PepR1, a CcpA-like transcription regulator of Lactobacillus delbrueckii subsp. lactis

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The PepR1 protein from Lactobacillus delbrueckii subsp. lactis DSM 7290 shares extensive homology with catabolite-control proteins from various Gram-positive bacteria. Expression of the subcloned pepR1 gene allowed for partial complementation of a ccpA defect in Staphylococcus xylosus. The influence of PepR1 on transcription of the prodidase gene pepQ, which is located adjacent to pepR1, was examined by use of lacZ reporter gene fusions in Escherichia coli. PepR1 stimulated transcription initiation at the pepQ promoter about twofold, and this effect required the integrity of a 14 bp palindromic cre-like sequence located 74 nt upstream of pepQ. In gel-mobility-shift assays, PepR1 specifically interacted with the pepQ promoter region and also with DNA fragments covering the promoters of the pepX, pepI and brnQ genes of Lb. delbrueckii subsp. lactis, which encode two additional peptidases and a branched-chain amino acid transporter, respectively. cre-like elements were identified in each of these DNA fragments. Catabolite control of PepQ was demonstrated in Lb. delbrueckii subsp. lactis. During growth with lactose the enzyme activity was twofold higher than in the presence of glucose, and corresponding differences were also detected in the level of pepQ transcription.

Keywords: Lactobacillus delbrueckii, PepR1 transcriptional regulator, peptidase Q, cre sequence, catabolite control

INTRODUCTION

Thermophilic lactic acid bacteria of the species Lactobacillus delbrueckii are involved in the fermentation of various dairy products, especially in the production of Italian-type hard cheeses (Olson, 1990). Due to numerous amino acid auxotrophies, growth of these bacteria in milk depends on the effective utilization of milk proteins (Juillard et al., 1995). This is achieved by the coordinate action of a cell-envelope-associated proteinase, various peptidases, and some peptide and amino acid transport systems (Mierau et al., 1997). We have previously isolated a series of 10 genes of the proteolytic system of Lb. delbrueckii subsp. lactis DSM 7290, a strain originally isolated from Emmental cheese. These genes, which code for peptidases with different specificities and an amino acid transporter, have been cloned and sequenced, and biochemical properties of most of the respective proteins have been established (Klein et al., 1995, 1997). From an economic point of view, it seems reasonable that the expression or activities of the various components of the proteolytic system are controlled in response to specific external or internal stimuli. Little is known, however, about the underlying mechanisms and the genetic functions mediating them.

In the vicinity of pepQ (Stucky et al., 1995a), one of the cloned peptidase genes of Lb. delbrueckii subsp. lactis, we recently detected an open reading frame (pepR1) which was suspected to encode a catabolite-responsive regulatory protein (Stucky et al., 1996). The pepR1 gene and its flanking regions were sequenced, and transcriptional start points were determined. The 152 bp intergenic region between the divergently running pepQ and pepR1 genes contains a 14 bp palindromic sequence which partly overlaps the −35 region of the pepR1 promoter. This palindrome is a highly conserved copy of the catabolite-responsive element cre, which is suspected to be involved in catabolite control of a number of genes in Gram-positive bacteria. The 37 kDa PepR1 protein has a typical helix–turn–helix DNA-binding motif and shares homology with a catabolite-control protein (CcpA), which is involved in the control of carbohydrate metabolism in Gram-positive bacteria. cre elements can be identified in the promoters of many CcpA-regulated genes, suggesting direct interactions of CcpA with these sequences (Hueck et al., 1994). Recently, in Strepto-
coccus mutans (Simpson & Russell, 1998), Lactococcus lactis (Luesch et al., 1998) and Lb. delbrueckii subsp. bulgaricus (Morel et al., 1999), two genes encoding CcpA and PepQ homologues were found to be arranged similarly to pepR1 and pepQ of Lb. delbrueckii subsp. lactis.

