Sugar Transport by the Marine Chitinolytic Bacterium

*Vibrio furnissii*

MOLECULAR CLONING AND ANALYSIS OF THE GLUCOSE AND N-ACETYLGLUCOSAMINE PERMEASES*

(Received for publication, January 5, 1996, and in revised form, August 19, 1996)

Carolyn L. Bouma$ and Saul Roseman$*

From the Department of Biology and the McCellum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218

Chitin catabolism by the marine bacterium *Vibrio furnissii* involves chemotaxis to and transport of N-acetyl-d-glucosamine (GlcNAc) and D-glucose. We report the properties of the respective permeases that complemented *E. coli* Glc−Man− mutants.

Although the *V. furnissii* Glc-specific permease (55,941 Da) shares 38% identity with *E. coli* II**Glc** (ptsG), it is 67% identical to MalX of the *E. coli* maltose operon (Reidl, J., and Boos, W. (1991) *J. Bacteriol.* 173, 4862–4876). An adjacent open reading frame encodes a protein with 52% identity to *E. coli* MalY. Glc phosphorylation requires only *V. furnissii* MalX and the accessory phosphoenolpyruvateglycosephosphotransferase system proteins. The *V. furnissii* equivalent of II**S** was not found in the 25,000 transformants screened. The GlcNAc/Glc-specific permease (52,884 Da) shares 47% identity with the N-terminal, hydrophobic domain of *E. coli* II**Man**, but is unique among II**Man** proteins in that it lacks the C-terminal domain and thus requires III**Glc** for sugar fermentation in vivo and phosphorylation in vitro. While there are similarities between the phosphoenolpyruvateglycosephosphotransferase system of *V. furnissii* and enteric bacteria, the differences may be important for survival of *V. furnissii* in the marine environment.

Chitin, a β,1→4-linked polymer of N-acetylglucosamine (GlcNAc), is one of the most abundant organic compounds in nature. Huge quantities of this highly insoluble polysaccharide are turned over annually in the aquatic biosphere, and marine bacteria such as *Vibrios* are major biological components of this ecologically indispensable process.

In previous papers (1–4), we reported that chitin degradation by one such organism, *Vibrio furnissii*, is extraordinarily complex, involving multiple signal transduction systems and many proteins. We proposed (4) that the means by which these cells locate chitin-containing organisms is by chemotaxis to components of the hemolymph and molt fluids including glucose, trehalose, GlcNAc and chitin oligosaccharides; all of these compounds are potent chemoattractants for *V. furnissii*.

Degradation of chitin by *V. furnissii* is initiated by chininas that hydrolyze it to soluble oligosaccharides, which are further hydrolyzed in the periplasmic space to GlcNAc and (GlcNAc)_2. Finally, each of these catabolites is taken up by specific transporters and converted intracellularly to Fru-6-P, NH₃, and acetate. The cytoplasmic membrane permeases are, therefore, essential components of the chitin catabolic cascade. The disaccharide permease is described in an accompanying paper (5), while the present report is concerned with the GlcNAc and Glc chemoreceptors/permeases.

We have presented evidence that the uptake and phosphorylation of GlcNAc, Glc, and Man in *V. furnissii* is mediated by the bacterial phosphoenolpyruvate:glycosephosphotransferase system (PTS).¹ While the complete PTS is required for both chemotaxis and transport, the sugar chemoreceptors/translocators are the membrane-associated Enzyme II complexes (for reviews see Refs. 6 and 7).² The Enzyme II complexes often show overlapping substrate specificities. For example, in *Escherichia coli* and *Salmonella typhimurium*, GlcNAc is recognized and taken up by II**Man** and Glc by the protein pair II**Glc**/III**Glc**, but both substrates are also taken up by the less specific, more complex mannose system, I**Man**. For this reason, definitive characterization of the specificities of the *V. furnissii* Enzyme II complexes requires that they be separated from one another.

In this and accompanying papers (8, 9), we describe the molecular cloning and characterization of genes and gene products from *V. furnissii* into *E. coli* that generate GlcNAc in the periplasmic space, and recognize and transport GlcNAc, Glc, and Man. The *V. furnissii* proteins are physiologically active in *E. coli*, and the deduced amino acid sequences of the proteins are similar to the corresponding proteins from the enteric bacteria (10), but they also show interesting and significant differences.

*¹ The abbreviations used are: PTS, phosphoenolpyruvate:glycosephosphotransferase system; MeGlc, methyl -glucoside; cAMP-CAP, cyclic AMP-catabolite activator protein complex; kb, kilobase pair(s); bp, base pair(s); ORF, open reading frame; PCR, polymerase chain reaction; PAGR, polyacrylamide gel electrophoresis.

*² The nomenclature of the Enzymes II is complicated by the different variants that have been identified, and two systems of nomenclature have been proposed (6, 56). In general, the sugar-specific, membrane-associated polypeptides are termed Enzyme II complexes. In the nomenclature used in this and the accompanying reports, the sugar receptors are designated “II” or Enzyme II if the integral membrane protein complex comprises a single polypeptide chain, such as II**Man**, or II**Glc**. When the complex contains more than one integral membrane protein, the sugar receptor is called “II**A**”, such as II-B**Man**. The sugar-specific, soluble phosphotransfer proteins are called “III**A**” or, if they are intrinsic membrane proteins, are designated “III**A**”. The terms II**A** and III are therefore functionally equivalent.
Glucose and GlcNAc Permeases of V. furnissii

MATERIALS AND METHODS

Biochemicals and Molecular Biological Reagents—Restriction enzymes and T4 DNA ligase were purchased from Life Technologies, Inc. or U. S. Biochemicals Corp. Radiochemicals were purchased from DuPont NEN or Amersham. The radiolabeled nucleotides were of the highest purity available and were obtained from Sigma or Aldrich.

