Restoration of hydrogenase activity in hydrogenase-negative strains of *Escherichia coli* by cloned DNA fragments from *Chromatium vinosum* and *Proteus vulgaris*

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DNA fragments from *Proteus vulgaris* and *Chromatium vinosum* were isolated which restored hydrogenase activities in both hydA and hydB mutant strains of *Escherichia coli*. The hydA and hydB genes, which map near minute 59 of the genome map, 17 kb distant from each other, are not structural hydrogenase genes, but mutation in either of these genes leads to failure to synthesize any of the hydrogenase isoenzymes. The smallest DNA fragments which restored hydrogenase activity to both *E. coli* mutant strains were 4.7 kb from *C. vinosum* and 2.3 kb from *P. vulgaris*. These fragments were cleaved into smaller fragments which did not complement either of the *E. coli* mutations. The cloned heterologous genes also restored formate hydrogenlyase activity but they did not restore activity in hydE, hupA or hupB mutant strains of *E. coli*. The cloned genes, on plasmids, did not lead to the synthesis of proteins of sufficient size to be the hydrogenase catalytic subunits. The hydrogenase proteins synthesized by hydA and hydB mutant strains of *E. coli* transformed by cloned genes from *P. vulgaris* and *C. vinosum* were shown by isoelectric and immunological methods to be *E. coli* hydrogenase. Thus, these genes are not hydrogenase structural genes.

**Introduction**

The synthesis of the enzyme hydrogenase in *Escherichia coli* requires a number of genes which have been characterized by mutational analysis. Four of these genes, hydA, hydB, hydE and hydF, have been mapped near minute 59 of the *E. coli* genome map and DNA fragments have been isolated which complemented the individual mutations (Lee *et al.*, 1985; Sankar *et al.*, 1985; Waugh & Boxer, 1986; Chaudhuri & Krasna, 1987; Sankar & Shanmugam, 1988a, b). Strains with mutations in these genes are devoid of all hydrogenase activities (reduction of viologen dyes with H₂, exchange reaction, growth on fumarate plus H₂, evolution of H₂ from formate) and lack the three hydrogenase isoenzymes (Ballantine & Boxer, 1985; Sawers *et al.*, 1985; Waugh & Boxer, 1986). The genetic map of the hydrogenase genes near minute 59 is shown in Fig. 1. The hydA and hydB genes are 17 kb distant from each other. The hydC and hydD genes, which map at minute 77, are also required for synthesis of all three isoenzymes (Wu & Mandrand-Berthelot, 1986). Mutations in the hydC and hydE genes can be overcome by growing cells in the presence of high concentrations of nickel (Waugh & Boxer, 1986; Wu & Mandrand-Berthelot, 1986; Chaudhuri & Krasna, 1987). The role of the hydA, hydB and hydF genes is not known. None of these genes are structural genes for hydrogenase but they are required for the synthesis of all hydrogenase isoenzymes.

To elucidate the role of the cloned *E. coli* hydrogenase genes near minute 59, DNA fragments were isolated from DNA libraries of hydrogenase-containing *Chromatium vinosum* and *Proteus vulgaris* and tested for their ability to restore the hydrogenase phenotype in hydA and hydB mutant strains of *E. coli*.

**Methods**

*Organisms and growth conditions.* Hydrogenase-positive *C. vinosum* and *P. vulgaris* were grown as previously described (Gitlitz & Krasna, 1975; Schengrund & Krasna, 1969) and the DNA isolated from these cells was used to prepare genomic libraries (Chaudhuri & Krasna, 1987, 1988). The wild-type hydrogenase-positive *E. coli* strain was K12W6 met bio (ATCC 25019). The hydrogenase-negative *E. coli* strains were SE-31 (hydB) and SE-19-1 (hydA) (Lee *et al.*, 1985), kindly supplied by
Dr K. T. Shanmugam. As positive controls, the hydA strain was transformed by plasmid pSE-201 and the hydB strain by plasmid pSE-128 (Sankar et al., 1985).

Bacteria were grown in complex or minimal medium containing appropriate antibiotics when required for selection as described previously (Chaudhuri & Krasna, 1987).

