Sugar Transport by the Marine Chitinolytic Bacterium
Vibrio furnissii

MOLECULAR CLONING AND ANALYSIS OF THE MANNOSE/GLUCOSE PERMEASE*

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We have previously reported that the chitin catabolic cascade in Vibrio furnissii involves multiple signal transducing systems, and that mono- and disaccharide chemoreceptors/transporters are essential components of some of these systems. This and the accompanying papers (Bouma, C. L., and Roseman, S. (1996) J. Biol. Chem. 271, 33457–33467; Keyhani, N. O., Wang, L.-X., Lee, Y. C., and Roseman, S. (1996) J. Biol. Chem. 271, 33409–33413) describe some of the sugar transporters. A 13-kilobase pair fragment of V. furnissii DNA was found to impart a Glc−, Man+ phenotype to Escherichia coli ptsG ptsM mutants, and encodes the mannone transporter, ptsM, of the phosphoenolpyruvate:glycose phosphotransferase system.

Unlike the E. coli mannone permease, V. furnissii IIMan is inactive with GlcNac and Fru, and is encoded by four genes rather than three. The gene order is manXYZW, where the product of manY corresponds to IIIPMan, manZ to the mannone receptor IIIBMan, and manX and manW to the single E. coli gene, manX (which encodes IIIMan, viz. IIAMan). Thus, in V. furnissii, the E. coli manX equivalent comprises two genes, which are separated in the genome by two other genes of the ptsM complex. Two additional open reading frames were detected in the V. furnissii DNA fragment. One encodes a GlcNac-6-P deacetylase, and the other is similar to aldolase.

The accompanying paper (1) discusses the relationships between the chitin catabolic cascade and the glucose and N-acetylglucosamine transporters/chemoreceptors in the marine bacterium Vibrio furnissii. The relevant proteins are the sugar-specific Enzyme II complexes of the bacterial phosphoenolpyruvate:glycose phosphotransferase system (PTS). In Enterobacteriaceae, such as Escherichia coli and Salmonella typhimurium, Glc and GlcNac are also substrates of the mannone complex, IIMan (2, 3). Indeed, in screening V. furnissii genomic libraries in E. coli, a V. furnissii IIMan equivalent was detected because it conferred a Glc− phenotype to E. coli ptsG ptsM mutants. We report here that V. furnissii ptsM differs from its E. coli homolog in the number of polypeptides required for its function, the gene order, and in its substrate specificity.

The cloned V. furnissii DNA fragment contained two additional open reading frames. The deduced amino acid sequences of these open reading frames indicate that one encodes a GlcNac-6-P deacetylase, and the other a Fru-1,6-diP aldolase.

MATERIALS AND METHODS

Biochemicals and Molecular Biology Reagents—Restriction enzymes and T4 DNA ligase were purchased from Life Technologies, Inc. or U. S. Biochemical Corp. Radiochemicals were purchased from DuPont NEN or Amersham. Commercial reagents and biochemicals were of the highest purity available, and were obtained from Sigma or Aldrich Chemical Co.

Bacterial Strains and Culture Media—The following bacterial and phage strains were employed: E. coli SR425, SR423 (4); LR2−175 (5); SA2600 (F−rpsL relA his−; Dr. S. Adhya, NCI, National Institutes of Health, Bethesda, MD); ZSC113 (manX ptsG22 fruA gih lacZ rha rpsL relA; Ref. 2); ZSC114 (manZ ptsG22 gih-7; Ref. 2); WA12127 (F− manXYZ thr leuB metB thi tonA supE hsdS lac−; Ref. 6); SK1592 (galP thi endA hsdR sbeB T1; Ref. 7); KL16−21−23 (8); SA2600 ΔptsC−rr; LR2−175 ΔptsC−rr; LR2−175 Δcrr; V. furnissii SR1514 (formerly V. fluvialis 1514; Ref. 9); and phage χiv and χiv hp (6). The genotypes of strains and plasmids not given here are shown in Table I or Table III. The plasmid pCAR-3 was a gift from Dr. C. A. Roessler (Department of Medical Biochemistry, Texas A&M College of Medicine, College Station, TX); E. coli LR2−175 was a gift from Dr. J. Lengeler (Universitat Osnabruck, Fachbereich Biologie/C hemie, Osnabruck, Germany); IBPC531 was a gift from Dr. J. Plumbridge (Institute de Biologie Physico-chimique, URA1139, Paris, France). For the preparation of tolueone-permeabilized cells, E. coli transformants were grown in M9 medium (10) supplemented with 0.1% casamino acids, 0.5% Man, and the appropriate antibiotic to A500 = 0.8. The accompanying article (1) describes culture conditions for transformations, transductions, plasmid preparations, sugar fermentation, and sugar phosphorylation assays.

