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Identification of iron-sulfur centers in the iron-molybdenum proteins of nitrogenase

(core extrusion/iron-molybdenum-sulfur enzymes/iron-molybdenum cofactor/^{19}F nuclear magnetic resonance spectroscopy)

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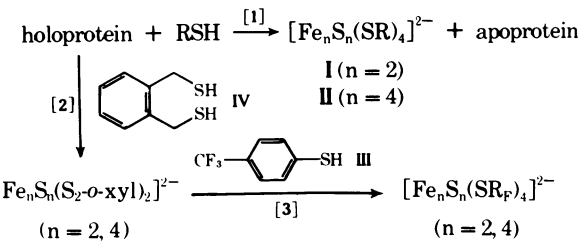
ABSTRACT **The core extrusion method has been applied to the determination of the type ([2Fe-2S], [4Fe-4S]) and number of iron-sulfur centers in the FeMo proteins of the nitrogenases from *Clostridium pasteurianum* and *Azotobacter vinelandii*. The method involves extrusion with *o*-xylyl-α,α'-dithiol, ligand exchange of the extrusion products with *p*-$CF_3C_6H_4SH$ (R_FSH), and identification and quantitation of the resultant $[Fe_nS_n(SR_F)_4]^{2-}$ complexes (n = 2,4) by ^{19}F NMR spectroscopy. In hexamethylphosphoramide/water, 4:1 (vol/vol), 49–56% of the Fe content was extruded as $[Fe_4S_4(SR_F)_4]^{2-}$, corresponding to 3.4–4.0 Fe_4S_4 cores per $\alpha_2\beta_2$ subunit complex. The extruded iron does not arise from the FeMo cofactor, separate examination of which detected no extrusion products, and corresponds to 90–103% of noncofactor iron. No significant quantity of Fe_2S_2 cores was extruded. These results indicate the presence of four [4Fe-4S] centers per $\alpha_2\beta_2$ subunit complex in preparations undepleted in iron. There are two main structural populations of iron atoms in these proteins, those in the cubane-type Fe_4S_4 cores and those in the FeMo cofactor.**

The core extrusion method (1) has proven to be of considerable utility in the identification of the type and number (n_d, [2Fe-2S]; n_t, [4Fe-4S]) of Fe-S centers in proteins and enzymes. The method is based on reaction **1**, which is conducted anaerobically with a large excess of thiol in a medium capable of unfolding protein structure. Fe_2S_2 or Fe_4S_4 cores of Fe-S centers are extruded in the form of synthetic analogues (**I** and **II**), which may be identified and quantitated on the basis of characteristic spectroscopic properties known from independently synthesized compounds (2, 3). Extrusion of small ferredoxin (Fd) proteins by reaction **1**, in which an aromatic thiol is usually employed, is essentially quantitative, as shown by spectrophotometric assay of products (4–7). This assay procedure is applicable in the absence of interfering protein chromophores (or when suitable corrective blanks are available)—e.g., in extrusion studies of hydrogenase (5, 8, 9), the Fe protein of nitrogenase (5, 10), trimethylamine dehydrogenase (11), and aconitase (12).



