The prpE gene of *Salmonella typhimurium* LT2 encodes propionyl-CoA synthetase

Alexander R. Horswill and Jorge C. Escalante-Semerena

Author for correspondence: Jorge C. Escalante-Semerena. Tel: +1 608 262 7379. Fax: +1 608 262 9865. e-mail: jcescala@facstaff.wisc.edu

Biochemical and genetic evidence is presented to demonstrate that the *prpE* gene of *Salmonella typhimurium* encodes propionyl-CoA synthetase, an enzyme required for the catabolism of propionate in this bacterium. While *prpE* mutants used propionate as carbon and energy source, *prpE* mutants that lacked acetyl-CoA synthetase (encoded by *acs*) did not, indicating that Acs can compensate for the lack of PrpE in *prpE* mutants. Cell-free extracts enriched for PrpE catalysed the formation of propionyl-CoA in a propionate-, ATP-, Mg²⁺- and HS-CoA dependent manner. Acetate substituted for propionate in the reaction at 48% the rate of propionate; butyrate was not a substrate for PrpE. The propionyl-CoA synthetase activity of PrpE was specific for ATP. GTP, ITP, CTP and TTP were not used as substrates by the enzyme. UV-visible spectrophotometry, HPLC and MS data demonstrated that propionyl-CoA was the product of the reaction catalysed by PrpE.

**Keywords:** propionyl-CoA synthetase, short-chain fatty acid catabolism, 2-methylcitric acid cycle enzymes, propionate catabolic genes

**INTRODUCTION**

Growth of *Salmonella typhimurium* on propionate as a sole carbon and energy source requires enzymes encoded by the *prp* locus (Hammelman *et al.,* 1996; Horswill & Escalante-Semerena, 1997). Five genes, *prpRBCDE*, make up this locus and four of these genes show DNA and amino acid sequence similarity to proteins with known biochemical activities (Fig. 1). PrpR is a member of the family of RpoN (α54) activators; PrpB is homologous to isocitrate lyases; PrpC is homologous to citrate synthases; and PrpE is homologous to acetyl-CoA synthetases. A similar region in *Escherichia coli* has recently been implicated in the breakdown of propionate by the methylicotic acid cycle and the PrpC homologue has been identified as methylcitrate synthase (Gerike *et al.*, 1998; Textor *et al.*, 1997). We have obtained evidence that *S. typhimurium* also catabolizes propionate via this pathway (data to be presented elsewhere).

The activation of propionate to propionyl-CoA is the proposed first step of the methylicotic acid cycle and of all other propionate breakdown pathways in bacteria (Horswill & Escalante-Semerena, 1997; Textor *et al.*, 1997). However, no evidence has been reported identifying a gene encoding propionyl-CoA synthetase (EC 6.2.1.17) activity. This activity has been attributed to the two routes of acetyl-CoA synthesis (Rhie & Dennis, 1995a, b; Van Dyk & LaRossa, 1987), i.e. the acetate kinase (AckA) and phosphotransacetylase (Pta) pathway, and the acetyl-CoA synthetase (Acs) pathway. In a previous paper, we proposed that PrpE catalysed the synthesis of propionyl-CoA during propionate breakdown (Horswill & Escalante-Semerena, 1997). However, no evidence to support this hypothesis was presented. The location of *prpE* downstream of the *prpBCD* operon strongly suggested that the putative propionyl-CoA synthetase activity was needed. Failure to isolate *prpE* mutants was attributed to the existence of an enzyme that can substitute for PrpE during growth on this carbon source (Horswill & Escalante-Semerena, 1997).

In this paper, we address the function of PrpE by demonstrating that: (i) *prpE* is part of the *prpBCD* operon; (ii) acetyl-CoA synthetase compensates for lack of PrpE; and (iii) PrpE has propionyl-CoA synthetase activity.