Construction and Screening of V. furnissii DNA Libraries—Genomic libraries of BamHI and HindIII fragments of V. furnissii 1514 DNA in the vector pBR322 were constructed and screened for plasmids conferring a Glc− phenotype to E. coli SR423 or SR425 as reported in the accompanying paper.

DNA Sequencing and Analysis—One strand of the SauI fragment of V. furnissii DNA contained in the plasmid pSHIS was sequenced by the Genetics Core Facility, Johns Hopkins Medical Institutions, Baltimore, MD. The complementary strand was sequenced by the dideoxy chain-termination method (11) with Sequenase T7 polymerase (U. S. Biochemical Corp.) as described in the accompanying article (1). The V. furnissii fda gene is truncated in pSHIS, and its sequence was therefore determined from pSH. Primers were designed as needed while sequencing, and were synthesized and purified according to the manufacturer’s recommendations (Applied Biosystems, Inc.). The analysis of DNA and amino acid sequences was carried out with the GCG sequence analysis package (Version 7, Genetics Computer Group, Madison, WI). The data bases searched for nucleotide and amino acid sequence similarities were: GenBank™ Release 79 and Swiss Protein Release 26.

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†The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U56015.

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1The abbreviations used are: PTS, phosphoenolpyruvate:glycose phosphotransferase system; kb, kilobase pair(s); nt, nucleotide(n).

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predicted amino acid sequences were aligned using CLUSTAL W version 1.5; similarities were shown by shading using BOXSHADE/DOS 2.7, kindly provided by Michael Cleave.

**Assay for λ Sensitivity—**E. coli transformants harboring plasmid clones of the V. furnissii man operon were scored for sensitivity to λvir by the cross-streak assay (6). Recipient cells were grown to an A600 = 0.8–1.0 with good aeration in LB supplemented with 0.2% maltose, 10 mM MgSO4, and the appropriate antibiotic. Complete lysis of the test strain was indicative of λ sensitivity (the ability of λvir DNA to penetrate the inner membrane via Enzyme IIMan).

In Vitro Transcription/Translation of Plasmid-encoded Polypeptides—Recombinant plasmids carrying V. furnissii DNA were used as templates in an in vitro transcription/translation system (Prokaryotic DNA-Directed Transcription/Translation System, Amersham). The plasmid-encoded gene products were labeled with [3H]leucine as directed by the manufacturer. The labeled polypeptides were separated by electrophoresis (15% SDS-PAGE; Ref. 12) and were visualized by autoradiography (Enlightning, DuPont NEN).

**Assay for Enzyme IPhom in Washed Membranes—**V. furnissii SR1514 and E. coli transformants were grown and harvested (1, 13), and membranes prepared as described (14). PTS Enzyme II activity was measured as reported (14), except that 25 µM HPs was used and IIIhom was omitted from the Enzyme IIhom assay mixture. Specific activities are expressed as nanomoles of sugar phosphate formed x min⁻¹ x mg of membrane protein⁻¹.

**Sugar Phosphorylation by Toluene-permeabilized Cells—**E. coli transformants harboring recombinant plasmids and V. furnissii SR1514 were grown, harvested, washed, and tolue-ne-permeabilized (13). Sugar phosphorylation was measured as described. Specific activities are expressed as nanomoles of sugar phosphate formed x min⁻¹ x mg of cell protein⁻¹.

