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In Vitro Incorporation of Molybdate into Demolybdoproteins in *Escherichia coli*

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When *Escherichia coli* was grown in the presence of tungstate, inactive forms of two molybdoenzymes, nitrate reductase and formate dehydrogenase, accumulated and were converted to their active forms upon incubation of cell suspensions with molybdate and chloramphenicol. The conversion to the active enzymes did not occur in cell extracts. When incubated with [^{99}Mo]molybdate and chloramphenicol, the tungstate-grown cells incorporated ^{99}Mo into protein components which were released from membranes by procedures used to release nitrate reductase and formate dehydrogenase and which migrated with these activities on polyacrylamide gels. Although neither activity was formed during incubation of the crude extract with molybdate, ^{99}Mo was incorporated into protein components which were released from the membrane fraction under the same conditions and were similar to the active enzymes in their electrophoretic properties. The in vitro incorporation of ^{99}Mo occurred specifically into these components and was equal to or greater than the amount incorporated in vivo under the same conditions. Molybdenum in preformed, active nitrate reductase and formate dehydrogenase did not exchange with [^{99}Mo]molybdate, demonstrating that the observed incorporation depended on the demolybdo forms of the enzymes. We conclude that molybdate may be incorporated into the demolybdo forms both in vivo and in vitro; some unknown additional factor or step, required for active enzyme formation, occurs in vivo but not in vitro under the conditions employed.

The in vitro reconstitution of several molybdoenzymes from their inactive demolybdo forms have been found to require the addition of a low-molecular-weight molybdenum-containing component. Nason et al. (7, 13, 14) demonstrated that *nit-1* mutants of *Neurospora crassa* accumulated an inactive form of nitrate reductase which was activated in vitro either by extracts of *nit-2* or *nit-3* mutants of *N. crassa* or by a low-molecular-weight fraction produced by acid treatment of several molybdoproteins. Similarly, inactive demolybo forms of nitrogenase from *Azotobacter vinelandii* (12) and of sulfite oxidase from rat liver (5) were activated by molybdenum-containing cofactor preparations derived from different sources including purified molybdoproteins. Molybdate ions would not replace the molybdenum cofactor except in the case of sulfite oxidase where a small, unstable portion of the demolybdoenzyme was activated by the free ion (6). The failure of molybdate to activate the demolybdoenzymes has generally been assumed to reflect a specific requirement for a molybdenum-containing cofactor in the activation process. Recently Pienkos et al. (15) have demonstrated that at least two distinct types of molybdenum cofactors can be prepared which specifically activate the demolybdo forms of different types of molybdoenzymes.

In *Escherichia coli,* nitrate reductase and formate dehydrogenase are molybdoprotein components (2, 21) of a membrane-bound, anaerobic electron transport pathway (4, 16, 17). When *E. coli* was grown in the presence of tungstate, inactive forms of both enzymes accumulated and the active enzymes were formed in the absence of protein synthesis when the tungstate-grown cells were incubated with molybdate (18, 20).

Little, if any, active nitrate reductase or formate dehydrogenase was formed when extracts of these cells were incubated with molybdate. These results suggested that, as in the cases of the other molybdoproteins which have been studied, molybdate is not incorporated directly into the demolybdoproteins but some process is required, perhaps molybdenum cofactor formation, which is lost during cell breakage. In initial attempts we have been unable to demonstrate significant activation of formate dehydrogenase

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or nitrate reductase in extracts of the tungstate-grown cells with preparations of the molybdenum cofactor prepared by acid treatment as described by others (7).

We have, therefore, investigated more directly the incorporation of molybdate into the demolybdoproteins by utilizing ^{99}Mo-labeled molybdate. Although active formate dehydrogenase and nitrate reductase are not formed in extracts of *E. coli*, ^{99}Mo is incorporated specifically into proteins which are electrophoretically similar to the two active enzymes.