**METHODS**

**Culture media and growth conditions.** Growth of *S. typhimurium* in rich and minimal media and the concentration of antibiotics were as described previously (Escalante-Semerena & Roth, 1987). The final concentrations of
A. R. HORSWILL and J. C. ESCALANTE-SEMERENA

Fig. 1. The prpRBCDE locus and methylcitric acid cycle of *S. typhimurium*. (a) Graphical representation of the prp locus showing homologues and predicted molecular mass of each protein (Horswill & Escalante-Semerena, 1997). (b) Propionate breakdown via the methylcitric acid cycle with the steps catalysed by proteins of the prp locus. Evidence for the assignment of PrpB, PrpC and PrpD catabolic steps will be presented elsewhere.

1382
Table 1. Strains and plasmids used

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype</th>
<th>Reference or source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli AJW805</td>
<td>ΔlacX74 thi-1 thr-1(amber) leuB6 metF159(amber) rpsL136 lacY acc::kan-1</td>
<td>A. Wolfe&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>DH5α/F&lt;sup&gt;−&lt;/sup&gt;</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; endA1 hsdR17 (r&lt;sup&gt;C&lt;/sup&gt; m&lt;sup&gt;C&lt;/sup&gt;) supE44 thi-1 recA1 gyrA (Nal&lt;sup&gt;R&lt;/sup&gt;) relA1 Δ(lacZYA-argF)U169 deoR [φ80lac lacZ]M15]</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>S. typhimurium†</td>
<td>hsdSA29 hsdSB121 hisD16 metA22 metE55 trpC2 ilv-404 galE719 H1-b H2-en,n,x (Fels2&lt;sup&gt;−&lt;/sup&gt;) fla-66 nml</td>
<td>Tsai et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>mutS::Tn10 galE496 metA22 metE55 xyl-404 (Fels2&lt;sup&gt;−&lt;/sup&gt;) H1-b nml H2 enx ilv hsdL6</td>
<td>S. Maloy via</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D. Downs&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>S. typhimurium†</td>
<td></td>
<td>K. Sanderson via</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J. Roth&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype</th>
<th>Reference or source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR6583</td>
<td>metE205 ara-9</td>
<td>This work</td>
</tr>
<tr>
<td>TR6583/pCP1-2</td>
<td>(T7 rpo&lt;sup&gt;+&lt;/sup&gt; kan&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>This work</td>
</tr>
<tr>
<td>TR6583/pBAD30</td>
<td>bla&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This work</td>
</tr>
<tr>
<td>JE4182</td>
<td>TR6583/pGP1-2 (T7 rpo&lt;sup&gt;+&lt;/sup&gt; kan&lt;sup&gt;+&lt;/sup&gt;) pPRP38 (prpE&lt;sup&gt;+&lt;/sup&gt; bla&lt;sup&gt;−&lt;/sup&gt;)</td>
<td>This work</td>
</tr>
<tr>
<td>JE4271</td>
<td>TR6583/pBAD30 bla&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This work</td>
</tr>
<tr>
<td>JE4287</td>
<td>TR6583/pGP1-2 (T7 rpo&lt;sup&gt;+&lt;/sup&gt; kan&lt;sup&gt;+&lt;/sup&gt;) pT7-6 bla&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This work</td>
</tr>
<tr>
<td>JE4288</td>
<td>acc::kan-1</td>
<td>This work</td>
</tr>
<tr>
<td>JE4289</td>
<td>prpE212::bla&lt;sup&gt;+&lt;/sup&gt; acc::kan-1</td>
<td>This work</td>
</tr>
<tr>
<td>JE4305</td>
<td>acc::kan-1 nrafA1::Tn10d(Tc)</td>
<td>This work</td>
</tr>
<tr>
<td>JE4312</td>
<td>Δ231 (acs)</td>
<td>This work</td>
</tr>
<tr>
<td>JE4354</td>
<td>JE3056/pPRP12-5.4 [prpBCD&lt;sup&gt;+&lt;/sup&gt; kan&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>This work</td>
</tr>
<tr>
<td>JE4358</td>
<td>JE4336/pPRP12-5.4 [prpBCD&lt;sup&gt;+&lt;/sup&gt; kan&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>This work</td>
</tr>
<tr>
<td>pPRP29</td>
<td>prpBCD&lt;sup&gt;+&lt;/sup&gt; cloned into pBR328 [bla&lt;sup&gt;+&lt;/sup&gt; cat&lt;sup&gt;−&lt;/sup&gt; (Ap&lt;sup&gt;−&lt;/sup&gt;, Cm&lt;sup&gt;−&lt;/sup&gt;)]</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>pPRP12-5.4</td>
<td>prpBCD&lt;sup&gt;+&lt;/sup&gt; in pSU39; kan&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Laboratory collection</td>
</tr>
</tbody>
</table>