**Enzyme assays.** The enzyme activities of interest were measured at 25 °C in washed cell suspensions. Hydrogenase activity was assayed by the deuterium exchange method and reduction of viologen dyes; formate dehydrogenase (FHL) activity was determined by manometric methods. Hydrogenase activity was assayed by reduction of methyl viologen by H₂ and growth on formate plus H₂. Strains SE-31 (hydA) and SE-19-1 (hydB) are Hyd⁻ and become Hyd⁺ when transformed by the cloned E. coli DNA fragments on plasmids (Sankar et al., 1985).

These strains were used when these strains were transformed by plasmid DNA libraries from C. vinosum or P. vulgaris. Hyd⁺ transformants were then grown on minimal medium plus glucose or on formate plus H₂ and assayed by deuterium exchange.

**Preparation of and transformation by genomic DNA libraries.** The methodology was essentially that described earlier (Chaudhuri & Krasna, 1987) for the cloning of and transformation by an E. coli DNA library. Chromosomal DNA from C. vinosum or P. vulgaris was partially digested with the restriction enzyme Sau3A, subjected to electrophoresis on 0.6% agarose, and fragments in the size range 4–20 kb isolated. These fragments were ligated to pBR322 which had been cleaved with BamHI and treated with calf intestinal phosphatase. Chromosomal DNA from C. vinosum (Chaudhuri et al., 1985) and all the transformed colonies were collected, grown in large volume and plasmid DNA isolated. These purified plasmid DNA preparations were the C. vinosum and P. vulgaris libraries used for gene selection.

These two libraries were used to transform hydA and hydB mutant strains of E. coli to ampicillin resistance (Ap⁰). A large number of these colonies were then grown on formate plus H₂ to enrich for Hyd⁺ cells. These cells were plated on agar and screened for the Hyd⁺ phenotype. A number of positive isolates were selected, and DNA was prepared from each and used to transform the mutant strains to the Hyd⁻ phenotype. In each case all Ap⁰ transformants were Hyd⁺ by all criteria including high levels of deuterium exchange in cells grown on formate plus H₂. The plasmids were mapped by restriction enzyme digestion. All the Hyd⁺ transformants from the respective genomic libraries were identical.

**Hybridization studies.** The cloned plasmid DNA fragments isolated from the different organisms were treated with appropriate restriction enzymes and purified by agarose gel electrophoresis to obtain unique fragments from each plasmid (free of vector plasmid DNA) that could be used as probes for hybridization to other plasmid digests or to chromosomal DNA digested with EcoRI. Hybridizations were carried out in 50% (v/v) formamide at 42 °C (Tm – 14 °C) overnight with the denatured labelled probe and the filters were washed in 2 x SSC/0.1% SDS at room temperature for 15 min and then at 65 °C (Tm – 25 °C) for 2 h with four changes of buffer (1 x SSC is 0.15 M-NaCl, 0.015 M-trisodium citrate, pH 7.0). Autoradiography was used to locate the bands. Washing the filters at 50, 37 or 25 °C gave the same results as washing at 65 °C.

**Synthesis of plasmid-coded proteins.** The maxicell procedure of Sancar et al. (1979) was followed, using a mixture of ¹⁴C-labelled amino acids and ³⁵S-labelled methionine. E. coli strain CSR603 (Sancar et al., 1979) was obtained from the E. coli Genetic Stock Center, Yale University.

**Characterization of hydrogenase proteins.** Membranes were isolated as previously described (Chaudhuri & Krasna, 1988) from parental strains of E. coli, P. vulgaris and C. vinosum as well as from hydA and hydB mutant strains of E. coli (SE-31 and SE-19-1) transformed by the different plasmids. The membranes were solubilized with Triton or Lubrol, and analysed by isoelectric chromatofocusing between pH 4 and pH 6 using Polybuffer PBE94 (Pharmacia). The solubilized membranes were also tested for reactivity with antibodies to E. coli hydrogenase isoenzyme 1 (Sawers et al., 1985). The antibodies were prepared by Dr K. T. Shanmugam and were a mixture of monoclonal antibodies to the separated 59 kDa subunit and the 28 kDa subunit.