MATERIALS AND METHODS

Culture. *E. coli* K-12 strain PK27 (3) was maintained on L-agar (8). PK27 was grown either on minimal salts medium supplemented with nutrient broth, KNO_3, and glucose (20) or on L-broth supplemented with 1.0% (wt/vol) KNO_3. Where noted, sodium tungstate was added to the growth medium.

Assay procedures. Nitrate reductase was assayed by using reduced methylviologen as the electron donor as previously described (19). One unit of enzyme is defined as the amount of enzyme necessary to produce 1 μmol of nitrite per min. Formate dehydrogenase was measured by using dichlorophenolindophenol reduction mediated with phenazine methosulfate (17). All enzyme assays were performed at 37°C.

Protein content was estimated in some cases from the turbidity of cell suspensions (9) or was determined by using the Folin phenol reagent (10).

Polyacrylamide gels. Polyacrylamide gels at pH 8.9 were prepared essentially by the procedure of Davis (1). To study heat-released membrane proteins, 7% acrylamide gels were used with stacking gels as previously described (20). The procedure to demonstrate detergent-released, membrane proteins utilized 5% acrylamide gels with 0.1% (wt/vol) Triton X-100 in gels and buffers (18). Bromophenol blue was used as a tracking dye.

Nitrate reductase activity was located on gels by the bleaching of reduced methylviologen in the presence of nitrate (11). Formate dehydrogenase activity was located by a technique utilizing phenazine methosulfate and *para*-Nitro Blue Tetrazolium (18). Proteins were stained with Coomassie brilliant blue.

Gels were fractionated into 2-mm sections by a Gilson fractionator. Each fraction was crushed and transported into a scintillation vial with 0.2 ml of water to which 10 ml of ACS scintillation cocktail (Amersham Corp.) was subsequently added. The amount of ^{99}Mo was determined in each fraction by using an LS-233 Beckman scintillation counter.

Materials. ^{99}Mo was obtained as Na_2MoO_4 (carrier free) from Union Carbide Corp., Sterling Forest Laboratories, Tuxedo, N.Y. Other materials used were obtained commercially and were analytical reagent grade.

RESULTS

Nitrate reductase and the inactive form of nitrate reductase which accumulates during growth on tungstate can be released from membranes of *E. coli* by heat treatment and identified after electrophoresis on polyacrylamide gels (20). To assess directly the possibility that molybdate is incorporated in vitro into the demolybdonitrate reductase, cells were grown in medium containing 1 mM tungstate and then harvested and washed in the presence of chloramphenicol. The cells were resuspended, and one-half of the suspension was incubated with [^{99}Mo]molybdate in the presence of chloramphenicol for 60 min. A crude extract was prepared from the remainder of the suspension and incubated under similar conditions. Finally, membrane fractions were prepared from each of the incubated samples and heat treated to release nitrate reductase or its inactive form. After electrophoresis of the released fractions on polyacrylamide gels, nitrate reductase activity was located on the gel prepared from the sample of incubated cells. The gels were crushed and fractionated, and radioactivity was determined in a scintillation counter (Fig. 1). No nitrate reductase activity was formed in the extract, whereas essentially normal levels of the activity were formed in the cell suspension upon incubation with molybdate. In contrast, ^{99}Mo was incorporated both in vitro and in vivo into components which migrated on the gels similarly to nitrate reductase activity. The profile of ^{99}Mo in the in vitro sample appeared somewhat broader than that in the in vivo sample, but the total radioactivity in the peak fractions was essentially the same. The samples applied to the gels contained essentially equal amounts of protein (Fig. 1), and the total radioactivity found in the peak fractions was 276 cpm for the in vivo sample and 267 cpm for the in vitro sample. Some ^{99}Mo was located at the top of each gel, but no other significant peaks of ^{99}Mo were observed.

