Cloning and Expression of a *Clostridium acetobutylicum* Alcohol Dehydrogenase Gene in *Escherichia coli*

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An alcohol dehydrogenase (ADH) gene from *Clostridium acetobutylicum* was cloned on a recombinant plasmid, pCADH100. *Escherichia coli* HB101, and an allyl alcohol-resistant mutant, HB101-adh1, containing this plasmid were unable to grow aerobically or anaerobically on agar media containing sublethal concentrations of allyl alcohol. *E. coli* HB101 and HB101-adh1 transformed with the plasmid pCADH100 produced increased levels of ethanol when grown anaerobically under alkaline conditions in the absence of nitrate. Cell extracts from aerobically and anaerobically grown *E. coli* HB101(pCADH100) and HB101-adh1(pCADH100) cells exhibited increased levels of NADP-dependent ADH activity with either ethanol or butanol as the substrate. The inability of *E. coli* HB101(pCADH100) to grow in the presence of allyl alcohol correlated with the appearance of an NADP-dependent ADH activity band on nondenaturing polyacrylamide gel electrophoresis with either ethanol or butanol as the substrate. The position of the cloned NADP-dependent ADH activity bands in *E. coli* HB101(pCADH100) cell extracts with either ethanol or butanol as the substrate coincided with the position of a single NADP-dependent ADH activity band in extracts of *C. acetobutylicum* cells. *E. coli* HB101(pCADH100) cell extracts prepared from both aerobically and anaerobically grown cells exhibited an additional protein band with an apparent Mr, of approximately 33,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis which was absent in cell extracts of *E. coli* HB101. A protein band with a similar apparent Mr, was observed in cell extracts of *C. acetobutylicum*, and in vitro transcription and translation experiments with pCADH100 produced a major protein product with a similar apparent Mr.

*Clostridium acetobutylicum* is a gram-positive endospore-forming obligate anaerobe which produces acetone, butanol, and ethanol from a variety of carbohydrate substrates. In batch culture, *C. acetobutylicum* produces hydrogen, carbon dioxide, acetate, and butyrate during the initial growth phase (acidogenic phase). The onset of solvent production involves a switch in the carbon flow from the acid-producing pathways to the solvent-producing pathways (solventogenic phase) and is accompanied by the appearance of solvent-forming enzymes (for a recent review, see reference 21). During solvent production acetyl coenzyme A (acyetyl-CoA) and butyryl-CoA are key intermediates for ethanol and butanol production, respectively. The pathway from butyryl-CoA to butanol requires two dehydrogenase activities: butyraldehyde dehydrogenase, which converts butyryl-CoA to butyraldehyde and requires NAD+ as a cofactor (11), and butanol dehydrogenase, which converts butyraldehyde to butanol. This enzyme has been reported to be dependent on NADPH* in both *C. acetobutylicum* and *Clostridium beijerinckii* (13, 17, 34). However, Andersch et al. (2) noted that in *C. acetobutylicum* DSM 1732 this enzyme was more reactive to NAD+. Although butanol dehydrogenase activity in solvent-producing clostridial cells has been reported, this enzyme has proved difficult to assay (2), and inactivation of the enzyme during extraction has hampered the study and characterization of these enzymes. Recently, Hiu et al. (17) described the development of an improved assay for determining butanol dehydrogenase activity and reported on the purification and characterization of two different alcohol dehydrogenase (ADH) enzymes from two strains of *C. beijerinckii*, one of which produced isopropanol in addition to ethanol and butanol. Hiu et al. (17) showed that the major alcohol-forming enzyme from the two strains differed significantly. Although butanol and ethanol dehydrogenase activities were present in both enzyme preparations, the enzyme which was purified from the isopropanol-producing strain also exhibited strong isopropanol dehydrogenase activity. This is unusual, since although most ADH enzymes which have been investigated normally display broad substrate specificity, they usually display high specificity toward either primary alcohols and aldehydes or secondary alcohols and ketones.

