Incorporation of Molybdenum into the Iron-Molybdenum Cofactor of Nitrogenase*

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The biosynthesis of the iron-molybdenum cofactor (FeMo-co) of dinitrogenase was investigated using 99Mo to follow the incorporation of Mo into precursors. 99Mo label accumulates on dinitrogenase only when all known components of the FeMo-co synthesis system, NifH, NifNE, NifB-cofactor, homocitrate, MgATP, and reductant, are present. Furthermore, 99Mo label accumulates only on the gamma protein, which has been shown to serve as a chaperone/insertase for the maturation of apodinitrogenase when all known components are present. It appears that only completed FeMo-co can accumulate on the gamma protein. Very little FeMo-co synthesis was observed when all known components are used in purified forms, indicating that additional factors are required for optimal FeMo-co synthesis. 99Mo did not accumulate on NifNE under any conditions tested, suggesting that Mo enters the pathway at some other step, although it remains possible that a Mo-containing precursor of FeMo-co that is not sufficiently stable to persist during gel electrophoresis occurs but is not observed. 99Mo accumulates on several unidentified species, which may be the additional components required for FeMo-co synthesis. The molybdenum storage protein was observed and the accumulation of 99Mo on this protein required nucleotide.

The iron-molybdenum cofactor (FeMo-co)1 of dinitrogenase (Fig. 1) constitutes the active site of the nif-encoded, molybdenum-containing dinitrogenase protein in Azotobacter vinelandii and other nitrogen-fixing organisms (1–3). FeMo-co can be isolated by extraction from the purified dinitrogenase protein (2), and the isolated cofactor can be used to activate FeMo-co-deficient forms of dinitrogenase (referred to hereafter as “apo-dinitrogenase”) that accumulate in strains unable to synthesize the cofactor (2, 4, 5). FeMo-co consists of Mo, Fe, and S atoms in a 1:7:9 ratio; in addition, the organic acid homocitrate is an integral component of the compound (6), serving as a nonprotein ligand to the molybdenum atom. The structure of FeMo-co

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1 The abbreviations used are: FeMo-co, iron-molybdenum cofactor of nitrogenase; Mo-Sto, molybdenum storage protein; NifB-co, iron- and sulfur-containing precursor to FeMo-co; DTH, sodium dithionite.

in the protein was determined by Kim and Rees (7) and Chan et al. (8).

Genetic studies have revealed that functional copies of the nifB, nifN, nifE, nifH, and nifV genes are required for synthesis of FeMo-co in vivo (9–11); the nifQ gene is also required under conditions of molybdenum limitation (12). The nifKD genes, which encode the subunits of dinitrogenase (NifKD), are not required for FeMo-co synthesis, and thus FeMo-co is not synthesized “in place” but rather is preformed and then transferred to its site in dinitrogenase (13). In the absence of NifKD, completed FeMo-co accumulates on a protein called gamma, which serves as a chaperone/insertase for the maturation of NifKD and for insertion of FeMo-co (14).

An in vitro FeMo-co synthesis system was devised to address the biochemical roles of these genes and to identify other factors required for FeMo-co synthesis (15). In this system, at least homocitrate, molybdenum (supplied as molybdate), MgATP, NifB-co, NifH (dinitrogenase reductase), reductant, and NifNE are required to achieve FeMo-co synthesis. Each of these components is available in a purified form, but when they are added together, very little FeMo-co synthesis is observed, suggesting that additional factor(s), yet to be discovered, are required.

Previous studies have shown that NifB-co contains iron and sulfur (16, 17). Purified, 55Fe- and 35S-labeled NifB-co has been shown to bind to NifNE in the absence of other factors, and the label can be shown to accumulate on the gamma protein and in dinitrogenase (17, 18). Thus NifB-co is believed to be the primary, if not sole, iron donor to FeMo-co.

The entry point of molybdenum into the pathway of FeMo-co synthesis has not been established. Pienkos and Brill (19) used 95MoO4− to study the incorporation of molybdenum in A. vinelandii cells in vivo. They observed the accumulation of molybdenum in a non-nif protein that they termed the molybdenum storage protein (Mo-Sto) and in the dinitrogenase protein in vivo. Ugalde et al. (13) noted the accumulation of 99Mo on a 65-kDa protein in K. pneumoniae in nifKD mutants; this protein may be the nifY gene product or an analog of the gamma protein. The accumulation of 99Mo on dinitrogenase and on possible precursors during in vitro FeMo-co synthesis was investigated by Hoover et al. (3) using various homologs of homocitrate in the synthesis system.

