Energy Transduction in *Escherichia coli*

GENETIC ALTERATION OF A MEMBRANE POLYPEPTIDE OF THE (CA\(^{++}\),MG\(^{++}\)) \(-\)ATPase COMPLEX

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Recent genetic analyses of the membrane components involved in energy transduction in *Escherichia coli* have concentrated on the (Ca\(^{++}\),Mg\(^{++}\)) \(-\)ATPase complex (EC 3.6.1.3). Many mutants have been described with altered biochemical properties and defects in energy-requiring processes such as oxidative phosphorylation, transhydrogenase activity, and active transport of several solutes.

This report describes the isolation of a mutant strain of *E. coli* that is defective in several energy-requiring processes. The strain BG-31 was obtained by "localized mutagenesis" using phage P1c1. The mutation maps at approximately 73.5 min on the *E. coli* chromosome. Reversion and suppression analyses indicate that the defect is the result of a single amber mutation. This strain is unable to utilize succinate, d-lactate, or malate for growth. Mutant cells are unable to couple the energy derived from the hydrolysis of ATP to the active transport of proline, although coupling of energy derived from electron transport to solute transport appears normal when examined in both cells and isolated membrane vesicles. Isolated membranes of the mutant are unable to couple the energy derived from the hydrolysis of ATP to transhydrogenase activity while they can utilize the energy generated from electron transport to drive transhydrogenase activity. Extracts of strain BG-31 have normal levels of (Ca\(^{++}\),Mg\(^{++}\)) \(-\)ATPase activity. The ATPase portion of the complex, bacterial F\(_{1}\) (BF\(_{1}\)), is poorly attached to the membrane portion of the complex. In *vitro* reconstitution of transhydrogenase activity with stripped membrane fractions and crude preparations of BF\(_{1}\) localize the defect in strain BG-31 to the membrane portion of the complex. Analysis of membranes of the strain BG-31 by acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate demonstrate the absence of a single polypeptide of molecular weight about 54,000 and the appearance of a new polypeptide of lower molecular weight, about 25,000. Analysis of a spontaneous revertant of BG-31 shows complete restoration of the parental phenotype including the gel patterns.

The characterization of this mutant provides the first demonstration of the consequences of a structural gene mutation on a polypeptide in the membrane portion of the complex and represents the initial stages in what we hope will be the biochemical definition and functional characterization of this important energy-transducing system.

A major area of recent research on energy transduction in biological membranes has been the genetic and biochemical analysis of the membrane-bound (Ca\(^{++}\),Mg\(^{++}\)) \(-\)ATPase complex of *Escherichia coli*. This complex is often considered to contain three parts analogous to the more thoroughly studied mitochondrial complex. The F\(_{1}\) portion is the most easily removed from the membrane, and contains five distinct polypeptides. A second portion is the stalk or that peptide or those peptides that join the F\(_{1}\) to the membrane. In mitochondria the so-called oligomycin sensitivity-conferring protein is presumably in the stalk. The protein nectar isolated from *Streptococcus faecalis* by Baron and Abrams (1) also performs this function. The remaining portion of the complex, the membrane sector, is the least well understood and may contain four or five distinct polypeptides. One of the interesting features of this ATPase complex is a striking phylogenetic conservation of structure. The structure of the F\(_{1}\) portion is basically the same in mitochondria, chloroplasts, yeast, and bacteria. The review by Senior (2) is an excellent discussion of the structural features of this complex, while Abrams and Smith (3) have compared the bacterial ATPases.

In functional terms, the complex was initially described as an ATPase activity and considered to operate in the final step in oxidative phosphorylation, namely the transphosphorylation reaction. The complex has, however, a bidirectional function. It can use the high energy state generated by respiration for the synthesis of ATP. It can also generate that same high energy state by the reverse reactions, hydrolysis of ATP. This latter reaction is particularly important in bacterial systems. In Mitchell's view, this is accomplished by a complex that serves as a reversible proton translocator while the so-called high energy state is a proton gradient (4). While there is consid-
erable evidence for this general function, the precise mecha-
nism is still a matter of conjecture (for a recent proposal by Mitchell, see Ref. 4).

