ing to trapped silylene (**22**, **24**) or silanone (**23**) was found. An even smaller amount of **7** (<2%) was identified by GCMS. When compared with the results of copyrolysis of **7** with excess dimethyldimethoxysilane (vide supra) under the same conditions, many of the numerous unidentified compounds formed can be attributed to redistribution reactions. No attempt was made to isolate the presumed trapping product or to provide positive identification of the redistribution products.

**Copyrolysis of 21 with 1,3-Butadiene.** Trisilane **21** (0.3448 g, $1.2 \times 10^{-3}$ mol) was pyrolyzed in a vertical flow system with 1,3-butadiene as the carrier gas. At 510 °C (35 mL/min butadiene flow rate), a mass recovery (after evaporation of volatiles) of 46% was obtained. No compound of $m/e$ 212 (corresponding to silylene trapping product) was found by GCMS.

**Acknowledgment.** The support of this work by the National Science Foundation is gratefully acknowledged.

**Registry No. 4**, 101347-32-4; **7**, 18146-00-4; 8, 101347-33-5; **9**, 18141-63-4; **11**, 71821-60-8; **12**, 7087-19-6; **13**, 101347-34-6; **15**, 16109-39-0; **17**, 101347-35-7; **18**, 101347-36-8; **21**, 101347-38-0; **25**, 101347-39-1; **26**, 762-72-1; **27**, 18163-07-0; $Me_3SiOMe$, 1825-61-2; $Me_2Si(OMe)OCH_2CH{=}CH_2$, 101347-37-9; allyl alcohol, 107-18-6; 1,1-dichlorotetramethyldisilane, 4518-99-4; 1,3-butadiene, 106-99-0; vinylcyclohexene, 25168-07-4; dimethyldimethoxysilane, 1112-39-6; 2,3-dimethylbutadiene, 513-81-5; 1-chloropentamethyldisilane, 1560-28-7; 1-methoxypentamethyldisilane, 18107-29-4; trimethylmethoxysilane, 1825-61-2; 2,2-dichloro-1,1,1,3,3,3-hexamethyltrisilane, 5181-42-0.

# *Communications to the Editor*

## EXAFS of *Klebsiella pneumoniae* Nitrogenase MoFe Protein from Wild-Type and *nif V* Mutant Strains

M. K. Eidsness,[§] A. M. Flank,[‡] B. E. Smith,[⊥] A. C. Flood,[‖] C. D. Garner,[‖] and S. P. Cramer*[†]

*Corporate Research Science Laboratories*
*Exxon Research and Engineering Co.*
*Annandale, New Jersey 08801*
*Department of Materials Science, Stanford University*
*Stanford, California 94305*
*LURE Batiment 209C, 91405 Orsay, France*
*AFRC Unit of Nitrogen Fixation*
*University of Sussex, Brighton, BN1 9RQ UK*
*Department of Chemistry, University of Manchester*
*Manchester, M13 9PL UK*

*Received October 15, 1985*

The enzyme nitrogenase catalyzes the biological reduction of $N_2$ to $NH_3$.[1] In *Klebsiella pneumoniae* a cluster of 17 genes in seven transcriptional units has been associated with nitrogen fixation.[2] The nitrogenase enzyme from the *nif V* mutants is relatively ineffective at dinitrogen reduction, is more efficient than the wild-type enzyme at HCN reduction,[3] and has its hydrogen evolution activity inhibited up to 80% by CO.[4,5] This altered substrate specificity has been shown to be associated with the iron-molybdenum cofactor, "FeMo-co", of the enzyme.[6] X-ray absorption spectroscopy has been a valuable tool for probing the molybdenum environment of wild-type nitrogenase,[7-11] and we report here similar studies on the Nif $V^-$ enzyme.[12,13]

The Mo K absorption edge regions for wild-type and Nif $V^-$ MoFe proteins are nearly identical. In both cases the first inflection point occurs at 20010.9 (5) eV, and the overall edge shape is the same within experimental error.[14] The extreme similarity in edge position and shape suggests that the Mo exists in the same oxidation state in both cases. On the basis of previous work with Mo,Fe,S cluster model compounds,[20] the edges also indicate that the number of sulfur donor ligands to molybdenum is four or five and most likely the same in both cases.

The EXAFS Fourier transforms of Nif $V^-$ and wild-type Kpl at 4 K are compared in Figure 1. Two strong peaks corresponding to Mo-S and Mo-Fe interactions are observed. These features are stronger and better resolved than in previously reported spectra because of the lower temperature and wide k range. Quantitation of these spectra[7-11] involved curve-fitting analysis,[21,24] the results

* To whom correspondence should be addressed.
† Exxon Research.
‡ LURE.
§ Stanford University.
⊥ University of Sussex.
‖ University of Manchester.