These results suggested that molybdate was incorporated into the demolybdo form of nitrate reductase in a crude extract without the appearance of nitrate reductase activity. However, this finding could be explained in several different ways. First, molybdenum incorporation into the demolybdoenzyme may occur directly from molybdate, but some additional step may be required for the formation of activity and this step may not occur in crude extracts under the conditions employed. Second, molybdenum incorporation may occur normally in the crude extract with the formation of active nitrate reductase, but nitrate reductase may be unstable in the crude extract under the conditions of the 60-min incubation. A third more trivial explanation is that, because the nitrate reductase is the major protein released by the heat treatment (11),

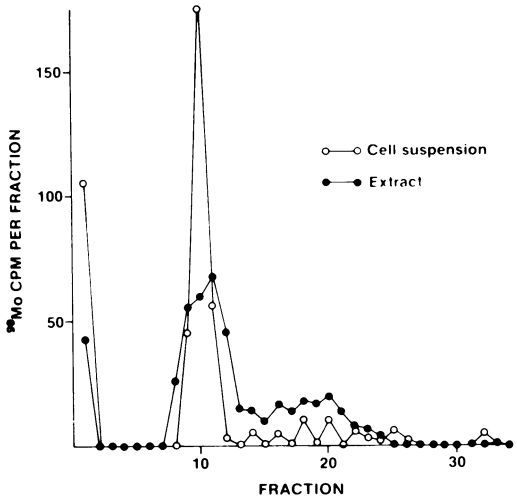

FIG. 1. *Incorporation of [^{99}Mo]molybdate into demolybdonitrate reductase in vivo and in vitro. Cells were grown on minimal salts medium supplemented with nutrient broth, KNO_3, glucose, and 1 mM sodium tungstate. When the bacteria reached about 70 Klett units (filter 54), chloramphenicol was added to a final concentration of 50 μg/ml. The cells were harvested by centrifugation and washed two times in 50 mM KPO_4 buffer, pH 7.0, containing 50 μg of chloramphenicol per ml. One-half of the suspension was incubated with 1 mM sodium molybdate containing 100 μCi of ^{99}Mo for 60 min at 37°C. The remaining half of the cells were broken in a French press at 15,000 lb/in^2; whole cells were removed by centrifugation for 5 min at 10,000 × g. Then the crude extract was incubated under the same conditions as the cell suspension. After the incubation, the bacteria of the cell suspension were fractured in the French press and centrifuged as in the preparation of the crude extract. The membrane fractions of both samples were prepared and heat treated, and the release proteins were run on polyacrylamide gels as previously described (11, 20). Identical volumes were used throughout the preparation of both samples, and an identical volume of each heat-released fraction was applied to each gel. Fractions were collected and assayed for nitrate reductase activity and ^{99}Mo content as described in the text.*

the apparent association of ^{99}Mo with the demolybdo form may represent relatively unspecific binding. The following experiments were conducted in an attempt to distinguish between these interpretations and to extend the observation to a second molybdoenzyme, formate dehydrogenase.

To facilitate a more detailed analysis of the activation of nitrate reductase in vivo and in vitro the activation was carried out in more concentrated cell suspensions than those employed above. Although the activation of demolybdonitrate reductase was much less efficient under these conditions, it permitted us to incubate extracts at higher protein concentrations which were, at the same time, comparable to the cell suspension being incubated in parallel. In addition, sufficient material was made available for the partial purification, separation, and analysis of both formate dehydrogenase and nitrate reductase.

When molybdate was added to cells which had been grown in L-broth plus nitrate and 10 mM tungstate and resuspended to 10% of their original volume with buffer plus chloramphenicol, a significant activation of nitrate reductase occurred upon incubation with molybdate (Fig. 2). Under these conditions the level of nitrate reductase reached a maximum in about 20 min at 37°C and decreased after that time. In this concentrated cell suspension, the maximum level reached was only 10 to 20% of that observed in cells grown in the absence of tungstate. When the cell suspension was broken in a French press and the crude extract was incubated under similar conditions, no increase in nitrate reductase activity occurred.