The *E. coli* complex has an $F_{2}$ component (BF$_{2}$) that is quite similar to that previously described for mitochondria and chloroplasts. It is easily removed from the membrane by washing with buffer of low ionic strength in the presence of EDTA (stripping procedure). It has five distinct polypeptides as reported initially by Bragg and Hou (5), and the molecular weights of the peptides appear to be approximately $\alpha=56,000$, $\beta=52,000$, $\gamma=30,000$, 5-20,000, and $\epsilon=10,000$. The ATPase activity of the complex is activated by Mg$^{2+}$, which also plays a role in attaching the $F_{2}$ to the membrane portion of the complex. The ATPase activity of the complex is inhibited by dicyclohexylcarbodiimide and Dio9, but the ATPase activity of the soluble BF$_{2}$ is not sensitive (6). Therefore a DCCD$^{-}$ sensitivity-conferring protein has been implicated as a component of the membrane portion of the complex. Removal (stripping) of BF$_{2}$ from the membrane results in energetic uncoupling that may be due to a proton leak through the membrane portion of the complex, the proton channel (7, 8). In *E. coli* this uncoupled state can be recoupled by treatment with DCCD or by reconstitution with BF$_{2}$ (9-13).

A host of mutants have now been isolated, particularly in *E. coli*, that have alterations in the ATPase complex. The first of such mutants was reported by Butlin et al. (14); these have been designated uric for uncoupled. This group observed that such mutants were incapable of coupling electron transport to ATP synthesis (oxidative phosphorylation). This original mutant, AN120, was also lacking ATPase activity. This same group later described another class of uric mutants that had normal ATPase activity (15).

Subsequently, a number of mutants both with and without ATPase activity, have been isolated by other workers (12, 16-21). The general phenotype of all uric mutants reported until now can be described as follows: (a) uric mutants are able to utilize fermentable carbon sources such as glucose or glycerol for growth but are unable to utilize carbon sources that yield energy primarily as a result of ATP synthesis via oxidative phosphorylation such as succinate, malate, lactate, etc.; (b) the mutants give low aerobic growth yields when grown on limiting amounts of glucose, or more simply the amount of cell mass produced per amount of glucose utilized is low; (c) uric mutants have normal respiratory activity; (d) the mutants have little or no detectable oxidative phosphorylation; (e) ATPase activity can be present or missing; (f) where determined all uric mutations so far described in *E. coli* are located at about 73.5 min on the chromosome and arc 20% to 50% co-transducible with the *ilv-C* locus and about 50% co-transducible with the asn locus (14, 15, 18, 20, 21).

This report described the physiological properties of the strain that has a mutation in a structural gene coding for a polypeptide of the membrane portion of the ATPase complex.

**Experimental Procedure**

**Materials**—The following materials were obtained from the indicated sources: Sepharose 4B from Pharmacia; [14C]choline from New England Nuclear; ATP, NAD, NADP, and alcohol dehydrogenase (yeast) from Sigma; DNase from Worthington. All other materials were obtained from commercial sources and were the highest purity available.

Enzyme Assays—Adenosine triphosphatase activity was determined as previously described (16). Incubation mixtures contained 0.05 M Tris-Cl buffer, pH 9.1, 1 mM MgCl$_{2}$, 2.5 mM ATP, and enzyme in a total volume of 1 ml. Assay mixtures were incubated for 15 min at 37°C. The addition of reagents for inorganic phosphate determination according to the method of Fiske and SubbaRow (23). All values reported in this paper were obtained from assays that gave linear responses with respect to increases in both time and enzyme concentration. Units of ATPase activity are expressed as micromoles of ATP hydrolyzed/min. Electron transport-driven and ATP-driven transhydrogenase activity were measured by a modification of the procedures of Fisher and Sanadi (24) and of Bragg and Hou (13). Incubation mixtures, prepared in cuvettes, contained 50 mM Tris-SO$_{4}$ buffer, pH 7.3, 5 mM MgCl$_{2}$, 700 mM sucrose, 0.1% bovine serum albumin, 0.1 mM dithiothreitol, 0.15 mM ethanol, 100 $\mu$g of alcohol dehydrogenase (325 units/mg), 15 $\mu$M NAD, and various amounts of protein as specified in the text in a total volume of 2 ml. Each sample was incubated for 10 min at 25°C, after which the cuvette was placed in a Gilford spectrophotometer, and the optical density at 340 nm was recorded by a Heathkit recorder. After the base-line stabilized, 1 to 2 min, the electron transport-driven reaction was initiated by the addition of 100 $\mu$l of 150 mM NaFDP, and the absorbance at 340 nm was measured at 4 to 5 min, the reaction rate decreased, presumably due to the depletion of oxygen in the cuvette. The initial linear portion of the recorder trace is used to calculate the rate of electron transport-driven transhydrogenase activity. In order to determine the ATP-driven reaction, 20 $\mu$l of a 50 mM solution of ATP is then added to the same cuvette with vigorous aeration, and the increase in optical density at 340 nm is recorded. The initial rate of this reaction represents the sum of the ATP-driven and the electron transport-driven activities, and the former is then calculated from the difference between the values measured before and after the addition of ATP.

