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Formation of the Formate-Nitrate Electron Transport Pathway from Inactive Components in *Escherichia coli*

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When *Escherichia coli* was grown on medium containing 10 mM tungstate, the formation of active formate dehydrogenase, nitrate reductase, and the complete formate-nitrate electron transport pathway was inhibited. Incubation of the tungstate-grown cells with 1 mM molybdate in the presence of chloramphenicol led to the rapid activation of both formate dehydrogenase and nitrate reductase, and, after a considerable lag, the complete electron transport pathway. Protein bands which corresponded to formate dehydrogenase and nitrate reductase were identified on polyacrylamide gels containing Triton X-100 after the activities were released from the membrane fraction and partially purified. Cytochrome b_1 was associated with the protein band corresponding to formate dehydrogenase but was not found elsewhere on the gels. When a similar fraction was prepared from cells grown on 10 mM tungstate, an inactive band corresponding to formate dehydrogenase was not observed on polyacrylamide gels; rather, a new faster migrating band was present. Cytochrome b_1 was not associated with this band nor was it found anywhere else on the gels. This new band disappeared when the tungstate-grown cells were incubated with molybdate in the presence of chloramphenicol. The formate dehydrogenase activity which was formed, as well as a corresponding protein band, appeared at the original position on the gels. Cytochrome b_1 was again associated with this band. The protein band which corresponded to nitrate reductase also was severely depressed in the tungstate-grown cells and a new faster migrating band appeared on the polyacrylamide gels. Upon activation of the nitrate reductase by incubation of the cells with molybdate, the new band diminished and protein reappeared at the original position. Most of the nitrate reductase activity which was formed appeared at the original position of nitrate reductase on gels although some was present at the position of the inactive band formed by tungstate-grown cells. Apparently, inactive forms of both formate dehydrogenase and nitrate reductase accumulate during growth on tungstate which are electrophoretically distinct from the active enzymes. Activation by molybdate results in molecular changes which include the reassociation of cytochrome b_1 with formate dehydrogenase and restoration of both enzymes to their original electrophoretic mobilities.

Nitrate reduction in *Escherichia coli* is catalyzed by a membrane-bound multienzyme system which includes formate dehydrogenase, cytochrome b_1, and nitrate reductase (11-13, 22, 23). The formation of the components of this pathway is under the control of at least seven genetic loci, the *chl* A-G genes (1, 9). Both nitrate reductase and formate dehydrogenase contain molybdenum (6, 27), and it has been shown that the *chl* D gene is involved in the incorporation of molybdenum into these enzymes (8, 25).

Tungstate competitively inhibits the formation of nitrate reductase and has been reported to cause the formation of inactive tungsten-containing protein analogues of the molybdoproteins in several different organisms (2, 14, 20, 21, 26). In *E. coli*, formate dehydrogenase and nitrate reductase activities were not formed when cells were grown on a medium containing tungstate (4). Sperl and DeMoss (25) showed that such cells contained an inactive form of nitrate reductase which was activated in the absence of protein synthesis when molybdate was added. Molybdenum was incorporated into the enzyme during activation, and at low molybdate concentrations the activation process was dependent on a functional *chl* D gene.

We show here that formate dehydrogenase as well as nitrate reductase is activated by the addition of molybdate to tungstate-grown cells and that the complete formate-nitrate electron transport pathway is restored to an active form in the absence of protein synthesis. A study of the nature of the inactive precursors and the molecular changes which occur during the activation process has been undertaken as an approach to defining some of the events involved in the assembly of this membrane-bound multienzyme complex.

MATERIALS AND METHODS

Culture. *E. coli* K-12, strain PK 27 (8), was maintained on L-agar (15) and grown on L-broth supplemented with 1.0% (wt/vol) KNO_3. Where noted 10 mM sodium tungstate was added to the growth medium.

Assay procedures. Nitrate reductase was assayed using reduced methylviologen as the electron donor as previously described (24). Formate dehydrogenase was measured by dichlorophenolindophenol reduction mediated by phenazine methosulfate (23). The complete formate-nitrate electron transport pathway was assayed by measuring nitrate reduction with formate as the electron donor (23). All enzyme assays were performed at 37 C.