In this study, the incorporation of 99Mo into protein-bound precursors of FeMo-co during in vitro FeMo-co synthesis was investigated. In vitro FeMo-co synthesis mixtures containing extracts and cofactors were mixed with 99MoO4− under anoxic conditions and following incubation to allow FeMo-co synthesis; the mixtures were electrophoresed on nondenaturing polyacrylamide gels. Protein bands containing 99Mo were detected by phosphorimage analysis. Where possible, 99Mo-labeled protein bands were identified by co-migration with purified proteins.
known to be involved in FeMo-co synthesis and by reaction with antibodies against those known proteins on immunoblots.

**Experimental Procedures**

**Strains and Growth Conditions**—A list of the Azotobacter vinelandii mutant strains used in this study is presented in Table I. In order to avoid dilution of the $^{99}$Mo with nonradioactively labeled molybdenum, all A. vinelandii strains in this work (except as specified) were grown and derepressed in medium containing tungsten in place of molybdenum as described previously (15). Cells grown in medium containing tungsten in place of molybdenum accumulate apodinitrogenase and all other identified components required for FeMo-co synthesis (19).

**Preparation of Extracts**—Cell-free extracts were prepared as described (20). Small molecules (i.e., homocitrate and nucleotides) were removed from the cell extracts by Sephadex G-25 column chromatography. Where necessary, crude extracts were chemically oxidized as described previously (21).

**Anoxic Native Gel Electrophoresis, Western Immunoblots, and Visualization of Radioactivity**—Gel electrophoresis, Western immunoblots, and phosphorimaging were performed as described previously (17).

**In Vitro FeMo-co Synthesis Assay**—Nine-ml serum vials were flushed with purified argon and rinsed with anoxic, 25 mM Tris-HCl (pH 7.4) containing 1.7 mM sodium dithionite (DTH). Complete FeMo-co synthesis reaction mixtures (defined below) were prepared by combining the following: 100 µl of anoxic 25 mM Tris-HCl (pH 7.4) containing 1.7 mM DTH, 10 µl of 10 µM Na$_2$MoO$_4$, 20 µl of 5 mM homocitrate (which had been treated with base to cleave the lactone, pH 8.0)) and 200 µl of an ATP-regenerating mixture (containing 3.6 mM ATP, 6.3 mM MgCl$_2$, 51 mM phosphocreatine, 20 units/ml creatine phosphokinase, and 6.3 mM DTH in 25 mM Tris-HCl, pH of the mixture was 7.4). These mixtures were incubated anoxically at room temperature for 10–15 min. Two hundred µl of the appropriate A. vinelandii cell-free extract, 10 µl of a solution containing an excess of NiIF-co as Fe and S donor for FeMo-co (16), and 0.1 mg of purified dinitrogenase reductase (10 µl) were added to the reaction mixture. One-half µl (carrier free) of Na$_3$MoO$_4$ (10 µl) (Nordion, Ontario, Canada) was prepared in 25 mM Tris (pH 7.4) containing 1.7 mM DTH was then added to the reaction. The total volume of each assay mixture was 560 µl. The vials were incubated in a rotary water-bath shaker at 30 °C for 30 min to allow time for FeMo-co synthesis and its subsequent insertion into apodinitrogenase. After this incubation (designated the “incubation phase” of the assay), samples to be applied onto native polyacrylamide gels were placed on ice until loading. To demonstrate that all components of the assay were functional, the synthesis of FeMo-co was monitored in duplicate vials using the acetylene reduction assay (17, 22) to detect newly formed active nitrogenase molecules present in the assay are activated by binding of the acetylene reduction assay (17, 22) to detect newly formed active nitrogenase. The purified in *vitro* FeMo-co synthesis reaction included NiIF, NiIF, homocitrate, Na$_3$MoO$_4$, MgATP, DTH, and NiFB-co. No apodinitrogenase was included in the experiments that employed the purified system; therefore, no label was detected at the position of dinitrogenase in those gels.