(1) (a) *Current Perspectives in Nitrogen Fixation*; Gibson A. H., Newton, W. E., Eds.; Australian Academy of Sciences: Canberra, 1981. (b) *Nitrogen Fixation: The Chemical-Biochemical Genetic Interfaces*; Müller, A., Newton, W. E., Eds.; Plenum: New York, 1983.
(2) (a) Brill, W. J. *NATO Adv. Sci. Inst. Ser. A*, **1983**, *63*, 231. (b) Dixon, R. A. *J. Gen. Microbiol.* **1984**, 2745-2755.
(3) Liang, Y. C.; Smith, B. E. *Advances in Nitrogen Fixation*; Veeger, C., Newton, W. E., Eds.; Martenus Nijhoff/Junk: The Hague, 1984; p 155.
(4) McLean, P. A.; Dixon, R. A. *Nature (London)* **1981**, *292*, 655-656.
(5) McLean, P. A.; Smith, B. E.; Dixon, R. A. *Biochem. J.* **1983**, *211*, 589-597.
(6) Hawkes, T. R.; McLean, P. A.; Smith, B. E. *Biochem. J.* **1984**, *217*, 317-321.
(7) Cramer, S. P.; Hodgson, K. O.; Gillum, W. O.; Mortenson, L. E. *J. Am. Chem. Soc.* **1978**, *100*, 3398-3407.
(8) Cramer, S. P.; Gillum, W. O.; Hodgson, K. O.; Mortenson, L. E.; Stiefel, E. I.; Chisnell, J. R.; Brill, W. J.; Shah, V. K. *J. Am. Chem. Soc.* **1978**, *100*, 3814-3819.
(9) Cramer, S. P.; Flank, A. M.; Weininger, M.; Mortenson, L. E. *J. Am. Chem. Soc.* **1986**, *108*, 1049-1055.
(10) Conradson, S. D.; Hodgson, K. O.; Burgess, B. K.; Newton, W. E.; Adams, M. W. W.; Mortenson, L. E., SSRL 1981 Activity Report, p VIII-26.
(11) Burgess, B. K.; Yang, S.-S.; You, C.-B.; Li, J.-G.; Friesnen, G. D.; Pan, W.-H.; Stiefel, E. I.; Newton, W. E.; Conradson, S. D.; Hodgson, K. O., ref. 1a, p 71.

(12) Large-scale cultures of the wild-type *Klebsiella pneumoniae* strain M5al[15] and the *nif V* mutant strain UNF1613[4] were grown and harvested as previously described. The MoFe proteins from both strains were purified and assayed for activity and Mo content by the methods described for the wild-type Kpl.[15,16] The EXAFS spectra of three different preparations of each protein were measured. The Kpl samples had specific activities between 1600 and 1750 units/mg (1 unit of activity - 1 nmol of $C_2H_2$ reduced/min) and activities/Mo between 255 and 315 units/mol of Mo. The Nif $V^-$ Kpl samples had specific activities between 1180 and 1550 units/mg and activities/Mo between 222 and 283 units/nmol of Mo. The oxidation state of the sample correspond to the EPR-active $S = {}^3/_2$ state.

(13) The X-ray absorption spectra were recorded on beam line VII-3 at the Stanford Synchrotron Radiation Laboratory (SSRL) during dedicated conditions (3.0 GeV and ~50 mA) by using Si(220) monochromator crystals. The protein data were measured in the fluorescence detection mode by using an array of sodium iodide scintillation detectors and zirconium filters.[17] Measurements at 4 and 100 K were made by using a continuous He flow Oxford Instruments cryostate (CF 1204) with aluminized Mylar windows. The spectra were calibrated, processed, and fit by using previously described procedures.[9,18,19]

(14) The XANES spectra were calibrated by using 20003.9 eV for the Mo foil first inflection point. The data are included as supplementary material.

(15) Eady, R. R.; Smith, B. E.; Cook, K. A.; Postgate, J. R. *Biochem. J.*, **1972**, *128*, 655-675.
(16) Smith, B. E.; Thorneley, R. N. F.; Yates, M. G.; Eady, R. R.; Postgate, J. R. *Proc. Int. Symp. Nitrogen Fixation, 1st, 1974* **1976**, 150-176.
(17) Cramer, S. P.; Scott, R. A. *Rev. Sci. Intrum.* **1981**, *52*, 395-399.
(18) Cramer, S. P.; Wahl, R.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1981**, *103*, 7721-7727.
(19) Cramer, S. P.; Solomonson, L. P.; Adams, M. W. W.; Mortenson, L. E. *J. Am. Chem. Soc.* **1984**, *106*, 1467-1471.
(20) Conradson, S. Ph.D. Thesis, Stanford University, Stanford, CA, 1984.