The membranes preparations were analysed by ELISA and by Western blotting. The proteins were treated with anti-hydrogenase 1 antibodies and then with anti-mouse-IgG antibodies conjugated with alkaline phosphatase and the colour developed with suitable substrates (Blake et al., 1984).

**Results and Discussion.**

**Restoration of the Hyd⁺ phenotype by a cloned DNA fragment from C. vinosum.**

From the C. vinosum library a single plasmid was isolated which restored hydrogenase activity in both hydA and hydB mutant strains. The plasmid, pSE3-1Chr (9.6 kb) had a chromosomal DNA insert of 5.2 kb; the restriction digest map is shown in Fig. 2. The results in Table 1 show that this plasmid restored deuterium exchange activity in both mutant strains. The deuterium exchange activity in the wild-type E. coli strain K12W6 was not affected by transformation by pSE3-1Chr. This plasmid did not restore activity to a hydE mutant strain of E. coli. An E. coli DNA fragment has been isolated (Waugh & Boxer, 1986; Chaudhuri & Krasna, 1987) which restored activity to both hydB and hydE mutant strains of E. coli. No DNA fragment from E. coli has been isolated which complements both hydA and hydB mutations. In E. coli the distance between these two genes is about 17 kb, based on reported genetic maps near minute 59 (Yerkes et al., 1984; Karube et al., 1984; Sankar et al., 1985; Kohara et al., 1987).

![Fig. 1. Location of the hyd genes near minute 59 in the E. coli genome map.](image-url)
Hydrogenase genes from *P. vulgaris* and *C. vinosum* 1155

Fig. 2. Restriction digest maps of plasmids from *C. vinosum*. The circular plasmids are represented in linear form, the heavy line being the vector plasmid and the light line the chromosomal DNA insert. The letters indicate the restriction sites: B/Sa, BamHI-Sau3A junction of vector and insert; H, HindIII; N, NruI; RI, EcoRI; S, Sall. There are no sites in the insert for CiaI, EcoRV, NdeI, NheI, PstI, PvuII, Scal and Sphi. The insert in pSE3-1Chr has one Ball site, one KpmI site, two AvaI sites, five StyI sites and five PvuI sites which have not been mapped precisely.

Table 1. Hydrogenase activity of *E. coli* mutant strains transformed by cloned DNA fragments

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>hyd genotype</th>
<th>Plasmid</th>
<th>Glucose grown</th>
<th>Fumarate + H₂ grown</th>
</tr>
</thead>
<tbody>
<tr>
<td>K12W6 Wild-type</td>
<td>None</td>
<td>0.7</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>KE3-31 hydB</td>
<td>None</td>
<td>&lt;0.02</td>
<td>NG</td>
<td></td>
</tr>
<tr>
<td>KE3-31 hydB</td>
<td>pSE3-1Chr</td>
<td>0.56</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>KE3-19-1 hydA</td>
<td>None</td>
<td>&lt;0.02</td>
<td>NG</td>
<td></td>
</tr>
<tr>
<td>KE3-19-1 hydA</td>
<td>pSE3-1Chr</td>
<td>0.38</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>KE3-31 hydB</td>
<td>pSE3-1PV</td>
<td>0.33</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>KE3-19-1 hydA</td>
<td>pSE3-1PV</td>
<td>0.73</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>KE3-31 hydB</td>
<td>pSE3-1PVSp</td>
<td>0.3</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>KE3-19-1 hydA</td>
<td>pSE3-1PVSp</td>
<td>0.4</td>
<td>4.7</td>
<td></td>
</tr>
</tbody>
</table>

NG, No growth.

* A unit of activity is defined as the appearance of 0.1% ²H₂H₂ in the gas phase from 10% ²H₂O and is equivalent to reaction with 0.287 μmol H₂ min⁻¹. These values are the mean of two separate determinations with different samples of intact cells. The maximum deviation from the mean was ±10%.

