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Cloning and Expression of the Transhydrogenase Gene of *Escherichia coli*

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Based on the rationale that *Escherichia coli* cells harboring plasmids containing the *pnt* gene would contain elevated levels of enzyme, we have isolated three clones bearing the transhydrogenase gene from the Clarke and Carbon colony bank. The three plasmids were subjected to restriction endonuclease analysis. A 10.4-kilobase restriction fragment which overlapped all three plasmids was cloned into the *PstI* site of plasmid pUC13. Examination of several deletion derivatives of the resulting plasmid and subsequent treatment with exonuclease *BAL* 31 revealed that enhanced transhydrogenase expression was localized within a 3.05-kilobase segment. This segment was located at 35.4 min in the *E. coli* genome. Plasmid pDC21 conferred on its host 70-fold overproduction of transhydrogenase. The protein products of plasmids carrying the *pnt* gene were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of membranes from cells containing the plasmids. Two polypeptides of molecular weights 50,000 and 47,000 were coded by the 3.05-kilobase fragment of pDC11. Both polypeptides were required for expression of transhydrogenase activity.

Pyridine nucleotide transhydrogenase (EC 1.6.1.1), found in the cytoplasmic membrane of *Escherichia coli* and in the inner membrane of mitochondria, catalyzes the reversible transfer of a hybrid ion equivalent between NAD^+ and $NADP^+$. Upon membrane energization by respiration or ATP hydrolysis, the rate of reduction of $NADP^+$ by NADH is increased severalfold (3, 8, 12, 25, 29).

Attempts have been made by several laboratories to isolate or identify the polypeptide composition of the bacterial enzyme. Liang and Houghton (21) partially purified the *E. coli* transhydrogenase by deoxycholate extraction of membranes followed by ion-exchange and gel filtration chromatography. Sodium dodecyl sulfate (SDS) gels showed two major protein bands of molecular weights 94,000 and 50,000 and several minor bands. When *E. coli* cells are grown on complex media containing high levels of amino acids, the synthesis of transhydrogenase is repressed (3). Using this observation, Liang and Houghton (21) attempted to determine which polypeptides were components of transhydrogenase by incorporating ^3H-labeled Casamino Acids in the initial repressive growth phase and nonrepressive levels of [^{14}C]leucine in the induction phase. SDS gels of the preparation indicated that the protein bands with the highest $^{14}C/^3H$ ratio corresponded to polypeptides of molecular weights 94,000 and 50,000. The relationships of the two polypeptides are not known. The largest polypeptide may represent a dimer of the 50,000-molecular-weight polypeptide. Alternatively, the smaller component may represent a proteolytic fragment of the larger component as the enzyme is sensitive to proteolytic degradation and the mitochondrial transhydrogenase is a single polypeptide of molecular weight 97,000 to 120,000 (1, 2, 15, 31). A third possibility is that the enzyme consists of two components analogous to the enzyme of *Rhodospirillum rubrum* (9, 10).

To identify the polypeptide structure of the *E. coli* transhydrogenase, we have isolated three clones bearing the transhydrogenase gene from the Clarke and Carbon colony bank (7). The *pnt* gene was subcloned into plasmid pUC13. Growth of cells containing the insert resulted in up to 70-fold amplification of transhydrogenase activity and the appearance of two polypeptides of molecular weights 50,000 and 47,000 associated with the membranes.

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MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* strain MV-12 is F^+ *recA* Δ*trp thr leu thi* and carries ColE1 plasmids prepared by Clarke and Carbon (7). The colony bank was provided by J. H. Weiner (University of Alberta). Strain JM83 (F^- *ara* Δ*lac pro rpsL* thi φ*80d lacZ* Δ*M15*) and plasmid pUC13 were provided by R. T. A. MacGillivray of this department. Strain W3110/ColE1 was used for the preparation of colicin E1 (27). Strains RH-5 (*argE3 lacY1 galK2 mtl-1 rpsL700 supE44 pnt*::Tn*5*) and AB1450 (*thi-1 ilvD16 argH1 metB1 hisG1 lac malA1 mtl-2 xyl-7 ara-13 gal-6 rpsL* tonA2 tsx-7 supE44 gltB13) were supplied by B. Bachmann (*E. coli* Genetic Stock Center).

Screening of the Clarke and Carbon collection for plasmids carrying the *pnt* gene. Each of the 2,112 clones from the Clarke and Carbon collection was grown at 37°C for 48 h in 12.5 ml of M9 medium (23) supplemented with 20 μg each of leucine, threonine, and tryptophan, 1 μg of thiamine, and 1 U of colicin E1 per ml. Cells were harvested by centrifugation at 12,000 × *g* for 5 min at 4°C. The supernatant was discarded and the cells were suspended in 2.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.2 mM dithiothreitol and 0.2 mM EDTA. The cells were broken by passage through an ice-cold French pressure cell (American Instrument Co., Silver Spring, Md.) at 1,400 kg/cm^2. Samples of 450 μl were used to measure transhydrogenase activity.