*1, Loyola University, Chicago, USA; 2, University of Wisconsin-Madison, USA; 3, University of Utah, USA.
† All S. typhimurium strains are derivatives of LT2.

Construction of prpE insertions and deletions. Plasmids pPRP50 and pPRP51 were transformed into S. typhimurium TR6583 (prpE<sup>+</sup>) and co-integrates were isolated and resolved as described by Hamilton et al. (1989). To isolate strains carrying prpE insertions from plasmid-containing strains, P22 phage grown on a pool of 10 independently resolved co-integrates was used as donor to transduce strain TR6583 to Ap or Km resistance. P22 crosses were plated on NB with Km for pPRP50 and NB with Ap for pPRP51, and incubated at 44 °C. Km<sup>−</sup> Cm<sup>−</sup> (for pPRP50) and Ap<sup>−</sup> Cm<sup>−</sup> (for pPRP51) transductants were saved for further analysis. A strain carrying insertion nrafA1::Tn10d16A17 (hereafter referred to as Tn10d(Tc); Way et al., 1984) was used to isolate an acs deletion by the method of Bochner et al. (1980) as modified by Maloy & Nunn (1981). A Km<sup>−</sup> Te<sup>−</sup> Acc<sup>−</sup> (JE4312) strain was isolated and saved for further experiments.

Sequencing of prpE insertions. The location of the Ap<sup>+</sup> and Km<sup>+</sup> resistance cassettes within prpE was verified by PCR amplification and sequencing. PCR reactions were prepared using one-tenth volume of boiled template, 50 pmol of each primer, 0.2 mM of each dNTP (Promega) and Pfu DNA polymerase (Stratagene) according to the manufacturer’s instructions. Reactions were performed in a GeneAmp PCR System 2400 (Perkin Elmer) using the following conditions: 30 cycles at 94 °C for 90 s, 50 °C for 30 s, 72 °C for 2 min. The 5' and 3' ends of the prpE212::bla<sup>+</sup> insertion were PCR-amplified using the following sets of primers: 5'-CGT-GGAGTTTACTGATGGAT-3' (in prpD) and 5'-GCATCTTTTTACTTGGAG-3' (in the bfa gene) to generate a 1.5 kb fragment; 5'-ATGGATGAACGAAATAGACAGA-3' (in the bfa gene) and S'-GCTCTTCATCGGTCTCTGA-3' (in prpE) to generate a 1.4 kb fragment. For prpE213::kan<sup>+</sup> the entire Km<sup>+</sup> cassette and flanking DNA was amplified as a 1.8 kb fragment using the prpD and prpE primers shown above. Amplified DNA was purified using the QIAquick PCR Purification kit (Qiagen). PCR sequencing reactions were prepared using the ABI PRISM Dye Terminator Cycle Sequencing kit (Perkin-Elmer) according to the manufacturer’s instructions. Reactions were purified in AutoSeq G-50 columns (Pharmacia Biotech), dried in a SpeedVac concentrator and subjected to chain termination sequencing. Sequences were analyzed using Sequencher software (Gene Codes Corp., Ann Arbor, MI).
trator (Savant Instruments) and sequenced at the Bio-
technology Center (University of Wisconsin-Madison, USA).