(21) The curve-fitting procedure[22] used empirical phase-shift and amplitude functions[18] for all Mo-X interactions except for the Mo-Fe amplitude function. This was better fit by using the theoretical Fe amplitude[23] and a scale factor of 0.345.

0002-7863/86/1508-2746$01.50/0 © 1986 American Chemical Society

**Table I.** Comparison of Wild-Type and Nif V⁻ Curve-Fitting Results

| sample | Mo-S | | | Mo-Fe | | | Mo-O,N | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $N^a$ | $R$,[b] Å | $\sigma$,[c] Å | $N$ | $R$, Å | $\sigma$, Å | $N$ | $R$, Å | $\sigma$, Å | function[e] |
| wild-type Kpl (4 K) | 4 | 2.370 | 0.056 | 3 | 2.691 | 0.023 | | | | 0.954 |
| | 5 | 2.371 | 0.066 | 3 | 2.694 | 0.022 | | | | 0.869 |
| | 4 | 2.370 | 0.056 | 3 | 2.691 | 0.023 | 0.98 | 2.153 | 0.050[d] | 0.839 |
| Nif V⁻ Kpl (4 K) | 4 | 2.363 | 0.055 | 3 | 2.694 | 0.027 | | | | 1.357 |
| | 5 | 2.365 | 0.065 | 3 | 2.697 | 0.024 | | | | 1.272 |
| | 4 | 2.363 | 0.055 | 3 | 2.694 | 0.027 | 0.75 | 2.180 | 0.050[d] | 1.280 |
| | 4.3 | 2.365 | 0.056[d] | 3.1 | 2.695 | 0.023[d] | | | | 1.337 |

[a] Coordination number. Values reported as integers were fixed during the refinement. [b] Mo-X distance. [c] Debye-Waller disorder term. [d] Fixed at reported value. [e] Defined as $[(\sum k^6(\chi_{obsd} - \chi_{calcd})^2)/(N_o - N_r)]^{1/2}$ where $\chi_{obsd}$ is the observed EXAFS, $\chi_{calcd}$ is the calculated EXAFS, $N_o$ is the number of data points, and $N_r$ is the number of parameters refined.

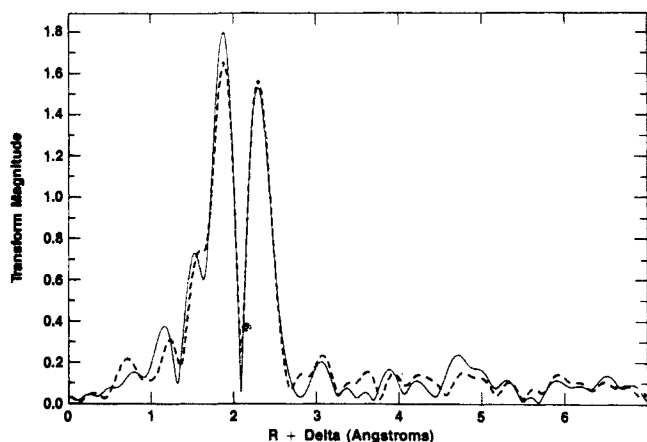


**Figure 1.** Fourier transforms of Mo EXAFS of Nif V⁻ MoFe protein (—) and wild-type MoFe protein (---) at 4 K. Transform range: $k$ = 4–16 Å$^{-1}$ $k^3$ weighting. The peaks are shifted from the true distances by about 0.4 Å. The Kpl sample was 182 mg/mL with a specific activity of 1600 units/mg and a Mo concentration of 1.13 mM. The Nif V⁻ Kpl sample was 128 mg/mL with a specific activity of 1515 units/mg and Mo concentration of 0.84 mM. Both proteins were in 25 mM Hepes pH 7.5 buffer, 100 mM NaCl, 0.1 mg/mL dithiothreitol, and 2 mM $Na_2S_2O_4$.

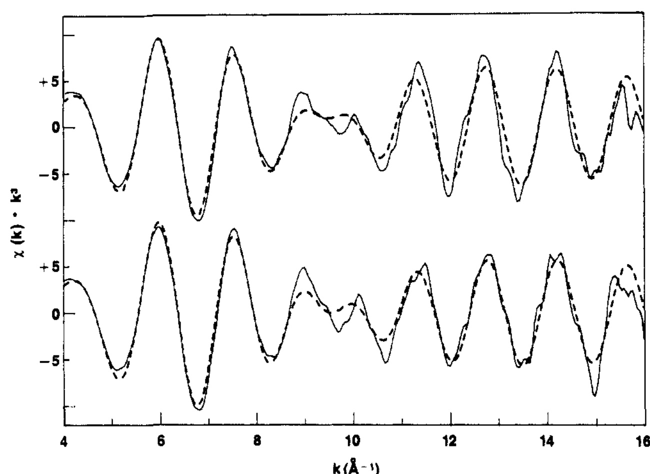


**Figure 2.** Gaussian smoothed EXAFS data (—) and best fit (---) for Nif V⁻ (top) and wild-type (bottom) MoFe proteins at 4 K. The fits use the values reported in Table I. The protein samples were those of Figure 1.