**Purification of Other Components**—FeMo-co was purified as described by Shah and Brill (2). Dinitrogenase and NiIF (dinitrogenase reductase) proteins were prepared as described previously (23). NiIF was purified as described in Ref. 14, was a gift from Mary Homer. Dinitrogenase reductase protein from *Clostridium pasteurianum* was a gift from Dr. Lance Seefeldt. Antibodies against various proteins were prepared at the UW antibody facility.

**Results and Discussion**

99$^Mo$ Is Detected on the Molybdenum Storage Protein—When 99$^Mo$-labeled molybdate is supplied to extracts capable of FeMo-co synthesis, two proteins become prominently labeled (Fig. 2, lane 2). These are the dinitrogenase protein and the molybdenum storage protein (Fig. 2, Mo-Sto). Mo-Sto was previously shown to accumulate 99$^Mo$ in *vivo* and the protein was purified by Pienkos and Brill (19). Mo-Sto is not co-regulated with nitrogenase, and the protein purified as an $\alpha$$_2$$\beta_2$ tetramer of 21 and 24 kDa subunits with approximately 15 Mo atoms/tetramer, although the Mo content of the protein decreased during purification. The protein labeled as Mo-Sto in Fig. 2 and elsewhere is proposed to be the same protein based on its accumulation of Mo, its position of migration on nondenaturing gels, and its expression in NH$_4$-grown cells. Based on its size and on results presented here, Mo-Sto would not appear to be the periplasmic molybdate-binding protein, ModA, or the molybdate-binding regulatory protein, ModE (24, 25). Mo-Sto is soluble, and thus it is not likely to be the product of the modB, modC, or modD genes, which encode the membrane-bound molybdate transport system that has been described in *A. vinelandii* and *Escherichia coli*.

**Nucleotide Is Required for the In Vitro Labeling of Mo-Sto**—Previously, it had been shown that Mo-Sto was 99$^Mo$-labeled during *in vitro* FeMo-co synthesis, and studies here expand that observation to show that either ATP or ADP is required for accumulation of 99$^Mo$ on Mo-Sto (Fig. 2) (26). In the absence of nucleotide, label does not accumulate on any protein observed by phosphorimaging. At this point, it is unknown whether the nucleotide is required to allow MoO$_4^{2-}$ to bind to Mo-Sto or if it is required to allow the removal of nonradioactive species (MoO$_4^{2-}$ or WO$_4^{2-}$) already bound to the protein; thereby making sites for binding of 99$^MoO_4^{2-}$ available. Previous studies showed that 185W accumulated in 185WO$_4^{2-}$-grown cells on a protein that copurified with Mo-Sto (19). The extracts used in experiments reported here were prepared from cells grown in the absence of Na$_2$MoO$_4$ and the presence of Na$_2$WO$_4$ to prevent the accumulation of molybdenum-containing intermediates in the FeMo-co synthetic pathway. Thus, it is possible that

**Table I**

| Strain | Relevant characteristics | Ref. |
|--------|--------------------------|------|
| UW45   | nif/B                    | 35   |
| DJ677  | Δnif/B::kan Δnif/DK      | 18   |
| DJ35   | Δnif/E                   | 36   |
| CA11.1 | Δnif/DIK Δnif/DKI::spc   | 37   |
| DJ224  | Δnif/W                   | 38   |
| UW97   | nif/H                    | 28   |
| CA12   | Δnif/DIK                 | 39   |
nucleotide is required to allow unlabeled W species to vacate sites for $^{99}$MoO$_4^-$ This question will be addressed in future studies.

ATP Is Required for $^{99}$Mo to Enter the FeMo-co Biosynthetic Pathway—Previous studies have shown that ATP is required for the biosynthesis of FeMo-co and for the insertion of FeMo-co. Results in Fig. 2 show that ATP (Fig. 2, lane 2), but not ADP (Fig. 2, lane 3), will support synthesis of FeMo-co when all other required factors are present. In the absence of nucleotide, $^{99}$Mo does not accumulate on any protein band and when ADP is supplied, Mo-Sto becomes labeled, but Mo does not enter the FeMo-co biosynthetic pathway. Thus, the requirement for nucleotide does not represent merely a requirement for label to pass through Mo-Sto, as that protein can be labeled in the absence of ADP.