For determining cytochrome b_1 levels, identical samples were either reduced with solid sodium dithionite or air oxidized and scanned in an Aminco-Chance DW-2 uv/vis spectrophotometer between 400 and 600 nm at room temperature. The concentration of cytochrome b_1 was calculated from the difference spectrum using the millimolar extinction coefficient of 60 at 428 nm or of 16 at 558 nm (10).

Protein content of cell suspensions (see Table 1 and Fig. 1) was estimated from the turbidity of the suspension as described by Lester and DeMoss (16). Protein in extracts was determined using the Folin-phenol reagent (18).

Enzyme activation experiments. Bacteria were grown in L-broth containing 0.1% (wt/vol) glucose, 1% (wt/vol) KNO_3, and 10 mM sodium tungstate harvested in late log phase of growth (80 Klett units using filter #54) and washed twice with 50 mM potassium phosphate buffer (pH 7.0) containing 50 μg of chloramphenicol per ml. The cells were either used directly or suspended in 50 mM phosphate buffer (pH 7.0), plus 20% (vol/vol) glycerol and frozen at −70 C under argon. Frozen cells were thawed and washed twice with 50 mM phosphate buffer (pH 7.0) to remove glycerol. The cells were suspended in 50 mM phosphate buffer (pH 7.0) containing 50 μg of chloramphenicol per ml and sparged with argon. Sodium molybdate (1 mM) and 1% (wt/vol) glucose were added, and the suspensions were incubated at 37 C. Samples were removed, cooled in an ice bath, and centrifuged at 8,000 × g for 10 min. Cells were suspended in cold 50 mM phosphate buffer (pH 7.0) containing 50 μg of chloramphenicol per ml and stored on ice until assayed.

Partial purification of components. The components of the formate-nitrate electron transport pathway were partially purified by the procedure of Enoch and Lester (6). Cells were broken by passage through a French pressure cell at 15,000 lb/in^2. In the subsequent steps of the procedure, the samples were maintained as much as possible under an argon atmosphere and at a temperature of 0 to 5 C. The membranes were separated from the bulk of the cytoplasmic proteins by addition of ammonium sulfate (230 g/liter) followed by centrifugation at 39,000 × g for 45 min. The nitrate reductase, formate dehydrogenase, and cytochrome b_1 were released by treatment of the membranes for 3 h at pH 8.0 in 0.1 M tris(hydroxymethyl)aminomethane (Tris)-chloride buffer with sodium deoxycholate (1 mg/mg of protein) and ammonium sulfate (42.9 ml of saturated solution per 100 ml of sample). The treated membranes were removed by centrifugation at 39,000 × g for 70 min. The supernatant was brought to 40% saturation with 16.7 ml of saturated ammonium sulfate in 0.1 M Tris-chloride (pH 7.0) per 100 ml of sample. The mixture was stirred for 30 min in an ice bath and centrifuged for 25 min at 39,000 × g. The supernatant was brought to 50% saturation by adding 20 ml of saturated ammonium sulfate in 0.1 M Tris-chloride (pH 7.0) to 100 ml of sample. This mixture was stirred for 30 min in an ice bath and subsequently centrifuged at 39,000 × g for 25 min. The pellet from this centrifugation was suspended in 0.1 M Tris-chloride (pH 7.2) and 0.5% (wt/vol) Triton X-100, gassed with argon, and stored at 4 C. This latter fraction is referred to as the "ammonium sulfate fraction" throughout this paper and was the fraction subjected to polyacrylamide gel electrophoresis and analysis.

Polyacrylamide gel electrophoresis. Polyacrylamide gels at pH 8.9 were prepared essentially by the procedure of Davis (3). Electrophoresis was carried out in separating gels containing 5% acrylamide with a 0.2-ml stacking gel containing 2.5% acrylamide. The electrophoresis buffer was 0.05 M Tris-0.38 M glycine (pH 8.3). The gels and buffer contained 0.1% (wt/vol) Triton X-100. The sample volume never exceeded 0.2 ml. The gels were subjected to electrophoresis for 10 min at 1 mA per gel followed by 120 min at 3 mA per gel. Bromophenol blue was used as a tracking dye, and the proteins were stained with Coomassie blue.

