural comparison between I and the corresponding tetraanion III (Table I) shows a significant shortening of the Mo–Fe and the Mo–S distances in the former and no significant differences within the Fe_6S_6 frameworks in the two structures. The data indicate that the highest occupied MO in III consists mainly of Mo and S atomic functions and is antibonding in character.

The isomer shifts (IS) and quadrupole splittings (Δ_{Eq}) in the ^{57}Fe Mössbauer spectra of I at 0.56 and 1.00 mm/s and of II at 0.59 and 1.06 mm/s, respectively (vs. Fe, $T = 92$ K), are quite similar to those of the corresponding tetraanions at 0.63, 1.00 mm/s and 0.62, 1.02 mm/s. These results, and the insignificant structural differences found in the Fe_6S_6 cores in both the 3- and 4- levels of the adducts, further reinforce the conclusion that the oxidation of the tetraanionic $Mo(CO)_3$ adducts is centered primarily on the Mo atoms. The isomer shifts in I, II, and their corresponding tetraanions are significantly larger by comparison to those in the "parent" $[Fe_6S_6(L)_6]^{3-}$ clusters (IS ~ 0.49 mm/s, Δ_{Eq} ~ 1.10 mm/s vs. Fe, $T = 125$ K). The data suggest that in the $[Fe_6S_6(L)_6(Mo(CO)_3)_2]^{n-}$ adducts the Fe_6S_6 cores assume an oxidation level lower than the one in the $[Fe_6S_6(L)_6]^{3-}$ clusters. In a formal sense both oxidation levels of the adducts can be described as containing the $[Fe_6S_6]^{2+}$ core. It appears that a reduction of the Fe_6S_6 core is important for the stability of either the 3- or the 4-levels of the adducts and may be able to stabilize adducts with Mo atoms in even higher formal oxidation states. The synthesis of the latter is an objective of future studies in our laboratory.

Acknowledgment. This research was supported by a grant from the National Institutes of Health (GM-26671).

Supplementary Material Available: Table of crystal and refinement data, positional and thermal parameters and intramolecular distances for I and II (6 pages); tables of structure factors for I and II (14 pages). Ordering information is given on any current masthead page.

---

(11) In CH_2Cl_2 solution the $Mo(CO)_3(CH_3CN)_3$ complex undergoes a one-electron irreversible oxidation on a Pt anode at 0.18 V vs. SCE.

(12) The infrared spectra reported were obtained in NaCl cells in CH_3CN solution.

(13) Crystal and refinement data for I and II can be found in the supplementary material.

Peptide Segment Coupling Catalyzed by the Semisynthetic Enzyme Thiolsubtilisin

T. Nakatsuka, T. Sasaki, and E. T. Kaiser*

Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, New York, New York 10021-6399

Received February 23, 1987

Stepwise solid-phase peptide synthesis[1] has developed in recent years to the stage where even the preparation of peptides greater than 100 amino acids in length has been undertaken.[2] Nevertheless, an alternative approach to the construction of very large peptides involving a combination of segment synthesis with segment condensation[3] remains very appealing. The segments that are the intermediates in this process can be purified and thoroughly characterized, reducing the need for laborious purification of the final product, which is often required for very large peptides produced by stepwise solid-phase synthesis. Further, the segment synthesis–condensation strategy is particularly adaptable to the preparation of analogues of naturally occurring peptides.

While the rapid synthesis of peptide segments of up to 10 amino acids is now feasible,[3,4] methods for the chemical couplng of such segments often suffer from problems such as racemization during

---

(1) Merrifield, R. B. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 799.

(2) Clark-Lewis, I.; Aebersold, R.; Ziltener, H.; Schrader, J. W.; Hood, L. E.; Kent, B. H. *Science* (*Washington, D.C.*) **1986**, *231*, 134.

(3) For example, a 44 amino acid peptide model of apolipoprotein A-I was recently prepared by this route. Nakagawa, S. H.; Lau, H. S. H.; Kezdy, F. J.; Kaiser, E. T. *J. Am. Chem. Soc.* **1985**, *107*, 7087.

(4) A p-nitrobenzophenone oxime derivative of polystyrene has been utilized in the facile preparation of peptide segments as described in: DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295; **1982**, *47*, 3258.

0002-7863/87/1509-3808$01.50/0 © 1987 American Chemical Society