Preparation of plasmid DNA. For large-scale preparations, plasmid DNA was isolated from cells amplified with chloramphenicol as described by Maniatis et al. (23). Plasmid DNA was extracted from lysozyme-Triton X-100 lysates and purified by isopycnic centrifugation in a cesium chloride-ethidium bromide density gradient (17, 18). The plasmid DNA was removed from the gradient and extracted with water-saturated isoamyl alcohol to remove the ethidium bromide, and the plasmid DNA was precipitated twice with ethanol in the presence of 0.3 M sodium acetate (pH 7.0).

TABLE 1. Transhydrogenase activity in membranes of selected strains from the Clarke-Carbon colony bank

Strain	Transhydrogenase activity (μmol/min per mg of protein)	
	LB medium	M9 medium
MV12(pLC14-12)[a]	0.04	0.18
MV12(pLC10-19)	0.32	1.33
MV12(pLC26-24)	0.38	1.52
MV12(pLC27-35)	0.42	1.80

[a] The level of transhydrogenase activity in this strain is typical of those strains not carrying the *pnt* gene on a ColE1 plasmid.

Ethanol-precipitated DNA was suspended to a final concentration of 0.5 to 1 mg/ml in 10 mM Tris-hydrochloride (pH 8.0) containing 1 mM EDTA.

For small-scale isolations, plasmid DNA was prepared from unamplified overnight cultures, using the alkaline lysis method (23).

Digestion with restriction enzymes and exonuclease BAL 31. The restriction endonucleases used for cleavage of plasmid DNA were *Pst*I, *Hin*dIII, *Eco*RI, *Sma*I, and *Xho*I from Amersham International Corp., Toronto, Ont.; *Sal*I and *Bst*EII from Boehringer Mannheim Biochemicals, Indianapolis, Ind.; and *Hpa*I and *Sst*I from Pharmacia P-L Biochemicals Ltd., Uppsala, Sweden. Exonuclease *BAL* 31 was from Boehringer Mannheim Corp. Digestions were carried out according to the instructions of the suppliers.

Conditions for partial digestion of plasmid DNA were established by adding serial dilutions of enzymes to the plasmid DNA and incubating at 37°C for 1 h. Reactions were terminated by the addition of one-sixth volume of concentrated loading buffer (0.25% bromophenol blue, 40% sucrose, 75 mM EDTA), and a sample was loaded onto a 0.5 to 1% agarose gel for electrophoresis (see below). Once the correct dilution of enzyme had been determined, partial digestion of plasmid DNA was carried out accordingly and the reactions were stopped by the addition of 0.5 M EDTA to a final concentration of 12 mM.

Ligations and transformations. The procedures described by Maniatis et al. (23) were used for ligation with T4 DNA ligase (Boehringer Mannheim), treatment with calf intestinal phosphatase (Boehringer Mannheim), filling recessed 3′ ends of double-stranded DNA with the Klenow fragment of *E. coli* DNA polymerase (Boehringer Mannheim), and transformation. Transformants were selected by their ability to grow on LB (23) plates containing ampicillin (100 μg/ml) which had been coated with 50 μl of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (2% in *N*,*N*′-dimethylformamide). Clones carrying an insert within the plasmid were identified as white colonies, whereas clones without inserts gave blue colonies.

Electrophoresis of DNA. Agarose slab gels (0.5 to 1%) were prepared and run in TBE buffer (0.09 M Tris, 0.09 M boric acid, 2 mM EDTA) at 60 V. Gels were stained in ethidium bromide (0.5 μg/ml), and DNA-containing bands were observed under UV light. Extraction of individual DNA bands for ligation was performed as described by Burns and Beacham (5), using low-melting-point agarose.

Preparation of membranes. Cells were grown with shaking at 37°C in 400 ml of LB media containing, where appropriate, 1 U of colicin E1, 25 μg of kanamycin, or 50 μg of ampicillin per ml, to an absorbance of 1.2 at 600 nm. The cells were harvested, washed with TED buffer (50 mM Tris-hydrochloride [pH 7.8], 1 mM dithiothreitol, 1 mM EDTA), suspended in 7 ml of TED buffer, and lysed by passage through an ice-cold French pressure cell at 1,400 kg/cm^2. Unbroken cells were removed by centrifugation at 12,000 × *g* for 10 min at 4°C. The supernatant was centrifuged at 180,000 × *g* for 2 h at 4°C. The supernatant obtained is referred to as the cytoplasmic fraction. The membranes were suspended in 5 ml of TED buffer. Samples were assayed for transhydrogenase activity and for protein by the method of Lowry et al. (22).