$^{99}$Mo Accumulates on the Dinitrogenase Protein—As shown in Figs. 2 and 3, $^{99}$Mo accumulates on the protein identified as the dinitrogenase protein when a complete in vitro FeMo-co synthesis mixture was used. This band has been identified as dinitrogenase by comigration with purified dinitrogenase. The amount of Mo incorporated into FeMo-co and inserted into dinitrogenase in a typical complete reaction mixture (for example, Fig. 3, lane 1) was estimated to be approximately 1.2 atoms of Mo per molecule of dinitrogenase. The amount of dinitrogenase present was estimated by quantitation of the protein band by comparison with known amounts of $^{99}$Mo blotted on a piece of filter paper. ImageQuant (Applied Biosystems) software was used in the measurement. Furthermore, an assay equivalent to that shown in Fig. 3, lane 1, exhibits >80% of the acetylene reduction activity of a mixture to which an excess of purified FeMo-co is added (e.g., Fig. 3, lane 6). When one or more components of the FeMo-co synthesis reaction mixture are left out of the assay, $^{99}$Mo does not accumulate on dinitrogenase, and acetylene reduction activity is not observed. As noted above, ATP is also essential for label to appear in the dinitrogenase band (Fig. 2). When any of the following are eliminated from the reaction mixture, accumulation of $^{99}$Mo on dinitrogenase is not observed: NifNE (Fig. 3, lane 4), NifB-co (Fig. 3, lane 5), and NiH (Fig. 3, lane 7). Furthermore, when an extract of nif-repressed (NH$_4^+$-grown), wild type A. vinelandii is used in the assay, only Mo-Sto is observed to accumulate $^{99}$Mo, and neither dinitrogenase nor any other protein is labeled. Finally, if the assay is saturated with unlabeled FeMo-co, $^{99}$Mo does not accumulate on dinitrogenase (Fig. 3, lane 6); this indicates that all the sites for FeMo-co on dinitrogenase are occupied and any $^{99}$Mo-labeled FeMo-co that is synthesized accumulates elsewhere. In the reaction mix used for Fig. 3, lane 6, the label accumulates on gamma protein and on an unidentified band (band B). The migration of gamma protein at this position has been established by comigration with purified gamma protein and by cross reaction with anti-gamma protein antibodies. When homocitrate is left out of the reaction mixture, label fails to accumulate as completed FeMo-co on gamma protein (Fig. 3, lane 8; in this reaction, both homocitrate and apodinitrogenase were left out of the mix). This result suggests that only completed FeMo-co is capable of being stably bound to gamma protein.

$^{99}$Mo Is Not Observed on NifNE—It is surprising that no $^{99}$Mo is detectable on NifNE in these experiments. From previous studies, it is known that NifNE migrates a little faster than dinitrogenase in the gel system employed here (17, 18). It is known that purified NifNE and NiFNE in an in vitro FeMo-co synthesis mixture will bind FeMo-co and that no additional factors are required for NifNE to bind FeMo-co (17, 18). Fe-labeled NifB-co is known to donate Fe atoms to FeMo-co (17). A significant degree of sequence similarity is observed between nifNE and nifKD, and the cysteine residue that serves as the ligand to FeMo-co in dinitrogenase is conserved in the deduced sequence of NifNE (27). Thus it has been proposed that NifNE is the scaffold upon which FeMo-co is completed. The results presented here do not support this hypothesis, as $^{99}$Mo is not observed at the position of the NifNE band under any condi-
tions. If Mo did bind to NiNE, accumulation of $^{99}$Mo on NiNE might have been expected in a mixture in which a component that performed a step in FeMo-co synthesis subsequent to NiNE was omitted from the reaction mixture; no reaction mixture lacking a single component of the assay mixture resulted in accumulation of $^{99}$Mo on NiNE (Fig. 3). Furthermore, binding of NiB-co to NiNE is not inhibited by FeMo-co (17).

It is very important to note that only those complexes of proteins with $^{99}$Mo that are sufficiently stable to remain intact during electrophoresis will be observed with the approach employed here. Therefore, it is possible that Mo does enter the pathway at the NiNE step but that the resulting intermediate is not stable during electrophoresis. Our working hypothesis is that Mo enters the FeMo-co synthesis pathway at a step subsequent to NiNE.