In order to avoid the problem of interfering protein chromophores we have developed a ^{19}F Fourier transform NMR procedure for identification and quantitation of extrusion products (7). In reaction **1** *p*-trifluoromethylbenzenethiol (R_FSH) (compound **III**) is the extrusion reagent, and assay is based on integration of the contact-shifted, fully resolved ^{19}F signals of the extrusion products. Although this procedure afforded quantitative extrusion and identification of [2Fe-2S] centers in milk xanthine oxidase (7, 13) and has led to identification of [2Fe-2S] and [4Fe-4S] centers in succinate dehydrogenase (14), results with the FeMo proteins of the nitrogenases from *Clostridium pasteurianum* and *Azotobacter vinelandii* were less satisfactory (7). By means of reaction **1** $\lesssim$25% of the total Fe content was extruded from the semireduced forms of these enzymes, or $\lesssim$50% of the Fe content not included in the dissociable FeMo-cofactor (FeMo-co) (15). Subsequently, we have found that extrusion of aconitase (12) by using R_FSH in reaction **1** was also unsatisfactory. However, employment of *o*-xylyl-α,α'-dithiol [*o*-xyl$(SH)_2$] (compound **IV**) as the extrusion reagent (reaction **2**) followed by ligand substitution with R_FSH (reaction **3**) afforded high recovery of the Fe-S center as $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$. This finding, together with prior demonstrations of extrusion of Fd proteins with *o*-xyl$(SH)_2$ (5, 6, 11) to yield $[Fe_nS_n(S_2\text{-}o\text{-xyl})_2]^{2-}$ (n = 2,4), has prompted us to reinvestigate the extrusion of FeMo proteins by sequential application of reactions **2** and **3** followed by ^{19}F NMR product analysis. Also included in this investigation is an examination of the behavior of FeMo-co under extrusion conditions.

MATERIALS AND METHODS

Reagents were prepared and solvents were purified as described (5, 7); *o*-xyl$(SH)_2$ was synthesized by a published method (16). FeMo proteins of the nitrogenases from *C. pasteurianum* and *A. vinelandii* were isolated and analyzed as described (7). Their analyses (g-atoms/mol based on the indicated molecular weight for the $\alpha_2\beta_2$ subunit complex) and activities (nmol of C_2H_2 reduced per min/mg of protein) were: *C. pasteurianum*, 220,000 daltons (17), 1.88 Mo, 28.4 Fe, 1466; *A. vinelandii*, 245,000 daltons (18), 2.01 Mo, 29.8 Fe, 1807. The FeMo-co was isolated from *A. vinelandii* FeMo protein and was analyzed as described (15). The *N*-methylformamide solution of the cofactor was concentrated by solvent removal under reduced pressure and was centrifuged to remove solid material. Analyses and activity of FeMo-co in the standard assay (15) were 4418 ± 78 nmol of Fe per ml, 683 ± 4 nmol of Mo per ml, and 261 nmol of C_2H_4 per min/nmol of Mo. In six preparations of FeMo-co the mean Fe-to-Mo g-atom ratio was 6.92 ± 0.14. The preparation and analysis of FeMo-co will be described in detail elsewhere (unpublished results).

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Abbreviations: Fd, ferredoxin; FeMo-co, iron-molybdenum cofactor; Me_6phosphoramide, hexamethylphosphoramide; *o*-xyl$(SH)_2$, *o*-xylyl-α,α'-dithiol; R_FSH, *p*-trifluoromethylbenzenethiol.

All core extrusion experiments were conducted under anaerobic conditions. FeMo proteins were utilized in their semireduced state (electron paramagnetic resonance signals of $g \approx 4.3$, 3.8, and 2.0). Typically, 0.1 ml of a FeMo protein solution containing 50 mM Tris·HCl (pH 7.4), 0.25 M NaCl, and *ca.* 0.5–1 mM $Na_2S_2O_4$ was diluted to 0.5 ml with hexamethylphosphoramide (Me_6phosphoramide) containing 25–40 mM *o*-xyl$(SH)_2$ and 0.2 μl of $C_6H_5CFCl_2$. After a 45- to 60-min reaction time at room temperature, 7–10 μl (50–70 μmol) of R_FSH was injected. Extrusion products were detected and quantitated at −15°C by ^{19}F Fourier transform NMR spectroscopy by using a Bruker HXS-360 spectrometer and described procedures (7). Final mole ratios in the extrusion solutions were [*o*-xyl$(SH)_2$]/[protein Fe] $\gtrsim$ 20 and [R_FSH]/[*o*-xyl$(SH)_2$] $\gtrsim$ 5. Control experiments monitored spectrophotometrically (12) (see below) showed that the latter ratio was sufficient to effect completion of reaction 3. FeMo-co was examined under conditions very similar to those resulting in extrusion of FeMo proteins, except that final solutions contained some proportion of *N*-methylformamide. Further experimental details are found in the figure legends; information in parentheses pertains to aqueous solutions prior to dilution with Me_6-phosphoramide. Quantitation of extrusion products is expressed by $n = C_i/C_{protein}$, where C_i is the concentration of $[Fe_nS_n(SR_F)_4]^{2-}$ (n = 2, $n = n_d$; n = 4, $n = n_t$) prior to the addition of standard in *N*,*N*-dimethylformamide solution.