Assay of transhydrogenase activity. The assay of transhydrogenase activity was based on the method of Kaplan (16). The reaction was carried out at 25°C in a mixture which contained 50 mM sodium phosphate buffer (pH 7.0), 1 mM KCN, 2 mM dithiothreitol, 0.5 mM EDTA, 1 mM 3-acetylpyridine-NAD$^+$, and 0.5 mM NADPH in a final volume of 1 ml. The reduction of 3-acetylpyridine-NAD$^+$ by NADPH was measured as an increase in the absorbance at 375 nm, using a Coleman 124 spectrophotometer.

Electrophoresis of proteins. SDS-polyacrylamide gel electrophoresis was performed on vertical slab gels of 10% polyacrylamide, with a stacking gel of 4% polyacrylamide. The discontinuous SDS buffer system of Laemmli (19) was used. Electrophoresis was carried out at a constant current of 40 mA for 2 h at room temperature. Gels were stained in 0.1% Coomassie blue in 25% isopropanol–10% acetic acid and destained in 10% acetic acid. Proteins labeled with [^{35}S]methionine were identified by autoradiography of dried gels.

P1 transduction. Bacteriophage P1 *vir* was obtained from P. Dennis of this department. The preparation of lysates and transductions were carried out as described by Miller (24).

In vitro protein synthesis. In vitro protein synthesis was carried out with a procaryotic DNA-directed translation kit obtained from Amersham International Corp. Proteins were translated in the presence of [^{35}S]methionine according to the instructions of the supplier.

RESULTS

Identification of the *pnt* plasmids. Mutants defective in the expression of transhydrogenase do not exhibit a readily detectable phenotype (14). This property ruled out any screening of an *E. coli* plasmid bank based on a recognizable phenotypic change when seeking the *pnt* locus. Therefore, we screened the Clarke and Carbon colony bank following the rationale that *E. coli* cells harboring multicopy plasmids containing the *pnt* gene should contain elevated levels of the enzyme. Among the approximately 2,100 clones of the Clarke and Carbon collection, clones pLC10-19, pLC26-24, and pLC27-35 contained 8- to 10-fold more transhydrogenase activity when compared with the other clones of the bank (Table 1). About 90% of the enzyme activity was found to be associated with the membranes of these cells. Expression of *E. coli* transhydrogenase is maximal when cells are grown in minimal media free of high levels of amino acids (3). Increased levels of transhydrogenase were observed when each of the *pnt*-bearing clones was grown in minimal M9 medium. Membrane prepared from cells grown in minimal medium exhibited a fourfold increase in transhydrogenase activity as compared with the transhydrogenase activity of membranes prepared from cells grown in LB medium (Table 1).

Restriction endonuclease analysis of the *pnt* plasmids. Restriction endonuclease analysis was performed on the three recombinant plasmids from the Clarke and Carbon colony bank to identify the regions of DNA containing the *pnt* gene.

An 8.7-kilobase region was found to be common to the inserts of all three plasmids (Fig. 1).

The region of the *E. coli* genome bearing the transhydrogenase gene has been physically mapped by Bouché (4). Comparison of the restriction endonuclease maps of the plasmids with that of the genome reveals that the plasmid inserts overlap a region of the genome between 35.2 and 35.7 min (Fig. 2). The overlap region common to all three plasmids includes the 35.4-min region which is the position mapped by Hanson and Rose (14) for the *pnt* gene.

Subcloning of the transhydrogenase gene into plasmid pUC13. Plasmid pLC26-24 was digested by restriction endonuclease *Pst*I to give four fragments (10.4, 5.9, 1.2, and 0.1 kilobases). The fragments were separated by gel electrophoresis in low-melting-point agarose, and the 10.4-kilobase fragment was excised from the gel. *Pst*I-digested pUC13, which had been dephosphorylated with calf intestinal phosphatase, was ligated with the 10.4-kilobase fragment and the ligated DNA was used to transform strain JM83. White ampicillin-resistant transformants were selected and screened for overproduction of transhydrogenase. Plasmid pDC1, containing the 10.4-kilobase *Pst*I fragment inserted into pUC13, amplified transhydrogenase activity 20-fold in JM83.

A 4.8-kilobase *Hin*dIII fragment of pDC1 was subcloned into the *Hin*dIII site of pUC13 as shown in Fig. 1 to yield plasmid pDC3. Transhydrogenase activity of JM83 carrying pDC3 was 50-fold greater when compared with JM83 harboring pUC13.