Bacterial Strains and Culture Media—The following bacterial strains were employed: E. coli SR423 and SR425 (11), LR2-175 (12), KPN15 and KPN6 (13), HB101 (14), and V. furnissii SR1514 (previously Vibrio flavidus 1514; Ref. 1). The relevant genotypes of strains and plasmids are given in Table I. The plasmid pKPl 1 and E. coli KPN15 were gifts from Dr. B. Waygood (University of Saskatchewan, Saskatoon, Canada); the strain LR2-175 was a gift from Dr. J. Lengeler (Universitat Osnabruck, Fachbereich Biologie/Chemie, Osnabruck, Germany); the strains IBPC531 and IPBC546 (15) were gifts from Dr. J. Plumbidge (Institut de Biologie Physio-chimique, URA1139, Paris, France); the strain SA2600 was a gift from Dr. S. Adhya (NCI, National Institutes of Health, Bethesda, MD). For transformations, transduction in these studies. More than 12,000 transformants were screened at 37°C in Luria broth (16). For sugar phosphorylation assays, E. coli strains were grown at 37°C in M9 medium (16) supplemented with 0.5% carbon source broth (16). For growth experiments in LR2-175, Medium A (17) was supplemented with Met (20 mg/ml) and Kan (12.5 mg/ml). For growth experiments in SA2600 and LR2-175 by the method of Silhavy (18). E. coli ptsG mutants—For growth experiments in LR2-175, Medium A (17) was supplemented with Met (20 mg/ml), His (22 mg/ml), Arg (22 mg/ml), and the appropriate antibiotic.

Construction and Screening of V. furnissii DNA Libraries—Restriction enzyme digestions, ligations, transformations, and agarose gel electrophoresis were carried out as described by Maniatis (16). Genomic DNA was prepared from V. furnissii 1514 by lysozyme-SDS treatment (18). Genomic DNA, digested with restriction enzymes, HindIII, was ligated into similarly digested, dephosphorylated pBR322. The ligation mixtures were transformed into E. coli HB101 and amplified by the method of Pulleyblank (19). The resulting recombinant plasmids were used to transform E. coli strains SR423 and SR425, screening for Glc Amp' colonies on MacConkey Glc/ampicillin agar. The plasmids pBR322 (vector) and pCB20 (carrying E. coli pslG) were used as negative and positive controls, respectively, for Glc fermentation defect in these studies. More than 12,000 transformants were screened at 37°C in MacConkey agar without lactose (Difco) was prepared with 1% sugar. An- at 37°C in M9 medium (16) supplemented with 0.5% carbon source broth (16). For sugar phosphorylation assays, E. coli strains were grown at 37°C in Luria broth (16). For growth experiments in LR2-175, Medium A (17) was supplemented with Met (20 mg/ml), His (22 mg/ml), Arg (22 mg/ml), and the appropriate antibiotic.

DNA Sequencing and Analysis—Nucleotide sequencing was performed on double-stranded plasmid templates by the dideoxy chain termination method with the Sequenase kit (U. S. Biochemical Corp.) and [α-32P]dATP (1000 Ci/mmol) as described by the manufacturer. Sequencing primers (clockwise EcoRI 17-mer and counterclockwise HindIII 17-mer) were obtained from Life Technologies, Inc. and Pharmacia Biotech Inc. The remaining primers were designed and synthesized as needed while sequencing the cloned E. coli DNA. The sequencing reactions were performed with Taq DNA polymerase (Perkin-Elmer), for 30 cycles of amplification (94°C, 30 s; 48°C, 1 min; 72°C, 2 min 30 s). The PCR products were ethanol precipitated, digested with BamHI and PstI, and were ligated into similarly digested pUC18. The resulting clones, the plasmids 3B1S-ORF1 and 3B1S-ORF2, were sequenced to confirm that no sequence errors were intro-duced during amplification.

Results

Complementation of E. coli Glc' Man' Mutants—Two libraries of V. furnissii genomic DNA, in the BamHI and HindIII sites of pBR322, were screened for plasmids that could comple-ment the Glc fermentation defect in E. coli strains SR423 or SR425. Five plasmids carrying V. furnissii DNA inserts of different molecular sizes were represented among the Glc' Amp' transformants. DNA hybridizations (data not shown) demonstrated that unique V. furnissii fragments were carried by four of these plasmids. Since no hybridization was observed between the cloned DNA fragments and E. coli genomic DNA (data not shown), these experiments also conclusively demonstrated that the plasmids did not contain E. coli genomic DNA, which might have occurred by recombination with the host strain during preparation or screening of the V. furnissii libraries. To determine the minimum size necessary for complementation of Glc fermentation, restriction fragments of these four Glc' plasmids were subcloned into pBB322, and screened for those that allowed Glc fermentation in SR423 or SR425. The restriction maps of two of the four clones (p3B1 and p5B16), and their subclones (p3B1S and p5B16H), are presented in Fig. 1.

Phenotypes of E. coli Transformants Carrying Glc' Plasmids—Various E. coli pts mutants were transformed with p3B1 (5.5-kb BamHI V. furnissii DNA), p3B1S (4-kb SalI V. furnissii DNA), p5B16 (6.8-kb BamHI V. furnissii DNA), p5B16H (2.2-kb HindIII V. furnissii DNA), and control plas-
Glucose and GlcNAc Permeases of V. furnissii

The plasmids p3B1 and p5B16 were isolated from libraries of V. furnissii DNA in pBR322 as described under "Materials and Methods." p3B1S and p5B16H were constructed by subcloning V. furnissii DNA fragments from p3B1 and p5B16 into pBR322. Thin lines represent vector DNA; open boxes represent V. furnissii genomic DNA. The following symbols are used for restriction sites:  A,  EcoRI;  B,  BamHI;  H,  BglII;  R,  BspI;  C,  ClaI;  X,  HindIII;  N,  NruI;  Nd,  NdeI;  PI,  PpuII;  PH,  PvuII;  Ps,  PstI;  RI,  EcoRI;  S,  SacI;  Sc,  ScaI;  and  X,  XhoI.

The clones were therefore tested in appropriate mutant strains (Table I). In addition, the plasmids p5B16 and p5B16H restored GlcNAc fermentation to the Glc fermentation defect in the strains ZSC113 (ptsG ptsM nagE::Cm, GlcNAc-6-P deacetylase) transformants carrying p3B1S, p5B16H, and control plasmids (p3B1 and p5B16 gave essentially the same results as p3B1S and p5B16H, respectively.) Table II summarizes these results. GlcNAc phosphorylation was not measured in SR425 transformants because of the wild-type plasmids when tested in the appropriate mutant strains (Table I).