**Sequencing of the acs::kan-1 insertion.** Strain JE4305 [acs::kan-1

*nrfAl::Tn10Δ(Tc)] was used to locate the Km resistance
cassette in acetyl-CoA synthetase. Primers 5'-TCCATTGCT-

GTGACAAAGG-3' for Tn10-L and 5'-ACCATATAAA-

ATCAGCATCC-3' for the Km resistance cassette were used
to PCR-amplify the chromosomal DNA between the two
insertions. The PCR amplification procedure used to locate
*prpE* insertions (described above) was also used to amplify a
2 kb DNA fragment between the *acs* and *nrfA* insertions.
Sequence was obtained (as described above) for both ends of
the amplified DNA fragment.

**Biochemical and spectroscopic techniques**

**Preparation of dialysed, cell-free extracts.** Cell pellets of strain

JE4184 grown as described above were resuspended in 40 ml
cold, 50 mM potassium phosphate buffer (pH 7.5) and the suspensions
were centrifuged at 10,500 g for 10 min at 4 °C.
The pellet was resuspended in 40 ml potassium phosphate buffer,
centrifuged as before and resuspended again in 10 ml potassium
phosphate buffer. Cells were kept on ice and broken by sonication
(10 min, 50% duty, setting 3) on a model 550 Sonic
Disembrator (Fisher Scientific). Cell debris was removed by
centrifugation in 50 ml Nalgene polypropylene copolymer
Oakridge tubes (Fisher Scientific) at 30,000 g for 1 h at 4 °C.
The supernatant was dialysed at 4 °C in Spectra/Por 1
Molecularporous dialysis membrane (Spectrum Medical Indus-
tries) against 1 l phosphate buffer (50 mM, pH 7.5, 4 °C).
The dialysis buffer was replaced after 2 and 4 h and then
allowed to dialyse an additional 16 h. The extracts (6 mg
protein ml⁻¹) were maintained at 4 °C for up to 1 week with no
detectable loss of activity. Strain JE4287 (pGP1-2 rop* kan¹;

pT7-6 bla¹) was used to establish the background acyl-CoA
synthetase activity in our *in vitro* assays. Increases in this
activity seen in extracts of strain JE4184 were relative to the
ones measured for extracts of strain JE4287.

**In vitro acyl-CoA synthetase assay.** The assay described by Brown

et al. (1977) was used to monitor the PrpE-dependent synthesis
of acyl-CoA compounds. The following were combined in a
1.5 ml reaction volume: HS-CoA, 0.75 μmol; ATP, 1.5 μmol;

MgCl₂, 7.5 μmol; hydroxylamine, 450 μmol; crude cell-free extract, 30 μg;

phosphate buffer, 75 μmol, pH 7.5. The mixture
was preincubated at 37 °C for 10 min and the reaction
was started with propionate (7.5 μmol). The reaction mixture
was incubated at 37 °C for 30 min and was stopped with
1.5 ml 20% FeCl₃/40% trichloroacetic acid/2 M HCl re-
gent. A reaction mixture without HS-CoA was prepared as
control for each condition tested. Colour was allowed to
develop for at least 10 min but no longer than 45 min.
Absorbance at 520 nm was measured using the no HS-CoA
controls as blanks. Detection of product was linear for 30 min
under these assay conditions. To assess the substrate specificity of PrpE, acetate and butyrate were substituted for propionate
at an equal concentration. Similarly, GTP, ITP, CTP and TTP
were substituted for ATP at an equimolar concentration.
Standard curves were prepared for the assay using propionyl-
CoA, acetyl-CoA and butyryl-CoA. A unit of activity [U] was
deﬁned as the amount of enzyme required for the synthesis of
1 μmol acyl-CoA min⁻¹.