**PCR Amplification of Cloned Sequences—**The genes of the V. furnissii man operon were amplified from genomic DNA by the polymerase chain reaction, and were cloned separately. The following primers were...
**Mannose/Glucose Permease of V. furnissii**

**TABLE II**

| Strain  | Plasmida | Growth Substrate | Membranes Specific activitya | Toluenized cells Specific activitya |
|---------|----------|------------------|-----------------------------|-----------------------------------|
|         |          |                  | Glc | Man | Glc | Man |
| LR2–175 | pBR322   | Lact + Glc       | 0.4 | 1   | 0.2 | 0.2 |
|         | p3H1S    | Glc              | 10  | 4   | 2.6 | 10.7|
| SR425   | pCAR-3   | Glc              | NT  | NT  | 12.6| 25.8|
|         | p3H1S    | Glc              | 1.6 | 0.3 | 0.2 | ND  |
|         | pCAR-3   | Man              | 5   | 1   | 1.9 | 2.2 |
|         | p3H1S    | Man              | 8   | 3   | 2.1 | 2.7 |
|         |          |                  | NT  | NT  | 19.2| 18.8|

a Plasmids are as detailed in footnote b of Table I.

b For sugar phosphorylation by membranes, specific activity is given in units of nmol sugar phosphate formed × min⁻¹ × mg membrane protein⁻¹; for toluenized cells, units are nmol sugar phosphate formed × min⁻¹ × mg cell protein⁻¹. ND, not detectable activity, NT, not tested.

The results summarized above therefore show that the cloned *V. furnissii* IIIP<sub>Man</sub> complex cross-reacts in vitro with *E. coli* phospho-HPr. As shown below, this is also true in *vitro*.

One of the characteristics of the *E. coli* ptsM gene complex is that it encodes the membrane protein Pel, or IIP<sub>Man</sub>. This protein is required for penetration of the inner membrane by phage λ DNA. Phage sensitivity assays conducted with SR425 transformants, however, showed that the clones containing *V. furnissii* DNA did not permit λ infection.

The phenotypic behavior of *E. coli* cells harboring the cloned *V. furnissii* DNA differed from those carrying pCAR-3, a plasmid carrying the complete *E. coli* ptsM locus (manXYZ). Despite allowing GlcNAc fermentation comparable to pCAR-3 transformants, the presumptive *V. furnissii* ptsM clones did not restore growth on minimal media with GlcNAc or Fru as the sole source of carbon. Transformation with the plasmid pCAR-3 allowed growth on minimal Man and GlcNAc, and weak growth on Fru (data not shown).**Sugar Phosphorylation in *Vitro*—**The phenotype conferred upon *E. coli* cells harboring the cloned *V. furnissii* transformants was 3–25-fold greater than obtained with controls, membranes isolated from strains transformed with the plasmid. Similarly, Man phosphorylation by p3H1S transformants membranes was 4–10-fold greater than background levels. The III<sup>Man</sup> subunit of the *V. furnissii* mannose permease was analyzed with a prokaryotic in vitro translation system. The [14C]leucine-labeled reaction products were separated by SDS-PAGE as described under "Materials and Methods." Lane 1, no DNA (control); lane 2, pUC19 (vector); lane 3, pAB (manX<sub>Y</sub>); lane 4, pAE (manXYZ); lane 5, pAG (manX); lane 6, p3H1S (manXYZ). The migration pattern of the standards is shown.

![Fig. 2. In vitro transcription/translation of plasmid-encoded polypeptides.](image-url)
FIG. 3. Nucleotide and deduced amino acid sequences of *V. furnissii* Enzyme II Man.

The sequence of the RNA-like strand is presented, with translations of the open reading frames given below. The potential ribosomal binding sites are indicated by double underlining, and putative promoter elements (235, 236) are shown in bold type with asterisks (*).
correlate with the phenotypic results presented in Table I.

While mannose and glucose were clearly phosphorylated by both the membrane and tonelized cell preparations, the activities were low compared to preparations obtained from cells transformed with the plasmid pCAR-3, which carries the E. coli mannose operon, i.e., the genes manXYZ. There are at least two possible explanations for these results; (a) cells transformed with pCAR-3 express more ptsM proteins than do cells transformed with p3H1S, or (b) the Vibrio ptsM proteins do not interact efficiently with E. coli phospho-HPr.