A single plasmid complementing both *hydA* and *hydB* mutations could be due to the plasmid containing both the individual *hydA* and *hydB* genes from *C. vinosum* which are homologous to those in *E. coli*. To decide whether pSE3-1Chr contained individual *hydA* and *hydB* genes, the plasmid was subcloned into smaller fragments by restriction enzyme digestion of the plasmid and self-ligation (Fig. 2). Removal of the 0.5 kb fragment at the right of the insert gave a plasmid with a 4.7 kb insert (pSE3-1ChrSS) which was as active as the original plasmid in restoring activity in both *hydA* and *hydB* mutant strains. The 2.2-2.5 kb insert at the left of the fragment (pSE3-1ChrS and pSE3-1ChrN) and the 2.9 kb insert at the right of the fragment (pSE3-1ChrH) failed to complement either the *hydA* or the *hydB* mutant strain, suggesting that the original 4.7 kb DNA fragment did not contain independently active *hydA* and *hydB* genes. Removal of the 0.4 kb fragment at the extreme left of the original insert (pSE3-1ChrRI) led to the loss of complementation for both *hydA* and *hydB* mutant strains. It is possible that the 0.4 kb BamHI/Sau3A-EcoRI insert fragment contains a single promoter for two genes with the first gene extending past the first NruI site.

The smallest active DNA fragment, 4.7 kb, could code for a protein of molecular mass 157 kDa; this is sufficiently large to be the hydrogenase protein, which was reported to be a dimer of two 50 kDa subunits (Gitlitz & Krasna, 1975). Sawers & Boxer (1986) reported that the subunit molecular masses of hydrogenase isoenzyme 1 were 64 and 35 kDa, and Ballantine & Boxer (1986) found that the subunit molecular masses of isoenzyme 2 were 61 and 35 kDa. The catalytic subunit was the one of higher molecular mass (Harker et al., 1986). It is, of course, possible that the DNA fragment cloned is a gene or genes whose effect in *E. coli* is the same as the combined effect of the individual *hydA* and *hydB* genes. This will be further discussed below.
Restoration of the Hyd⁺ phenotype by a cloned DNA fragment from P. vulgaris

From the P. vulgaris library a single plasmid was isolated which restored hydrogenase activity in both hydA and hydB mutant strains. The plasmid, pSE3-1PV (8.4 kb) had a chromosomal DNA insert of 4.0 kb; the restriction digest map is shown in Fig. 3. The results in Table 1 demonstrate that this plasmid restored deuterium exchange activity in both mutant strains. The activity in E. coli strain K12W6 (wild-type) was not affected by the P. vulgaris plasmid and this plasmid did not restore activity to a hydE mutant strain of E. coli.

The plasmid was subcloned into smaller fragments by restriction enzyme digestion, purification of the fragment of interest, and ligation to pBR322 treated with the same restriction enzyme. Treatment of pSE3-1PVSp with EcoRV gave a 2.7 kb fragment containing the 0.4 kb Sphl–EcoRV fragment of pBR322. Treatment of pSE3-1PV with PvuI gave a 1.1 kb fragment containing the original insert (pSE3-1PVSpRV) gave plasmids containing a repeat of 0.4 kb or 1.1 kb pBR322 fragment, respectively.

The restriction maps of the subclones are shown in Fig. 3. The 2.7 kb Sphl–Sphl insert (pSE3-1PVSp) was as active as the 4.0 kb insert in restoring activity in both hydA and hydB mutant strains (see Table 1). Removal of a 0.4 kb fragment at the left of this insert (pSE3-1PVSpRV) did not affect the complementation. Removal of the 2.5 kb DNA fragment at the right of the original insert (pSE3-1PVpV) led to the loss of all activities. The smallest active DNA fragment, 2.3 kb, could code for a protein of molecular mass 77 kDa. The native molecular mass of the P. vulgaris hydrogenase was reported to be 115 kDa (Schengrund & Krasna, 1969); the subunit composition has not been determined.