$^{99}$Mo Accumulates on the Gamma Protein—The gamma protein serves as a chaperone/insertase for the maturation of apodinitrogenase and the insertion of FeMo-co (14, 28). It was first observed as a protein that co-purified with apodinitrogenase from nifB mutants (5). Subsequent work has shown that gamma protein is associated with the nifKD gene products in extracts of nifB mutants. Gamma protein becomes associated with apodinitrogenase in the presence of NiFH and MgATP (apodinitrogenase from nifH mutants accumulates as an $\alpha_2\beta_2$ tetramer of nifK and nifD gene products) and is required for the insertion of FeMo-co (28). Free gamma protein exists as a dimer and binds purified FeMo-co in the absence of any additional factors; upon binding FeMo-co, gamma dimers dissociate into monomeric gamma:FeMo-co units (14). In the in vitro FeMo-co synthesis system, $^{99}$Mo accumulates at the position of the gamma protein in a system that includes all components required for FeMo-co synthesis, but lacks the apodinitrogenase (Fig. 3, lane 2). When the apodinitrogenase is available, most of the label accumulates on dinitrogenase as a result of insertion of completed FeMo-co into the apodinitrogenase. In these experiments, the position of gamma protein with FeMo-co bound was confirmed by co-electrophoresis with purified material. $^{99}$Mo is not observed at the position of the gamma protein if NiNE, NiB-co, NiFH, ATP, or homocitrate is excluded from the reaction mixture (Fig. 3, lanes 4, 5, 7, and 8; Fig. 2, lane 1). Because purified gamma protein will bind FeMo-co, these results are consistent with the hypothesis that the label accumulating at the position of gamma protein represents completed FeMo-co bound to gamma protein. It is possible that the label represents an immature form of FeMo-co bound to gamma protein, but there are no data to suggest that hypothesis. Accumulation of $^{99}$Mo on gamma protein does not occur when an extract of nif-repressed, wild type A. vinelandii is used in the assay (Fig. 3, lane 3) even though that extract contains gamma protein; expression of gamma protein is not co-regulated with the nif regulon (14). Thus, the accumulation of $^{99}$Mo on the gamma protein requires production of the nif regulon that perform known steps of FeMo-co synthesis. Note that the assay with the NEH$_2$-grown extract did contain added ATP, homocitrate and NiB-co; these plus the gamma protein are not sufficient to allow accumulation of $^{99}$Mo on any protein in the extract other than Mo-Sto. When excess, unlabeled FeMo-co is added to the complete FeMo-co synthesis mixture, a decreased amount of $^{99}$Mo is observed on gamma protein (Fig. 3, lane 6), indicating that most of the sites are occupied by the added FeMo-co.

What Is the Role of NiFH in FeMo-co Synthesis?—NiFH (dinitrogenase reductase) plays several roles in nitrogen fixation. In addition to serving as the unique electron donor to dinitrogenase for N$_2$ reduction, NiFH is required for both FeMo-co synthesis and insertion (10, 28, 29). In the absence of NiFH, no $^{99}$Mo accumulates on any protein other than Mo-Sto in the in vitro FeMo-co synthesis system (Fig. 3, lane 7); thus, Mo does not appear to enter the FeMo-co synthesis pathway in the absence of NiFH. In the gel system employed here, NiFH migrates in the region of the protein bands labeled C and D in Fig. 3. At this time, it is not possible to state definitively that one or the other of these $^{99}$Mo-labeled bands contains NiFH. Identification of the proteins forming bands C and D will require at least partial purification of the labeled proteins, and this is being pursued at this time. It is interesting that a $^{55}$Fe-labeled band is observed in the same region of the gel as bands C and D when $^{55}$Fe-labeled NiB-co is used in the in vitro system (i.e., lanes 2 and 5 of Fig. 3 of Ref. 17). Like bands C and D, that $^{55}$Fe-labeled band appeared in reactions lacking homocitrate, consistent with the hypothesis that they represent FeMo-co precursors.