Although Fru and GlcNAc are substrates of E. coli II^Man, phosphorylation of these compounds by membranes from p3H1S transformants was not detectable (data not shown). Membranes from the transformants were also tested with 2-deoxyglucose and methyl α-D-glucopyranoside. These analogues are commonly used with E. coli cells and membranes to distinguish between ptsM and ptsG, respectively (17). No phosphorylation of either compound was detected, although 2-deoxyglucose is an excellent substrate for E. coli II^Man.

In Vitro Translation of p3H1S—To assess the number and molecular weights of polypeptides encoded by p3H1S, the plasmid was translated in a prokaryotic in vitro system (Fig. 2). In addition to the vector-encoded β-lactamase, four peptides were translated from p3H1S, with apparent molecular masses of 30.5, 26.5, 17.5, and 16.5 kDa. An additional band was observed with an apparent molecular mass of 42 kDa. The results in Fig. 2 are discussed further below.

DNA Sequence Analysis—The 4.6-kb V. furnissii DNA fragment of p3H1S was sequenced (11) by the dideoxy chain-termination method. Four open reading frames with identity to the subunits of E. coli Enzyme II^Man were identified. To be consistent with the established nomenclature of the Man PTS (4, 18), we have designated these genes manX, manY, manZ, and manW, respectively. A fifth open reading frame (manD) overlapping the terminal 11 nucleotides of manW, encodes a polypeptide with GlcNAc-6-P deacetylase activity. Finally, a sixth open reading frame, which overlaps the last 10 nucleotides of manD, resembles several bacterial Fru 1,6-P aldolases and was designated manF. Fig. 3 shows that these six open reading frames are closely linked and may be co-transcribed. The termination codon of manX is 15 nucleotides from the initiation codon of manY, the putative ribosomal binding site and initiation codon of manZ are within manY, and manW begins 57 nucleotides from the termination codon of manZ. A putative ribosomal binding site was identified for each coding region (Fig. 3), based upon identity with the E. coli ribosomal binding site (19). A potential promoter (20) for the man operon is also highlighted in Fig. 3. An 8-nt inverted repeat sequence is located between manZ and manW. Following manF are two GC-rich regions of dyad symmetry preceding a series of T residues, a sequence that could function as a transcriptional terminator in the mRNA.

Identification of V. furnissii Enzyme II^Man Subunits—In order to correlate the V. furnissii man genes with the polypeptides observed in Fig. 2, the man genes were amplified from genomic DNA, individually and in various combinations, and were analyzed by in vitro translation. The plasmids were also used in attempts to complement E. coli ptsM mutants of defined genetic defects.

Table III shows that Man fermentation was possible by the E. coli ptsM mutants only when all four V. furnissii Enzyme II^Man subunits were present. V. furnissii ManW and ManX, the homologs of E. coli III^Man, did not complement an E. coli III^Man mutant whether the two V. furnissii subunits were present individually or were co-transformed.

The clones of the V. furnissii Enzyme II^Man subunits were used as templates for in vitro translation in order to assign the observed products to their respective open reading frames. The results (Fig. 2) indicate that ManY (27 kDa) corresponds to the 26-kDa labeled polypeptide and ManZ (31.7 kDa) to the 30.5-kDa labeled peptide. ManW (15.9 kDa) and ManX (17.2 kDa), however, migrate as 17.5- and 16.5-kDa proteins, respectively. The apparent molecular masses of the translation products agreed reasonably well with the predicted values, except that the product of manW migrated somewhat more slowly than expected.

Identification of GlcNAc-6-P Deacetylase and Fructose 1,6-BisP Aldolase—The faint, 42-kDa band observed in p3H1S transcription/translation reactions corresponds closely to the predicted molecular mass (43 kDa) of the ManD gene product. Only the plasmid p3H1S directed the in vitro synthesis of this polypeptide. Furthermore, a functional GlcNAc-6-P deacetylase is encoded by p3H1 and p3H1S, since both plasmids com-