Localization of components on gels. Nitrate reductase activity was located on gels by the bleaching of reduced methyl viologen in the presence of nitrate (K. Lund and J. A. DeMoss, submitted for publication). Formate dehydrogenase activiy was located by placing the gel in a test tube with a 10-ml capacity. To the gel was added 0.3 ml of 3-mg/ml phenazine methosulfate, 0.05 ml of 5 M sodium formate, and 8.7 ml of 0.1 M Tris-chloride buffer (pH 8.3), containing 0.04% (wt/vol) para-nitro blue tetrazolium. The gels were maintained in an argon atmosphere and incubated at room temperature in the dark. A dark brown band developed in that area of the gel where formate dehydrogease was located. Using a spectrophotometer (Gilford 2400-S) with a linear transport accessory, the cytochrome b_1 was located by scanning the gels at 428 nm, the Soret peak for reduced

cytochrome b_1, before and after reduction with sodium dithionite. Where cytochrome b_1 was present on the gels an increase in absorbance occurred upon reduction with dithionite.

RESULTS

In preliminary experiments, *E. coli* was grown into late exponential phase under anaerobic conditions in L-broth supplemented with 10 mM tungstate. These cells lacked formate dehydrogenase and nitrate reductase activities but, when suspended in L-broth supplemented with 50 μg of chloramphenicol per ml and 1 mM molybdate, formed formate dehydrogenase, nitrate reductase, and the formate-nitrate electron transport pathway. To examine the requirements for this activation process, cells grown in tungstate were washed in phosphate buffer, resuspended in phosphate buffer containing 20% glycerol, and stored at −70 C prior to use. When these cells were resuspended in buffer plus molybdate and chloramphenicol, little activation was observed (Table 1). However, the addition of 1% glucose resulted in the maximum activation observed with these cells. Addition of other constituents of the L-broth such as amino acid mixtures produced no further stimulation. During the incubation, nitrate reductase and formate dehydrogenase activities increased approximately 10-fold; cytochrome b_1 levels, however, remained essentially unchanged.

The kinetics of activation of the components are shown in Fig. 1. Formate dehydrogenase and nitrate reductase increased continuously over the incubation period and at approximately equivalent rates while the level of cytochrome b_1 changed very little. The full chain activity began to increase only after a 30-min lag, suggesting that restoration of the complete formate-nitrate electron transport chain involves more than activation of the two enzymes.

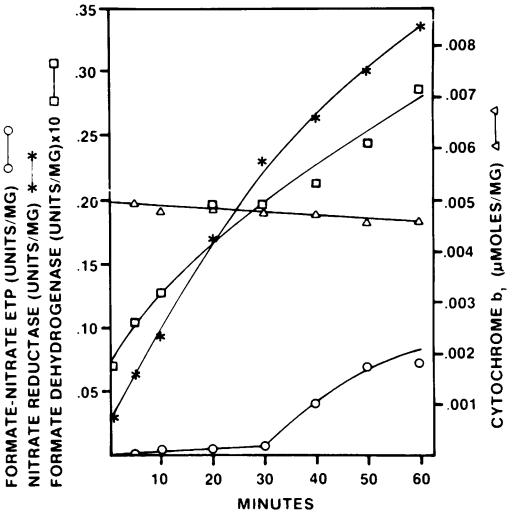

FIG. 1. *Kinetics of activation of the formate-nitrate electron transport pathway (ETP) by molybdate in the presence of chloramphenicol. Cells were grown in L-broth containing 1% potassium nitrate and 10 mM sodium tungstate. The cells were washed and suspended (0.3 mg of protein per ml) in 50 mM phosphate buffer (pH 7.0) containing 1% glucose, 50 μg of chloramphenicol per ml, and 1 mM sodium molybdate. Incubation temperature was 37 C. Samples were removed and enzyme activities and cytochrome b_1 content were determined as described in Materials and Methods.*

TABLE 1. *Formation of activities of the nitrate reductase complex in the absence of protein synthesis*

Incubation[a]	Activity	Units[b]/mg of protein	
		0 min	60 min
Buffer only	F-N ETP	0.000	0.000
	Nitrate reductase	0.035	0.096
	Cytochrome b_1	0.130	0.115
	Formate dehydrogenase	0.009	0.006
Buffer + glucose	F-N ETP	0.000	0.011
	Nitrate reductase	0.051	0.444
	Cytochrome b_1	0.124	0.115
	Formate dehydrogenase	0.002	0.051

[a] Frozen cells previously grown in tungstate and prepared as described in Materials and Methods were suspended in 50 mM phosphate buffer (pH 7.0) containing 50 μg of chloramphenicol per ml and 1 mM molybdate with and without glucose. The suspensions were incubated under argon at 37 C and samples were assayed for the formate-nitrate electron transport pathway (F-N ETP) and its components as described in Materials and Methods.