Hybridization studies

To determine whether the cloned DNA fragments from C. vinosum and P. vulgaris which restored hydrogenase activity to both hydA and hydB mutant strains of E. coli contained individual hydA or hydB genes homologous to those of E. coli, Southern blot hybridizations were carried out among the different plasmids as well as to chromosomal DNA digests of the three organisms of interest (see Methods for details). [The restriction digest maps of hydA and hydB are given by Sankar et al. (1985) and the map of hydE by Chaudhuri & Krasna (1987).] The results are summarized in Table 2. The E. coli hydB probe did not hybridize to the E. coli hydA probe nor to plasmids pSE3-1Chr or pSE3-1PV; it only hybridized to E. coli chromosomal DNA. The E. coli hydA probe did not hybridize to plasmids pSE3-1Chr or pSE3-1PV; it only hybridized to E. coli chromosomal DNA. A probe prepared from plasmid pSE3-1PV (2.7 kb Sphl–Sphl fragment) did not hybridize to pSE3-1Chr and only hybridized to P. vulgaris chromosomal DNA. Plasmid pSE3-1Chr only hybridized to C. vinosum chromosomal DNA. None of these plasmids hybridized to the cloned hydE gene (Chaudhuri & Krasna, 1987) or hupB gene (Chaudhuri & Krasna, 1988).

It is clear that the cloned DNA fragments from C. vinosum and P. vulgaris which complement E. coli strains with mutations in hydA and hydB did not hybridize to each other, nor to the cloned E. coli hydA and hydB genes.
described above were assayed for hydA controls for the restoration of hydB which has been ascribed to isoenzyme 3 (Ballantine & Boxer, 1985; Sawers et al., 1985). Plasmids pSE-128 and pSE-201 contain cloned the C. vinosum exchange reaction. The cloned DNA fragments from C. vinosum hydrogenase isoenzymes and are devoid of hydrogenase activity as measured by the deuterium exchange reaction. The cloned DNA fragments from C. vinosum (data not shown).

Restoration of FHL activity

hydA and hydB mutant strains of E. coli lack all hydrogenase isoenzymes and are devoid of FHL activity, which has been ascribed to isoenzyme 3 (Ballantine & Boxer, 1985; Sawers et al., 1985). The transformants described above were assayed for FHL activity (Table 3). Plasmids pSE-128 and pSE-201 contain cloned E. coli hydB and hydA genes (Sankar et al., 1985) and serve as controls for the restoration of FHL activity in the two mutant strains. The P. vulgaris plasmid, pSE-3-1PV, restored FHL activity in both mutant strains, whereas the C. vinosum plasmid, pSE-3-1Chr, restored FHL activity in the hydB mutant but not in the hydA mutant.

Lack of complementation of Hup" mutants

Hup" mutant strains of E. coli show a hydrogen-uptake-negative phenotype (failure to reduce viologen dyes with H2 and to grow on fumarate plus H2), but express normal hydrogenase activity as measured by the deuterium exchange reaction. The cloned DNA fragments from C. vinosum or P. vulgaris did not complement hupA mutations at minute 65 (Lee et al., 1985) or hupB mutations at minute 17 (Chaudhuri & Krasna, 1988) (data not shown).

Synthesis of plasmid-encoded proteins

Since the molecular masses of the polypeptides encoded by the P. vulgaris and C. vinosum plasmids might indicate whether the cloned genes were structural genes, the

<table>
<thead>
<tr>
<th>Table 2. Southern hybridization among different DNA fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probe</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>E. coli hydB†</td>
</tr>
<tr>
<td>E. coli hydA‡</td>
</tr>
<tr>
<td>P. vulgaris hydAB§</td>
</tr>
<tr>
<td>pSE3-1Chr</td>
</tr>
</tbody>
</table>

* † Good hybridization under the conditions outlined in Methods; ‡ no hybridization; ND, not done.
† 2.8 kb SalI–SalI fragment from plasmid pSE-130 (hydB).
‡ 3.9 kb EcoRI–PstI fragment from plasmid pSE-201 (hydA).
§ 2.7 kb SphI–SphI fragment from plasmid pSE-3-1PV (hydAB).