Cells grown in L-broth plus nitrate without added tungstate or molybdate also exhibited an initial increase in nitrate reductase activity when incubated with molybdate under the same conditions, although the increase was superimposed on a high background of activity (Fig. 2). Cell breakage resulted in a 25% reduction in the background activity, and no increase occurred in the extract upon incubation with molybdate and chloramphenicol. Cells grown in L-broth plus nitrate supplemented with 0.1 mM molybdate possessed a significantly higher initial specific activity than the unsupplemented cells and yielded no additional increase in activity when incubated with molybdate in the presence of chloramphenicol. When these cells were broken, no significant reduction in nitrate reductase activity was observed. During the incubation with molybdate and chloramphenicol, there was a slow decline of enzyme activity in both the extract and the whole cells.

These results indicated that some demolybdo form of the enzyme accumulated in cells grown in L-broth without molybdate supplementation and that this form was activated by incubation with molybdate in intact cells but not in the crude extracts. The addition of 0.1 mM molybdate to the growth medium apparently prevented this accumulation by allowing all precursor to be converted to active enzyme. In addition, the slow decline of preformed nitrate reductase activity in extracts indicated that the failure to produce nitrate reductase in extracts of tungstate-grown cells was not due to a more rapid

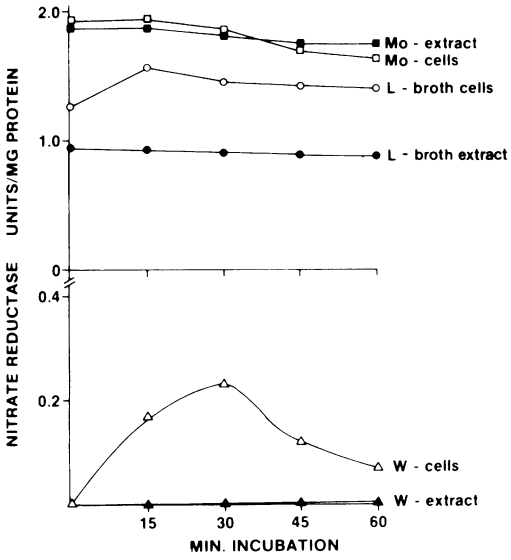

FIG. 2. *Nitrate reductase activation in vivo and in vitro during incubation with molybdate. Cells were grown in L-broth unsupplemented (L) or in the presence of 10 mM tungstate (W) or 1 mM molybdate (Mo). When the culture reached about 70 Klett units (filter 54), the bacteria were harvested by centrifugation and suspended at one-tenth their original volume in 50 mM KPO_4 buffer, pH 7.2, containing 0.1 M NaCl and 50 μg of chloramphenicol per ml. To make the extract, one-half of each suspension was fractured in a French press. Cell suspensions and extracts were incubated with 1% glucose and 1 mM molybdate at 37°C under an argon atmosphere. Samples were removed at the indicated times, stored on ice, and assayed for nitrate reductase activity after the completion of the experiment. Protein concentrations of cell suspensions and extracts were 0.12 mg/ml for L, 0.10 mg/ml for W, and 0.13 mg/ml for Mo.*

inactivation of nitrate reductase.

To establish that molybdate is incorporated specifically into demolybdoproteins in extracts, an experiment similar to that described in the legend to Fig. 2 was conducted with [^{99}Mo]-molybdate. At the end of the incubation with molybdate, membrane fractions were prepared, treated with deoxycholate, and fractionated with ammonium sulfate as described above. The ammonium sulfate fractions prepared contained both nitrate reductase and formate dehydrogenase as well as apparent demolybdo forms of these enzyme, all of which can be visualized on polyacrylamide gels (18). This made it possible to assess the incorporation of molybdenum into demolybdonitrate reductase prepared by release from the membrane by a procedure other than heat treatment and to investigate the possible incorporation of molybdate into demolybdoformate dehydrogenase at the same time.