The fermentation patterns of the transformants suggested that p3B1 and p3B1S might encode the V. furnissii Enzyme IIIC, and p5B16 and p5B16H the V. furnissii Enzyme IIICα. The clones were therefore tested in ptsI and ptsI-crr deletion mutants to confirm that they encoded membrane permeases of the PTS. PtsI and ptsI-crr strains should remain pleiotropically negative for PTS sugar fermentation when transformed by plasmids encoding PTS permeases. The results (Table I) showed that p3B1 and p3B1S require Enzyme I and IIICα for fermentation, as does E. coli IIICα. Interestingly, the putative GlcNAc permease encoded by p5B16 and p5B16H required both Enzyme I and IIICα for fermentation, in contrast to its E. coli counterpart (encoded by pKPl.1) which does not require IIICα. Negative fermentation results were obtained by transformation of S. typhimurium SB2950 (17), which carries a ptsHi-crr deletion (data not shown), confirming the requirement for these three soluble PTS proteins by the cloned permeases.

E. coli strain LR2-175 transformants carrying p3B1S and p5B16H grow on minimal media with Glc as the sole carbon source with generation times of 65 to 75 min (data not shown). Transforms carrying p5B16 or p5B16H grow on GlcNAc minimal media (generation time, 68 min).

The plasmids p5B16 and p5B16H were examined further for their ability to complement E. coli nag mutants (Table I). E. coli IBPC331 (nagA::Cm, GlcNAc-6-P deacetylase) transformants carrying p5B16 fermented GlcNAc while p5B16H transformants did not. Another nagA mutant, E. coli KPN15 (13) was also complemented by p5B16 (data not shown). Neither p5B16 nor p5B16H complemented the nagB (GlcNH₂-6-P deaminase) defect in IBPC546. It therefore appears that p5B16 encodes a PTS-dependent GlcNAc/Glc permease and GlcNAc-6-P deacetylase, while p5B16H encodes only the permease.

Enzyme II Activity in Membranes from E. coli Transformants—PTS-dependent sugar phosphorylation was measured with membranes prepared from E. coli SR425 and LR2-175 transformants harboring p3B1S, p5B16H, and control plasmids. (p3B1 and p5B16 gave essentially the same results as p3B1S and p5B16H, respectively.) Table II summarizes these results. GlcNAc phosphorylation was not measured in SR425 transformants because of the wild-type nagE allele in this strain.

Sugar phosphorylation by control membranes (from pBR322 transformants) was very low. Membranes from p3B1S transformants phosphorylated Glc at 20–60-fold rates greater than the controls, thereby explaining the Glc⁻ phenotype. In the one case tested, transformants of LR2-175, the membranes also showed an increased rate of phosphorylation of GlcNAc over controls, but this rate is comparable to that of LR2-175 transformants expressing cloned E. coli IIICα from the plasmid pCB20 (data not shown). Thus, the apparent in vitro nonspecificity of the IIICα system either does not function in vivo, or the rate is insufficient to permit growth on GlcNAc.

Membranes from p5B16H transformants phosphorylated...
both Glc and GlcNAC, and in these cases, the increases ranged from 300- to 1000-fold over control sugar phosphorylation rates. The rate-limiting proteins in the assays were the membranes, and growth of the transformant on GlcNAC yielded an increased specific activity compared to membranes from Glc-grown cells. The results therefore agreed with those expected from the phenotypes of the transformants. However, there is a quantitative inconsistency in Table II. The ratio of GlcNAC to Glc phosphorylation varied with the sugar used for cell growth, although the ratio should be constant for a single Enzyme II. This quantitative effect may result from the difficulties associated with measuring membrane-bound II<sup>new</sup> activities. Also, bacterial membranes change in composition depending on the carbon source used for growth (30). Perhaps the V. furnissii II<sup>new</sup> protein inserts differently into E. coli membranes of similar composition, giving rise to kinetic differences with respect to its sugar substrates.

Other radioactive sugars were tested as potential substrates in both systems. There was little to no detectable phosphorylation of the following labeled sugars (1 mM) by membranes from transformants of LR2-175: fructose, mannose, sucrose, 2-deoxyglucose, and MeGlc. The latter result was surprising, since to this point the results indicated that p3B1S encoded the equivalent of IIGlc, and MeGlc is an excellent sugar for this enzyme. Fermentation was scored on MacConkey agar with 1% sugar and the appropriate antibiotic. +, dark red colonies; -, light pink/white colonies; +/-, red colonies. NT = not tested.

**Table I**

| Strain   | Genotype<sup>a</sup> | Plasmid<sup>b</sup> | Phenotype<sup>c</sup> |
|----------|------------------------|----------------------|-----------------------|
|          |                        |                      |                       |
|          |                        |                      | Glc | Man | GlcNAC | Gal | Fru | Mtl |
| SR423    | galIH endA hsdR sbcB   | pBR322               | +   | +   | -     | +   | +   |
|          | ptsM (manXYZ) ptsG T1<sup>R</sup> | p3B1, p3B1S       | +   | +   | +     | +   | +   |
|          |                        | p5B16, p5B16H       | +   | -   | +     | +   | +   |
|          |                        | pCB20               | +   | -   | -     | +   | +   |
|          |                        | 3B1S-ORF1           | +   | -   | -     | +   | +   |
|          |                        | 3B1S-ORF2           | -   | -   | -     | +   | +   |
| SR425    | galIH endA hsdR sbcB   | pBR322               | +   | -   | -     | +   | +   |
|          | ptsM (manXYZ) ptsG T1<sup>R</sup> | p3B1, p3B1S       | +   | -   | -     | +   | +   |
|          |                        | p5B16, p5B16H       | +   | -   | -     | +   | +   |
|          |                        | pCB20               | -   | -   | -     | +   | +   |
|          |                        | 3B1S-ORF1           | -   | -   | -     | +   | +   |
|          |                        | 3B1S-ORF2           | -   | -   | -     | +   | +   |
| LR2–175  | galIP63 manI161 manI162 | pBR322             | +   | -   | -     | +   | +   |
|          | (ptsM) nagE167         | p3B1, p3B1S         | +   | -   | -     | +   | +   |
|          |                        | p5B16, p5B16H       | +   | -   | -     | +   | +   |
|          |                        | pCB20               | -   | -   | -     | +   | +   |
|          |                        | 3B1S-ORF1           | -   | -   | -     | +   | +   |
|          |                        | 3B1S-ORF2           | -   | -   | -     | +   | +   |
| LR2–175  | Δ(ptsI-crr)            | LR2–175 Δ(ptsI-crr) | +   | -   | -     | +   | +   |
|          |                        | Km<sup>i</sup>      | +   | -   | -     | +   | +   |
| SA2600   | F<sup>−</sup> His<sup>−</sup> rpsL relA Δ | pBR322              | +   | -   | -     | +   | +   |
|          | (ptsI-crr) Km<sup>i</sup> | p3B1, p3B1S         | +   | -   | -     | +   | +   |
|          |                        | p5B16, p5B16H       | +   | -   | -     | +   | +   |
|          |                        | pCB20               | -   | -   | -     | +   | +   |
|          |                        | 3B1S-ORF1           | -   | -   | -     | +   | +   |
|          |                        | 3B1S-ORF2           | -   | -   | -     | +   | +   |
| LR2–175  | Δ(ptsI-crr)            | LR2–175 Δ(ptsI-crr) | +   | -   | -     | +   | +   |
|          |                        | Km<sup>i</sup>      | +   | -   | -     | +   | +   |
| IBPC546  | thI-1 argG6 argE3 his-4 mtl-1 | pBR322             | +   | -   | -     | +   | +   |
|          | xyl-5 rpsL ΔacX74      | p3B16               | +   | -   | -     | +   | +   |
|          | nagB::Kan             | p5B16H              | +   | -   | -     | +   | +   |
|          |                        | pKP1.1              | +/−| +   | −     | −   | −   |
| IBPC531  | thI-1 argG6 argE3 his-4 mtl-1 | pBR322             | +   | -   | -     | +   | +   |
|          | xyl-5 rpsL ΔacX74      | p3B16               | +   | -   | -     | +   | +   |
|          | nagA::Cm              | p5B16H              | +   | -   | -     | +   | +   |
|          |                        | pKP1.1              | +/−| +   | −     | −   | −   |