**Chromatography and spectroscopy.** For HPLC analysis of the
PrpE reaction, we used a modification of the procedure
described by Hosokawa et al. (1986) to separate propionyl-
CoA from reaction mixture substrates. The reaction mixture
was resolved using reverse-phase HPLC with a Prodigy 5
ODS-2 column (250 x 4.60 mm; Phenomenex). The column
was developed immediately after injection of the sample with
an 80 min convex gradient (Waters curve 3) of acetoniitre/water (10:90) containing 0.2 M ammonium acetate.
The flow rate was 1 ml min⁻¹ and column temperature
was maintained at 35 °C. Elution was monitored at 260 nm on a
Waters model 990 Plus photodiode array detector (Millipore).

For MS of the PrpE product, HPLC fractions containing the
PrpE product were combined and solvent was removed in a
SpeedVac concentrator. Negative ion electrospray spectra
were obtained by resuspending samples in 50 % acetonitrile
and analysing them with a Perkin Elmer Sciex API 365 triple
quadrupole spectrometer equipped with an ion spray source.
Authentic propionyl-CoA (Sigma) was subjected to the same
manipulations and used as positive control.

**RESULTS AND DISCUSSION**

**Construction and discussion of prpE mutants**

Chromosomal insertions *prpE212::bla¹* and

*prpE213::kan¹* were constructed by gene replacement and the location of both antibiotic markers within *prpE*
was verified by sequencing. On propionate medium, *prpE* mutants and the *prpE¹* strain grew with very
similar doubling times, i.e. 6.6 h for the mutant and 6.1 h
for the wild-type. This result suggested that an alterna-
tive function compensated for the lack of PrpE. Since
bacterial acetyl-CoA synthetases often activate pro-
ionate (Maruyama, 1982; Preston et al., 1990; Priefert
& Steinbüchel, 1992), we inactivated the *acs* gene which
encodes acetyl-CoA synthetase. An *E. coli* *acs::kan¹*
insertion was moved into *S. typhimurium* (Kumari et al.,
1995) and the location of the Km resistance cassette was
verified by PCR amplification of chromosomal DNA
between *acs::kan¹* and *nrfAl::Tn10Δ(Tc) in strain
JE4305, followed by sequencing of the amplified DNA.
The *acs* mutant JE4288 grew in propionate medium
with a doubling time of 6.2 h. However, the *acs prpE*
double mutant strain JE4289 failed to grow on propio-
nate. Plasmid pPRP54 (*P. putida prpE¹*) allowed strain
JE4313 (*metE205 ara-9 Δ1231acs prpE213::kan¹*) to
grow in propionate medium containing arabinose with a
doubling time of 8.3 h. This rate of growth was identical
to the one measured for the *acs prpE* strain JE4271.
Plasmid pPRP54 also complemented growth of *acs*
murants on low acetate (10 mM) when arabinose was
included in the medium, indicating that PrpE can synthesize acetyl-CoA.

On the basis of these data we conclude that acetyl-CoA
synthetase can compensate for the lack of propionyl-
CoA synthetase activity in *prpE* mutants. PrpE and Acs
appear to be the only propionyl-CoA synthetase ac-

activities in the cell capable of supporting growth on propionate.
In *S. typhimurium*, the acetate kinase (*ackA*) and phosphotransacetylase (*pta*) pathway of acetyl-CoA synthesis does not appear to be a major contributor (if at all) to the synthesis of propionyl-CoA, since *ackA* mutants of this bacterium are not affected in their ability to grow on propionate (data not shown). These results are consistent with reports that propionate is not a substrate for AckA (Fox & Roseman, 1986).

### prpE is part of the prp operon

To determine if *prpE* was cotranscribed with *prpBCD*, the polarity of a *prpB* insertion on *prpE* was assessed. For this purpose, plasmid pPRP12-5.4 (Horswill & Escalante-Semerena, 1997) was introduced into a tester strain lacking AcS. When the growth of strains JE4354 (*prpB121::Tn10d(Tc)/pPRP12-5.4*) and JE4358 (*prpB121::Tn10d(Tc) Δ1231 (acs)/pPRP12-5.4*) was compared, strain JE4358 failed to utilize propionate as carbon and energy source. The polar effect of the *prpB121::Tn10d(Tc)* element on *prpE* lends strong support to the idea that *prpE* is part of the *prp* operon.