In the present communication we show that PepR1 fulfills a CcpA-like physiological function, that it modulates transcription from the pepQ promoter, and that it specifically binds to DNA sequences derived from several genes of the proteolytic system.

METHODS

Bacterial strains, plasmids and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Media used were LB medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl) for Escherichia coli, B medium (1% peptone, 0.5% yeast extract, 0.5% NaCl, 0.1% K2HPO4) for Staphylococcus xylosus and CDM (chemically defined medium; Ledesma et al., 1977) or MRS medium (De Man et al., 1960) for Lb. delbrueckii. Antibiotics and supplements were added to the media as required: kanamycin at 40 µg ml⁻¹, ampicillin at 200 µg ml⁻¹, tetracycline at 30 µg ml⁻¹, chloramphenicol at 20 µg ml⁻¹, erythromycin at 2.5 µg ml⁻¹ and thymidine at 100 µg ml⁻¹.

Recombinant DNA techniques and DNA sequencing. Standard techniques were applied for the cloning, PCR amplification and Southern blotting of DNA, and for purification of DNA fragments from gels (Sambrook et al., 1989). Plasmid DNA was prepared by the method of Birnboim & Doly (1979) except that, in the case of Sta. xylosus, lysostaphin (0.1 mg ml⁻¹ final concentration) was added to the lysis mixture. Restriction endonucleases, nucleic acid modifying enzymes (Boehringer Mannheim and New England Biolabs) and ULTma DNA polymerase (Perkin Elmer) were used as recommended by the manufacturers. Escherichia coli and Sta. xylosus were transformed by electroporation (Dower et al., 1988; Brückner, 1997) using a Gene Pulser (Bio-Rad). Primers for PCR and nucleotide sequencing were purchased from MWG-Biotech. Automated sequencing with an Applied Biosystems model 373A sequencer was performed on both strands of the respective DNA segment. Synthetic primers were designed from the known sequences of the vector and the DNA insert. Nucleotide and amino acid sequences were analysed by the Microgene (Beckman), Huser (Geniussnet) and PC/Gene (IntelliGenetics) programs.

Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Relevant features</th>
<th>Reference/source</th>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
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<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DH5×</td>
<td>endA recA hsdR gyrA</td>
<td>Hanahan (1983)</td>
</tr>
<tr>
<td>CM89</td>
<td>Δ(pepD-phoA) pepA pepB pepN pepQ</td>
<td>Miller &amp; Schwartz (1978)</td>
</tr>
<tr>
<td>TB1</td>
<td>Δ(lac-proAB) rpsL (860 lacZ∆M15) hsdR</td>
<td>Johnston et al. (1986)</td>
</tr>
<tr>
<td><strong>Lactobacillus delbrueckii subsp. lactis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSM 7290</td>
<td>Isolated from Emmental cheese</td>
<td>Klein et al. (1995)</td>
</tr>
<tr>
<td><strong>Staphylococcus xylosus</strong></td>
<td></td>
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</tr>
<tr>
<td>C2a</td>
<td>Wild-type</td>
<td>Götz et al. (1983)</td>
</tr>
<tr>
<td>TX154</td>
<td>C2a ccpA::ermB</td>
<td>Egter &amp; Brückner (1996)</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRB473</td>
<td>Ap' Cm' Km' Bm', CoE1 origin, pUB110 origin</td>
<td>Egter &amp; Brückner (1996)</td>
</tr>
<tr>
<td>pR1473</td>
<td>pRB473 fused to 1330 bp fragment containing pepR1 from pUR1</td>
<td>This study</td>
</tr>
<tr>
<td>pFPN13</td>
<td>pFN476 fused to 1325 bp PCR product containing pepR1</td>
<td>Stucky et al. (1996)</td>
</tr>
<tr>
<td>pMS1</td>
<td>Km', pepQ pepR1+, pSC105 origin</td>
<td>Stucky et al. (1995a)</td>
</tr>
<tr>
<td>pMC1871</td>
<td>Tc', lacZ, CoE1 origin</td>
<td>Shapira et al. (1983)</td>
</tr>
<tr>
<td>pMCPQ2</td>
<td>pMC1871 fused to 123 bp PCR product containing pepQ upstream region</td>
<td>This study</td>
</tr>
<tr>
<td>pMCPQ3</td>
<td>pMC1871 fused to 123 bp PCR product containing mutated pepQ upstream region</td>
<td>This study</td>
</tr>
<tr>
<td>pUR1</td>
<td>Ap', pepR1+, CoE1 origin</td>
<td>Stucky et al. (1996)</td>
</tr>
<tr>
<td>pR1Mal</td>
<td>pMal-c2 fused to 1012 bp PCR product containing pepR1</td>
<td>This study</td>
</tr>
<tr>
<td>pBR322</td>
<td>Ap' Tc', CoE1 origin</td>
<td>Sutcliffe (1979)</td>
</tr>
<tr>
<td>pJK433</td>
<td>Km', pepX', pSC105 origin</td>
<td>Meyer-Barton et al. (1993)</td>
</tr>
<tr>
<td>pJK502</td>
<td>Km', pepI', pSC105 origin</td>
<td>Klein et al. (1994)</td>
</tr>
<tr>
<td>pKS1</td>
<td>Km', brnQ', pSC105 origin</td>
<td>Stucky et al. (1995b)</td>
</tr>
</tbody>
</table>
Plasmid construction. To subclone pepR1, the gene, together with 102 nt upstream and 229 nt downstream sequence, was excised from pUR1 as a 1330 bp KpnI-BamHI fragment and inserted between the BamHI and KpnI sites of the shuttle vector pRB473. The resulting plasmid, pR1473, initially identified in transformants of E. coli DH5α, was introduced into Sta. xylosus C2a and TX154.

Transcriptional fusions of the pepQ promoter region (P_{pepQ}) with a promoterless lacZ reporter gene were constructed by cloning a 123 bp fragment, covering the first 18 nt of the pepQ coding sequence and 105 upstream nt (see Fig. 3b) and a variant of this fragment (P_{pepQ}^u) with a CG → AT exchange at positions 81/82 upstream of the pepQ start codon, into the Smal site of the plasmid pMC1871 vector, leading to the plasmids pMCPQ2 and pMCPQ3. The 123 bp fragments were amplified by PCR from plasmid pMS1, using the primers 5’- TAATTTGTCTAAATTTCTGAGCAATTC-3’ and 5’- CATGAGAACATTCTTGCTGACATGG(→ TA) CTTACAG-3’ (underlined nucleotides were exchanged in the primer used for construction of pMCPQ3).

To construct a pepR1::malE gene fusion (di Guan et al., 1988), a 1019 bp DNA fragment including the pepQ gene fusion (di Guan et al., 1988), the pepR1 gene (positions 153 to +17 from the first nt of the start codon), the pepX gene (positions -288 to -5 from the first nt of the start codon), the lacZ gene (positions -127 to +59 from the first nt of the start codon), and the brrN gene (positions -171 to +12 from first nt of the start codon) was amplified by PCR from the plasmids pMS1 (Stucky et al., 1995a), pJK433 (Meyer-Barton et al., 1993), pJK502 (Klein et al., 1994) and pKSI (Stucky et al., 1995b) by using the primer pairs 5’- AATTACGCTATCTATCATGAAAATC-3’/5’- TTAATTGTTTTAGATATTTTC-3’ and 5’- GCTTTTC-3’/5’- GCTTTTCCTCTACTATCTAC-3’. As a control, an internal fragment of the bla gene (positions +586 to +732 from the first nt of the start codon) was amplified from pBR322 by using the primers 5’- CTCTAATTCTACGCGGCCGAC-3’ and 5’- CAATATATGACGCGAGACGCGACGAC-3’. PCR products were non-radioactively labelled as follows. To generate 3’- recessed ends, the DNA samples (1 µg) were combined with 1 U T4 DNA polymerase in 20 µl reaction volumes, containing 0.1 mM dithiothreitol, 1 mM Tris/HCl, pH 7.5, 1 mM MgCl2, 5 mM NaCl and 0.1 mM DTT, and incubated for 10 min at 37 °C. After addition of 1 µl 1 M ethanol (each) dATP, dCTP and dGTP, DNA ends were filled in during a further incubation at 37 °C for 10 min. Reactions were stopped by heating at 75 °C for 10 min, and the DNA was precipitated with ethanol and redissolved in 20 µl water.