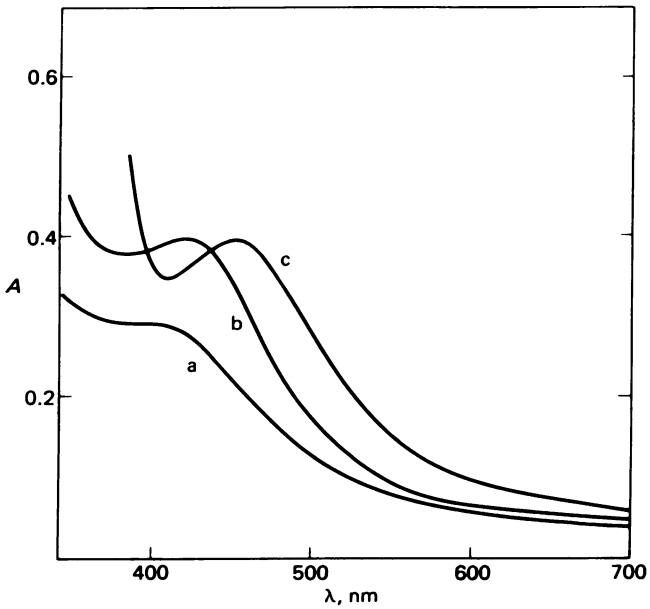

FIG. 1. Core extrusion of *C. pasteurianum* Fd_{ox} in 4:1 (vol/vol) Me_6phosphoramide/H_2O (50 mM Tris·HCl, pH 8.5); l = 1 cm at 25°C. Curves: a, 11 μM Fd_{ox}; b, 11 μM Fd_{ox} plus 10 mM *o*-xyl$(SH)_2$ after ≈60 min; c, solution b plus 24 mM R_FSH after 10 min; n_t = 1.96.

RESULTS AND DISCUSSION

Extrusion of FeMo Proteins. As one control experiment for the extrusion procedure used for the nitrogenase FeMo proteins, the sequential reaction of *C. pasteurianum* Fd_{ox} with *o*-xyl$(SH)_2$ and R_FSH in 4:1 (vol/vol) Me_6phosphoramide/H_2O is illustrated in Fig. 1. Reaction **2** affords the species $[Fe_4S_4(S_2$-*o*-xyl$)_2]^{2-}$,[§] which in reaction **3** is fully converted to the well-characterized complex $[Fe_4S_4(SR_F)_4]^{2-}$ (λ_{max} = 452 nm) (7). Spectrophotometric quantitation yields n_t = 1.96, in excellent agreement with the established presence of two [4Fe-4S] centers in this protein. Quantitative extrusion of the single [2Fe-2S] center in spinach Fd_{ox} by this procedure is presented elsewhere (12). When reactions **2** and **3** were applied to the clostridial FeMo protein, the final absorption spectrum was suggestive of the formation of some $[Fe_4S_4(SR_F)_4]^{2-}$ but was not sufficiently well resolved to permit secure identification and quantitation of extrusion products. Inconclusive results by using reaction **1** with benzenethiol have been obtained by ourselves (7) and others (10, 19).