---

<sup>a</sup> Strains SR423, SR425, and LR2–175 are ptsM mutants. The ptsM locus has been renamed (11), and contains the genes manXYZ, manMW, and manZ. The relevant manXYZ genotype, if known, is given. Δ(ptsI-crr) and Δcrr strains were constructed by P1 transduction as described under “Materials and Methods.”

<sup>b</sup> The following plasmids were used: p3B1, pBR322 with 5.5-kb BamHI V. furnissii DNA; p3B1S, pBR322 with 4-kb SalI V. furnissii DNA; p5B16, pBR322 with 6.8-kb HindIII V. furnissii DNA; p5B16H, pBR322 with 2.2-kb HindIII V. furnissii DNA; p5B16 ORF1, nucleotides 7–2284 of p3B1S in the BamHI/PstI sites of pUC18; 3B1S-ORF2, nucleotides 2188–3574 of p3B1S in the BglII/PstI sites of pUC18; pCB20, pBR322 with E. coli p5B16H (20); pDS20, E. coli ptsII (8); and pKP1.1, E. coli nagABCDEF (13).

<sup>c</sup> Fermentation was scored on MacConkey agar with 1% sugar and the appropriate antibiotic. +, dark red colonies; −, light pink/white colonies; +/-, red colonies. NT = not tested.
Glucose and GlcNAc Permeases of *V. furnissii*

*PTS-mediated sugar phosphorylation by membranes from *E. coli* transformants harboring *V. furnissii* PTS permease genes*

Membranes prepared from cells grown in M9 medium containing the indicated carbon source were assayed for sugar phosphorylation in the presence of *E. coli* Enzyme I, HPr, and III<sup>Glc</sup> as described under “Materials and Methods.” *PTS-mediated sugar phosphorylation by membranes from *E. coli* transformants harboring *V. furnissii* PTS permease genes*

| *E. coli* strain | Plasmid | Phenotype | Cell growth | Specific activity<sup>a</sup> |
|-----------------|---------|-----------|-------------|--------------------------|
|                 |         | Glc       | GlcNAc      | Gutar                    |
| LR2–175         | pBR322  | –         | –           | 0.4                      |
|                 | p3B1S   | +         | –           | 10<sup>b</sup>           |
|                 | p3B16H  | +         | +           | 125<sup>c</sup>          |
|                 | p5B16H  | +         | +           | 1250<sup>c</sup>         |
| SR425           | pBR322  | –         | –           | 1.6<sup>b</sup>          |
|                 | p3B1S   | +         | +           | 31<sup>d</sup>           |

<sup>a</sup> Specific activity = nmol sugar phosphate formed/min/mg membrane protein.

<sup>b</sup> In the absence of added III<sup>Glc</sup>, the specific activity was 1.8 nmol min<sup>-1</sup> mg membrane protein<sup>-1</sup>.

<sup>c</sup> In the absence of added III<sup>Glc</sup>, the specific activity was 5 nmol min<sup>-1</sup> mg membrane protein<sup>-1</sup>.

<sup>d</sup> ND, not determined.

The latter comprises an N-terminal membrane-bound domain and a cytoplasmic C-terminal domain, which is the functional equivalent of III<sup>Glc</sup> and accepts the phosphoryl group directly from phospho-HPr.

The requirement for III<sup>Glc</sup>, both in *vitro* and in *vivo* (Tables I and II) were clarified by the experiments described below.

**In Vitro Transcription/Translation of Plasmid-encoded Proteins**—To further characterize the cloned genes from *V. furnissii*, the plasmid-encoded gene products were analyzed by *in vitro* transcription/translation. The plasmids p3B1S, p5B16H, and pBR322 were translated in a prokaryotic cell-free system and the labeled polypeptides were separated by SDS-PAGE as described under “Materials and Methods.” The plasmid p3B1S produced labeled products with apparent molecular masses of 53 and 44.5 kDa as well as the vector-encoded β-lactamase (Fig. 2). A strongly labeled product with an apparent molecular mass of 49.5 kDa and β-lactamase was produced from the plasmid p5B16H (it should be noted that the background varies with this *in vitro* system). The isolates p3B1 and p5B16, carrying larger *V. furnissii* genomic DNA fragments, did not give clear results with this system, making it difficult to determine the number of gene products they express.