The fraction prepared by this procedure from cells grown in the presence of 10 mM tungstate and subsequently incubated with [^{99}Mo]molybdate and chloramphenicol exhibited levels of both formate dehydrogenase and nitrate reductase activities (Table 1) which were significantly lower than those found in the fraction from cells grown with 0.1 mM [^{99}Mo]molybdate but not further incubated (Table 1). When extracts of the tungstate-grown cells were incubated in the same way, little or none of either activity was present in the fraction (Table 1). Control cells grown on L-broth and an extract of these cells were incubated with [^{99}Mo]molybdate under similar conditions (Table 1) and fractionated by the same procedure. The fraction from the cell suspension exhibited specific activities of the

TABLE 1. *Activities of nitrate reductase and formate dehydrogenase after incubation with [^{99}Mo]molybdate in vivo and in vitro*

Growth supplement	Material incubated with [^{99}Mo]molybdate[b]	Ammonium sulfate fraction[c]		
		Protein (mg/ml)	Formate dehydrogenase (U/mg of protein)	Nitrate reductase (U/mg of protein)
[^{99}Mo]molybdate	None	4.1	0.781	4.12
Tungstate	Cells	4.2	0.058	0.552
Tungstate	Extract	5.2	0	0.038
None (control)	Cells	3.8	0.231	1.22
None	Extract	4.1	0.100	1.01

[a] Cells were grown to about 70 Klett units on L-broth unsupplemented or supplemented with either 1 mM sodium [^{99}Mo]molybdate (43.2 mCi/mmol) or 10 mM sodium tungstate. Cells were harvested, and extracts were prepared as described in the legend to Fig. 2.

[b] Where indicated, the samples were incubated for 60 min with 1 mM sodium [^{99}Mo]molybdate and 1% glucose under argon at 37°C.

[c] Membrane proteins were prepared and fractionated by ammonium sulfate after treatment with deoxycholate as previously described (18).

two enzymes somewhat lower than those of the cells grown on molybdate. The nitrate reductase activity was somewhat reduced after incubation of the extract, and formate dehydrogenase was reduced by at least 50%.

The ammonium sulfate fractions described in Table 1 were subjected to electrophoresis on 5% polyacrylamide gels containing 0.1% Triton X-100. Gels were divided on a Gilson gel fractionator, and radioactivity was determined to assess the association of ^{99}Mo with formate dehydrogenase and nitrate reductase as well as with their inactive forms (Fig. 3). Duplicate gels were stained with Coomassie brilliant blue (Fig. 4). The fraction prepared from cells grown on [^{99}Mo]molybdate yielded three radioactivity peaks on the gel (Fig. 3A). Two of the peaks corresponded to nitrate reductase and formate dehydrogenase activities, as determined on parallel gels, as well as to two of the major bands visualized on the gels stained for protein (Fig. 4A). Considerable radioactivity remained at the top of the gel, and an additional peak of radioactivity was observed toward the bottom which could not be correlated with any specific protein band. The gel from the fraction prepared from incubated, tungstate-grown cells (Table 1) contained less radioactivity but exhibited peaks which corresponded to the same three peaks, with little activity remaining at the top of gel (Fig. 3B). Quantitatively, the two peaks corresponding to the enzymes appeared to contain approximately 25% of the radioactivity found on a gel displaying proteins from the control cells. Only a small amount of radioactivity was associated with the rapidly migrating component corresponding to the peak seen in the [^{99}Mo]-molybdate-grown cells. The fraction prepared from the incubated extract of the tungstate-grown cells (Table 1) contained still higher levels of radioactivity in the two peaks corresponding to formate dehydrogenase and nitrate reductase, even though little of either enzyme activity was present in this fraction (Fig. 3C). There was no radioactivity associated with a faster migrating peak in this case.