In view of the importance of ADHs in the solvent-producing pathways of *C. acetobutylicum*, we have investigated the cloning and expression of an NADP-dependent butanol-ethanol dehydrogenase gene in *Escherichia coli*.

**MATERIALS AND METHODS**

**Bacteria, plasmids, and growth conditions.** *C. acetobutylicum* P262 (1, 20) was used as the source of DNA. *E. coli* HB101 (leuB6 trp-38 metE70 recA13 supE44) (5) was used as the recipient strain for recombinant plasmids. Plasmid pEcoR251, obtained from M. Zabeau, Plant Genetic Systems, Ghent, Belgium, is a positive selection vector containing the *E. coli* EcoRI gene under the control of the *λ* rightward promoter, the ampicillin resistance gene, and the pBR322 origin of replication. It was derived from the pCL plasmids described by Zabeau and Stanley (43). Plasmid pEcoR251 was used to prepare a genomic library of *C. acetobutylicum* chromosomal DNA (44). *C. acetobutylicum* P262 was grown under strict anaerobic conditions in *Clostridium* basal medium (CBM) (30) as described by Alcock et al. (1). *E. coli* was grown in Luria medium (28) or in complex medium broth (pH 8.0) supplemented with 1% glucose as described by Clark and Cronan (7).

**Isolation of ADH-deficient mutants of *E. coli* HB101.** Mutants of *E. coli* HB101 defective in NAD-dependent ADH activity were isolated following mutagenic treatment with
ethyl methanesulfonate by the procedure described by Carlton and Brown (6). Mutants defective in ADH activity were selected by growing mutagenized cells on agar plates made from complex medium (pH 8.0) supplemented with 1% glucose as described by Clark and Cronan (7) and 400 mM allyl alcohol. The plates were incubated under anaerobic conditions for 48 h at 37°C. Colonies which were resistant to allyl alcohol were isolated and characterized.

Preparation of DNA. Plasmid DNA was prepared by the alkaline lysis method of Ish-Horowicz and Burke (19). C. acetobutylicum cellular DNA was prepared by the method of Marmur (29), modified (44) to overcome the high nuclelease activity exhibited by C. acetobutylicum (41). E. coli cellular DNA was prepared as described by Maniatis et al. (28).

Cloning of a C. acetobutylicum P262 adh gene. E. coli HB101 cells were transformed with plasmid DNA isolated from a pooled C. acetobutylicum P262 genomic library (44) in which Sau3A I endonuclease fragments (4 to 7 kilobases [kb]) of C. acetobutylicum P262 chromosomal DNA were ligated with pEcoR251 which had been restricted with BglII endonuclease. Recombinant pEcoR251 plasmids were selected on Luria agar containing ampicillin (50 μg/ml). Colonies were replica plated onto Luria agar containing ampicillin or ampicillin plus 200 mM allyl alcohol and incubated anaerobically. Colonies unable to grow on ampicillin plus allyl alcohol were screened for ADH activity.

Restriction mapping. Recombinant plasmids harboring putative adh genes were characterized by restriction mapping by using standard procedures (28).

DNA hybridization. Cellular DNA from C. acetobutylicum and E. coli was digested with BglII endonuclease. The digested DNA fragments were fractionated by electrophoresis in 0.8% (wt/vol) agarose gels in Tris-acetate buffer and transferred bidirectionally to two GeneScreen nitrocellulose filters (New England Nuclear Corp., Boston, Mass.) (39, 40). Plasmid DNA probes were prepared by nick translation with [α-32P]dCTP (32).

In vitro transcription and translation. A procaryotic DNA-directed in vitro transcription and translation kit (no. N380; Amersham International, Amersham, England) was used as specified by the manufacturers for in vitro transcription and translation of plasmid DNA.

Ethanol and acetate determinations. The concentration of ethanol and acetate in culture supernatants was determined by using gas chromatography as described previously (26).