**Nucleotide Sequencing and Deduced Amino Acid Sequence Analysis of p3B1S**—The *V. furnissii* DNA fragment carried by p3B1S was sequenced (21) by the dideoxy chain-termination method (GenBank accession number U65013). The largest open reading frame in the 3997-bp SalI fragment (ORF 1, Fig. 3) extended from nucleotide 647 to 2218 and was preceded by a potential ribosomal binding site (32) centered 10 nucleotides upstream from the initiation codon. A second open reading frame was found between nucleotides 2273 and 3472 (ORF 2, Fig. 3). A potential promoter (positions 510–534) was also identified by its similarity to the *E. coli* consensus sequence (33). However, no sequences with strong identity to the consensus CAP binding sequence (34) or a region with the structural characteristics of a p-independent terminator (35) were found.

The deduced amino acid sequence of ORF 1 predicted a protein of 523 amino acids with a calculated molecular mass of 55,941 Da. This molecular mass is similar to the Mr<sup>r</sup> of the larger *in vitro* transcription/translation product of p3B1S (Fig. 2). Similarity searches (36, 37) of protein and nucleic acid data bases revealed homology between this *V. furnissii* protein and *E. coli* II<sup>Glc</sup> (38% identity), *E. coli* II<sup>Man</sup> (34% identity), and *Bacillus subtilis* II<sup>Glc</sup> (37% identity). Surprisingly, the protein showed the greatest identity with the recently reported (10) *E. coli* MalX (67%). Its hydropathic profile also resembles that of *E. coli* MalX (data not shown), and its hydrophobicity is reflected in its amino acid composition (data not shown). An alignment of *V. furnissii* MalX with *E. coli* MalX is shown in Fig. 4. These sequences are well conserved, with 33% identical residues between the three proteins. Previous studies have suggested functional roles for several regions of high sequence identity among the PTS permeases (reviewed in Refs. 6 and 7). A putative site of phosphorylation (His-211 in *E. coli* II<sup>Glc</sup>) was identified among the PTS membrane permeases by sequence comparison (6). Recent evidence obtained by mass spectrometry and biochemical analysis of phosphorylated peptides indicates that Cys-421 of *E. coli* II<sup>Glc</sup> is phosphorylated (38, 39). These residues are conserved among *E. coli* II<sup>Glc</sup>, MalX, and the *V. furnissii* Glc permease. Additionally, four of the six residues of *E. coli* II<sup>Glc</sup> recently described to be important for sugar translocation (Met-17, Gly-149, Lys-150, Ser-157, His-339, and Asp-343; Ref. 40) are conserved in the *V. furnissii* homolog.

Translation of ORF 2 from p3B1S predicted a 399-amino acid protein with a molecular mass of 45,359 Da. This molecular mass compares favorably with that of the smaller polypeptide obtained by *in vitro* transcription/translation (Fig. 2). Since ORF 1 of p3B1S is similar to *E. coli* MalX, it is not surprising that the deduced amino acid sequence of ORF 2 shared 52% identity with *E. coli* MalX (10). The deduced sequence of the *V. furnissii* MalY-like gene product is aligned with the *E. coli* MalY sequence in Fig. 5. *E. coli* MalY has recently been identified as a β-cystathionase, an essential enzyme in methionine catabolism (41). Thus, *E. coli* MalY complements *E. coli* metC mutants. Dr. Boos and associates have also shown that the *V. furnissii* malY analogue (i.e. p3B1S) reported here likewise complements *E. coli* metC mutants (41). Thus, the *V. furnissii* DNA fragment encodes a functional MalY protein, thereby
FIG. 3. Nucleotide and deduced amino acid sequences of the V. furnissii Glc permease. The coding strand of the 3993-bp SalI fragment of V. furnissii DNA from p3B1S is presented, and the deduced amino acid sequences of the two major open reading frames are given. Putative promoter elements (−35 and −10) are underlined. Ribosomal binding sites are indicated by double underlines.
providing even stronger evidence that the adjacent gene is indeed \textit{V. furnissii} malX and not \textit{ptsG}. It should be noted that \textit{V. furnissii} grows very well on maltose and exhibits chemotaxis to this sugar (4).

Since two open reading frames were discovered in the \textit{V. furnissii} SalI fragment in p3B1S, and both were expressed \textit{in vitro}, we wished to determine whether both gene products were required for Glc transport and phosphorylation \textit{in vivo}. Each reading frame was amplified by PCR with the addition of BamHI and PstI sites, and was ligated into pUC18. As shown in Table I, 3B1S-ORF1 alone was sufficient for Glc fermentation. A \textit{ptsG ptsM glk} strain, ZSC113 (28), was also able to ferment Glc when transformed with p3B1S or 3B1S-ORF1, but not when transformed with 3B1S-ORF2 (data not shown). Therefore, glucokinase is not required for Glc fermentation in cell transformants of \textit{V. furnissii} MalX.SR425 transformants carrying p3B1S or 3B1S-ORF1 grew well on Glc as the sole carbon source (doubling time, 65 min), whereas transformants of 3B1S-ORF2 did not (no detectable growth after 3 h).

Neither the deduced amino acid sequence nor any portion of the nucleotide sequence of p3B1S showed similarity to \textit{E. coli} IIIGlc, a result consistent with the \textit{in vivo} and \textit{in vitro} requirements of p3B1S transformants for III\textsuperscript{Glc} (Tables I and II).

**FIG. 4. Alignment of \textit{V. furnissii} and \textit{E. coli} MalX.** The sequences were aligned as described under “Materials and Methods.” ECMALX, \textit{E. coli} MalX sequence (1); VFMALX, \textit{V. furnissii} MalX sequence. Identical amino acids are indicated by dark shading, similar amino acid residues by light shading.

**FIG. 5. Alignment of \textit{V. furnissii} MalY-like protein and \textit{E. coli} MalY.** The sequences were aligned as described under “Materials and Methods.” VFMALEY, \textit{V. furnissii} MalY sequence; ECMALY, \textit{E. coli} MalY sequence (1). Identical amino acids are indicated by dark shading, similar amino acid residues by light shading.