Localization of the *pnt* gene in pDC3. Plasmid pDC3 was subjected to restriction endonuclease analysis, using the restriction endonucleases *Hpa*I, *Bst*EII, *Xho*I, *Sma*I, and *Sal*I. After the locations of the restriction endonuclease sites had been established, various segments of the 4.8-kilobase

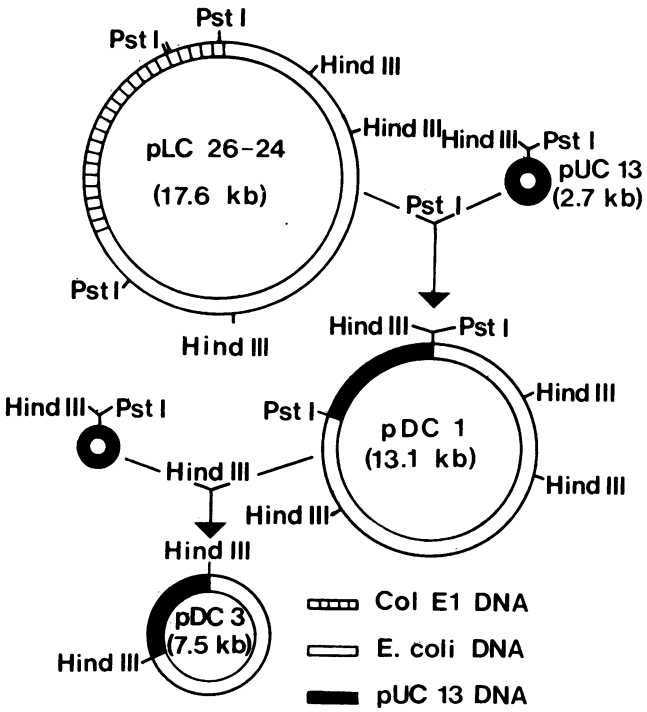

FIG. 1. Subcloning of DNA carrying the *pnt* gene. The plasmids and relative positions of the restriction sites are drawn approximately to scale. kb, Kilobase.

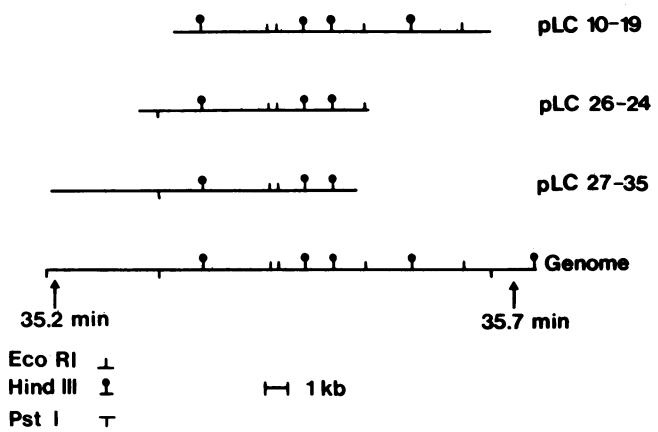

FIG. 2. Comparison of restriction endonuclease maps of ColE1 plasmid inserts with a region of the *E. coli* genome. The restriction map of the *E. coli* genome was determined by Bouché (4). kb, Kilobase.

*Hin*dIII inserts of pDC3 or pDC4 were removed. Plasmids pDC3 and pDC4 differ only in the orientation of the insert in the pUC13 vector. Plasmid pUC13 contains a single *Eco*RI site. The plasmids in Fig. 3 are drawn so that this site is closest to one end of the insert. The constructed plasmids were used to transform JM83. The cells were grown and their transhydrogenase levels were determined (Fig. 3). Deletion of a 0.55-kilobase *Hpa*I-*Sma*I fragment from pDC3 or a 1.6-kilobase *Hpa*I-*Sma*I fragment from pDC4 to give plasmids pDC8 and pDC9, respectively, resulted in loss of enhanced expression of transhydrogenase activity. These results demonstrated that at least the 2.65-kilobase fragment bounded by the *Hpa*I restriction sites was essential for the expression of enzyme activity. During the construction of pDC9 from pDC4, one clone was isolated which contained plasmid pDC11. This was missing 0.75 kilobases of DNA between the *Hpa*I and *Hin*dIII sites (Fig. 3). There was no *Hpa*I site at this point in pDC4 so the cleavage may have been made by another enzyme contained in the *Hpa*I preparation. This plasmid conferred a 70-fold amplification of transhydrogenase activity in the membranes of JM83 (Fig. 3). The gene was further localized by treatment with the exonuclease *BAL* 31. Plasmid pDC11 contains a single *Hin*dIII site at one junction of the pUC13 and insert DNA (Fig. 3) and a single *Sst*I site within vector pUC13 at the other junction. The plasmid was first cleaved with *Sst*I and then treated with *BAL* 31 for different lengths of time. The *BAL* 31-treated inserts were cleaved with *Hin*dIII and then ligated into the *Hin*dIII and *Hin*cII sites of pUC13. The resulting plasmids were used to transform JM83. The smallest plasmid still retaining the transhydrogenase gene was then isolated, and the process described above was repeated for the other end of the insert by first cleaving with *Hin*dIII followed by digestion for various lengths of time with *BAL* 31. The digested inserts were cleaved with *Sst*I and then ligated into the *Sst*I and *Hin*cII sites of pUC13. The resulting plasmid, pDC21, contained a 3.05-kilobase insert. Inserts 50 to 100 base pairs smaller than the insert of pDC21 did not exhibit transhydrogenase activity. Plasmid pDC21 conferred a 70-fold amplification of transhydrogenase activity in the membranes of JM83 (Fig. 3).