[b] Units for F-N ETP, nitrate reductase, and formate dehydrogenase are micromoles per minute. Units for cytochrome b_1 are micromoles × 10^2.

Since the activation occurred in the presence of chloramphenicol, these results indicated that inactive precursors were converted to active formate dehydrogenase and nitrate reductase. To investigate the nature of the inactive precursors, the following procedures were utilized to identify on polyacrylamide gels the protein bands which correspond to active formate dehydrogenase and nitrate reductase. Formate dehydrogenase and nitrate reductase were released from membrane preparations with deoxycholate and partially purified by ammonium sulfate precipitation by the procedure of Enoch and Lester (6) as outlined in Materials and Methods. Samples of the ammonium sulfate fraction were subjected to electrophoresis on 5% acrylamide gels containing 0.1% Triton X-100, and both enzymes were assayed directly on the

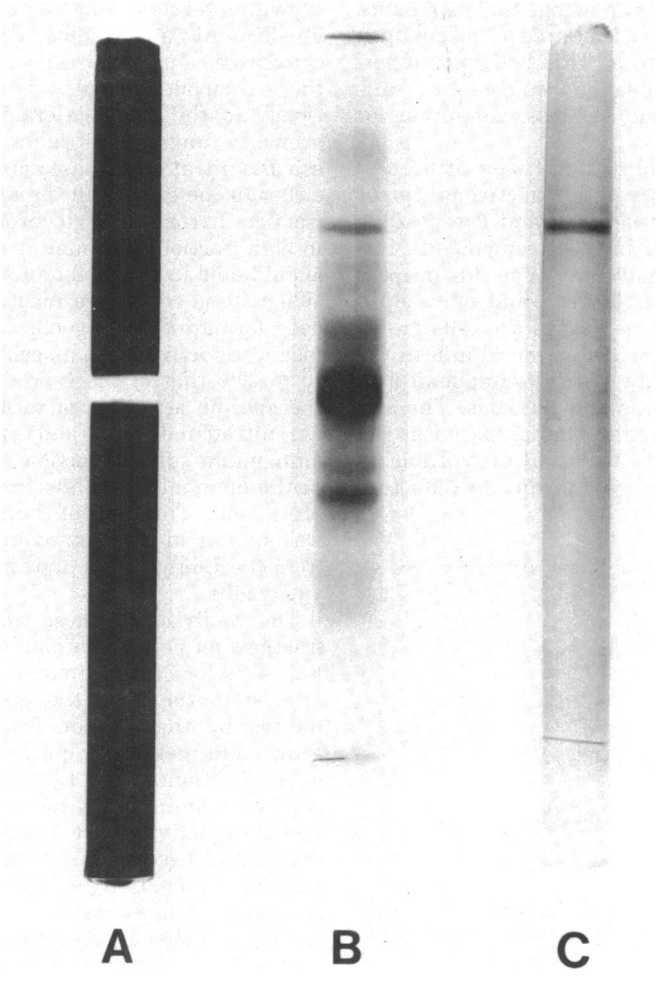

FIG. 2. *Identification of formate dehydrogenase and nitrate reductase on 5% polyacrylamide gels containing 0.1% Triton X-100. Identical samples containing 60 μg of protein of the ammonium sulfate fraction from cells grown in L-broth were applied to gels and were run as described in Materials and Methods. The gels were stained for nitrate reductase (A), formate dehydrogenase (C), and protein (B) as described in Materials and Methods.*

gels (Fig. 2). Formate dehydrogenase activity corresponded to a well-defined band of protein on the gels stained with Coomassie blue. Nitrate reductase exhibited a broad band of activity which corresponded to a diffuse protein band. The distribution of the enzyme activities on gels was confirmed by the results shown in Fig. 3. A gel was crushed with a Gilson gel fractionator; individual fractions were assayed for formate dehydrogenase (23) and nitrate reductase (24). The formate dehydrogenase activity spanned only 3 gel fractions and nitrate reductase activity was present in 13 gel fractions. The positions of the activities corresponded to the same protein bands identified by the activity stains in Fig. 2.