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**Table III**

| Strain | Relevant genotype | Plasmid | Phenotype |
|--------|------------------|---------|-----------|
| SR425  | ptsG manYZ       | pUC19   | −         |
|        | p3HIS            | + +     |           |
|        | pAB + pCD        | + +     |           |
|        | pAE + pFD        | + +     |           |
|        | pAB              | − −     |           |
|        | pCD              | − −     |           |
|        | pAE              | − −     |           |
|        | pFD              | − −     |           |
|        | pAG              | − −     |           |
|        | pAG + pFD        | − −     |           |
|        | pAG + pCD        | − −     |           |
|        | pAG + pFD        | − −     |           |
|        | pAG + pCD        | − −     |           |
| ZSC113 | ptsG manX        | pUC19   | −         |
|        | p3HIS            | + +     |           |
|        | pAB + pCD        | + +     |           |
|        | pAE + pFD        | + +     |           |
|        | pAB              | − −     |           |
|        | pCD              | − −     |           |
|        | pAE              | − −     |           |
|        | pFD              | − −     |           |
|        | pAG              | − −     |           |
|        | pAG + pFD        | − −     |           |
|        | pAG + pCD        | − −     |           |
|        | pAG + pCD        | − −     |           |

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^a^ See footnote a of Table I. Complete genotypes are given under "Materials and Methods."  
^b^ The following plasmids were used: p3H1S, V. furnissii manXYZW; pAB, V. furnissii manXY; pCD, V. furnissii manZ; pAE, V. furnissii manYZ; pFD, V. furnissii manW; pAG, V. furnissii manX. Construction of these plasmids is described under "Materials and Methods."  
^c^ Fermentation was scored on MacConkey agar with 1% Glc or Man, where + + designates red colonies and − − designates light pink/white colonies.  
^d^ Identical results were obtained with comparable transformants of E. coli. Only the plasmid p3H1S directed the in vitro synthesis of this polypeptide. Furthermore, a functional GlcNAc-6-P deacetylase is encoded by p3H1 and p3H1S, since both plasmids com-
Mannose/Glucose Permease of V. furnissii

A gene encoding a potential V. furnissii Fru 1,6-bisP aldolase overlaps manD (Fig. 3), and we have named this gene manF. The deduced amino acid sequence of this protein shares up to 41% identical residues with Class II aldolases from E. coli (21), Rhodobacter sphaeroides (22), and Bacillus subtilis (23). More interestingly, its sequence is most similar to GatY, which is encoded by the E. coli gat operon and reportedly carries ketose bisphosphate aldolase activity (24). An alignment of ManF with E. coli GatY and B. subtilis aldolase is given in Fig. 5. Whether V. furnissii manF directs the synthesis of a functional aldolase was not determined in this study.

DISCUSSION

We have proposed that (a) chemotaxis to G1c and trehalose may be the mechanism by which marine (and terrestrial?) bacteria locate chitin-producing organisms (13), and (b) chemotaxis to and transport of GlcNAc are essential for functioning of the chitin catabolic cascade in V. furnissii (9). An accompanying paper (1) describes the cloning and characterization of a GlcNAc transporter from this organism. Both are PTS sugars in V. furnissii, and the respective Enzyme II complexes serve as the sugar receptors in both chemotaxis and uptake. However, bacteria often use more than one system for chemotaxis/transport to a given sugar. The IIMan complex of E. coli serves as the sugar receptors in both chemotaxis and uptake. The IIMan complex of E. coli NagA, NagB, and NagC (27) and Klebsiella pneumoniae Sor (1-sorbose) Enzyme II complexes (28, 29), which consist of four proteins each. The sequences of these proteins are similar to the three proteins of the E. coli IIMan complex. The latter is encoded by three adjacent genes (4, 15) designated manX (encodes IIMan, also called IIBMan), manY (encodes IIPMan), and manZ (encodes IIBMan). The protein IIPMan (and also possibly IIPMan) is required for phage λ DNA penetration of the E. coli inner membrane (4). However, the V. furnissii homolog does not permit penetration of the cytoplasmic membrane by phage λ DNA.

E. coli IIMan transfers the phosphoryl group from phospho-HPr to Man, Glc, 2-deoxyglucose, Fru, GlcNAc, GlcNH2, and other sugars, concomitant with their translocation (25, 29). By sharp contrast, the V. furnissii IIMan complex apparently phosphorylates only Man and Glc.