#### Overexpression of *prpE*

Plasmid pPRP38 was used to overexpress *prpE*. Plasmids pPRP38 or pT7-6 (vector-only control) were each transformed into strain JE4182, which carried plasmid pPRP381 or pT7-6 (vector-only control) were each transformed into strain JE4182, which carried plasmid pPRP381 or pT7-6 (vector-only control) were each transformed into strain JE4182, which carried plasmid pPRP381 or pT7-6 (vector-only control) were each transformed into strain JE4182, which carried plasmid pPRP381 or pT7-6 (vector-only control). For this purpose, plasmid pPRP12-5.4 (Horswill & Escalante-Semerena, 1997) was introduced into a tester strain lacking AcS. When the growth of strains JE4354 (*prpB121::Tn10d(Tc)/pPRP12-5.4*) and JE4358 (*prpB121::Tn10d(Tc) Δ1231 (acs)/pPRP12-5.4*) was compared, strain JE4358 failed to utilize propionate as carbon and energy source. The polar effect of the *prpB121::Tn10d(Tc)* element on *prpE* lends strong support to the idea that *prpE* is part of the *prp* operon.

**Table 2. PrpE activity and substrate specificity in dialysed, crude cell-free extracts**

<table>
<thead>
<tr>
<th>Assay conditions</th>
<th>Specific activity [\text{mU} \text{(mg protein)}^{-1}]</th>
<th>Relative activity (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>630</td>
<td>100</td>
</tr>
<tr>
<td>– ATP</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>– Mg²⁺</td>
<td>92</td>
<td>15</td>
</tr>
<tr>
<td>– Propionate</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>– PrpE</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Complete with acetate‡</td>
<td>300</td>
<td>48</td>
</tr>
<tr>
<td>Complete with butyrate§</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>pT7-6 with propionate</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>pT7-6 with acetate</td>
<td></td>
<td>52</td>
</tr>
</tbody>
</table>

* A unit of activity (U) is defined as the amount of enzyme that generates 2 μmol product min⁻¹.
† Relative activities were determined by dividing specific activities by that of the PrpE reaction with propionate [630 mU (mg protein)⁻¹].
‡ Acetate substituted for propionate.
§ Butyrate substituted for propionate.

An equal amount of dialysed cell-free extract of strain JE4287 (vector-only control) was substituted for cell-free extract of the *prpE*⁺-overexpressing strain JE4184.

3 and 4). Under these overexpression conditions, PrpE remained mostly soluble, however a small amount was lost as inclusion bodies (lane 2). Overexpression of *prpE* to higher levels using plasmid pPRP45 (*prpE*⁺ in pT7-7) with the T7 ribosome-binding site resulted only in a large increase of insoluble protein (data not shown).

### Acyl-CoA synthetase activity

Table 2 presents data which demonstrate that PrpE has acyl-CoA synthetase activity. Enzyme activity depended upon the addition of propionate or acetate, HS-CoA, ATP, Mg²⁺ and PrpE present in crude cell-free extract. Propionate was the preferred substrate of PrpE. The activity of the enzyme with propionate as substrate was assigned the arbitrary value of 100%. PrpE synthesized acetyl-CoA at 48% of the rate of propionate. Most of this activity was attributed to PrpE since the control extract displayed only 8% activity (Table 2); butyrate was not a substrate for PrpE. The enzyme displayed high specificity for ATP, GTP, ITP, CTP and TTP failed to substitute for ATP in the reaction. The specificity for ATP contrasts to that of the acetyl-CoA synthetase (ADP-forming) activity of *Pyrococcus furiosus*, which can use GTP and ITP very effectively (Glasmacher et al., 1997). At present, we do not know if PrpE is an acyl-CoA (ADP-forming), or acyl-CoA (AMP-forming) synthetase. A careful analysis of the products of the PrpE reaction is necessary.