Identification of the *pnt* gene products. During the course of this study, we found that the *pnt* gene products irreversibly aggregated when solubilized in SDS gel electrophoresis

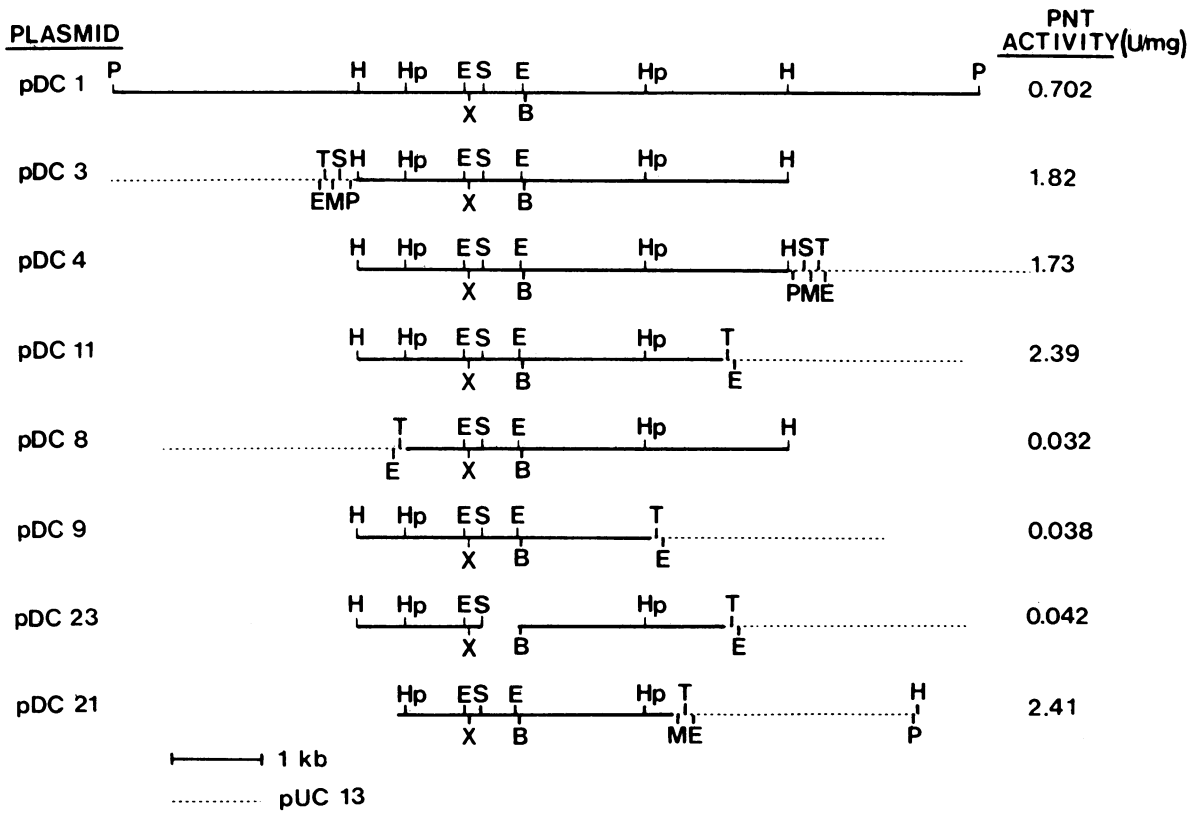

FIG. 3. Restriction endonuclease maps of plasmids containing the *pnt* gene and transhydrogenase activities of membranes prepared from cells harboring each of the plasmids. Plasmids were constructed and transformed into JM83. Membranes were prepared from transformants grown in LB medium and assayed for transhydrogenase (PNT) activity. The transhydrogenase activity of membranes prepared from JM83(pUC13) was 0.035 U/mg of protein. Symbols: P, *Pst*I; H, *Hin*dIII; B, *Bst*EII; Hp, *Hpa*I; E, *Eco*RI; S, *Sal*I; X, *Xho*I; T, *Sst*I; M, *Sma*I. Solid lines are inserted DNA. kb, Kilobase.

sample buffer at 100°C and did not enter SDS-polyacrylamide gels during electrophoresis. However, two protein products of molecular weight 50,000 and 47,000 were observed in the gels of membranes from JM83(pDC11), but not in the gels of membranes from JM83(pUC13), when solubilization in the SDS sample buffer was carried out at 37°C (Fig. 4).