Gel-retardation assays. Of the individual promoter-containing DNA fragments, 2 µg were incubated with 16 µM purified PepR1 protein for 20 min at room temperature in 20 µl reaction volumes containing 20 mM Tris/HCl, pH 8.0, 20% (v/v) glycerol, 1 mM EDTA, 200 mM KCl, 1 mM DTT, 2 µg coli TBL1(pR1Mal) by the addition of IPTG (0.3 mM final concentration) at OD<sub>600</sub> 0.5. Two hours after induction, bacteria were harvested by centrifugation (20 min, 4 °C, 3000 g), washed in buffer A (20 mM Tris/HCl, pH 7.4, 200 mM NaCl, 1 mM EDTA), resuspended in 10 ml of the same buffer per gram of cells (weight), and disrupted by ultrasonication. Debris was removed by centrifugation for 30 min at 4 °C and 10000 g, and the supernatant, after adjusting its protein content to 2.5 mg ml⁻¹ (Spector, 1978), was subjected to affinity chromatography through an amylose resin (New England Biolabs). The column (2.5 x 10 cm, 15 ml bed volume, equilibrated with buffer A) was loaded with the extract and washed with 8 vols buffer A. The fusion protein was eluted with 10 mM maltose in the same buffer at a flow rate of 1 ml min⁻¹ and, of 30 2 ml fractions collected, those containing the protein peak were pooled. The resulting solution, containing 15 mg protein at a concentration of 1-1.1 mg ml⁻¹, was treated with 0.5 µg factor Xa for 14 h at 22 °C to cleave the PepR1–MalE fusion. The sample was dialysed against 20 mM Tris/HCl, pH 8.0, 25 mM NaCl, and subjected to ion-exchange chromatography on a Q-Sepharose Fast Flow (Pharmacia Biotech) column (1 x 10 cm, 5 ml bed volume), equilibrated with buffer B (10 mM Tris/HCl, pH 8.0, 25 mM NaCl). After application of the protein solution, the column was washed with 5 vols buffer B and the proteins were eluted with a gradient of 25–500 mM NaCl (25 ml each) in 20 mM Tris/HCl, pH 8.0. Fractions (1 ml) containing the separated PepR1 protein were identified by analysing aliquots (5 µl) on SDS-polyacrylamide gels.