Preparation of cell extracts. Cell extracts of E. coli were prepared from overnight cultures (200 ml) grown either aerobically or anaerobically as described by Clark and Cronan (7). Cell extracts of C. acetobutylicum were prepared from solvent-producing cells grown anaerobically in tryptone-yeast extract-glucose (TYA) medium (18). Cell extracts were prepared under anaerobic conditions by the procedure of Clark and Cronan (7) and stored at −70°C to preserve activity.

Protein concentrations in the extracts were determined by the biuret method as described by Gornall et al. (15).

ADH and aldehyde dehydrogenase assays. ADH activity was determined spectrophotometrically at 340 nm by measuring the rate of NAD+ or NADP+ reduction at 20°C. The reaction mixture contained 12 mM sodium PP, buffer (pH 8.5), NAD+ or NADP+ (75 nmol), ethanol or n-butanol (0.35 mM), enzyme preparation (0.5 to 2.0 mg of protein), and distilled water to give a final volume of 1.0 ml. Enzyme activities were expressed as nanomoles of NAD(P)H per minute per milligram of protein.

ADH activity was also assayed by nondenaturing discontinuous polyacrylamide gel electrophoresis (PAGE) as described by Rodbard and Chrambach (33) and Singer and Finnerty (38). ADH activity was localized on the nondenaturing gels by staining with a solution containing 0.1 M glycine–NaOH buffer (pH 9.0), 1 mM NAD+ or NADP+, 1 mM p-iodonitrotetrazolium violet, 0.1 mM phenazine methosulfate, and 3% (vol/vol) ethanol or n-butanol as substrates (13). Gels were incubated in the dark at 22°C for 1 h. A dark-red formazan precipitate was formed at the sites of enzyme activity.

Aldehyde dehydrogenase activity was determined as described by Clark and Cronan (7) by using acetaldehyde or butyraldehyde diluted 10-fold with methanol (17) as substrates.

SDS-PAGE. Sodium dodecyl sulfate SDS-polyacrylamide gel electrophoresis (PAGE) was carried out by the method of Laemmli (27) with 50 μg of proteins from crude cell extracts per lane. Gels low-molecular-weight standards were used as markers.

RESULTS

Cloning of a C. acetobutylicum adh gene. Aldehydes in which the keto group is conjugated with a double or triple bond are potent protein-alkylating agents. When the corresponding alcohols (allyl, propargyl, and butynyl) are incubated with ADH in the presence of a suitable cofactor (NAD+ or NADP+), they are converted to lethal alkylating aldehydes (31). Allyl alcohol has been used as a suicide substrate for the selection of ADH-deficient mutants of E. coli (27) and C. acetobutylicum (11, 34). E. coli HB101 was unaffected by 200 mM allyl alcohol when grown aerobically, but was inhibited by 50 mM allyl alcohol when grown anaerobically (Table 1). Susceptibility to allyl alcohol has been shown to correlate with the expression of ADH activity in E. coli (27).

In this study, we isolated ADH-defective mutants of E. coli HB101 which were resistant to 400 mM allyl alcohol when cells were grown aerobically and anaerobically on complex agar medium containing 1% glucose (Table 1). One of these mutants (HB101-adh1) was shown to lack NAD-dependent ethanol dehydrogenase activity, although some residual NAD-dependent butanol dehydrogenase activity remained (Table 2). The NADP-dependent alcohol dehydrogenase activity appeared to be unaffected in this mutant.