**Nucleotide Sequencing and Deduced Amino Acid Sequence Analysis of p5B16H**—The 2.2-kb HindIII fragment of p5B16H was also sequenced (GenBank accession no. U65014). The major open reading frame, indicated in Fig. 6, begins at nucleotide 140, terminates at nucleotide 1630, and is preceded by sequences similar to the \textit{E. coli} ribosomal binding site (32) and −35 and −10 promoter elements (33). The predicted polypeptide is 496 amino acid residues in length with a molecular mass of 52,894 Da. This is similar to the apparent molecular mass of the major \textit{in vitro} transcription/translation product (Fig. 2). Hydrophatic analysis (34) showed the permease to be primarily hydrophobic, resembling the hydrophobic domain of \textit{E. coli} II\textsuperscript{Glc} (13). The predicted amino acid composition of the permease also showed a high proportion of hydrophobic amino acids (data not shown). FASTA and BLAST searches (36, 37) of the GenBank and Swiss Protein data bases revealed 48% identity (67% similarity) between \textit{E. coli} II\textsuperscript{Glc} (13) and the permease encoded by p5B16H. Other sequences showing similarity were \textit{E. coli} II\textsuperscript{Glc} (37%) and \textit{B. subtilis} II\textsuperscript{Glc} (41%). The plasmids p5B16 and p5B16H therefore carry the \textit{V. furnissii} homolog of nagE. An alignment of the \textit{V. furnissii} and \textit{E. coli} GlcNAc permeases is presented in Fig. 7. Several extensive regions of the two enzymes are highly conserved (residues 256–273, 295–330, and
424–445, for example). However, the C-terminal, hydrophilic IIIGlc-likedomain of E. coli IINag is absent from the V. furnissii protein. The hydropathic profile of V. furnissii IINag (data not shown), the transformant phenotypes (Table I), and the invitro sugar phosphorylation data (Table II) are consistent with this. The residues surrounding the His phosphorylation site proposed by Meadow et al. (6) are identical in 9 out of 13 positions in the V. furnissii and E. coli GlcNAc permeases. A sequence, IDACITRL (residues 432–439), homologous to the phosphorylation site of E. coli IIGlc identified by Meins et al. (43), is also present in the V. furnissii GlcNAc permease. No similarity to E. coli IIIGlc or the E. coli IIIGlc-like domain of E. coli IINag was detected within the 2205-nucleotide V. furnissii DNA fragment in p5B16H.

The sequences upstream of the initiation codon and beyond the termination codon of V. furnissii nagE were analyzed for potential regulatory signals. Several potential promoter sequences were detected, and the one with the greatest identity to the consensus sequence for E. coli promoters (33) is highlighted in Fig. 6. The E. coli nag regulon is coordinately controlled by repressor (NagC) and cAMP-CAP binding to an operator located between nagE and nagBACD (15, 44, 45). The operator overlaps the −35 region of the nagE promoter (45). A site was identified upstream of V. furnissii nagE (Fig. 6), which is identical in eight of nine positions to the consensus sequence for NagC binding (TAATTTTTTTTTXCXXA; Ref. 45), and which overlaps the −35 region of the putative promoter sequence. No strong CAP binding sites were detected. A putative ρ-independent terminator (35) with a GC-rich region of dyad symmetry was found within 100 bp of the nagE termination codon (Fig. 6).

**DISCUSSION**

The PTS is widely distributed in the eubacteria, including Gram-negative, facultative anaerobes such as E. coli and S. typhimurium. The families Enterobacteriaceae and Vibrionaceae are closely related, and Vibrio is one of the most widespread of the bacterial genera (46). The PTS was found in all Vibrio species tested (47–49). Kubota et al. (50, 51) reported that Glc, trehalose, Fru, Man, and mannitol are PTS sugars in Vibrio parahaemolyticus, and separated four fractions from extracts (by gel filtration) that corresponded in their activities to Enzyme I, HPr, IIGlc, and IIIGlc. Sucrose is a PTS sugar in Vibrio alginolyticus (52), and the Scr operon has been cloned and sequenced.

In V. furnissii, chemotaxis to and the transport of GlcNAc and Glc are part of a complex series of physiological events that we have designated the chitincatabolic cascade (1–4). We have previously reported (4) that in this organism, the following are PTS sugars: GlcNAc, Glc, mannitol, mannose, fructose, trehalose, and sucrose. Each of these carbohydrates is a potent...
VFNAG  1 HNIGTCKVGMADVTHTIAWALSHGUGVY23456DNWGA789GSALQGINKPQGQ12345
        1 HNIGTCKVGMADVTHTIAWALSHGUGVY23456DNWGA789GSALQGINKPQG12345
ECNAG  61 SWLAVGAVSGVSHDGIASSALGAVYPPY94644PRG--CDL--VLL--VLFAGAAL--23
        61 SWLAVGAVSGVSHDGIASSALGAVYPPY94644PRG--CDL--VLL--VLFAGAAL--23
VFNAG  121 QYIVLVDLRMELNYTTPPLVPALARRPVQGKYNFRQGSF94644PRG--CDL--VLL--VLFAGAAL--23
        121 QYIVLVDLRMELNYTTPPLVPALARRPVQGKYNFRQGSF94644PRG--CDL--VLL--VLFAGAAL--23
ECAH  158 EAYAGCVWVSSA2GAMLLPAPLLPPGLQ94644PRG--CDL--VLL--VLFAGAAL--23
        158 EAYAGCVWVSSA2GAMLLPAPLLPPGLQ94644PRG--CDL--VLL--VLFAGAAL--23
VFNAG  321 AAVGCFHVQVSGVSHDGIASSALGAVYPPY94644PRG--CDL--VLL--VLFAGAAL--23
        321 AAVGCFHVQVSGVSHDGIASSALGAVYPPY94644PRG--CDL--VLL--VLFAGAAL--23
ECAH  325 CFVWVSSA2GAMLLPAPLLPPGLQ94644PRG--CDL--VLL--VLFAGAAL--23
        325 CFVWVSSA2GAMLLPAPLLPPGLQ94644PRG--CDL--VLL--VLFAGAAL--23
VFNAG  397 EYVQGCHSN2NDACIERTLRRVSMIDDEK3FGAGCV2GKE8SNDLVL2PL23
        397 EYVQGCHSN2NDACIERTLRRVSMIDDEK3FGAGCV2GKE8SNDLVL2PL23
ECAH  416 EYVQGCHSN2NDACIERTLRRVSMIDDEK3FGAGCV2GKE8SNDLVL2PL23
        416 EYVQGCHSN2NDACIERTLRRVSMIDDEK3FGAGCV2GKE8SNDLVL2PL23
VFNAG  476 IILGQKRHDM2NEKEETN2V2E2QGS3
        476 IILGQKRHDM2NEKEETN2V2E2QGS3
ECAH  495 ILLGQKRHDM2NEKEETN2V2E2QGS3
        495 ILLGQKRHDM2NEKEETN2V2E2QGS3

**FIG. 7. Alignment of V. furnissii and E. coli Enzyme II**<sup>NC</sup> <sup>seq</sup> sequences. The sequences were aligned as described under "Materials and Methods." VFNAG, V. furnissii II<sup>N</sup>C<sup>seq</sup>; ECNAG, E. coli II<sup>N</sup>C<sup>seq</sup>. Identical amino acids are indicated by dark shading, similar amino acid residues by light shading.

chemoattractant except fructose. The non-PTS sugars include glycerol, galactose, maltose, and N,N’-diacetylcysteine. Galactose is a weak chemoattractant (unlike in *E. coli*), whereas both disaccharides are potent attractants.