Preparation of promoter-containing DNA fragments. DNA fragments covering the promoter regions of the pepQ gene (positions –153 to +17 from the first nt of the start codon), the pepX gene (positions –288 to –5 from the first nt of the start codon), the pepL gene (positions –127 to +59 from the first nt of the start codon), and the brrN gene (positions –171 to +12 from first nt of the start codon) were amplified by PCR from the plasmids pMS1 (Stucky et al., 1995a), pJK433 (Meyer-Barton et al., 1993), pJK502 (Klein et al., 1994) and pKSI (Stucky et al., 1995b) by using the primer pairs 5’- AATTACGCTATCTATCATGAAAATC-3’/5’- TTAATTGTTTTAGATATTTTC-3’ and 5’- GCTTTTC-3’/5’- GCTTTTCCTCTACTATCTAC-3’. As a control, an internal fragment of the bla gene (positions +586 to +732 from the first nt of the start codon) was amplified from pBR322 by using the primers 5’- CTCTAATTCTACGCGGCCGAC-3’ and 5’- CAATATATGACGCGAGACGCGACGAC-3’. PCR products were non-radioactively labelled as follows. To generate 3’- recessed ends, the DNA samples (1 µg) were combined with 1 U T4 DNA polymerase in 20 µl reaction volumes, containing 0.1 mM dithiothreitol, 1 mM Tris/HCl, pH 7.5, 1 mM MgCl2, 5 mM NaCl and 0.1 mM DTT, and incubated for 10 min at 37 °C. After addition of 1 µl 1 M ethanol (each) dATP, dCTP and dGTP, DNA ends were filled in during a further incubation at 37 °C for 10 min. Reactions were stopped by heating at 75 °C for 10 min, and the DNA was precipitated with ethanol and redissolved in 20 µl water.
Bacterial and 1 ng sonicated herring sperm DNA. The samples were loaded at 100 V on 6% polyacrylamide gels in 40 mM Tris/HCl, pH 8.0, 20 mM acetic acid, 2 mM EDTA, 3% (v/v) glycerol and run at 150 V for 2.5 h. After blotting the DNA to positively charged nylon membranes, bands were visualized by chemiluminescence using a commercially available digoxigenin detection kit (Boehringer Mannheim).

**Northern analysis.** Total RNA (10 µg), prepared from culture aliquots of *Lb. delbrueckii* subsp. *lactis* according to Chomczynski (1994), was separated on 1.2% agarose, 1% formaldehyde gels and blotted to positively charged nylon membranes (Boehringer Mannheim) (Sambrook et al., 1989). A *pepQ*-specific probe of 1107 bp was amplified by PCR from plasmid pMS1 by using the primers 5′-ATGAATTAGACAAATTACAAAACTGCTTGCAAGAAAAGG-3′ and 5′-TTATTCCTTAAGCTGCAAGACCTATTCACTCTTGCTA-3′. After random-primed labelling with [α-32P]dCTP (High Prime kit; Boehringer Mannheim), the probe was used in Northern hybridizations as described by Church & Gilbert (1984).

**RESULTS AND DISCUSSION**

**PepR1 complements a ccpA mutation**

On the basis of sequence comparisons, PepR1 of *Lb. delbrueckii* subsp. *lactis* DSM 7290 falls into the LacI/GalR family of transcription regulators (Weickert & Adhya, 1992). PepR1 shares significant sequence identity with CcpA proteins from *Lactobacillus casei* ATCC 393 (50.5%) (Monedero et al., 1997), *Bacillus megaterium* (44.8%) (Hueck et al., 1995), *Sta. xylosus* (44.7%) (Egeter & Brückner, 1996), *Bacillus subtilis* (43.9%) (Henkin et al., 1991), and with the CcpA homologue RegM from *Str. mutans* (49.6%) (Simpson & Russell, 1998). To check the functional significance of these similarities, we subcloned the *pepR1* gene into the *E. coli*/*Bacillus* shuttle vector pRB473. The resulting plasmid, pR1473, was used to perform complementation studies in a *ccpA* knock-out mutant of *Sta. xylosus* TX154, and expression of the *malRA* operon, known to be subject to CcpA-dependent catabolite repression in this organism (Egeter & Brückner, 1996), was examined by assaying the α-glucosidase activity of MalA. A wild-type strain, *Sta. xylosus* C2a, showed about fivefold repression of α-glucosidase in the presence of 25 mM glucose; this repression was completely abolished in the *ccpA* mutant (Table 2). Expression of the cloned *pepR1* gene from plasmid pR1473 in the *ccpA* mutant was sufficient to relieve the deregulation of α-glucosidase to an extent that allowed for almost threefold repression of α-glucosidase by glucose. This clearly indicated that PepR1 of *Lb. delbrueckii* subsp. *lactis* DSM 7290 can interact in a CcpA-like manner with elements of the signal-transduction chain, which mediate catabolite control in Gram-positive bacteria (Hueck & Hillen, 1995). It is therefore possible that PepR1 is not only operative in *Sta. xylosus* but that it might be the functional equivalent of CcpA in *Lb. delbrueckii*. The fact that PepR1 achieved only partial complementation of the CcpA defect in *Sta. xylosus* is most probably related to structural differences between the two proteins. These differences may account for less specific interactions of PepR1 with the HPr component of the phosphotransferase system (Deutscher et al., 1995) and with DNA target sites (cre sequences) (Fujita et al., 1995) in the heterologous *Sta. xylosus* host. In Fig. 3b, the upstream sequence of *Sta. xylosus* *malRA*, including the putative CcpA interaction site cre (Egeter & Brückner, 1996), is compared with upstream regions of *Lb. delbrueckii* genes that were found to specifically bind PepR1 (see below).