Cell Growth—Cells for all experiments were grown in a simple salts medium that contained 0.05 M potassium phosphate buffer, pH 7.5, 1 mM MgCl$_{2}$, and 0.2% ammonium sulfate. Glucose was used as carbon and energy source at 0.7% final concentration, and thiamine was added as a growth supplement at 10 $\mu$g/ml. In genetic selection procedures some strains required isolucine and valine at a concentration of 10 $\mu$g/ml.

Erlenmeyer flasks (2-liter size), containing 600 ml of the above medium, were inoculated in the evening, and cells were grown overnight at 37°C with shaking at 200 rpm on a New Brunswick gyratory shaker. Cells were harvested in late log phase of growth by centrifugation and washed with either the medium salts solution (transport medium) or 0.05 M Tris-Cl buffer, pH 7.8, and resuspended in 0.05 M Tris-Cl, buffer, pH 7.8, and 0.01 M MgCl$_{2}$ (preparation of extracts and transport vesicles). Solid media for screening mutants and for testing various carbon sources for growth were prepared by including 1.5% agar in the above recipe. Mutant strains grown in liquid culture were tested for the appearance of revertants by spreading 0.1 ml of the culture on an agar plate containing the requisite amino acids. Carbon sources other than glucose were used at a final concentration of 0.5%.

Isolation of Strain BG-31—All bacterial strains used in this report were derived from *E. coli* K12 strain 1100 which contained a mutation in the *ilv-C* locus.

The *E. coli* strain, BG-31, was isolated by "localized mutagenesis" using phage Pi1c. The procedure was essentially that described by Murga and Yanofsky (25) and will only be outlined. Use of this technique is possible since it is known that the uric mutations co-

transduce with the *ilv-C* locus with a frequency of 20 to 50%. The technique has the advantage of ensuring that the mutations obtained are in a particular region of the chromosome and eliminates the possibility of mutations at other sites contributing to the observed phenotype. Phage Pi1c were mutagenized with hydroxylamine, and the mutagenized phage were used as donors in a transduction experiment with 1100 *ilv-C* as the recipient. The transduction mixture was spread on minimal glucose plates and incubated for 3 days at 37°C. The colonies that formed in the absence of isolucine and valine (*ilv-C* transductants) were then picked and put on master plates containing glucose as carbon source. After 1 day at 37°C the colonies on the master plate were replicate plated to a minimal plate containing succinate as carbon source. Those colonies that failed to grow on succinate were purified by repeated single colony isolation and saved for further study. Before biochemical analysis, the mutation

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\* The abbreviation used is: DCCD, dicyclohexylcarbodiimide.
in strain BG-31 was transferred by transduction into a fresh isolate of
1100 ilv- by using P1C1 grown on strain BG-31 as donor and 1100
ilv- as recipient. The selection and screening are the same as in the
initial isolation. In this second transduction an ilv+ succinate+ transdant was taken as the parental strain and an ilv- succinate-
strain was taken as BG-31. Both were purified by repeated single
colony isolation. A revertant of BG-31 was then selected by spreading
about 10^4 cells on a plate containing succinate as carbon source
followed by incubation for 4 days at 37°C. Several colonies were
picked and purified by repeated single colony isolation, and one
was retained for further study. Strain BG-31 was tested for the presence
of an amber mutation by spreading 10^4 cells on a plate containing
succinate as carbon source and 1% methionol and a pinch of bromphenol blue. Samples contain-
ing 2 mg/ml of protein were incubated at 100°C for 2 min. Twenty
microliters were applied to each slot of the gel and run at a current of
20 mA for about 3 hours. Protein was detected by staining with
Coomassie blue according to the procedure of Fairbanks et al. (33).