Table 3. FHL activity in mutant strains transformed by plasmids

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>hyd genotype</th>
<th>Plasmid</th>
<th>FHL activity* [nmol H2 evolved min(^{-1}) (mg protein(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>K12W6</td>
<td>Wild-type</td>
<td>None</td>
<td>42</td>
</tr>
<tr>
<td>SE-31</td>
<td>hydB</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>SE-31</td>
<td>hydE</td>
<td>pSE-128</td>
<td>154</td>
</tr>
<tr>
<td>SE-31</td>
<td>hydB</td>
<td>pSE3-1Chr</td>
<td>94</td>
</tr>
<tr>
<td>SE-31</td>
<td>hydB</td>
<td>pSE-3-1PV</td>
<td>116</td>
</tr>
<tr>
<td>SE-19-1</td>
<td>hydA</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>SE-19-1</td>
<td>hydA</td>
<td>pSE-201</td>
<td>94</td>
</tr>
<tr>
<td>SE-19-1</td>
<td>hydA</td>
<td>pSE3-1Chr</td>
<td>10</td>
</tr>
<tr>
<td>SE-19-1</td>
<td>hydA</td>
<td>pSE-3-1PV</td>
<td>148</td>
</tr>
</tbody>
</table>

* These values are the mean of two separate determinations with different samples of intact cells. The maximum deviation from the mean was ±10%.

and it is unlikely that they are simply carrying individual genes homologous to hydA and hydB genes. The cloned genes could be structural genes for hydrogenase or genes whose effect on E. coli is the same as the combined effect of hydA and hydB genes, though there is no strong homology in the DNA sequences.

maxicell procedure of Sancar et al. (1979) was used to identify the subunit molecular masses of these polypeptides. We had previously shown (Chaudhuri & Krasna, 1987) that a plasmid containing the E. coli hydB, hydE and hydF genes led to the synthesis of polypeptides of molecular masses 41, 36 and 30 kDa, the 36 kDa protein being the product of hydE. Sankar & Shanmugan (1988a, b) identified the 30 kDa protein as the product of the hydB gene and the 41 kDa protein as the product of the hydF gene.

Fig. 4 shows the maxicell results for the plasmids used in the present study. The plasmid vector, pBR322, coded for three polypeptides of molecular masses 43, 33, and 28 kDa (Sancar et al., 1979); the former two were seen only in longer exposures. Plasmid pSE-201, containing the hydA gene, led to the synthesis of a protein of subunit molecular mass 44 kDa. Plasmid pSE-128, used as the source of the hydB gene, led to the synthesis of proteins of molecular masses 72 and 78 kDa. (The 30 kDa protein product of the hydB gene was not discernible in this
Fig. 4. Autoradiograph of an SDS-polyacrylamide gel of polypeptides expressed in maxicells (E. coli CSR603) by plasmids derived from E. coli, P. vulgaris and C. vinosum, A, pBR322; B, pSE-201; C, pSE-128; D, pSE3-1Chr; E, pSE3-1ChrSS; F, pSE3-1PV; G, pSE3-1PVSpRV.

experiment.) Sankar et al. (1988) reported that the 72 and 78 kDa proteins were products of the fde and fhfA genes. Plasmid pSE3-1Chr led to the synthesis of polypeptides of molecular masses 33, 38 and 44 kDa (the last seen only in longer exposures) while the smaller plasmid pSE3-1ChrSS led to the synthesis of proteins of molecular masses 38 and 44 kDa. Plasmid pSE3-1PV led to the synthesis of polypeptides of molecular masses 33 and 40 kDa and the smaller plasmid pSE3-1PVSpRV led to the synthesis of polypeptides of the same molecular masses (seen only in longer exposures).

Characterization of hydrogenase in mutant strains transformed by plasmids

If the hydrogenase genes isolated from P. vulgaris and C. vinosum are genes which restore the ability of hydA and hydB mutant strains of E. coli to synthesize the hydrogenase isoenzymes, then the enzymes synthesized should be characteristic of those of E. coli. On the other hand, if the cloned genes are structural genes, then the hydrogenases synthesized should be characteristic of those found in P. vulgaris or C. vinosum. The active enzymes synthesized by the mutant E. coli strains transformed by the cloned genes from the two heterologous organisms were thus studied in detail.