Quantitative core extrusion results for the two FeMo proteins in Me_6phosphoramide/H_2O, 4:1 (vol/vol), obtained by ^{19}F NMR analysis of the products of reaction 3, are collected in Table 1. Shown in Fig. 2 *left* and *right* are representative ^{19}F NMR spectra of the extrusion solutions initially containing *C. pasteurianum* FeMo protein and *A. vinelandii* FeMo protein, respectively, after completion of reaction. The signals at 6.3 ppm arose from $[Fe_4S_4(SR_F)_4]^{2-}$, as shown by separate measurement of this species in the same medium (7) and by the coincidence of these signals with those of the added standard. No significant signal intensity at 3.7 ppm, the characteristic chemical shift of $[Fe_2S_2(SR_F)_4]^{2-}$ (7), was observed in any case. Quantitation of the 6.3-ppm signals by integration with those from solutions with added standard (7) revealed that 49–56% of total Fe was extruded in experiments 2–4, 6, and 7.

[§] Although the presence of a Fe_4S_4 core in this unisolated species is not in question, its exact formulation is uncertain; other possibilities include $[Fe_4S_4[S(SH)$-*o*-xyl$]_4]^{2-}$ and oligomeric $[Fe_4S_4$-$(S_2$-*o*-xyl$)_2]_n^{2n-}$.

Table 1. Core extrusion results for nitrogenase FeMo proteins and FeMo cofactor

Species initial conc., mM	Exp.	Initial conc., mM [Fe]	Initial conc., mM [Mo]	Extruded $[Fe_4S_4(SR_F)_4]^{2-}$, mM[a]	Extruded [Fe], mM	n_t	Noncofactor Fe extruded, %[b]
C. pasteurianum FeMo[c]							
0.062[d]	1	1.45	0.110	0.0734	0.294	1.2	43
0.041	2	1.16	0.077	0.141	0.564	3.4	90
0.041	3	1.16	0.077	0.161	0.644	3.9	103
0.041	4	1.16	0.077	0.144	0.576	3.5	92
A. vinelandii FeMo[c]							
0.060[d]	5	1.79	0.121	0.0743	0.297	1.2	31
0.053	6	1.58	0.107	0.212	0.848	4.0	101
0.053	7	1.58	0.107	0.198	0.792	3.7	94
A. vinelandii FeMo-co[e]	8	0.442	0.068	Undetectable	0	–	–

[a] Either none or only a trace amount of $[Fe_2S_2(SR_F)_4]^{2-}$ was detectable in any solution.
[b] See text.
[c] In Me_6phosphoramide/H_2O, 4:1 (vol/vol).
[d] Data from ref. 7.
[e] In Me_6phosphoramide/*N*-methylformamide/H_2O, 7:1:2 (vol/vol) (see Fig. 4).