Because cytochrome b_1 has been reported to be associated with both formate dehydrogenase and nitrate reductase (5, 6, 17) and is a component of the formate-nitrate electron transport pathway (22, 27), the gels were scanned at 428 nm, the Soret peak for reduced cytochrome b_1 (5, 17), before and after reduction with dithionite. A comparison of the protein profile and the scans at 428 nm (see Fig. 5A) showed that cytochrome b_1 was coincident with the protein peak corresponding to formate dehydrogenase. Surprisingly little, if any, cytochrome b_1 was

present where the nitrate reductase was located. Rather than increasing, the absorbance at 428 nm decreased in that area of the gel upon reduction by dithionite. This behavior apparently reflects the nonheme iron content of this enzyme (7; Lund and DeMoss, submitted for publication).

The above procedures have been utilized to investigate the nature of the inactive precursor in cells grown on tungstate and the possible changes which occur in these components during activation by molybdate. For this purpose cells were grown on L-broth and on L-broth plus 10 mM tungstate. Part of the cells grown on tungstate were incubated for 60 min in the presence of molybdate and chloramphenicol as described in Materials and Methods. The ammonium sulfate fraction was prepared as described for each of the three cultures (Table 2). Formate dehydrogenase and nitrate reductase

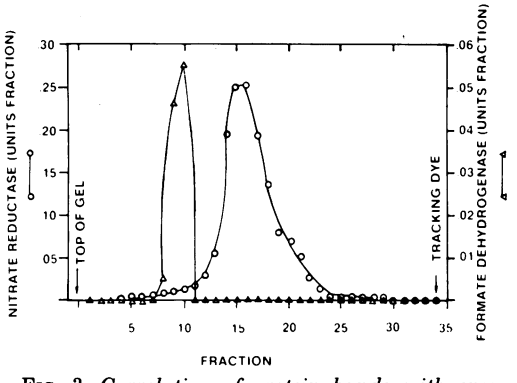

FIG. 3. *Correlation of protein bands with enzymatic activities. Identical samples of the ammonium sulfate fraction from cells grown on L-broth were run on 5% acrylamide gels containing 0.1% Triton X-100. The gel was divided into 2-mm fractions with a Gilson gel fractionator. The fractions in 50 mM phosphate buffer (pH 7.0) were incubated overnight at 4 C and were assayed for nitrate reductase and formate dehydrogenase.*

were each purified about 20-fold from cells grown in L-broth, whereas cytochrome b_1 was purified only about 5-fold. Presumably not all cytochrome b_1 in the cells is associated with these components and it is therefore not enriched to the same extent. After cells were grown in tungstate, little formate dehydrogenase and nitrate reductase activity was detected either in the cells or in the ammonium sulfate fraction. In contrast, cytochrome b_1 was present in both fractions although it was only enriched about 2-fold in the ammonium sulfate fraction. When these cells were incubated with molybdate, formate dehydrogenase and nitrate reductase were activated to a spcific activity of only 10 to 20% that of the L-broth cells. However, the specific activities of formate dehydrogenase, nitrate reductase, and cytochrome b_1 in the ammonium sulfate fraction were 50% of those in the ammonium sulfate fraction from the L-broth cells. The ratio of the three components was similar in the ammonium sulfate fraction from the L-broth cells and the molybdate-activated cells.

The analyses of these ammonium sulfate fractions on polyacrylamide gels are shown in Fig. 4. When equal amounts of protein were applied to the gels, the protein pattern exhibited by the fraction from the tungstate-grown cells (gel B) was quite different from that of the fraction for the L-broth grown cells (gel A). The protein which corresponded to nitrate reductase activity (NR, gel A) was greatly reduced, and a second faster migrating band was present in increased amounts (n, gel B). In the fraction from molybdate-activated cells (gel C), the original band was only partially restored and nitrate reductase activity was found in both locations in the gel (NR_1, NR_2, gel C). The major portion of the nitrate reductase activity appeared to be associated with NR_1. The protein band corresponding to formate dehydrogenase appeared to undergo a similar change as

TABLE 2. *Activities present in cells and in ammonium sulfate fractions before and after molybdate activation*[a]

Culture conditions	Fraction	Protein (mg/ml)	Formate dehydrogenase (μmol/min/mg)	Nitrate reductase (μmol/min/mg)	Cytochrome b_1 (μmol/mg × 10)
L-broth	Cells	5.81	0.45	0.98	1.41
	AS fraction	5.04	9.87	15.48	6.94
L-broth + tungstate	Cells	6.21	0.00	0.01	1.82
	AS fraction	3.50	0.38	0.23	2.60
Molybdate activated	Cells	4.09	0.13	0.12	2.24
	AS fraction	2.38	5.91	8.84	5.25

[a] Cells were grown in L-broth without or with 10 mM tungstate. Molybdate-activated cells were prepared from cells grown on tungstate as described in Materials and Methods. Cells and ammonium sulfate (AS) fractions were prepared and assayed as described in Materials and Methods.