Reaction mixtures lacking Mg²⁺ showed 15% PrpE activity, which was attributed to Mg²⁺ present in the
crude extracts. Consistent for the need for Mg$^{2+}$, the propionyl-CoA synthetase activity of PrpE was sensitive to EDTA in the reaction mixture (data not shown).

**HPLC purification of PrpE product**

The PrpE product was purified by reverse-phase HPLC. The reaction product eluted as unresolved peaks from 39 to 44 min (data not shown), which were collected and analysed by MS. Authentic propionyl-CoA standard dissolved in 50 mM phosphate buffer pH 7.5 had the same elution profile, retention times and UV-visible spectrum when the acetonitrile/ammonium acetate solvent system was used (data not shown). The propionyl-CoA peak was not seen when cell-free extract of strain JE4287 (vector-only control) was substituted for cell-free extract of strain JE4184 (pT7-6 prpE$^+$) in the reaction mixture (data not shown). If the solvent system was changed to phosphate buffer with methanol (Corkey et al., 1981), both the product of the PrpE reaction and authentic propionyl-CoA eluted as a single peak (data not shown). This result suggested that multiple peaks observed in the acetonitrile/ammonium acetate solvent system were a mixture of different salts of the CoA moiety. The phosphate/methanol solvent system was not used due to difficulties in removing phosphate buffer from the PrpE product.

**MS of PrpE product**

Negative ion electrospray mass spectra were obtained for authentic propionyl-CoA and for the product of the PrpE-catalysed reaction (Fig. 3a and b). The molecular mass of propionyl-CoA is 823 Da at the neutral state. Fig. 3(a) shows the negative molecular ion (M-1) for authentic propionyl-CoA at $m/z$ 822.2. Peaks at $m/z$ 766.2, 860.2 and 898.0 were assigned to CoA-1, the potassium salt of the negative molecular ion (M-1+K$^+$) and the di-potassium salt of the negative molecular ion (M-1+2K$^+$), respectively. Two other prominent peaks ($m/z$ 742.2 and $m/z$ 686.2) were presumed to be fragments of propionyl-CoA, but were not identified. Fig. 3(b) shows that the spectrum obtained for the HPLC-purified product of the PrpE-catalysed reaction displayed the same peaks. This result confirmed that propionyl-CoA was the product of the PrpE reaction.

**Implications of the amino acid sequence of PrpE**

Moyed & Lipmann (1957) first reported propionyl-CoA synthetase activity in extracts of an unidentified soil bacterium. This paper reports the first identification of a gene encoding propionyl-CoA synthetase. In light of the homology of prpE to genes encoding acetyl-CoA synthetases, it is possible that genes annotated in
databases as encoding acetyl-CoA synthetases in other organisms may actually encode propionyl-CoA synthetases. For example, according to BLASTP sequence analysis (Altschul et al., 1997), homologues in E. coli (GenBank accession no. U73857), Lysobacter (Y07914) and Pseudomonas putida (U24215) are 89, 58 and 54% identical to PrpE, respectively. However, AcS of E. coli (P27550) is only 37% identical to PrpE and 36–40% identical to the PrpE homologues from E. coli, Lysobacter and P. putida. These differences raise the possibility that these homologues are likely to be propionate-specific acyl-CoA synthetases.

ACKNOWLEDGEMENTS

This work was supported by NSF grant MCB 9724924 and UW-Madison Graduate School project 970146 to J. C.-E.-S. A.R.H. was supported by an NIH biotechnology training grant GM08349 and an NSF predoctoral fellowship. We thank A. J. Wolfe, S. R. Kushner and D. Downs for plasmids and strains. We thank Amy Harms of the Biotechnology Center of the University of Wisconsin-Madison for obtaining the mass spectra.

REFERENCES


Received 24 November 1998; revised 15 February 1999; accepted 5 March 1999.