Solubilized membranes were prepared from wild-type P. vulgaris, C. vinosum and E. coli, and from hydA and hydB mutant strains of E. coli transformed by plasmids pSE3-1PV and pSE3-1Chr; the preparations were analysed by isoelectric chromatofocusing and reactivity with antibodies to E. coli hydrogenase isoenzymes. On isoelectric chromatofocusing most of the hydrogenase preparations had pI values of 5.1–5.3, the only exception being the enzyme from C. vinosum which had a pI value of 4.5–4.7. This would suggest that the E. coli mutant strains transformed by plasmid pSE3-1Chr synthesize E. coli hydrogenases.

Sawers et al. (1985) and Ballantine & Boxer (1985) showed that E. coli has at least two hydrogenase isoenzymes and that antibodies to the individual isoenzymes do not cross-react with the different isoenzymes. To conclusively demonstrate that the hydrogenase protein synthesized in E. coli mutants transformed by the cloned genes from P. vulgaris and C. vinosum is E. coli hydrogenase, the solubilized membranes were tested for reactivity with antibodies to E. coli hydrogenase 1. The results from a Western blot are shown in Fig. 5; similar results were obtained by ELISA methods. The hydB mutant strain does not react with the antibodies (data not shown). The hydrogenase from P. vulgaris cross-reacts with antibodies to the E. coli hydrogenase as does the E.
coli hydB mutant transformed by the *P. vulgaris* cloned DNA fragment. Thus, no definite conclusion can be drawn as to the nature of the hydrogenase synthesized by the *E. coli* mutant strain transformed by the *P. vulgaris* DNA fragment. However, from the data presented above on the restoration of all hydrogenase activities, and the relatively small size of the proteins encoded by the *P. vulgaris* DNA fragment, it seems probable that the hydrogenase protein synthesized by the *E. coli* hydB mutant transformed by the *P. vulgaris* DNA fragment is *E. coli* hydrogenase.

It is also clear from Fig. 5 that the proteins in *C. vinosum* do not cross-react with antibodies to *E. coli* hydrogenase 1 while the *E. coli* hydB mutant transformed by the cloned DNA fragment from *C. vinosum* synthesizes *E. coli* hydrogenase 1. This demonstrates conclusively that the cloned DNA fragment is not a structural hydrogenase gene of *C. vinosum*.

**Concluding remarks**

This study has shown that DNA fragments from *P. vulgaris* and *C. vinosum* (which do not hybridize to each other) restore activity to two distinct mutant strains of *E. coli* (hydA and hydB) with mutant genes 17 kb distant from each other. The individual hydA and hydB genes have been shown to be required for the synthesis of all hydrogenase isoenzymes in *E. coli*. It was also demonstrated that the two heterologous DNA fragments do not contain individual hydA and hydB genes homologous to those of *E. coli*. Rather they contain a gene (or genes) whose protein product (or products) in *E. coli* has the same positive effect as the combined effects of the products of the *E. coli* hydA and hydB genes. The precise mechanism of this effect is not known, but it is likely that both the hydA and hydB gene products (as well as those of the genes cloned from *P. vulgaris* and *C. vinosum*) affect another gene or genes which lead to synthesis of all hydrogenase isoenzymes. A similar conclusion was arrived at by Sankar & Shanmugam (1988b) with regard to the *E. coli* hydF and jhlA genes.

DNA fragments from *Clostridium butyricum* and *Citrobacter freundii* have been shown to complement *E. coli* hydA mutations (Karube et al., 1983; Kanayama et al., 1986). In the former case the DNA fragment hybridized to *E. coli* DNA and in the latter case hybridization results were not reported.

*Note added in proof*

It has recently been demonstrated in this laboratory that plasmids pSE3-1Chr and pSE3-1PV also restore all hydrogenase activities in a hydF mutant strain of *E. coli* (Sankar & Shanmugam, 1988b). These plasmids, however, do not restore formate hydrogenlyase activity in a jhlA mutant strain of *E. coli* (Sankar et al., 1988). The position of the hydF gene, immediately to the left of the hydB gene, is shown in Fig. 1. The jhlA gene (2 kb) lies immediately to the right of the hydB gene.

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the authors and do not necessarily reflect the view of the DOE or the NIH.

References