FIG. 4. Alignment of ManD with E. coli GlcNAc-6-P deacetylase. VPEMND, V. furnissii GlcNAc-6-P deacetylase; ECNAGA, E. coli GlcNAc-6-P deacetylase. Alignments were obtained as described under “Materials and Methods.” Identical amino acids are indicated by dark shading, similar amino acid residues by light shading.

FIG. 5. Alignment of V. furnissii ManF with E. coli GatY and B. subtilis Fru 1,6-bisP aldolase. V. furnissii aldolase (MANF), B. subtilis (FBA) and E. coli GatY (GATY) were compared as described under “Materials and Methods.” Identical amino acids are indicated by dark shading, similar amino acid residues by light shading.

IIMan complex.

The Enzyme II complexes of the PTS comprise from one to as many as four individually coded polypeptide chains (for reviews, see Refs. 25 and 26). The most complex are the B. subtilis fructose or levulose (27) and Klebsiella pneumoniae Sor (1-sorbose) Enzyme II complexes (28, 29), which consist of four proteins each. The sequences of these proteins are similar to the three proteins of the E. coli IIMan complex. The latter is encoded by three adjacent genes (4, 15) designated manX (encodes IIMan, also called IIBMan), manY (encodes IIPMan), and manZ (encodes IIBMan). The protein IIPMan (and also possibly IIPMan) is required for phage λ DNA penetration of the E. coli inner membrane (4). However, the V. furnissii homolog does not permit penetration of the cytoplasmic membrane by phage λ DNA.

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Nucleotide sequence analysis of the cloned V. furnissii DNA revealed six closely linked open reading frames (Fig. 3). The first four showed identity with the subunits of the E. coli Man permease. The other two encoded proteins similar to GlcNAc-6-P deacetylase and fructose 1,6-bisP aldolase.

Four separate proteins were encoded by the first four open reading frames in the plasmid p3H1S. All four proteins were required for sugar phosphorylation, and they functioned, both in vivo and in vitro, with E. coli phospho-HPs as the phosphoryl donor. By marked contrast, individual subunits of the V. furnissii permease were unable to substitute for their E. coli IIman homologs, i.e. a functional IIMan complex was observed only

2 As noted earlier in this paper, the gene designations are based on the recommendations of B. Bachmann (18). The PTS nomenclature system is described in recent reviews (25, 26). An alternative has been proposed for both the genetic and protein nomenclatures (26, 30); the designation III is supplanted by IIA, and the nomenclature is based on similarity of the functional domains of the PTS permeases. In the latter system, the proteins encoded by the three E. coli ptsM genes were designated as follows: manX, IAB; manY, IIC; manZ, IID.
when the transformants expressed the three E. coli or the four V. furnissii proteins. Apparently there is insufficient homology between the V. furnissii and E. coli permeases to allow inter-subunit complementation. It is, however, important to note that the V. furnissii proteins formed functional II Man complexes when transcribed from different plasmids. From this it appears that the integral membrane components of the V. furnissii II Man complex properly fold and recognize one another after being separately expressed. It will be interesting to determine whether this association takes place before or after insertion into the membrane.

Enzyme II complexes comprise three functional domains: a hydrophilic domain, which possesses the first phosphorylation site and accepts the phosphoryl group from HPr; a second hydrophilic domain containing another phosphorylation site; and a hydrophobic transmembrane domain, which binds and transports the sugar. The domains may be in a single or in separate proteins. In the E. coli II Man complex, there are two transmembrane domains, II A Man and II B Man, and a hydrophilic subunit, II C Man. II C Man consists of two functional subdomains linked by an Ala-Pro-rich hinge peptide; each subdomain contains a phosphorylation site (16). In the phosphotransfer reactions, II A Man is phosphorylated at residue His-10 by phospho-HPr. The phosphoryl group is then transferred to His-175, in the second functional domain of II B Man (16, 31). Finally, transfer of the phosphoryl group to the sugar then requires the two membrane proteins, II A Man and II B Man (4, 32). The two subdomains of II C Man are complementary when separated in vitro by protease digestion, or in vivo by molecular genetic techniques (16). Recently, Stolz et al. (31) concluded from a study of II C Man point mutants that His-86 is required for phosphotransfer between His-10 and His-175 and that His-86 can substitute for His-10 provided that it is on the same II C Man polypeptide as His-175.