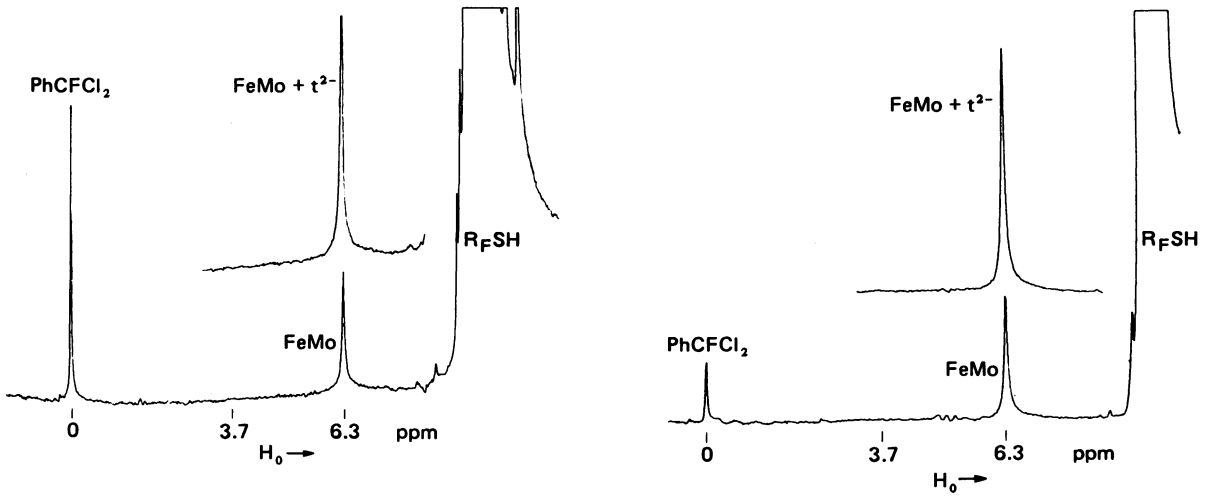

FIG. 2. (*Left*) ^{19}F Fourier transform NMR spectra (338 MHz) of Me_6phosphoramide/H_2O, 4:1 (vol/vol) (50 mM Tris·HCl, pH 7.4/0.25 M NaCl/≈0.5 mM $Na_2S_2O_4$) containing 41 μM *C. pasteurianum* FeMo protein at −15°C after completion of the core extrusion reaction (lower spectrum) and after addition of $[Fe_4S_4(SR_F)_4]^{2-}$ (t^{2-}) standard solution in *N,N*-dimethylformamide (upper spectrum). Spectra were acquired with 5000 pulses (17 min). (*Right*) Conditions were the same as for *Left* except that $Na_2S_2O_4$ was at ≈1 mM and 53 μM *A. vinelandii* FeMo protein was used.

Origin and Quantitation of Extruded Fe. In order to ascertain the origin of the extruded Fe, the FeMo-co isolated from the *A. vinelandii* FeMo protein was subjected to extrusion conditions very similar to those employed with the FeMo proteins. The ^{19}F NMR spectrum resulting from this experiment is shown in Fig. 3. In view of the lower Fe concentration (experiment 8 of Table 1), the spectral acquisition time was twice as long as that normally employed with FeMo protein extrusions. Comparison of the upper and lower spectra in Fig. 3 reveals that FeMo-co *does not yield* detectable quantities of either $[Fe_2S_2(SR_F)_4]^{2-}$ or $[Fe_4S_4(SR_F)_4]^{2-}$ under these conditions. Similarly, neither species was detected upon attempted extrusion in *N*-methylformamide solution. These observations are consistent with the report that benzenethiol does not disrupt FeMo-co (20) and further strengthens the conclusion drawn from spectroscopic evidence (20, 21) that the cofactor does not contain conventional [2Fe-2S] and [4Fe-4S] centers. By using the mean analytical value of 6.92 g-atom of Fe per g-atom of Mo, 90–103% of noncofactor Fe was calculated to have been extruded from the FeMo proteins in five experiments. Given the estimated ±15% error in the quantitation procedure (7) and an expected low determinate error, we conclude that essentially all noncofactor Fe has been extruded.¶ This result contrasts sharply with those of two typical experiments (1 and 5 of Table 1) which employed reaction 1 with R_FSH as the extrusion reagent (7). In these cases only 43% and 31%, respectively, of noncofactor Fe (or 20% and 17% of total Fe) was recovered as $[Fe_4S_4(SR_F)_4]^{2-}$. The results of this and previous work (7) are, however, consistent in that the great majority of Fe displaced from the semireduced proteins occurs in the form of Fe_4S_4 clusters.

Values of n_t, the number of [4Fe-4S] centers per protein molecule, fall in the range 3.4–4.0 and have been obtained with protein preparations having ≈30 ± 2 g-atom of Fe per mol, which is thought to apply to the most highly purified preparations (22). If normalized to the high end of this range, n_t values are 3.8–4.4. We conclude that protein preparations with a full complement of noncofactor Fe contain four [4Fe-4S] centers per molecule [i.e., per $\alpha_2\beta_2$ subunit complex (17, 18, 23)]. We find reasonable the proposed correlation (22) of extrudable [4Fe-4S] centers with the four Fe_4S_4 (P) clusters having unconventional electronic properties whose existence has been deduced from Mössbauer spectroscopic analysis (22). A second major Mössbauer spectral component, M^{EPR}, is associated with FeMo-co (20–22). A minority component S, accounting for ≈6% of total Fe absorption (22), cannot be identified on the basis of our extrusion results.