These results demonstrated that in crude extracts [^{99}Mo]molybdate was incorporated into proteins which migrate on gels to positions which correspond to nitrate reductase and formate dehydrogenase. Because other proteins which stain with Coomassie brilliant blue were present on these gels (Fig. 4) and had no associated radioactivity, the incorporation of ^{99}Mo occurred specifically into the demolybdo forms of these enzymes. Although their mobilities were similar, neither the radioactivity peaks nor the Coomassie brilliant blue bands from the tung-

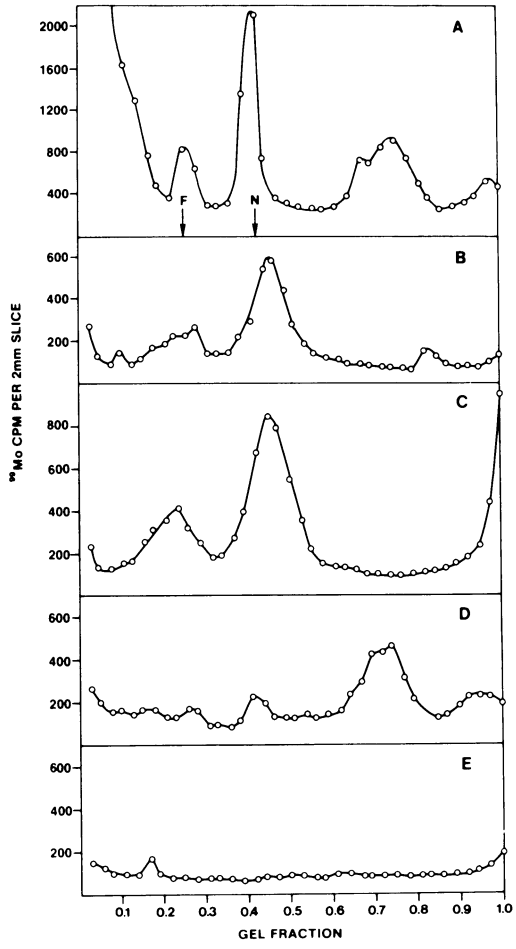

FIG. 3. *Incorporation of ^{99}Mo into membrane proteins of whole cells and extracts. Cells were grown in L-broth unsupplemented (L) or in the presence of 10 mM tungstate (W) or 1 mM [^{99}Mo]molybdate (control cells). The bacteria were grown, harvested, and resuspended as described in the legend to Fig. 2; cell suspensions and extracts were made of L and W cells and were incubated with ^{99}Mo at 37°C for 60 min. Cells grown in ^{99}Mo were maintained on ice and used as control cells for measurement of maximum ^{99}Mo incorporation. Whole cells were then fractured in a French press. Membranes from the extracts were precipitated with ammonium sulfate and treated with ammonium sulfate and deoxycholate (2, 18). By using previously described procedures (5, 18), proteins released from the membranes were partitioned with ammonium sulfate. The proteins precipitating between 40 and 50% saturation of ammonium sulfate contained nitrate reductase (N) and formate dehydrogenase (F) activities and were subjected to polyacrylamide gel electrophoresis as described in the text. The amount of protein loaded on the gels was 82 μg from control cells (A), 85 μg from W extract (B), 103 μg from W cell suspension (C), 82 μg from L extract (D), and 77 μg from L cell suspension (E).*