To establish that the two protein products of molecular weights 50,000 and 47,000 were plasmid encoded, an in vitro transcription/translation system was used to determine which proteins were formed with plasmid pDC11 as template. The proteins, labeled with [^{35}S]methionine, were separated by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography of the dried gels. Two radioactive polypeptides of molecular weights 50,000 and 47,000 were observed to be products of the in vitro translation of pDC11 but not of pUC13 (Fig. 5). These two products correspond to the two polypeptides amplified in membranes of JM83(pDC11). The lower-molecular-weight radioactive polypeptides are products of the pUC13 vector DNA.

Two polypeptides of a combined molecular weight of 97,000 would require the coding capacity of 2.7 kilobases of DNA. This agrees with the observation that at least a 3.05-kilobase insert is required for the expression of enzyme activity. To establish whether or not both polypeptides were needed for transhydrogenase activity, the products of the various plasmids (Fig. 3) were examined by SDS-polyacrylamide gel electrophoresis (Fig. 6). The expression of both polypeptides was observed in JM83 membranes containing either pDC11 or pDC21, and amplification of transhydrogenase activity was observed in both cases. Similar results were obtained with plasmids pDC1, pDC3, and pDC4 (data not shown). Neither of the two polypeptides was observed in the membranes of cells containing pUC13 or pDC8. Only one membrane-bound protein was observed as a product of plasmid pDC9. No increase in transhydrogenase activity was detected in these membranes, indicating that the 50,000-molecular-weight polypeptide alone is not capable of transhydrogenation.

The possibility that the 47,000-molecular-weight polypeptide alone is responsible for transhydrogenase activity was examined by constructing plasmid pDC23 from pDC11. Plasmid pDC11 was digested with *Sal*I and *Bst*EII, and the recessed 3′ ends of the DNA were filled in before ligation, using the Klenow fragment of *E. coli* DNA polymerase. The resulting plasmid, pDC23, was used to transform strain JM83. Membranes prepared from the transformed strain contained the 47,000-molecular-weight polypeptide and a polypeptide of molecular weight 42,000 (Fig. 6). (The latter may be a proteolytic digestion product of the 47,000-molecular-weight polypeptide or a translation product of the residual DNA resulting from the 0.45-kilobase deletion.) No increase in transhydrogenase activity was detected in these membranes (Fig. 3), indicating that the 47,000-molecular-weight polypeptide alone is not capable of transhydrogenation.

Further evidence that the 50,000- and 47,000-molecular-weight polypeptides compose the transhydrogenase mole-

cule and are not merely stimulating expression of the chromosomally encoded transhydrogenase gene comes from complementation tests with strain RH-5. In this strain transposon Tn5 is inserted in the *pnt* locus with the result that there is complete loss of transhydrogenase activity (14). Since RH-5 transformed poorly, the defective *pnt* locus was transduced into AB1450, using bacteriophage P1. This strain was then transformed with plasmids pUC13, pDC9, pDC23, and pDC21. Transhydrogenase activity was restored to the recipient by pDC21 only, indicating that both polypeptides