RESULTS AND DISCUSSION

Properties of the Mutant BG-31

Growth Properties—The growth properties of BG-31 are
essentially those reported for other unc type mutants (12,
16-21). It fails to grow on carbon sources that require the
oxidative synthesis of ATP for energy production such as suc-
cinate, α-lactate, and malate. It does grow reasonably well on
fermentable carbon sources such as glucose, glycerol, galactose,
lactose, maltose, and pyruvate. The mutant retains its sens-
sitivity to the antibiotic Neomycin in contrast to unc mutants
that have been selected as Neomycin-resistant. It is interesting
that in our experience only those unc mutants selected on
Neomycin are resistant to the antibiotic suggesting that this
selection procedure may bias the type of mutants obtained.
It was largely for this reason we have employed the phage
mutagenesis procedure which avoids any selection pressure.
The revertant strain BG-31-R has essentially the same growth
properties as the parental strain.

Map Location—The isolation procedure for BG-31 ensures that
the defect occurs in the unc region of the chromosome.
In separate experiments we have determined that the lesion in
this strain is approximately 20% co-transducible with the ilv-C
locus. The defect is the result of a single point mutation as
indicated by a reversion frequency of about one in 10^4 Twelve
revertants were screened for their ability to grow on various
carbon sources, and all appeared indistinguishable from the
parental strain.

Since we hoped to be able to demonstrate an altered poly-
peptide by a technique that discriminates on the basis of size,
we screened each mutant isolated for the presence of nonsense
or chain-terminating mutations. This was done by treating
each strain with phage ø60 that carried the amber suppressor
su-3. Strain BG-31 was one of several strains tested that gave
a positive test when treated with this phage.

(Ca^++,Mg++)-ATPase Activity—The ATPase activity of
strain BG-31 was examined in extracts at various steps in the
fractionation procedure. The results are presented in Table I.
Compared to the parental strain and the revertant, BG-31 has
essentially normal amounts of ATPase activity if the crude
sonic extract is examined. However, as the membranes of
BG-31 are isolated and washed, only about 40% of the initial
activity is recovered in the washed membrane fraction. This
is in marked contrast to a recovery of over 80% of the activity
in washed membranes of the parental and revertant strains.
The loss of activity from the membrane fraction of strain BG-31
is dependent on the volume of buffer used in the sonication
and wash steps and can be varied from 10% with small volumes
to as high as 80% with larger volumes. From this initial

Transport Experiments—Cells were grown, harvested, and washed
as described above. Transport systems containing 5 ml of minimal
sugars solution, 0.2% glucose, 1 mM KCN (when present), and cells
(0.3 to 0.4 mg of protein) were incubated for 15 min at 23°C, and
the reaction was terminated by the addition of 0.1 ml 0.5 mM
(3) [Proline
(233 Ci/mol) was used at a final concentration of 5 μM and α-lactate at a final
concentration of 10 mM.

Polyacrylamide Gel Electrophoresis—Denatured samples of mem-
branes from each strain were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodeyl sulfate, using the dis-
continuous buffer system of Laemmli (30), a slab gel apparatus
similar to that described by Studier (31) and according to the procedure
of Ames (32). Samples were prepared by incubation of the membranes
in a solution that contained 1% sodium dodeyl sulfate, 2 mM urea, and
1% mercaptethanol and a pinch of bromphenol blue. Samples contain-
ing 2 mg/ml of protein were incubated at 100°C for 2 min. Twenty
microliters were applied to each slot of the gel and run at a current of
20 mA for about 3 hours. Protein was detected by staining with
Coomassie blue according to the procedure of Fairbanks et al. (33).
observation, it seems clear that the attachment of BF1 to the membrane is altered in this strain. The revertant strain has recovered the binding capacity and appears similar to the parental strain.