Enhanced sensitivity to allyl alcohol was used to select clones of E. coli HB101 harboring putative adh genes from C. acetobutylicum. A gene library of C. acetobutylicum P262 was established in E. coli HB101 by insertional inactivation in the BglII cloning site of the EcoRI gene of pEcoR251 (44). Recombinant plasmid DNA prepared from pools of clones containing C. acetobutylicum DNA was used to transform E. coli HB101. Ap' transformants were replica plated onto
Luria agar plus ampicillin and Luria agar plus ampicillin plus allyl alcohol (200 mM). We isolated an E. coli HB101 Ap⁻ transformant which was sensitive to allyl alcohol (Table 1) and was unable to grow on allyl alcohol plates under aerobic conditions. The strain contained a pEcoR251 recombinant plasmid designated pCADH100. The E. coli HB101-adh1 allyl alcohol-resistant mutant strain was transformed with pCADH100, and the transformants were inhibited by 10 mM allyl alcohol under aerobic and anaerobic conditions (Table 1). The plasmid origin of the allyl alcohol sensitivity was confirmed by retransformation of E. coli HB101 and HB101-adh1. Allyl alcohol sensitivity was always associated with transformation to Ap⁻.

Restriction endonuclease mapping of pCADH100. The restriction endonuclease map of pCADH100 was obtained by complete single or double digestions with a variety of restriction endonucleases (Fig. 1).

DNA homology. The origin of the 3.2-kb DNA insert in pCADH100 was determined by the method of Southern (40) and by DNA hybridization with two α-32P-labeled probes derived from pCADH100 digested with BglII endonuclease. Probes X and H corresponded, respectively, to the 1.2- and 2.0-kb BglII restriction fragments on the insert in pCADH100 (Fig. 1). C. acetobutyllicum cellular DNA was partially digested with BglII and hybridized with probes X and H (Fig. 2). Probe X showed strong homology with the 3.3- and 5.3-kb bands, and probe H showed strong homology with the 2.0- and 5.3-kb bands. Hybridization of probe X to a 3.3-kb BglII chromosomal fragment indicates that the BglII restriction site at the left-hand end of the insert was reconstructed during ligation of the chromosomal Sau3A1 end with the BglII vector end. Hybridization of both probes X and H to a partial 5.3-kb BglII chromosomal fragment indicates the existence of a BglII restriction site at 2.1 kb from this Sau3A1 site on the chromosome. Hybridization of PstI-restricted pEcoR251 with probes X and H (Fig. 2) indicates some vector contamination of these probes. However, pEcoR251 has no homology with C. acetobutyllicum DNA (data not shown). Probes X and H did not show any homology with E. coli chromosomal DNA either unrestricted or restricted with BglII.

Ethanol production. When E. coli is grown anaerobically under alkaline conditions in the absence of nitrate with glucose as a substrate, approximately equimolar amounts of ethanol and acetate are produced in addition to a number of other end products (9). Ethanol is produced through the conversion of acetaldehyde by means of an inducible NAD-dependent ADH enzyme (7).

E. coli HB101(pCADH100) showed an increase of 64% in ethanol production and a proportionate drop in acetate production when compared with wild-type E. coli HB101 (Table 3).

The E. coli HB101-adh1 mutant, which lacked NAD-dependent ethanol dehydrogenase activity (Table 2), was still capable of producing significant amounts of ethanol when compared with wild-type E. coli HB101, presumably owing to the presence of NADP-dependent ADH activity. E. coli HB101-adh1(pCADH100) showed an increase of 116% in ethanol production, whereas the level of acetate production remained unaltered compared with that of E. coli HB101-adh1.

ADH activity. Cell extracts from aerobically and anaerobically grown E. coli HB101, HB101(pCADH100), HB101-adh1, and HB101-adh1(pCADH100) cells were assayed spectrophotometrically at 340 nm for ADH activity under aerobic conditions with ethanol or butanol as the substrate and NAD⁺ or NADP⁺ as cofactor (Table 2). The NADP-dependent specific activities of ADH in extracts of aerobically and anaerobically grown E. coli HB101(pCADH100) cells were two- to fivefold greater than in the control E. coli HB101 cell extracts when ethanol or butanol was used as the substrate. In contrast, the specific activities of NAD-dependent ADH in extracts of E. coli HB101 and E. coli HB101(pCADH100) cells exhibited little or no difference (<10% variation) when ethanol or butanol was used as the substrate.