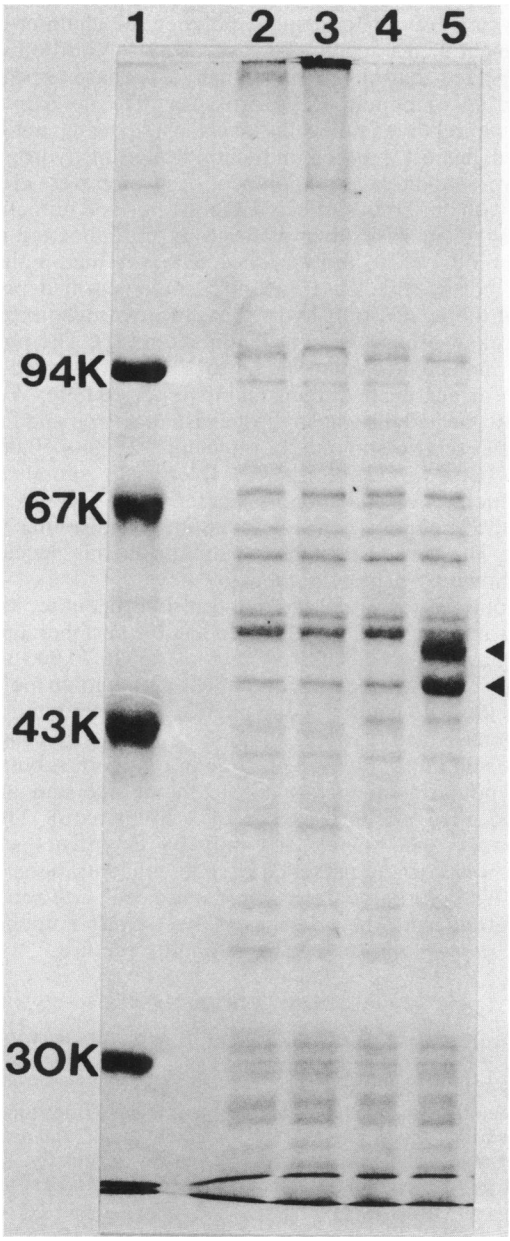

FIG. 4. SDS-polyacrylamide gel electrophoresis of membranes of JM83 containing either pUC13 or pDC11. Membranes were solubilized in SDS sample buffer at either 37°C for 10 min or 100°C for 3 min before electrophoresis. Lane 1, Molecular weight markers; lane 2, JM83(pUC13) membranes solubilized at 100°C; lane 3, JM83(pDC11) membranes solubilized at 100°C; lane 4, JM83(pUC13) membranes solubilized at 37°C; lane 5, JM83(pDC11) membranes solubilized at 37°C.

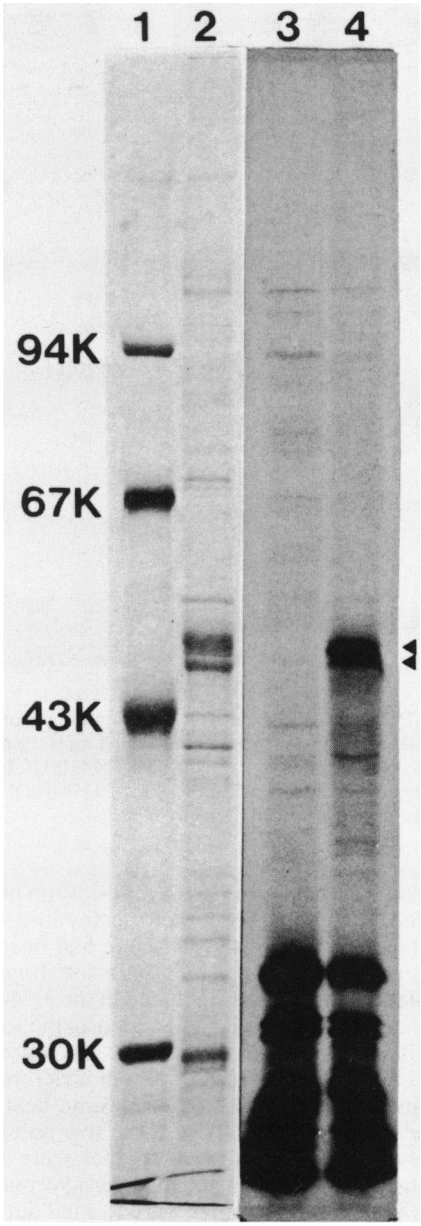

FIG. 5. Autoradiograph of SDS-polyacrylamide electrophoresis gel of [^{35}S]methionine-labeled products, using plasmids pUC13 (lane 3) and pDC11 (lane 4) as templates in an in vitro transcription/translation system. Plasmid DNA was added to a final concentration of 100 μg of DNA per ml. The reaction mixture was incubated at 37°C for 40 min. The reaction was terminated by cooling to 0°C. Samples of the reaction mixture were mixed with an equal volume of electrophoresis sample buffer, and 10 μl was used for electrophoresis. The polypeptides of lanes 1 (molecular weight markers) and 2 [membrane polypeptides of JM83(pDC11)] were stained with Coomassie blue.

are part of the enzyme. A specific activity of 1.94 U/mg of protein was observed in membranes prepared from the recipient.

DISCUSSION

Difficulties in the identification and purification of the transhydrogenase of *E. coli* are the results of it being a relatively minor membrane protein. However, three plas-

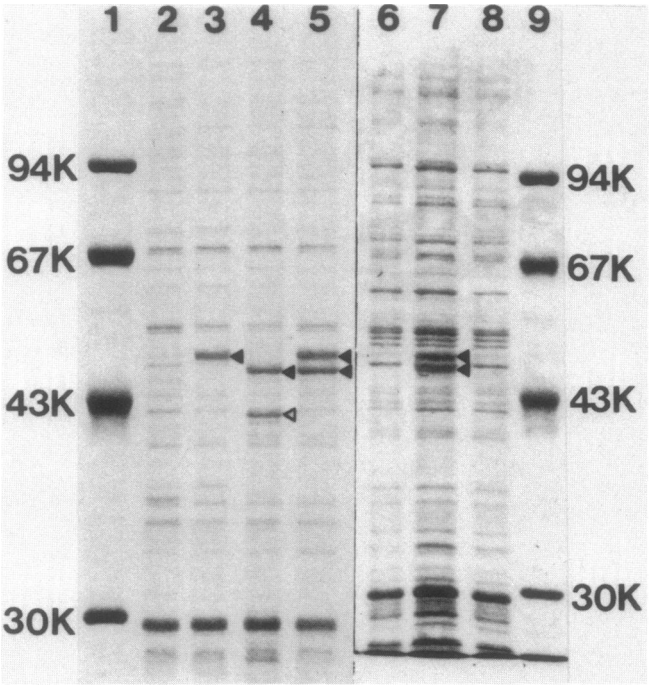

FIG. 6. SDS-polyacrylamide gel electrophoresis of membrane fractions of JM83 carrying hybrid plasmids. Lanes 1 and 9, Molecular weight markers; lanes 2 and 8, JM83(pUC13); lane 3, JM83(pDC9); lane 4, JM83(pDC23); lane 5, JM83(pDC21); lane 6, JM83(pDC8); lane 7, JM83(pDC11).