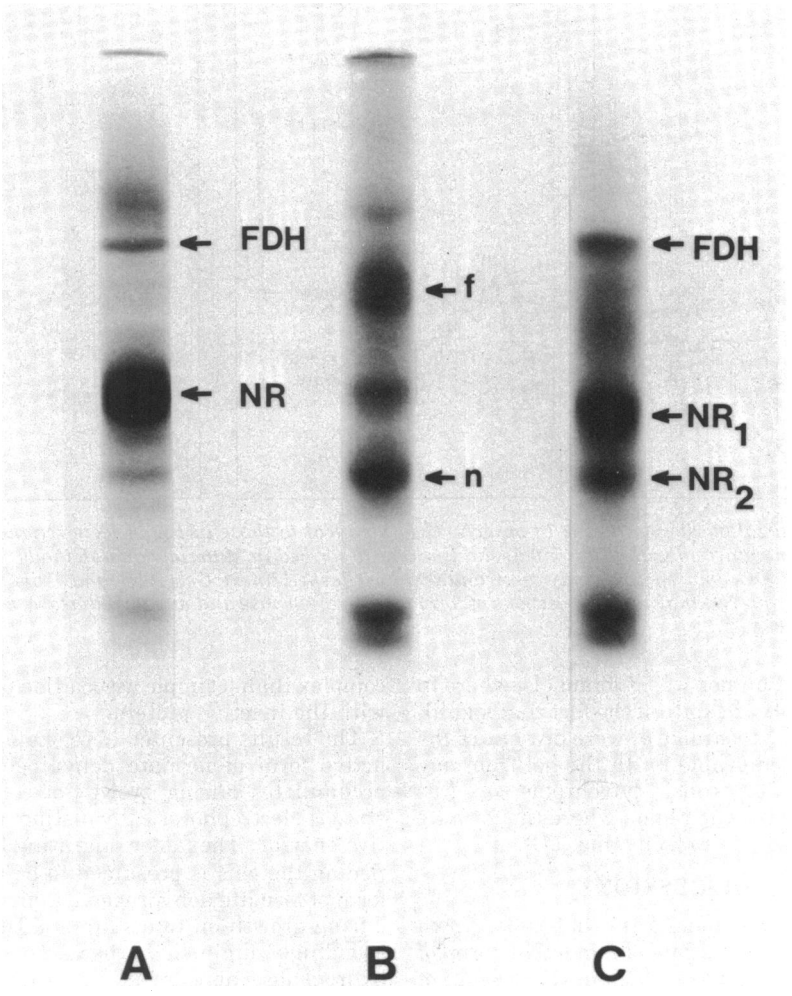

FIG. 4. *Analysis of ammonium sulfate fractions on polyacrylamide gels. Samples were run on 5% acrylamide gels containing 0.1% Triton X-100 and stained for protein with Coomassie blue. Gel A, Ammonium sulfate fraction (120 μg of protein) prepared from cells grown on L-broth; gel B, ammonium sulfate fraction (120 μg of protein) prepared from cells grown on L-broth containing 10 mM tungstate; gel C, ammonium sulfate fraction (114 μg of protein) prepared from cells grown in tungstate and then incubated with 1 mM molybdate in the presence of chloramphenicol as described in Materials and Methods. In each case identical gels were stained for nitrate reductase and formate dehydrogenase. FDH and NR indicate the position of formate dehydrogenase and nitrate reductase activities, respectively. f and n indicate inactive proteins which appear or increase as the result of growth on tungstate.*

the result of growth on tungstate. In the fraction from the tungstate-grown cells (gel B), the protein band corresponding to formate dehydrogenase (FDH, gel A) was extremely depressed and a diffuse band (f, gel B) migrating somewhat faster was present. In the fraction from molybdate-activated cells, the activity and the protein band appeared in the original position (FDH, gel C).