of which are illustrated in Figure 2 and Table I. In both cases, the main features can be reproduced by assuming four to five sulfur atoms at 2.37 (4) Å and two to four Fe atoms at 2.69 (3) Å. Although the EXAFS can be interpreted in terms of various coordination numbers, this ambiguity arises from systematic errors which are canceled in a direct comparison of spectra. To facilitate this comparison, a fit was done constraining the Debye-Waller factors for the Nif V⁻ EXAFS to the same values obtained for the wild-type enzyme. After optimization, the Nif V⁻ Kpl EXAFS gave coordination numbers within 9% of the wild-type values. Furthermore, the Mo-S and Mo-Fe distances were the same to within 0.01 Å. In both cases, a small improvement to the fits was obtained by including a Mo-O,N component at 2.18 (5) Å.

The close similarity of the Mo EXAFS from wild-type and Nif V⁻ proteins narrows the range of structural variations conceivable for the Nif V⁻ defect. There is no evidence in the EXAFS for a change in the number or distance of S or Fe neighbors. If it is assumed as a working model that molybdenum is bridged primarily or completely by sulfide ions to iron in the nitrogenase FeMo-co, then the current data suggest that the core cluster arrangement in the vicinity of molybenum is not changed in the Nif V⁻ mutation. On the basis of chemical analogy with other molybdenum clusters,[29] it is unlikely that a sulfide bridge could be added, subtracted, or replaced by an oxo bridge or other group without significantly changing the Mo-Fe distance. A change in the overall number of sulfur ligands is also dictated against, from the results of the EXAFS amplitude analysis. Conceivably, the mutation involves the substitution of one non-sulfur ligand for another non-sulfur ligand to Mo outside the core of the Mo,Fe,S cluster.[26] Alternatively, the structural change is more closely associated with an Fe site(s) in FeMo-co.

**Acknowledgment.** We thank S. Harrison for growth of large-scale cultures, Carol Gormal for skilled technical assistance, and the staff of SSRL for their aid in making this work possible. SSRL is supported by the Department of Energy, Office of Basic Energy Science, and the National Institutes of Health, Biotechnology Resource Program, Division of Research Resources. We are grateful to the S.E.R.C. for the award of a studentship (to A.C.F.).

**Registry No.** Mo, 7439-98-7.

**Supplementary Material Available:** Pictures of the absorption edge region and printouts of the raw X-ray absorption data for *Klebsiella pneumoniae* wild-type and Nif V⁻ MoFe protein EXAFS (4 pages). Ordering information is given on any current masthead page.

(22) Eccles, T. K. Ph.D. Thesis, Stanford University, Stanford, CA, 1977.

(23) Teo, B.-K.; Lee, P. A. *J. Am. Chem.* **1979**, *101*, 2815-2832.

(24) In addition, an alternative procedure,[25-27] based upon the Daresbury suite of EXAFS programs, was used to interpret a representative selection of the EXAFS data. This data analysis used the single-scattering spherical-wave method for calculating the EXAFS, with phase shifts derived from ab initio calculations; the refinement and quality of fit were accomplished as described previously.[28] The only significant disagreement between the two data analysis results involved the Debye-Waller factors; $\sigma$ values were 2-3 times higher in the Daresbury analysis. A detailed comparison will be presented elsewhere.

(25) Lee, P. A.; Pendry, J. B. *Phys. Rev. B* **1975**, *11*, 2795-2811.

(26) Perutz, M. F.; Hasnain, S. S.; Duke, P. J.; Sessler, J. L.; Hahn, J. E. *Nature (London)* **1982**, *295*, 535-538.

(27) Gurman, S. J.; Binsted, N.; Ross, I. *J. Phys. C* **1984**, *17*, 143-151.

(28) Blackburn, N. J.; Hasnain, S. S.; Diakun, G. P.; Knowles, P. F.; Binsted, N.; Garner, C. D. *Biochem. J.* **1983**, *213*, 765-768.

(29) For example, in the case of dinuclear Mo compounds, the Mo-Mo distances tend to be progressively longer proceeding from di-$\mu$-oxo through oxo, sulfido to di-$\mu$-sulfido complexes. Stiefel, E. I. *Prog. Inorg. Chem.* **1977**, *22*, 1.