**Sensitivity of ATPase Activity to Dicyclohexylcarbodiimide**

- Dicyclohexylcarbodiimide is a relatively specific inhibitor of the ATPase activity in a variety of systems and is effective only when the F1 portion is associated with the membrane portion of the complex (6). There is also evidence, particularly in mitochondrial systems, that DCCD reacts specifically with one polypeptide in the membrane portion of the complex (34, 35). The ATPase activity of strain BG-31 is insensitive to inhibition by DCCD over a wide concentration range (see Fig. 1). One possible explanation of this insensitivity is that the strain is defective in the DCCD reactive protein. A more likely possibility would be that the poor attachment of BF1 to the membrane renders it insensitive to the effects of DCCD. A final resolution of these alternatives must await direct DCCD binding experiments.

**Transhydrogenase Activity**—The functional state of the isolated membranes can be conveniently tested by measuring energy-dependent transhydrogenase activity. Energy can be provided for this reaction by both electron transport and hydrolysis of ATP by the ATPase. These two types of transhydrogenase activity are measured in BG-31, and the results are presented in Table II. This strain has nearly normal levels of the electron transport-dependent activity but is totally lacking the ATP-driven activity. This result is similar to that reported for a number of similar mutants (13, 20). The revertant strain has complete restoration of the ATP-driven activity. Thus it appears that even though this strain is capable of normal rates of ATP hydrolysis it cannot couple the resultant energy to this energy-dependent process.

It is also interesting to note that these strains show neither a decrease in respiratory-driven transhydrogenase as a consequence of removal of BF1 nor a stimulation of this activity by treatment with dicyclohexylcarbodiimide. This is in contrast with results obtained with another strain (13). This inconsistency may be due to a strain difference and/or a difference in the procedures used to remove BF1. This question is of considerable importance since removal or abnormal attachment of BF1 to the membranes has been shown to increase proton permeability and result in reduced energy coupling (9, 13). We are currently attempting to resolve this difference.

The transhydrogenase system allows us to perform in vitro complementation tests to attempt to localize the defect in this strain to the membrane portion of the BF1 portion of the complex. This can be done because it is possible to remove the BF1 portion from the membrane by the stripping procedure.

### Table I

Ca++,Mg++-ATPase activity of strains 1100, BG-31, and BG-31-R

The fractions were prepared and assayed as described under “Experimental Procedure.”

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fraction</th>
<th>ATPase activity (total units)</th>
<th>Protein (total milligrams)</th>
<th>Specific activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1100 (parent)</td>
<td>Sonic extract</td>
<td>374</td>
<td>536</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Membranes</td>
<td>300</td>
<td>188</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>Washed membranes</td>
<td>300</td>
<td>1/4</td>
<td>1.66</td>
</tr>
<tr>
<td>BG-31 (mutant)</td>
<td>Sonic extract</td>
<td>400</td>
<td>594</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Membranes</td>
<td>280</td>
<td>214</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>Washed membranes</td>
<td>170</td>
<td>192</td>
<td>0.80</td>
</tr>
<tr>
<td>BG-31-R (revertant)</td>
<td>Sonic extract</td>
<td>490</td>
<td>556</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Membranes</td>
<td>440</td>
<td>248</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Washed membranes</td>
<td>490</td>
<td>216</td>
<td>2.10</td>
</tr>
</tbody>
</table>

**Fig. 1 (left).** The effect of dicyclohexylcarbodiimide on the ATPase activity of membranes from strains 1100, BG-31, and BG-31-R. Approximately 30 μg of the membrane fraction from each strain were incubated in 1.0 ml of 0.05 M Tris-Cl buffer, pH 7.5, 1 mM MgCl2, and the indicated amounts of DCCD. The DCCD was added from an ethanol stock solution, and the highest concentration of ethanol was 0.12%, which in itself was not inhibitory. After preincubation of this mixture for 10 min at 25°C, the assay was initiated by the addition of ATP to a final concentration of 2.5 mM. O, strain 1100; ●, strain BG-31-R; and □, strain BG-31.

**Fig. 2 (center).** Reconstitution of ATP-driven transhydrogenase activity in strains 1100, BG-31, and BG-31-R. The stripped membrane fraction, approximately 1 mg, and EDTA extract (containing BF1 amount shown on figure) were added to the transhydrogenase incubation mixture, prior to the addition of NADP, and incubated for 15 min at 25°C. The assays were then conducted as described under “Experimental Procedure.” O, membranes (1100) + BF1 (1100); ●, membranes (1100) + BF1 (BG-31); □, membranes (1100) + BF1 (BG-31-R); △, membranes (BG-31) + BF1 (1100); and A, membranes (BG-31) and BF1 (1100).