Gels identical to those shown in Fig. 4 were scanned for cytochrome b_1 prior to staining with Coomassie blue. The scan of protein bands and the scan of absorbance at 428 nm before and after reduction with dithionite are compared in Fig. 5. Cytochrome b_1, identified by the increase in absorbance at 428 nm upon reduction, was present only at the position of formate dehydrogenase in the fraction from L-broth cells (Fig. 5A). None was associated with the faster migrating band in the gel from the tung-

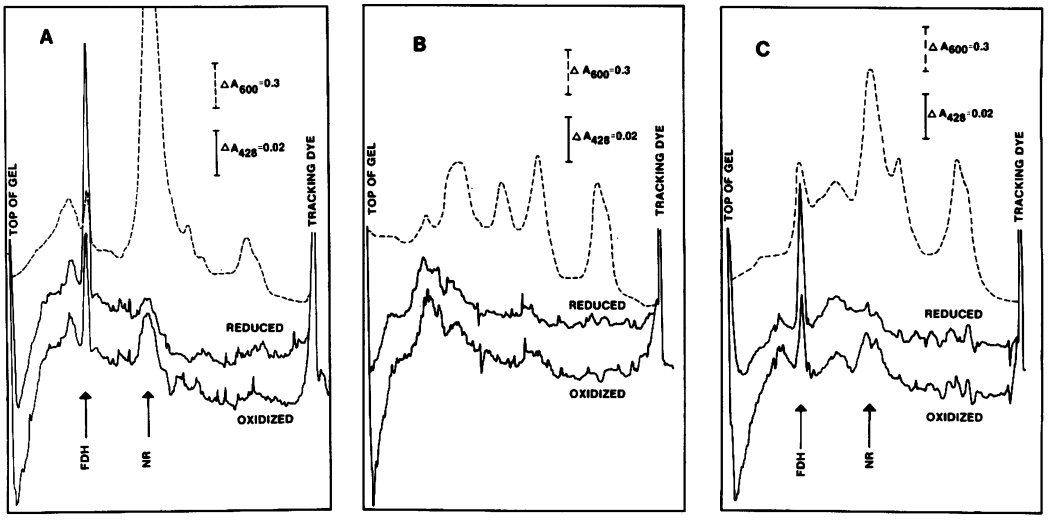

FIG. 5. *Localization of cytochrome b_1 on gels. Gels identical to those in Fig. 4 were scanned at 428 nm before and after reduction with dithionite (solid lines) as described in Materials and Methods. The gels were then stained with Coomassie blue and scanned at 600 nm (dashed lines). Gels A, B, and C are as designated in Fig. 4. FDH and NR indicate the positions of formate dehydrogenase and nitrate reductase, respectively, as determined in Fig. 4.*

state cell fraction nor was it found elsewhere in the gel (Fig. 5B), in spite of the fact that significant levels of cytochrome b_1 were present in the fraction applied (Table 2). In the gel from molybdate-activated cells, cytochrome b_1 was again localized in the region where the formate dehydrogenase was present (Fig. 5C).

DISCUSSION

These results demonstrate that cells grown on tungstate accumulate an inactive form of formate dehydrogenase which in the absence of protein synthesis can be converted in vivo to an active form by incubation of the cells with molybdate. As shown previously (25), an inactive form of nitrate reductase accumulates and is activated under similar conditions. Both formate dehydrogenase and nitrate reductase are molybdoproteins (6, 27), and tungstate presumably prevents the formation of the active enzymes by interfering with the incorporation of molybdenum (4, 25). As a result, addition of molybdate permits the formation of the active enzyme in the presence of chloramphenicol. Sperl and DeMoss (25) have shown that molybdenum incorporation into the nitrate reductase accompanies the activation and, as shown here, the activation process in whole cells requires glucose under anaerobic conditions. Sperl and DeMoss (25) were unable to convert the inactive form of nitrate reductase to active form by adding molybdate to cell-free extracts of nitrate reductase and concluded that activation is more complex than a simple association of molybdate with the inactive protein.