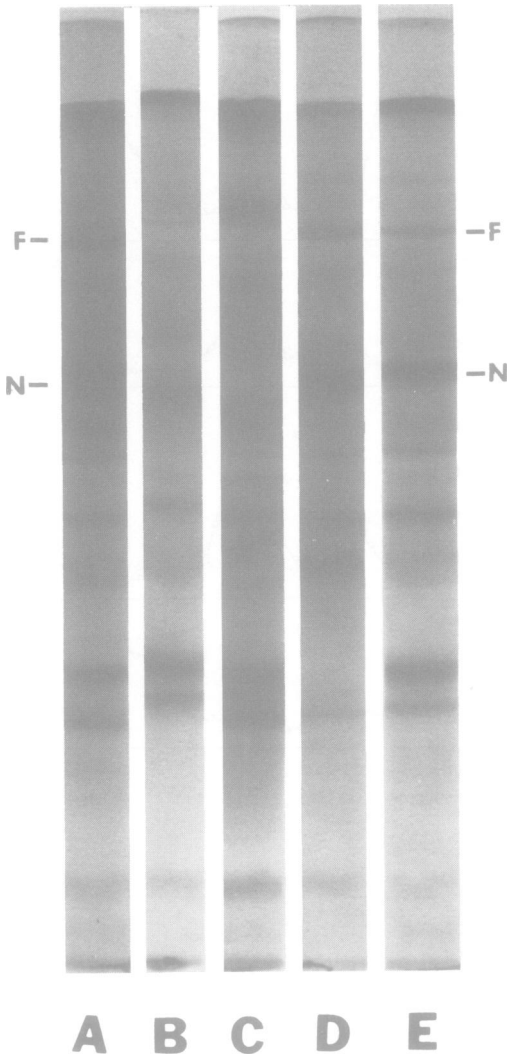

FIG. 4. *Polyacrylamide gels stained with Coomassie brilliant blue. Unstained gels identical to gels A and E were stained for formate dehydrogenase and nitrate reductase as previously described (18), and the corresponding protein bands are indicated for formate dehydrogenase (F) nitrate reductase (N). Gels A through E correspond to the preparations for Fig. 3A through E, as described in the legend to Fig. 3.*

state-grown cells and the extracts from these cells appeared to correspond precisely to the same bands in the control cultures (Fig. 3 and 4).

To determine whether the two molybdoenzymes can exchange their molybdenum with [^{99}Mo]molybdate under the conditions which resulted in incorporation into the demolybdoproteins, a control culture grown in L-broth was incubated with [^{99}Mo]molybdate both in cell suspension and in crude extract (Table 1). Only very small amounts of radioactivity were associated with the peaks corresponding to the formate dehydrogenase and nitrate reductase in the cell suspension, although a rather large amount of the faster running peak of radioactivity was found during the incubation (Fig. 3). In the cell extracts, essentially no radioactivity was associated with any of the pertinent components, although significant amounts of enzyme activity (Table 1) and the corresponding Coomassie brilliant blue-staining bands (Fig. 4E) were present in the fractions. These results demonstrate that little if any exchange occurs between the preformed molybdoenzymes and [^{99}Mo]molybdate either in whole cells or in crude extracts.

DISCUSSION

Molybdate is apparently incorporated into the demolybdo forms of both nitrate reductase and formate dehydrogenase in crude extracts of *E. coli,* although neither enzymatic activity is formed as a result of that incorporation. The inactive, ^{99}Mo-labeled products formed in vitro were present in the membrane fraction and could be released and fractionated by the same procedures used for purifying the active enzymes. On polyacrylamide gels the ^{99}Mo-labeled products migrated similarly, although not identically, to the migration of active nitrate reductase and formate dehydrogenase, while other proteins on the gel were not significantly labeled. In addition, a ^{99}Mo-labeled protein was released from the membrane fraction by the heat release procedure utilized for active nitrate reductase, and the labeled component migrated on polyacrylamide gels to the same position as the active enzyme. The amount of ^{99}Mo incorporated during incubation of the extract of tungstate-grown cells was at least as great as into the active enzymes formed in the cell suspension and was approximately 25% of the amount of ^{99}Mo found associated with two enzymes in a similar mass of cells grown in the presence of [^{99}Mo]molybdate. This level of incorporation and the fact that no significant exchange occurred between [^{99}Mo]molybdate and preformed nitrate reductase or formate dehydrogenase appear to rule out the possibility that Mo incorporation occurred by exchange with a small amount of molybdoenzyme present in the extracts of tungstate-grown cells. Thus, the incorporation of molybdate in vitro occurs at significant levels, appears to be relatively specific, and depends on the presence of demolybdo forms of the molybdoenzymes.

