Clostridium tetani encodes a phosphocarrier protein, HPr

The phosphocarrier protein HPr is an essential component of the phosphoenolpyruvate (PEP) dependent phosphotransferase system (PTS) which plays an important role in the transport and phosphorylation of carbon substrates in a variety of species of clostridia including Clostridium acetobutylicum, Clostridium beijerinckii, Clostridium longisporum and Clostridium perfringens (Mitchell, 1998). In addition to its role in PTS transport, HPr also plays a pivotal regulatory role in low-GC Gram-positive bacteria. In Bacillus subtilis and other low-GC Gram-positive bacteria, HPr may be phosphorylated on residue Ser-46 by a metabolite-activated ATP-dependent kinase/phosphorylase, and in this form it can interact with other regulatory elements, such as the catabolite control protein CcpA, to effect gene regulation (reviewed by Stülke & Hillen, 2000). Carbon catabolite repression by glucose has been demonstrated in a number of clostridia and it has recently been established that the HPr in C. acetobutylicum is subject to phosphorylation by an ATP-dependent kinase/phosphorylase (Tangney et al., 2003). Furthermore, the elements of the catabolite repression system proposed for other low-GC Gram-positive bacteria have been identified from genome sequence analysis of this organism and it now seems likely that this mechanism also operates in C. acetobutylicum.

The Clostridium tetani genome has recently been sequenced and analysed, and a database of the predicted protein complement has been published (Brüggemann et al., 2003). Although relatively deficient in PTSs, the genome includes a putative PTS enzyme I, together with one complete PTS enzyme II permease which is closely related (66% identity) to the glucose PTS of C. acetobutylicum. However, no HPr protein was identified, implying that the PTS does not operate to catalyse sugar uptake in C. tetani. The genome also encodes a putative ATP-dependent HPr kinase/phosphorylase and a CcpA homologue, both of which are required for the aforementioned HPr-mediated mechanism of carbon catabolite repression. In light of these observations, the lack of a putative HPr protein is both curious and unexpected.

In order to resolve this discrepancy, we investigated the C. tetani genome sequence and uncovered a single putative ptsH gene, encoding HPr, that is not recorded in the C. tetani database. The proposed C. tetani ptsH gene is situated as an unidentified ORF between a putative aspartate aminotransferase (CTC01294) and an NAD+ -specific glutamate dehydrogenase (CTC01295). Significantly, we observed that the C. acetobutylicum ptsH gene is also preceded by a putative aspartate aminotransferase. The C. tetani ptsH gene encodes a protein of 85 aa with a predicted molecular mass of 8864 Da. It shares 72% identity and 82% similarity with the HPr (86 aa) from C. acetobutylicum (Tangney et al., 2003).
An alignment of these two proteins is integrated into Fig. 1 as part of a multiple alignment of the deduced amino acid sequence of the \emph{C. tetani} HPr with HPr sequences from representative species of six other disparate genera of low-GC Gram-positive bacteria. As can be seen, the proteins share considerable homology throughout their length, with the sequences surrounding the strictly conserved phosphorylatable residues His-15 and Ser-46 being particularly well conserved between the \emph{C. tetani} sequence and the other HPr proteins (Fig. 1).

We therefore propose that the identified sequence is indeed a \emph{ptsH} gene and that \emph{C. tetani} encodes an archetypal HPr which is likely to play a role in PTS transport and gene regulation in this organism. As a further observation, it is worth noting that in most bacteria \emph{ptsH} is in an operon with \emph{ptsI}, which encodes the other general component of the PTS, enzyme I. However, we have observed that this may not be the case in clostridia as the putative \emph{C. tetani} \emph{ptsH} is not linked to \emph{ptsI} and the same is true for \emph{C. acetobutylicum}, \emph{C. perfringens} and apparently also \emph{C. beijerinckii} (unpublished observations). The implications of separation of these genes with regard to metabolic regulation remain to be established.

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\textbf{Brüggemann, H., Bäumer, S., Fricke, W. F. \& 8 other authors (2003).} The genome sequence of \emph{Clostridium tetani}, the causative agent of tetanus disease.\emph{Proc Natl Acad Sci U S A} 100, 1316–1321.


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\textbf{Fig. 1.} Multiple alignment of the amino acid sequence of the proposed \emph{Clostridium tetani} HPr (Cte) with HPr sequences from low-GC Gram-positive bacteria. Identical residues in the two clostridial sequences (Cte and Cac) are shown in bold font; residues that are identical in all sequences are within black boxes; conserved residues are shaded grey. The phosphorylation sites at His-15 and Ser-46 are indicated (*). Abbreviations (with accession numbers in parentheses) are as follows: \textbf{Bsu}, \emph{Bacillus subtilis} (A46238); \textbf{Efa}, \emph{Enterococcus faecalis} (A25053); \textbf{Lmo}, \emph{Listeria monocytogenes} (AF030824); \textbf{Lsa}, \emph{Lactobacillus sakei} (AF172726); \textbf{Sau}, \emph{Staphylococcus aureus} (X93205); \textbf{Smu}, \emph{Streptococcus mutans} (A44562); \textbf{Cac}, \emph{Clostridium acetobutylicum} (AY196477). The \emph{C. tetani} sequence has been submitted to GenBank with the accession number DAA02130.