### Table 2. Specific activity of ADH in E. coli cell extracts

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sp act (nmol/min per mg of protein) of ADH*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAD⁺ plus ethanol</td>
</tr>
<tr>
<td></td>
<td>Anaerobic</td>
</tr>
<tr>
<td>E. coli HB101</td>
<td>11.05</td>
</tr>
<tr>
<td>E. coli HB101(pCADH100)</td>
<td>11.32</td>
</tr>
<tr>
<td>E. coli HB101-adh1</td>
<td>0.0</td>
</tr>
<tr>
<td>E. coli HB101-adh1(pCADH100)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* NAD- and NADP-dependent specific activities were assayed with ethanol or butanol as substrates. Standard errors of the mean were < 10% of reported values.

![FIG. 1. Restriction endonuclease map of pCADH100.](http://aem.asm.org/ Downloaded from October 19, 2017 by guest)
E. coli fourfold increase found for dehydrogenase aldehyde acetobutylicum cells of when assayed with propanol of cell adhl(pCADH100) BglII-restricted little induced No (7) butanol ADH activity extracts of HB101-adhl(pCADH100) DNA H shows an HB101(pCADH100) agarose gel which was transferred bidirectionally to GeneScreen and hybridized with probes X (lanes A, B, C, and D) and H (lanes E, F, G, and H). Lanes: A and E. aceto butylicum cellular DNA partially digested with BglII; B and F, pEcoR251 digested with PstI; C and G, hybridization controls (unlabeled probe X fragments); D and H, hybridization controls (unlabeled probe H fragments). No hybridization occurred between unrestricted or BglII-restricted E. coli HB101 chromosomal DNA and probes X and H (data not shown).

Although extracts of aerobically grown E. coli HB101 and HB101(pCADH100) cells showed low levels of NAD-dependent ADH activity with ethanol as the substrate, significant levels of NAD-dependent ADH activity were obtained with butanol as the substrate. The absence of NAD-dependent butanol ADH activity in E. coli HB101-adhl and HB101-adhl(pCADH100) cell extracts indicates that the anaerobically induced NAD-dependent ADH reported by Clarke and Cronan (7) may also be expressed under certain conditions of aerobic growth.

The NAD-dependent specific ADH activity in extracts of E. coli HB101-adhl(pCADH100) cells showed a two- to fourfold increase over that in extracts of E. coli HB101-adhl cells with either ethanol or butanol as the substrate. Extracts of E. coli HB101-adhl and HB101-adhl(pCADH100) cells showed little or no NAD-dependent ADH activity (Table 2).

Extracts of E. coli. HB101(pCADH100), and C. acetobutylicum cells showed no detectable ADH activity when assayed with NAD⁺ or NADP⁺ as the cofactor and methanol, isopropanol, or acetone as the substrate, although when propanol was used as the substrate, results similar to those found for ethanol and butanol were obtained (data not shown).

Aldehyde dehydrogenase activity. No differences in the aldehyde dehydrogenase activities in extracts of E. coli

<table>
<thead>
<tr>
<th>Strain</th>
<th>pH</th>
<th>A₄₅₀</th>
<th>Ethanol</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli HB101</td>
<td>5.2</td>
<td>0.76</td>
<td>0.83</td>
<td>1.28</td>
</tr>
<tr>
<td>E. coli HB101(pCADH100)</td>
<td>5.2</td>
<td>0.77</td>
<td>1.36</td>
<td>0.90</td>
</tr>
<tr>
<td>E. coli HB101-adhl</td>
<td>6.1</td>
<td>0.60</td>
<td>0.50</td>
<td>0.37</td>
</tr>
<tr>
<td>E. coli HB101-adhl(pCADH100)</td>
<td>6.1</td>
<td>0.63</td>
<td>1.08</td>
<td>0.38</td>
</tr>
</tbody>
</table>