**Table 2. Complementation of a ccpA defect in *Sta. xylosus* by pepR1 of *Lb. delbrueckii* subsp. *lactis***

<table>
<thead>
<tr>
<th><em>S. xylosus</em> strain (plasmid)</th>
<th>α-Glucosidase specific activity</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− Glucose</td>
<td>+ Glucose</td>
</tr>
<tr>
<td>C2a(pRB473)</td>
<td>12.7 ± 1.6</td>
<td>2.40 ± 0.3</td>
</tr>
<tr>
<td>TX154(pRB473)</td>
<td>11.36 ± 0.5</td>
<td>11.16 ± 0.5</td>
</tr>
<tr>
<td>TX154(pR1473)</td>
<td>11.98 ± 0.6</td>
<td>4.30 ± 0.1</td>
</tr>
</tbody>
</table>

**PepR1 regulates pepQ expression**

From the fact that the *pepR1* and *pepQ* genes are closely linked, we previously suspected that PepR1 might exert a regulatory effect on the expression of *pepQ* (Stucky et al., 1996). This was supported by the observation that in initial attempts to identify *pepQ* in a plasmid library of the DSM 7290 genome, only plasmids which, in addition to *pepQ*, contained at least the 5′ part of *pepR1* (nt 1–470) were able to complement a prolidase mutation in *E. coli* (Stucky et al., 1995a). This part covers the region (nt 19–84) that encodes the predicted DNA-binding motif of PepR1. To investigate a possible influence of PepR1 on *pepQ* transcription, we fused a DNA fragment (position −77 to +45 from the transcription-start site; see Stucky et al., 1995a), carrying the assigned *pepQ* promoter (*P*<sub>pepQ</sub>), with the promoterless *lacZ* gene of the vector pMC1871. Transcription of the *P*<sub>pepQ</sub>::*lacZ* fusion from the resulting construct pMPQ2 was monitored by measuring β-galactosidase activity in transformants of *E. coli* expressing *pepR1* from its own promoter in the compatible plasmid pFNP13 (Stucky et al., 1996). Fig. 1 shows that PepR1 activated the expression of the *P*<sub>pepQ</sub>::*lacZ* fusion about twofold in this system, as compared with a control culture containing the unmodified vector pFN476 instead of pFNP13. This effect remained nearly constant throughout the growth of the bacteria and therefore
NTNWCA-3 lacZ from the modified promoter region almost completely abolished transcription activation the original cre Guan by constructing an N-terminal fusion with MalE (di susceptible promoters or genes, we purified the protein Ppr1 interacts with promoter regions pepQ like element plays an essential role in this interaction. In addition, cre may also be involved in some kind of autoregulation of PepR1, since the element was found to overlap the assigned –35 region of the pepR1 promoter (Stucky et al., 1996).