**Fig. 3 (right).** Binding of BF1 (1100) to stripped membranes of strains 1100, BG-31, and BG-31-R. Various amounts of EDTA extract (BF1) from strain 1100 were incubated for 15 min at 23°C with 1 mg of stripped membranes isolated from each of the three strains tested in 1.0 ml of 0.05 M Tris-Cl buffer, pH 7.5, containing 1 mM MgCl2. The mixture was subjected to centrifugation at 150,000 × g for 60 min, after which the supernatant solution was removed and the membrane pellet was washed by resuspension in the incubation buffer and resedimented as above. The washed membrane pellet was reconstituted and the amount of ATPase activity determined, as described under “Experimental Procedure.” In all incubations the total recovery of added membrane protein and added ATPase activity were >90%. Symbols same as in Fig. 1.
with concomitant loss of the ATP-driven transhydrogenase activity. If the EDTA extract containing BF, (or pure BF,) is then added back to the stripped membranes under appropriate conditions, ATP-driven transhydrogenase activity can be re-

stored (26). It should be pointed out that the demonstration of normal ATPase activity does not rule out the possibility of an alteration in BF, (27). As shown in Fig. 2, BF, isolated from strain BG-31 is as effective as BF, isolated from the parental strain in restoring ATP-driven transhydrogenase activity to stripped membranes of the parental strain. In contrast, stripped membranes of BG-31 are inactive for reconstitution regardless of the source of BF,_. It seems clear that the defect in BG-31 resides in the membrane portion of the complex. Membranes isolated from the revertant have fully restored capacity for reconstitution.

Binding of BF, to Stripped Membranes—The evidence that BF, of strain BG-31 is poorly attached and that membranes isolated from BG-31 are inactive in transhydrogenase re-

constitutions suggested that rebinding of BF, to the membrane may be defective in this strain. This was tested directly in binding experiments, and the results are shown in Fig. 3. Membranes of BG-31 do not bind appreciable amounts of any BF, relative to membranes of the parental strain. It therefore appears that the membrane defect in strain BG-31 results in poor attachment of BF, as determined both by the ease with which it is lost during fractionation and by direct binding. The revertant strain has recovered the ability to bind BF,.

Transport Studies—The effect of a number of unc mutations on the active transport of a variety of solutes has been examined previously (10, 12, 16, 17, 20, 21, 36). It was largely from such studies that it became clear that energy coupling to active transport of a variety of solutes has been examined

on the active transport of a variety of solutes has been examined previously (10, 12, 16, 17, 20, 21, 36). It was largely from such studies that it became clear that energy coupling to active transport of solutes in Escherichia coli could occur both from electron transport and the hydrolysis of glycolytically-

generated ATP. It seems likely that energy from both sources is used to generate a proton motive force which is the direct

source of energy for solute accumulation (37). In order to assess the effect of the membrane defect in BG-31 on solute transport we have examined the uptake of [14C]proline by cells and isolated membrane vesicles. The data presented in Fig. 4 demonstrate that cells of BG-31 are able to transport proline at rates equivalent to the parent. If, however, the cells are treated with KCN to inhibit electron transport activity, the solute transport capacity is abolished. In contrast, the parental strain is only inhibited about 50% by treatment with KCN. The amount of transport retained by the parental strain in the presence of the electron transport inhibitor is a reflection of the energization of the transport by ATP. It is therefore clear that the mutant cannot utilize ATP for transport.

We have examined the transport capacity of isolated mem-

brane vesicles where the only source of energy is provided by an exogenous substrate of the electron transport system, D-lactate. As shown in Fig. 5 the electron transport-driven uptake of proline appears normal in membrane vesicles.