The results presented here show that the inactive form of formate dehydrogenase which accumulates during growth of cells on tungstate is electrophoretically distinct from the active enzyme. The faster migrating band identified on the gels is presumed to be the inactive form of formate dehydrogenase since it appears during growth on tungstate and then is correspondingly diminished when active formate dehydrogenase appears at the original position after activation with molybdate in the presence of chloramphenicol. The inactive protein band is more diffuse and lacks any detectable cytochrome b_1 although cytochrome b_1 is associated with the band of active formate dehydrogenase from cells grown on normal media and from cells which had been grown on tungstate and then activated with molybdate. It is possible that cytochrome b_1 was associated with the inactive form of formate dehydrogenase but was dissociated or denatured during electrophoresis. In any case, cytochrome b_1 was not detected anywhere on the gels from tungstate-grown cells, in spite of the fact that significant amounts of cytochrome b_1 were present in the fraction applied to the gels. These results suggest that, during growth on tungstate, an inactive form of formate dehydrogenase accumulates in the membranes. The inactive form is not associated with cytochrome b_1 and is electrophoretically different from active formate

dehydrogenase. Upon molybdate addition to whole cells, this inactive form is apparently converted to an active form which is associated with cytochrome b_1.

Sperl and DeMoss (25) observed that, when the proteins were released from the membrane fraction by heating, an inactive protein was present in the fraction derived from membranes of tungstate-grown cells which was electrophoretically identical to active nitrate reductase. Using deoxycholate to solubilize the proteins, we showed here that on gels containing Triton X-100 active nitrate reductase migrates as a diffuse band and that in the fraction from tungstate-grown cells the equivalent protein band is considerably reduced in amount. As in the case of formate dehydrogenase, a faster migrating band appeared as the result of growth on tungstate. After activation with molybdate, nitrate reductase activity and corresponding protein bands were present in two areas of the gels. The major band of activity was coincident electrophoretically with the activity from L-broth-grown cells and a minor band of activity located in the region of the presumed inactive form of the enzyme. The molecular basis for the diffuseness of the nitrate reductase band or the function of two distinct bands of activity in the fraction from the activated cells is unknown. Heat-released nitrate reductase has been shown to be heterogeneous in its subunit composition and to dissociate to smaller active enzyme species upon dilution (Lund and DeMoss, submitted for publication); the deoxycholate-solubilized enzyme may exhibit similar properties. In any case, growth on tungstate appears to produce an inactive form of reductase with altered electrophoretic properties and activation with molybdate results in an enzyme with the original electrophoretic properties.

Several lines of evidence indicate that cytochrome b_1 is physically associated with nitrate reductase in *E. coli*. Enoch and Lester (5, 6) demonstrated that nitrate reductase purified to near homogeneity from deoxycholate-treated membranes contained significant levels of cytochrome b_1. MacGregor found that, after nitrate reductase was released from membranes by Triton X-100 treatment, cytochrome b_1 was precipitated along with nitrate reductase by specific antibodies to purified nitrate reductase (19). It was therefore surprising to find no cytochrome b_1 associated with nitrate reductase on the polyacrylamide gels, particularly since the procedure used to prepare the samples was identical to the initial purification steps of Enoch and Lester (6). It is possible that either the heme group or the cytochrome b_1 protein dissociated from nitrate reductase during the electrophoresis or that cytochrome b_1 was denatured under these conditions. However, this loss would have to occur under conditions in which active cytochrome b_1 remained associated with formate dehydrogenase. At present, we do not understand the reason for this apparently anomalous behavior of cytochrome b_1.

The activation of formate dehydrogenase and nitrate reductase upon molybdate addition is accompanied by the restoration of the complete formate-nitrate electron transport pathway. However, as shown in Fig. 1, the activity of the complete pathway begins to appear at a time well after the appearance of significant amounts of the component enzyme activities. This observation indicates that the activity of the complete pathway depends on more than the presence of active formate dehydrogenase and nitrate reductase. This lag may involve accumulation or association of additional components such as cytochrome b_1 or a quinone (5), or it may involve the time required to assemble the active components into a specific complex in the membrane.

Growth in the presence of tungstate prevents the formation of the active molybdoprotein components of this pathway and therefore the assembly of an active form of this multienzyme electron transport pathway. Although the inactive components accumulated in the membrane may or may not be intermediates in the normal sequence of assembly, it is clear that the subsequent activation by molybdate provides an opportunity for defining the molecular events involved in the conversion of inactive, membrane-bound precursors into active components and an effective multienzyme structure.

ACKNOWLEDGMENTS

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