The genes that encode the Glc and GlcNAc Enzyme II complexes and the proteins that serve both as the chemoreceptors and translocators of their respective sugar substrates are the subjects of this paper.

The soluble *E. coli* PTS proteins (Enzyme I, HPr, and III<sup>Glc</sup>) cross-reacted with *V. furnissii* membranes in sugar phosphorylation assays in vitro (47). Therefore, it seemed likely that the *V. furnissii* Enzymes II would be functionally expressed in *E. coli* transformants, and based on this rationale, Glc- Man<sup>+</sup> *E. coli* strains SR423 and SR425 (11) were used for screening *Bam*II and *Hind*III libraries (in the plasmid vector pBR322) of *V. furnissii* genomic DNA. Four plasmids carrying nonhomologous DNA fragments each permitted the mutants to ferment Glc, and each isolate encoded a permease capable of transporting Glc. The accompanying article (8) reports a PTS-dependent Man complex; the second Glc transporter, which is PTS-independent, will be reported elsewhere. The present paper describes a plasmid that encodes a *V. furnissii* Glc-specific PTS permease, and one that encodes a *V. furnissii* GlcNAc/Glc permease.

We had expected that some of the Glc<sup>+</sup> *E. coli* transformant colonies would express the *V. furnissii* homolog of II<sup>Glc</sup>, which is widespread in enterobacteria. It functions in concert with the cytoplasmic protein III<sup>Glc</sup> (also called IIIA<sup>Glc</sup>), and translocates/phosphorylates Glc and the analogue MeGlc. II<sup>Glc</sup> has also been assigned an important role in regulating the "glucose effect" (6, 7).

Based upon complementation tests (Table I) and in vitro sugar phosphorylation assays, it appeared that the plasmid p3B1S encoded *V. furnissii* II<sup>Glc</sup>, although it showed little activity with MeGlc or with other Glc analogues that were tested. Searches of protein sequence data bases also suggested this interpretation, since one of the *V. furnissii* open reading frame in p3B1S is 98% identical to *E. coli* ptsG. But to our surprise, the same reading frame is 67% identical to *E. coli* malX.

The maltose regulon (53) comprises a complex array of genes and proteins. Reidl and Boos (10) cloned an operon from *E. coli* consisting of malX and malY that are under the control of a repressor encoded by malI. *E. coli* MalX is 35% identical to *E. coli* II<sup>Glc</sup>.

That the first open reading frame (ORF 1) in p3B1S is the *V. furnissii* equivalent of malX received further support by comparing the sequences of the other open reading frames in the plasmid with the data bases. ORF 2 encodes a functional malY-like protein, and another (partial) open reading frame (complementary to nucleotides 1–411, Fig. 3) shares 47% identity with the N-terminal portion of *E. coli* MalI. Thus, the sequence data provide strong evidence that the gene cloned from *V. furnissii* is the analogue of *malX*, not ptsG.

The roles of MalX and MalY in regulation of the *E. coli* Mal system have been investigated (10). Our results are consistent with the previous study in that the II<sup>Glc</sup>-like (MalX) gene product does not require MalY for Glc fermentation (Table I). The authors suggested (based on phenotypic behavior of mutants) that the malX gene product normally transports Glc (and maltose) by facilitated diffusion, but that under certain conditions, it could phosphorylate Glc; biochemical studies of MalX PTS activity were not reported. We show here that this protein functions like other Enzymes II of the PTS in Glc translocation (Tables I and II); it requires Enzyme I and II<sup>Glc</sup> as well as MalY for Glc fermentation (Table I). 

Unlike p3B1S, cells transformed with the plasmid p5B16H fermented GlcNAc as well as Glc. It is likely that Glc is a true substrate of the *V. furnissii* GlcNAc permease since (a) we
selected Glc-fermenting transformants on rich medium, and (b) membranes from the transformants rapidly phosphorylated Glc, close to the same rate as GlcNac (Table II). Previous reports (54) have shown that E. coli II\textsuperscript{Nag}, when transformed into a pts\textsuperscript{G} pts\textsuperscript{M} cry\textsuperscript{Nag} background, allows only slow growth on Glc and there are no reports that Glc is rapidly translocated by E. coli II\textsuperscript{Nag}.

Thus, V. furnissii II\textsuperscript{Nag} is apparently unique in its ability to translocate both Glc and GlcNac. This permease also appears to be the sole transporter of GlcNac; a V. furnissii mutant defective in this protein is unable to take up GlcNac or to show taxis to this compound, although the cells behave normally on the disaccharide (GlcNac)\textsubscript{2} (4).

The plasmid pSB16H contains the V. furnissii homolog of nag\textsuperscript{E}, the gene that encodes the GlcNac permease, II\textsuperscript{Nag}. However, V. furnissii nag\textsuperscript{E} is unique compared to all such genes that have been reported (6, 7). (a) The size of the in vitro transcription/translation product from pSB16H (M\textsubscript{r} = 49,500) compares favorably with the deduced amino acid sequence (52,594 Da) and is substantially smaller than the 69 kDa of E. coli II\textsuperscript{Nag} (2). (b) The deduced amino acid sequence of the V. furnissii gene shows 47% identity (67% similarity) to the N-terminal domain of E. coli II\textsuperscript{Nag} (Fig. 7). The C-terminal II\textsuperscript{Glc}-like domain of E. coli II\textsuperscript{Nag}, however, is absent from the V. furnissii permease, and no cry\textsuperscript{Nag}like sequence was found in the pSB16H nucleotide sequence. (c) Unlike the other known proteins encoded by the nag\textsuperscript{E} gene from other species, all of which interact directly with phospho-HPr, the V. furnissii GlcNac permease requires a III\textsuperscript{Nag} equivalent both in vivo (Table I) and in vitro (Table II).