**PepR1 interacts with promoter regions**

To investigate whether PepR1 can directly interact with susceptible promoters or genes, we purified the protein by constructing an N-terminal fusion with MalE (di Guan et al., 1988) (Fig. 2). Verification of the fusion by sequencing the respective region of the hybrid plasmid pr1Mal revealed a T at position 212 of the pepR1 coding sequence instead of the previously reported C (Stucky et al., 1996). After cleavage of the fusion protein and removal of the MalE moiety (Fig. 2), purified PepR1 was used to perform gel-mobility-shift assays with a 172 bp DNA fragment containing the pepQ promoter region. This DNA–protein complex was almost completely retarded in the gel, whereas an arbitrary control DNA fragment was not shifted (Fig. 3a).

Since CcpA proteins of other Gram-positive bacteria are global regulators, modulating the expression of numerous genes or operons (Hueck et al., 1995), we also checked the affinity of PepR1 for the upstream and 5′-terminal regions of other genes of the proteolytic system of Lb. delbrueckii subsp. lactis DSM 7290. In gel-retardation assays (Fig. 3a), PepR1 was found to specifically interact with promoter-containing fragments of two additional peptidase genes, pepX and pepl (Meyer-Barton et al., 1993; Klein et al., 1994), and of the brnQ gene, which encodes a branched-chain amino acid transporter (Stucky et al., 1995b). Under the conditions used, PepR1 showed distinct affinity for the pepI and brnQ fragments whereas the pepX fragment was only partially shifted.

Interaction of PepR1 with multiple promoter regions from the chromosome of Lb. delbrueckii strongly suggested that the protein is involved in the coordinate transcriptional control of a set of susceptible genes. This set seems to include various functions related to the production and uptake of protein cleavage products. Searching for a physical basis for specific PepR1–DNA recognition, we found cre-like sequences in all of the PepR1-binding DNA fragments (Fig. 3b). They match the cre consensus for B. subtilis (Weickert & Chambliss, 1990) in at least 10 out of 14 positions and are located at distances of 16, 39 and 22 nt upstream of the assigned –35 regions of the pepQ, pepI and brnQ promoters, or between the –35 and –10 regions of the pepX promoter. An additional cre-like element overlaps the translation-start codon of pepI. Together with the clear effect of altering the cre-like sequence upstream of pepQ on PepR1-dependent control of the pepQ promoter (see Fig. 1), the occurrence of potential cre sites in additional PepR1-binding DNA sequences indicates that cre is directly involved in PepR1–DNA interactions. Differences between the primary sequences of individual cre elements and between their locations relative to the transcription-start sites of the respective genes may contribute to determining the efficiency of PepR1 binding and action. In general, cre sites of genes activated by CcpA proteins are located upstream of the
promoters, whereas for genes repressed by CcpA they are found within the promoters or at the 5’ end of the respective genes or operons (Henkin, 1996). The location of a cre-like sequence upstream of the pepQ promoter (Fig. 3a) and the stimulating effect of PepR1 on pepQ transcription (Fig. 1) are in agreement with this general principle. From this, one might expect that pepX (where cre is within the promoter) would be repressed whereas brnQ (where cre is upstream of the promoter) would be activated by PepR1.

**PepQ is subject to catabolite control**

To examine whether the expression or the activity of pepQ or its product are effectively modulated by the nature of the carbon source, we cultivated *Lb. del-

*brueckii* subsp. *lactis* DSM 7290 in different media in the presence of either glucose or lactose (Fig. 4a). Fig. 4b shows the specific PepQ activities measured in cell-free extracts prepared at different times during growth in chemically defined medium supplemented with 2% Casamino acids (Difco). During the exponential, late-exponential and stationary phases, the specific PepQ activities determined in the presence of glucose were 1.8-, 2- and 1.7-fold higher than those measured with lactose. This effect was reproducible in a number of experiments, and it was similar when other culture media (chemically defined medium without Casamino acids or MRS medium) were used. As estimated from Northern blots (Fig. 4c), the amount of pepQ transcripts correspondingly varied in response to the carbon source used. This indicated that the observed differences in
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