Collectively considered, these results suggest a model in which NiB-co, synthesized by NiFH, is donated to NiNE. In a MgATP- and NiFH-dependent step, molybdenum is incorporated and accumulates on the protein bands marked as C and D. Homocitrate is added last either as a prerequisite to transfer of completed FeMo-co to gamma protein or as the final step of FeMo-co synthesis on gamma protein. FeMo-co accumulates on gamma protein until NiFH-mediated association of gamma protein and apodinitrogenase occurs, whereupon gamma protein transfers FeMo-co to apodinitrogenase. At some point, perhaps when MoO$_4^{2-}$ enters the pathway, it must be reduced from Mo(VI) to Mo(IV), the formal oxidation state proposed to be in the position of completed FeMo-co into the apodinitrogenase. In these experiments, the migration of the proteins forming bands C and D when $^{55}$Fe-labeled band appeared in reactions lacking homocitrate, very little active dinitrogenase is formed. The incorporation of $^{99}$Mo into components of the purified system was investigated, and the results are shown in Fig. 4. No $^{99}$Mo accumulated in any band in the absence of NiB-co (Fig. 4, lane 1), NiFH (lane 2), NiNE (lane 3), or ATP (lane 4). In the complete purified system, a small amount of label accumulated in the region of bands C and D, similar to the bands C and D of Fig. 3. In the absence of homocitrate, $^{99}$Mo accumulated in a band that moves more slowly than band C and D; this band was not observed in the crude system. Although complexes of citrate with MoO$_4^{2-}$ and homocitrate with vanadate have been reported (30–32), MoO$_4^{2-}$:homocitrate did not form a species that migrates as a distinct band in our gel system; for example, such a band would be expected in Fig. 4, lanes 1–4. It is difficult to avoid the conclusion that one or more of the purified proteins added to the system is responsible for the $^{99}$Mo-labeled bands seen in lanes 5 and 6. These bands are in the approximate position of NiFH, and this protein is the best candidate for these bands, although we lack a positive demonstration of NiFH at exactly these positions. From other work, it is known that binding of metal clusters to proteins can affect the migration of the protein on gels (18).

Time Course of $^{99}$Mo-Labeling of Mo-Sto and Dinitrogenase—Fig. 5 shows a time course of labeling of Mo-Sto and
dinitrogenase using a crude extract of *A. vinelandii* strain UW45 (nifB) plus added NifB-co, homocitrinate, and MgATP. Mo-Sto becomes labeled first, and dinitrogenase fails to show marked labeling until the 10 min time point. More importantly, it appears that the entry of Mo into the FeMo-co biosynthetic pathway is a slow step in the process, as there is no significant accumulation of label on gamma protein or bands C and D. Any Mo that makes it into the system is rapidly converted to completed FeMo-co that is inserted into dinitrogenase.

*Use of NifH from *C. pasteurianum*—NifH from *C. pasteurianum* was used in place of NifH from *A. vinelandii* to supplement a crude extract of *A. vinelandii* strain UW97 (nifH) that lacks NifH. NifH from *C. pasteurianum* is known to form an inhibitory complex with *A. vinelandii* dinitrogenase that is unable to reduce substrates (33). It was thought that *C. pasteurianum* NifH might fail to function in FeMo-co synthesis or might form an inhibitory complex with a component of FeMo-co synthesis, leading to accumulation of an intermediate in the pathway. As seen in Fig. 6, *C. pasteurianum* NifH functions effectively in FeMo-co synthesis in the crude *A. vinelandii* system, indicating that it possesses the structural features required for this role of NifH. Some 99Mo accumulation on gamma protein is observed in this experiment, indicating that the *C. pasteurianum* NifH might not be fully effective in mediating the association of gamma protein with *A. vinelandii* dinitrogenase. Previously, we have shown that VnfH from *A. vinelandii* will substitute for NifH from *A. vinelandii* in FeMo-co synthesis and gamma protein-dependent insertion (34).

The results presented here show that 99Mo label accumulates on dinitrogenase only when all known components of the FeMo-co synthesis system are present. Furthermore, 99Mo label only accumulates on the gamma protein, which has been shown to be a chaperone/insertase for the maturation of apo-dinitrogenase when all known components are present. It appears that only completed FeMo-co can accumulate on the gamma protein. Using all known components in purified form, very little FeMo-co synthesis was observed, and therefore, additional factors are required for optimal FeMo-co synthesis. 99Mo was not observed on NifNE under any conditions tested, suggesting that Mo enters the pathway at some other step, although it remains possible that a Mo-containing precursor of FeMo-co that is not sufficiently stable to persist during gel electrophoresis occurs but was not observed. 99Mo accumulates on several unidentified species, which may be the additional components required for FeMo-co synthesis. A nucleotide dependence for accumulation of Mo on Mo-Sto was demonstrated.

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