We do not yet know whether V. furnissii nag\textsuperscript{E} functions in the cell with a specific (previously undescribed) III\textsuperscript{Nag}, or whether it functions with III\textsuperscript{Glc} in V. furnissii as it does in the E. coli transformants and in vitro. If so, the gene that encodes a specific III\textsuperscript{Nag}, it was not found in pSB16 and therefore is not linked to nag\textsuperscript{E} on the V. furnissii chromosome.

The E. coli nag operon is inducible by growth on GlcNac and is stimulated by cAMP-CAP (15, 45, 55), and the V. furnissii nag operon may be similarly regulated. E. coli LR2-175 transformants carrying pSB16H, grown on GlcNac, gave 3 times greater Enzyme II\textsuperscript{Nag} activity than Glc-grown cells (Table II). The effect of cAMP-CAP on expression of the cloned genes was not tested.) Qualitatively similar results were obtained in sugar phosphorylation experiments with membranes (Table II) and tolue-nermethylated cells of wild-type V. furnissii (44). Because of the observed inducibility of GlcNac phosphorylation, we believe that the V. furnissii nag promoter is present on pSB16 and pSB16H.

It is noteworthy (Table I) that the plasmid pSB16 complemented E. coli nag\textsuperscript{A} mutants, defective in GlcNac-6-P deacetylase, but not a nagB strain, defective in GlcNH\textsubscript{2}-6-P deaminase. This result indicates genetic linkage of V. furnissii nag\textsuperscript{E} and nag\textsuperscript{A}, analogous to the gene order of the E. coli nag operon (13); it seems likely that the remaining V. furnissii nag genes are in close proximity to the ones that we have cloned. Preliminary sequencing results show that pSB16 carries a nagC-like gene.

Certain functional domains have been defined in the Enzymes II, assignments that are based primarily upon sequence alignments and a few biochemical studies (6, 7). These domains seem to be conserved among the sugar permeases described here. For example, of the six residues of E. coli II\textsuperscript{Glc} recently reported to be important in sugar translocation but not phosphorylation (40), four are conserved in the V. furnissii Man\textsubscript{V} Glc permease and five are conserved in V. furnissii II\textsuperscript{Nag}. The region surrounding the residue His-211 of E. coli II\textsuperscript{Glc}, a puta-

Acknowledgments—We are especially grateful to Dr. Winifred Boos for help in identifying the malX and malY genes. Dr. J. Lengeler, Dr. B. Waygood, Dr. J. Plumbridge, and C. Y. Wong generously provided bacterial strains, plasmids and phage. Dr. N. Meadow and P. Coyle provided homogeneous Enzyme I, HPr (E. coli, and III\textsuperscript{Glc} (S. typhimurium). Expert technical assistance was given by Glenn Weiss and John Statler. We thank Dr. J. Stoll for critical review of the manuscript.

REFERENCES
1. Yu, C., Lee, A. M., Basler, B. L., and Roseman, S. (1991) J. Biol. Chem. 266, 24260–24267
2. Bassler, B. L., Gibbons, P. J., Yu, C., and Roseman, S. (1991) J. Biol. Chem. 266, 24268–24275
3. Bassler, B. L., Yu, C., Lee, Y. C., and Roseman, S. (1991) J. Biol. Chem. 266, 24276–24286
4. Yu, C., Bassler, B. L., and Roseman, S. (1993) J. Biol. Chem. 268, 9405–9409
5. Keyhani, N., Wang, L. X., Lee, Y. C., and Roseman, S. (1990) J. Biol. Chem. 267, 33429–33433
6. Meadow, N. D., Fox, D., and Roseman, S. (1990) Annu. Rev. Biochem. 59, 529–542
7. Postma, P. W., Lengeler, J. W., and Jacobson, G. R. (1993) Microbiol. Rev. 57, 543–594
8. Bouma, C. L., and Roseman, S. (1996) J. Biol. Chem. 271, 33488–33475
9. Keyhani, N., and Roseman, S. (1996) J. Biol. Chem. 271, 33425–33432
10. Reidl, J., and Bons, W. (1991) J. Bacteriol. 173, 4862–4876
11. Williams, N., Fox, D., Shear, C., and Roseman, S. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8904–8908
12. Sprenger, G. A., and Lengeler, J. W. (1984) J. Bacteriol. 157, 39–45
13. Peri, K. G., Goldie, H., and Waygood, E. B. (1990) Biochem. Cell Biol. 68, 125–137
14. Boyer, H. W., and Roulland-Dussaine, D. (1969) J. Mol. Biol. 41, 459
15. Plumbridge, J. A. (1991) Mol. Microbiol. 5, 2053–2062
16. Manaatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Cordaro, J. C., and Roseman, S. (1972) J. Bacteriol. 112, 17–29
18. Silhavy, T. J., Berman, M. L., and Enquist, L. W. (1978) Experiments with Gene Fusions, pp. 137–139, Cold Spring Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Patil-Shankar, D., Michalak, M., Daisley, S. L., and Glick, R. (1983) Mol. Biol. Rep. 9, 191–195
20. Bouma, C. L., Meadow, N. D., Stever, E. W., and Roseman, S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 930–934
21. Sanger, F., Nicola, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
22. Anderson, S. (1981) Nucleic Acids Res. 9, 3015–3027
23. Meadow, N. D., and Roseman, S. (1982) J. Biol. Chem. 257, 14526–14537
24. Waygood, E. B., and Meadow, N. D. (1982) Methods Enzymol. 90, 423–431
25. Robillard, G. T., Doiijewaard, G., and Lolkema, J. (1979) Biochemistry 18, 2954–2960
26. Beneski, D. A., Nakazawa, A., Weigel, N., Hartman, P. E., and Roseman, S. (1982) J. Biol. Chem. 257, 14492–14498
27. Markwell, M. A., Haas, S. M., Bieber, L. L., and Tolbert, N. E. (1978) Anal. Biochem. 87, 206–210
28. Curtis, S. J., and Epstein, W. (1975) J. Bacteriol. 122, 1189–1199
29. Henderson, P., Giddens, R., and Jones-Mortimer, M. (1977) Biochem. J. 162, 309–320