Although both nitrate reductase and formate

dehydrogenase were inactivated at greater rates in extracts than in cell suspensions, the rates were insufficient to explain the failure to obtain active enzyme formation in vitro. It therefore seems likely that some critical step(s) in the formation of the active enzymes, other than Mo incorporation, does not occur in the crude extract under the incubation conditions. In whole cells this step(s) would appear to be dependent on the presence of Mo or on the prior incorporation of Mo. Several observations suggest that the defective step(s) may involve the addition of nonheme iron to the molybdoproteins. We have recently found that a purified preparation of demolybdonitrate reductase isolated from tungstate-grown cells lacks the spectral characteristics of typical nonheme iron proteins or of isolated, active nitrate reductase, although its subunit structure is similar to that of active nitrate reductase (Scott and DeMoss, unpublished data). In addition, Pienkos et al. (15) have recently demonstrated that nitrogenase component I, a nonheme iron molybdoprotein, is activated by an Fe-containing molybdenum cofactor but not by a molybdenum cofactor which does not contain Fe. However, no direct evidence has been obtained to support the idea that nonheme iron incorporation is essential for the activation of demolybdonitrate reductase. In experiments not described here, addition of various forms of iron during the incubation of the crude extract from tungstate-grown cells with molybdate failed to stimulate the formation of active nitrate reductase.

The faster migrating peak of ^{99}Mo on the gels of the ammonium sulfate fractions may be involved in some way in the activation process. This peak is not associated with an observable Coomassie brilliant blue-staining band, but it is distinct from free molybdate which migrates ahead of the tracking dye. This component is present in normally grown cells and is formed when tungstate-grown cells or L-broth-grown cells are incubated with molybdate, but it is not formed in the extracts under the same conditions. Because its presence is correlated with formation of the active enzymes but not necessarily with molybdenum incorporation, it will be of some interest to determine its nature and behavior during enzyme activation.

It is possible that the failure to form the active enzymes in vitro results from extreme instability of the demolybdoproteins under the conditions of extract preparation. It is difficult to rule out this possibility, although the use of different breakage procedures and the addition of various stabilizing agents such as reducing agents, protease inhibitors, and substrates have not significantly increased the levels of the activities formed in the in vitro incubations. In any case, it is clear that the ability to specifically incorporate molybdate is retained by the demolybdoproteins in extracts, and this process provides a direct experimental approach for the study of molybdate incorporation into molybdoproteins.

Whether the direct incorporation of molybdate into the demolybdoproteins represents a normal step in the assembly of nitrate reductase and formate dehydrogenase cannot be ascertained at the present time. Many previous studies with other molybdoproteins have focused on the formation of active enzymes and have demonstrated generally that small-molecular-weight molybdenum cofactors are required for the activation of the demolybdoenzymes in vitro, while molybdate is ineffective (7, 12, 14). These cofactors have invariably been isolated from molybdoproteins or from crude extracts which presumably contain molybdoproteins, and no reports of de novo synthesis of the molybdenum cofactors from molybdate have appeared. However, Jones et al. (6) demonstrated that molybdate activates in vitro a fraction of the demolybdosulfite oxidase accumulated by tungstate-fed rats and that molybdenum is incorporated during that process. The in vitro incorporation of molybdenum observed here may be similar to that process, although no enzyme activity is formed. The results presented here suggest that the normal pathway of assembly of molybdoenzymes may involve the sequential addition of molybdate and other small-molecular-weight components, such as nonheme iron and sulfide, and leave open the possibility that molybdenum cofactors are products of molybdoproteins rather than their normal biosynthetic precursors.

ACKNOWLEDGMENTS

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