Extrusion Conditions. The superiority of reactions 2 and 3 compared to reaction 1 alone in achieving a significantly greater extent of extrusion is evident from the data presented in Table 1 and elsewhere (7). The cause of this improvement is not obvious; it seems unlikely that *o*-xyl$(SH)_2$ should possess a better

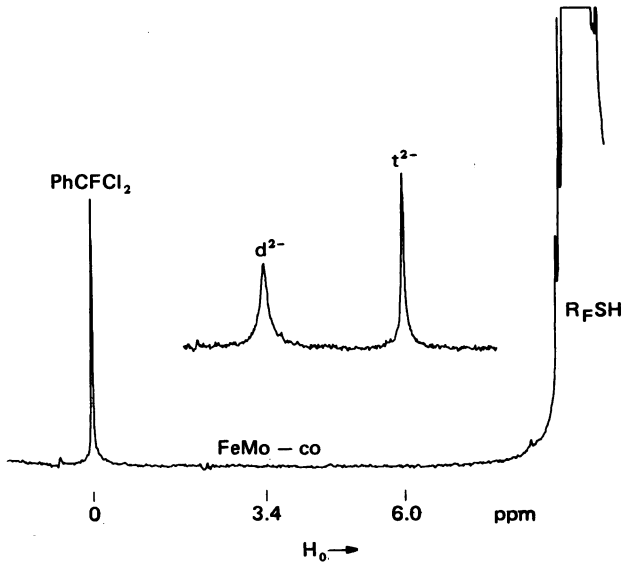

FIG. 3. ^{19}F Fourier transform NMR spectra (338 MHz) of Me_6-phosphoramide/*N*-methylformamide/H_2O, 7:1:2 (vol/vol) (50 mM Tris·HCl, pH 7.4) solutions at −15°C. Lower spectrum: *A. vinelandii* FeMo-co ([Fe] = 0.442 mM and [Mo] = 0.068 mM) in the presence of 10 mM *o*-xyl$(SH)_2$ and 102 mM R_FSH (added 45 min later); spectral acquisition (10,300 pulses, 35 min) began 15 min after addition of R_FSH. Upper spectrum: 0.1 mM $[Fe_2S_2(SR_F)_4]^{2-}$ (d^{2-}) plus 0.1 mM $[Fe_4S_4(SR_F)_4]^{2-}$ (t^{2-}) added in *N,N*-dimethylformamide solutions (2292 pulses, 7.8 min).

¶ If the ratio 8 g-atom of Fe per g-atom of Mo for FeMo-co reported by Shah and Brill (15) is used, apparent values of 104–118% noncofactor Fe extruded are calculated.

ability than R_FSH to penetrate a protein matrix, folded or unfolded.[‖] If full extrusion is kinetically controlled, the dithiol could hasten the reaction by providing higher local concentrations of incoming thiol and general acid, both of which should accelerate the rate-determining step of bound thiolate protonation (24). Another possibility is that *o*-xyl$(SH)_2$ is a superior extrusion reagent for Fe_nS_n cores not wholly attached to the protein by the conventional Fe-S-Cys bonds found in Fd proteins. It is noted that in the two cases in which the dithol has been more effective than R_FSH (or C_6H_5SH) the Fe-S centers (22, 25) do not appear to exhibit the standard properties of Fd centers. The difficulty in extruding these centers may in part result from an unusual protein environment, which is reflected in their spectroscopic properties. In the FeMo proteins one or two [4Fe-4S] centers are, however, readily displaced by R_FSH in reaction **1** (7). These centers may be, in effect, those absent from the metal-depleted protein that has been separated from the clostridial FeMo protein (26), and, thus, they are inherently more labile.