mids were isolated from the Clarke and Carbon colony bank which conferred an amplification of transhydrogenase activity on the host cells. That the *pnt* gene had been cloned is shown by the following. (i) An 8.7-kilobase fragment common to all three plasmids was included in the 35.4-min region of the *E. coli* genome previously mapped as the locus for the *pnt* gene. (ii) Transhydrogenase activity was repressed when the plasmid-bearing cells were grown in a rich medium (3). (iii) Analysis of membranes of the plasmid-bearing strains showed the amplified expression of the two polypeptides of molecular weights 50,000 and 47,000 which were observed in partially purified preparations of the transhydrogenase of *E. coli* (6, 21). Similar polypeptides were formed during in vitro transcription/translation of pDC11 DNA. (iv) Transhydrogenase activity was restored to a transhydrogenase-defective mutant when transformed with plasmid pDC21.

Subcloning of the *pnt* gene into pUC13 resulted in up to 70-fold amplification of transhydrogenase activity. Initial attempts to identify the protein products by SDS-polyacrylamide gel electrophoresis failed because heating at 100°C in the presence of SDS gel electrophoresis sample buffer caused aggregation of the proteins so that they did not enter the gel. The *rodA* gene product (26), lactose permease (28), and glycerol phosphate permease (20) are other proteins which aggregate when boiled in SDS. All four proteins which behave in this manner are cytoplasmic membrane proteins.

Both polypeptides are needed for the expression of transhydrogenase activity. Deletion of a 1.6-kilobase *Hpa*I-*Hin*dIII fragment from pDC4 to give pDC9 resulted in the loss of the 47,000-molecular-weight polypeptide from the membranes of plasmid-bearing cells. Deletion of the 0.55-kilobase *Hpa*I-*Hin*dIII fragment from the 4.8-kilobase *Hin*dIII insert of pDC3 to give pDC8 resulted in the loss of both polypeptides. The 50,000-molecular-weight polypeptide was lost when a 0.45-kilobase *Sal*I-*Bst*EII fragment was deleted from pDC11. No transhydrogenase activity was associated with any of these plasmids. These results suggest that the promoter region is found in the 0.60-kilobase *Hpa*I-*Xho*I region followed by the regions coding for the 50,000- and 47,000-molecular-weight polypeptides, respectively.

The structure of the *E. coli* transhydrogenase differs markedly from the well-studied transhydrogenase of the bovine heart mitochondria and *R. rubrum*. The bovine heart mitochondrial transhydrogenase has been purified to homogeneity and consists of a single polypeptide chain of molecular weight 97,000 to 120,000 (1, 2, 15, 31). Voordouw et al. (30) reported that the *E. coli* transhydrogenase appeared to contain an immunologically cross-reactive polypeptide of the same relative molecular mass when using antibodies directed against purified mitochondrial transhydrogenase. However, we did not find polypeptides which were cross-reactive with antibodies directed against purified mitochondrial transhydrogenase when we tried to reproduce the results of Voordouw et al. (unpublished data). A high-molecular-weight polypeptide observed in partially purified preparations of the *E. coli* transhydrogenase (6) was also repressed when cells were grown in a rich medium (21). The polypeptide of molecular weight 90,000 to 100,000 observed previously is an aggregate of the subunits reported here. We have recently purified the transhydrogenase of *E. coli* and found it to consist of two subunits of molecular weights 50,000 and 47,000 (D. M. Clarke and P. D. Bragg, submitted for publication). The two subunits aggregate on storage, resulting in the appearance of a polypeptide of molecular weight 97,000. The presence of both subunits in this species has been shown by peptide mapping.

In contrast to mitochondrial transhydrogenase, the enzyme from *R. rubrum* consists of a soluble peripheral protein factor having a molecular weight of about 70,000 and an integral membrane-bound component of unknown molecular weight (9, 10). Neither component alone exhibited transhydrogenase activity (11). The *E. coli* transhydrogenase differs from the *R. rubrum* enzyme in that both subunits are tightly bound to the cytoplasmic membrane and are not released even in the presence of 4 M urea (6). The two components can be released only by detergents such as deoxycholate in the presence of high concentrations of salt (6, 21). Therefore, the transhydrogenase of *E. coli* appears to be different from the enzymes of beef heart mitochondria and *R. rubrum* with respect to subunit structure.

ACKNOWLEDGMENTS

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