Polypeptide Analysis of Membranes of BG-31—Although many unc mutants have been described and a variety of physiological studies have been conducted there is to our knowledge no direct evidence that any of the mutations occur in the structural genes coding for the various polypeptide components of the complex. We have examined membranes of BG-31 in order to determine if an altered

TABLE II

Respiratory and ATP-driven transhydrogenase activity of membranes from strains 1100, BG-31, and BG-31-R

Assays were conducted as described under "Experimental Procedure," using 0.3 to 1.0 mg of the appropriate membrane fraction. Dicyclohexylcarbodiimide, when used, was included in the preincubation at a final concentration of 12 $\mu$m.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Membrane fraction</th>
<th>Transhydrogenase activity (nmol NADPH formed/min/mg membrane protein)</th>
<th>Respiratory-driven</th>
<th>ATP-driven</th>
</tr>
</thead>
<tbody>
<tr>
<td>1100</td>
<td>Membranes</td>
<td>20.9</td>
<td>22.9</td>
<td></td>
</tr>
<tr>
<td>1100</td>
<td>Membranes + DCCD</td>
<td>22.1</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>1100</td>
<td>Stripped membranes</td>
<td>16.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1100</td>
<td>Stripped membranes</td>
<td>18.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BG-31</td>
<td>Membranes</td>
<td>17.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BG-31</td>
<td>Membranes + DCCD</td>
<td>18.2</td>
<td>0</td>
<td></td>
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<tr>
<td>BG-31</td>
<td>Stripped membranes</td>
<td>15.7</td>
<td>0</td>
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<td>BG-31</td>
<td>Stripped membranes</td>
<td>15.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BG-31-R</td>
<td>Membranes</td>
<td>23.7</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>BG-31-R</td>
<td>Membranes + DCCD</td>
<td>20.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BG-31-R</td>
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<td>0</td>
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</tr>
<tr>
<td>BG-31-R</td>
<td>Stripped membranes</td>
<td>17.8</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Transport of [14C]proline by strains 1100, BG-31, and BG-31-R. Transport experiments were conducted as described under "Experimental Procedure." ○, strain 1100; ■, strain BG-31-R; and □, strain BG-31. Transports shown in a were carried out in the absence of KCN, and those shown in b in the presence of 1 mm KCN.

Fig. 5. Transport of [14C]proline by membrane vesicles of strains 1100, BG-31, and BG-31-R. Transports were conducted as described under "Experimental Procedure." ○, strain 1100; ■, strain BG-31; and □, strain BG-31-R.
gene product could be detected. We have used polyacrylamide slab gels run in the presence of sodium dodecyl sulfate for this analysis since this technique provides good separation of membrane polypeptides and easy comparison of different strains. Since we have concentrated on nonsense mutations we would expect to see an alteration in the size of a specific polypeptide. The gel patterns obtained are presented in Fig. 6. Stripped membranes of BG-31 appear to be missing a polypeptide of molecular weight approximately 54,000. In addition, a new peptide with a molecular weight of approximately 25,000 appears in the pattern and presumably represents that fragment of the polypeptide synthesized prior to the chain-terminating mutation. It is most significant that the gel pattern of the revertant is indistinguishable from that of the parent. We therefore conclude that BG-31 possesses a nonsense mutation in a structural gene that codes for a polypeptide of about 54,000 molecular weight that is a component of the membrane portion of the ATPase complex. This is further supported by analysis of the gel pattern for the supressed strain which shows partial restoration of the original polypeptide and diminution of the 25,000 Mₚ polypeptide as one would predict (data not shown). We make this interpretation with some caution, however, since other explanations, although unlikely, are possible. Since unc strains are generally quite defective for production of energy, their physiological state is quite different from that of the parental strain, and it is possible that both the disappearance of the 54,000 molecular weight peptide and the appearance of the 25,000 molecular weight species are the result of physiological regulation and are not a reflection of the mutational event. In order to minimize this possibility we have examined the membranes from one unc⁻ ATPase⁻ strain and three unc⁻ ATPase⁺ strains and have detected no differences in the gel patterns from that obtained with membranes from the parental strain. We feel therefore that the defect observed in BG-31 is not the result of an altered physiological state.

CONCLUSIONS

We have isolated and partially characterized a mutant strain of E. coli that has an altered polypeptide constituent of the membrane portion of ATPase complex. This defect results in poor attachment of BF, to the membrane portion of the complex, resistance of the ATPase activity to the inhibitory effects of DCCD, and the inability to couple energy from the hydrolysis of ATP to either transhydrogenase activity or solute accumulation. The interrelationship of these functional parameters, however, makes it impossible to assign a specific function to this membrane component. We are continuing to analyze other mutants in the membrane portion of the complex with the eventual goal of defining the complex and assigning specific functional roles to each polypeptide component.

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