SUMMARY

Extrusion studies of *C. pasteurianum* and *A. vinelandii* FeMo proteins have demonstrated the existence of 3.4–4.0 [4Fe-4S] centers, interpreted as four [4Fe-4S] centers per $\alpha_2\beta_2$ complex in preparations undepleted in Fe. These centers do not derive from FeMo-co and, with the cofactor, represent the two major structural populations of Fe atoms in these proteins. No significant quantity of Fe_2S_2 cores was extruded, in agreement with earlier observations of their extrusion only after protein oxidation (7). Although the extrusion method establishes the presence of [4Fe-4S] centers containing Fe_4S_4 cores in the semireduced FeMo proteins, it does not directly identify the terminal ligands bound to the Fe atoms. Nor does the method convey information concerning environmental features of these centers in the protein matrix. Evidently there are as yet unelucidated factors, perhaps both intrinsic and extrinsic to these centers, that serve to alter their Mössbauer (22), electron paramagnetic resonance (22), and chiroptical (27) properties from those of $[Fe_4S_4(S\text{-}Cys)_4]$ clusters in Fd proteins.

The extrusion results, results from Mössbauer (21, 22), electron paramagnetic resonance (20, 22), magnetic circular dichroism (27), and x-ray absorption spectroscopy (28, 29), and the synthesis of Fe-Mo-S clusters (30–34) containing Mo environments similar by the extended x-ray absorption fine structure criterion to that in the FeMo proteins and cofactor contribute to an emerging molecular description of the metal sites in the nitrogenase enzyme complex.