FIG. 2. Hybridization of [α-³²P]dCTP-labeled probes X and H with C. acetobutylicum DNA. Probes X and H corresponded to the 1.2- and 2.0-kb BglII restriction fragments on the insert in pCADH100 (Fig. 1), respectively. The figure shows an autoradiogram of the agarose gel which was transferred bidirectionally to GeneScreen and hybridized with probes X (lanes A, B, C, and D) and H (lanes E, F, G, and H). Lanes: A and E. acetobutylicum cellular DNA partially digested with BglII; B and F, pEcoR251 digested with PstI; C and G, hybridization controls (unlabeled probe X fragments); D and H, hybridization controls (unlabeled probe H fragments). No hybridization occurred between unrestricted or BglII-restricted E. coli HB101 chromosomal DNA and probes X and H (data not shown).

HB101 and E. coli HB101(pCADH100) cells were observed when NAD⁺ plus CoA or NADP⁺ plus CoA were used as cofactors and acetaldehyde or butyraldehyde was used as the substrate (data not shown).

PAGE ADH activity gels. ADH activity in extracts of anaerobically grown C. acetobutylicum P262 cells and anaerobically and aerobically grown E. coli HB101 and HB101(pCADH100) cells was determined by using non-denaturing PAGE gels with NAD⁺ or NADP⁺ as the cofactor and ethanol or butanol as the substrate.

When NAD⁺ was used as the cofactor, C. acetobutylicum cell extracts produced a single major band of activity with an Rf of ca. 0.44 when butanol was used as the substrate (Fig. 3) and a weaker single band of activity at the same approximate Rf value when ethanol was used as the substrate (Fig. 4). When NADP⁺ was used as the cofactor and butanol was used as the substrate, extracts of anaerobically grown E. coli HB101 cells always produced a single band of activity with an Rf of ca. 0.40 (Fig. 3). In addition to this constant band of activity, there was a variation in the intensity and number of activity bands from E. coli HB101 control cell extracts with Rf values between 0.23 and 0.29 (Fig. 3). Since this variation occurred in many different experiments and since the Rf values of the activity bands were very different from the Rf values of the C. acetobutylicum activity band, this variation was disregarded. Extracts of aerobically grown E. coli HB101 control cells showed similar NADP⁺-dependent activity band profiles (data not shown). Extracts from anaerobically and aerobically grown E. coli HB101(pCADH100) cells always produced the activity band with an Rf of ca. 0.40 and an activity band with an Rf of ca. 0.44 which corresponded in position to the activity band obtained with C. acetobutylicum P262 cell extracts (Fig. 3).

When NADP⁺ was used as the cofactor and ethanol was used as the substrate, no distinct ADH activity bands were observed with extracts of either anaerobically grown (data not shown) or aerobically grown E. coli HB101 cells (Fig. 4). However, with extracts of aerobically grown E. coli HB101(pCADH100) cells, a strong band of activity was observed which corresponded in position to the band of activity with an Rf of ca. 0.44 that was obtained with C.
acetobutylicum cell extracts (Fig. 4). With extracts of anaerobically grown *E. coli* HB101(pCADH100) cells, a similar pattern of activity was observed, but the intensity of the activity band was lower (data not shown).

When NAD\(^+\) was used as the cofactor with either ethanol or butanol as the substrate, no detectable NAD-dependent ADH activity was observed with *C. acetobutylicum* cell extracts. Extracts of anaerobically grown *E. coli* HB101 and HB101(pCADH100) cells both exhibited strong bands of NAD-dependent activity with an *R\(_f\)* of ca. 0.04 with either ethanol or butanol as the substrate. However, no additional activity bands were observed with the *E. coli* HB101 (pCADH100) cell extracts. When ethanol was used as the substrate and NAD\(^+\) was used as the cofactor with extracts of aerobically grown *E. coli* HB101 and HB101(pCADH100) cells, no activity bands were observed (data not shown). However, when butanol was used as the substrate and NAD\(^+\) was used as the cofactor with extracts of aerobically grown *E. coli* HB101 and HB101(pCADH100) cells, identical ADH activity bands with an *R\(_f\)* of ca. 0.25 were observed.