The deduced amino acid sequences of the V. furnissii II Man complex shared identity with the four subunits of B. subtilis Enzyme IIA (from 25.7% to 26.1% identity) and K. pneumoniae Enzyme IIB (from 19% to 36.6% identity). But the greatest identity was with E. coli Enzyme IIB (Fig. 6). We have designated the V. furnissii proteins ManW (15.9 kDa, 30% identity with the N-terminal domain of E. coli III Man), ManX (17.2 kDa, 37% identity with the C-terminal domain of E. coli III Man), ManY (27 kDa, 28% identity with E. coli IIB Man), and ManZ (31.7 kDa, 31% identity with E. coli IIB Man). The two phosphorylation sites in E. coli III Man (ManX) are conserved in the V. furnissii proteins, with the homolog of E. coli His-10 located in V. furnissii ManW and the homolog of E. coli His-175 located in V. furnissii ManX. As expected, the
Ala-Pro-rich motif that links the functional domains of E. coli III\(^{\text{Man}}\) (ManX) is not present in the V. furnissii permease. Perhaps more interestingly, there is no His residue corresponding to His-86 of E. coli III\(^{\text{Man}}\) in V. furnissii ManW. Thus, when the two subdomains of E. coli III\(^{\text{Man}}\) are separated, His-86 appears to be unnecessary for phosphoryl transfer.

Our data therefore suggest that the single gene, manX, which encodes III\(^{\text{Man}}\) in E. coli, is a fusion product of two genes, manX and manW. Alternatively, the reverse could have occurred, separating one gene into two genes in V. furnissii. This separation also occurs in the B. subtilis lev (27) and K. pneumoniae sor (29) equivalents of E. coli manX. However, in lev and sor, the genes encoding each of the III-like protein domains are adjacent to one another, unlike the separation observed in the V. furnissii man complex.

The DNA fragment p3H15 contains two open reading frames besides manXYZW, but neither is essential for Man phosphorylation by the PTS. One open reading frame, designated manD, encodes a protein with identity to the E. coli enzyme, GlcNAc-6-P deacetylase (33). The enzymes required for GlcNAc catabolism in E. coli are encoded by the nag operon; nagA encodes GlcNAc-6-P deacetylase. The deacetylase is the first enzyme in the GlcNAc dissimilation pathway after the sugar enters the cell as GlcNAc-6-P via the PTS (34). It appears that V. furnissii ManD is functional in E. coli since it complementates nagA mutants. The reason for linkage of manD with the man operon is unclear, however, since the V. furnissii nag operon also encodes a GlcNAc-6-P deacetylase (see accompanying article; Ref. 1). The deduced sequences of the two V. furnissii deacetylases are only 29% identical (data not shown). One possibility for the linkage of manD with ptsM in V. furnissii is that the true substrate of the deacetylase is N-acetylmannosamine-6-P. N-Acetylmannosamine is a substrate of E. coli ptsM (35), and linking it with a deacetylase would convert external ManNac to internal mannosamine-6-P. There are, of course, other deacetylases for derivatives of GlcNAc, such as the NodB proteins and chinT deacetylases. However, the identity/similarity of these sequences to ManD is relatively small (data not shown).

Another open reading frame (manF) linked with manXYZW and manD potentially encodes a protein that is similar to several bacterial aldolases. ManF is not required for mannose uptake. Further analysis, such as transcriptional mapping in wild-type V. furnissii, is required to conclusively demonstrate the co-expression of manXYZW, manD, and manF.

In sum, the V. furnissii ptsM operon has been identified. While it is similar to E. coli ptsM, it exhibits interesting and potentially important differences that may reflect different physiological roles in the two organisms. For example, in E. coli, ptsM is an alternate path for GlcNAc uptake but apparently not in V. furnissii. These results explain our previous observation that a mutation in nagE (which encodes the membrane receptor, II\(^{\text{Nag}}\)) prevents V. furnissii from taking up or exhibiting chemotaxis to GlcNAc (9). In view of the complexity of the chitin catabolic cascade in this Vibrio (9, 13, 36, 37), it may be essential that GlcNac uptake and phosphorylation be limited to a single permease that is maintained under stringent control.

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