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1. Holm, R. H. (1977) in *Biological Aspects of Inorganic Chemistry*, eds. Addison, A. W., Cullen, W. R., Dolphin, D. & James, B. R. (Wiley-Interscience, New York), pp. 71–111.
2. Holm, R. H. & Ibers, J. A. (1977) in *Iron-Sulfur Proteins*, ed. Lovenberg, W. (Academic, New York), Vol. III, pp. 205–281.
3. Holm, R. H. (1977) *Accounts Chem. Res.* **10**, 427–434.
4. Que, L., Jr., Holm, R. H. & Mortenson, L. E. (1975) *J. Am. Chem. Soc.* **97**, 463–464.
5. Gillum, W. O., Mortenson, L. E., Chen, J.-S. & Holm, R. H. (1977) *J. Am. Chem. Soc.* **99**, 584–595.
6. Averill, B. A., Bale, J. R. & Orme-Johnson, W. H. (1978) *J. Am. Chem. Soc.* **100**, 3034–3043.
7. Wong, G. B., Kurtz, D. M., Jr., Holm, R. H., Mortenson, L. E. & Upchurch, R. G. (1979) *J. Am. Chem. Soc.* **101**, 3078–3090.
8. Erbes, D. L., Burris, R. H. & Orme-Johnson, W. H. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4795–4799.
9. Hatchikian, E. C., Bruschi, M. & Le Gall, J. (1978) *Biochem. Biophys. Res. Commun.* **82**, 451–461.
10. Orme-Johnson, W. H. & Davis, L. C. (1977) in *Iron-Sulfur Proteins*, ed. Lovenberg, W. (Academic, New York), Vol. III, pp. 15–60.
11. Hill, C. L., Steenkamp, D. J., Holm, R.H. & Singer, T. P. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 547–551.
12. Kurtz, D. M., Jr., Holm, R. H., Ruzicka, F. J., Beinert, H., Coles, C. J. & Singer, T. P. (1979) *J. Biol. Chem.* **254**, 4967–4969.
13. Kurtz, D. M., Jr., Wong, G. B. & Holm, R. H. (1978) *J. Am. Chem. Soc.* **100**, 6777–6779.
14. Coles, C. J., Holm, R. H., Kurtz, D. M., Jr., Orme-Johnson, W. H., Rawlings, J., Singer, T. P. & Wong, G. B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3805–3808.
15. Shah, V. K. & Brill, W. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3249–3253.
16. Mayerle, J. J., Denmark, S. E., DePamphilis, B. V., Ibers, J. A. & Holm, R. H. (1975) *J. Am. Chem. Soc.* **97**, 1032–1045.
17. Huang, T. C., Zumft, W. G. & Mortenson, L. E. (1973) *J. Bacteriol.* **113**, 884–890.
18. Swisher, R. H., Landt, M. L. & Reithel, F. J. (1977) *Biochem. J.* **163**, 427–432.
19. Orme-Johnson, W. H., Davis, L. C., Henzl, M. T., Averill, B. A., Orme-Johnson, N. R., Münck, E. & Zimmerman, R. (1977) in *Recent Developments in Nitrogen Fixation*, eds. Newton, W., Postgate, J. R. & Rodriquez-Barrueco, C. (Academic, New York), pp. 131–178.
20. Rawlings, J., Shah, V. K., Chisnell, J. R., Brill, W. J., Zimmermann, R., Münck, E. & Orme-Johnson, W. H. (1978) *J. Biol. Chem.* **253**, 1001–1004.
21. Huynh, B. H., Münck, E. & Orme-Johnson, W. H. (1979) *Biochim. Biophys. Acta* **527**, 192–203.
22. Zimmermann, R., Münck, E., Brill, W. J., Shah, V. K., Henzl, M. T., Rawlings, J. & Orme-Johnson, W. H. (1978) *Biochim. Biophys. Acta* **537**, 185–207.
23. Lundell, D. J. & Howard, J. B. (1978) *J. Biol. Chem.* **253**, 3422–3426.
24. Dukes, G. R. & Holm, R. H. (1975) *J. Am. Chem. Soc.* **97**, 528–533.
25. Ruzicka, F. J. & Beinert, H. (1978) *J. Biol. Chem.* **253**, 2514–2517.
26. Zumft, W. G. & Mortenson, L. E. (1973) *Eur. J. Biochem.* **35**, 401–409.
27. Stephens, P. J., McKenna, C. E., Smith, B. E., Nguyen, H. T., McKenna, M.-C., Thomson, A. J., Devlin, F. & Jones, J. B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2585–2589.
28. Cramer, S. P., Hodgson, K. O., Gillum, W. O. & Mortenson, L. E. (1978) *J. Am. Chem. Soc.* **100**, 3398–3407.
29. Cramer, S. P., Gillum, W. O., Hodgson, K. O., Mortenson, L. E., Stiefel, E. I., Chisnell, J. R., Brill, W. J. & Shah, V. K. (1978) *J. Am. Chem. Soc.* **100**, 3814–3819.
30. Wolff, T. E., Berg, J. M., Warrick, C., Hodgson, K. O., Holm, R. H. & Frankel, R. B. (1978) *J. Am. Chem. Soc.* **100**, 4630–4632.
31. Wolff, T. E., Berg, J. M., Hodgson, K. O., Frankel, R. B. & Holm, R. H. (1979) *J. Am. Chem. Soc.* **101**, 4140–4150.
32. Wolff, T. E., Berg, J. M., Power, P. P., Hodgson, K. O., Holm, R. H. & Frankel, R. B. (1979) *J. Am. Chem. Soc.*, in press.
33. Christou, G., Garner, C. D., Mabbs, F. E. & King, T. J. (1978) *J. Chem. Soc., Chem. Commun.*, 740–741.
34. Christou, G., Garner, C. D., Mabbs, F. E. & Drew, M. G. B. (1979) *J. Chem. Soc., Chem. Commun.*, 91–93.

[‖] The last sentence in the abstract of ref. 7 on this point is an error in proof and should be disregarded.