**Sodium dodecyl sulfate-PAGE.** *E. coli* HB101(pCADH100) cell extracts prepared from both aerobically and anaerobically grown cells exhibited an additional protein band on SDS-PAGE which was absent in *E. coli* HB101 cell extracts. This band corresponded to a protein band with an apparent *M\(_r\)* of approximately 33,000 (Fig. 5). A protein band with a similar apparent *M\(_r\)* was observed in *C. acetobutylicum* cell extracts (Fig. 5).

In vitro transcription and translation experiments with pCADH100 produced a major protein product with an apparent *M\(_r\)* of approximately 33,000 in addition to other polypeptides (Fig. 6).

**DISCUSSION**

*E. coli* produces ethanol as a major fermentation product by utilizing a NAD-specific ADH enzyme which is expressed only under anaerobic conditions (27). In addition, broad-specificity ADH enzymes which exhibit both NAD and NADP specificity have been reported to be associated with the uptake of alcohols by *E. coli* (16).
The cloned NADP-dependent ADH enzyme in *E. coli* cells shows a broad substrate specificity to primary alcohols and aldehydes, but not to secondary alcohols and ketones. *Clostridium acetobutylicum* cell extracts showed no ADH activity with NAD\(^+\) as the cofactor and methanol, ethanol, propanol, butanol, isopropanol, or acetone as the substrate. However, when NADP\(^+\) was used as the cofactor, ADH activity was observed with ethanol, propanol, or butanol as the substrate, but not with methanol, isopropanol, or acetone as the substrate. These findings support previous reports which indicated that the butanol dehydrogenases from solvent-producing clostridia are NADP-specific enzymes (13, 17, 34). At present, little is known about the ADH enzymes from *C. acetobutylicum* owing to difficulties encountered in extracting and assaying the enzymes (2, 3, 11). Butanol dehydrogenases from the solvent-producing clostridia have been reported to be partially inactivated under aerobic conditions (11, 17). However, the cloned NADP-specific ADH enzyme extracted under aerobic conditions from aerobically and anaerobically grown *E. coli* cells retained detectable activity both in the spectrophotometric assay system and in PAGE under aerobic conditions.

Cell extracts of *E. coli* transformed with pCADH100 exhibited an additional protein band on sodium dodecyl sulfate-PAGE with an apparent *M*\(_{r}\) of approximately 33,000, which was absent in wild-type cells. A protein band with a similar apparent *M*\(_{r}\) was present in *C. acetobutylicum* cell extracts. pCADH100 in in vitro transcription and translation experiments produced a polypeptide with an apparent *M*\(_{r}\) of approximately 33,000. It is interesting that other NADP-dependent ADH enzymes with subunits of similar apparent *M*\(_{r}\) have been reported for *Leuconostoc mesenteroides* (37) and *Thermoanaerobium brockii* (24). Most ADH enzymes described for both procaryotes and eucaryotes have subunits (usually two or four) with an apparent *M*\(_{r}\) of approximately 35,000 to 40,000, and Jörnvall (22) has suggested that even closely related ADHs may exhibit extensive variations in structure.

A number of NADP-dependent ADH genes have been cloned and sequenced from eucaryotes (4, 10, 12, 14, 42), and recently Conway et al. (8) have reported the cloning and sequencing of the NAD-dependent *adhB* gene from *Zymomonas mobilis*. The *adh* gene from *C. acetobutylicum* described in this study appears to be the first NADP-dependent *adh* gene which has been cloned; it should provide an alternative approach to the characterization and study of this key enzyme.

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