





Figure 2. Vertical view of a putative  $\alpha$ -helix of the carboxyl-terminal region of the melibiose transporter of *S. typhimurium*. A region from Gly-437 to Thr-454 is shown. The region from Gly-437 to Arg-452 is predicted to form an  $\alpha$ -helix structure. Amino acid residues which are involved in the interaction with  $\text{IIA}^{\text{Glc}}$  are circled.

### Direct Evidence for the Interaction Between Sugar Transporter and $\text{IIA}^{\text{Glc}}$ Component of Glucose-PTS

The role of  $\text{IIA}^{\text{Glc}}$  in the inducer exclusion is now well established based on various experimental observations. In the study of the lactose transport system, considerable biochemical evidence for the interaction between the transport protein and  $\text{IIA}^{\text{Glc}}$  have been obtained. The first evidence for the involvement of  $\text{IIA}^{\text{Glc}}$  in the regulation of the lactose transport system has been reported by Dills *et al.* (1982). They demonstrated that the partially purified preparation of  $\text{IIA}^{\text{Glc}}$  loaded into *E. coli* membrane vesicles possessing high activity of lactose transport inhibited the lactose uptake. In addition, in the presence of enzyme I and HPr, intravesicular PEP effaced the inhibition. The results have been later confirmed by Misko *et al.* (1987). It has been reported that in the presence of methyl- $\alpha$ -glucoside, a non-metabolizable analog of glucose, the activity of lactose transport decreased in membrane vesicles. When the vesicles were prepared from *ptsI*<sup>-</sup>, *ptsH*<sup>-</sup>, or *crr*-negative strain, methyl- $\alpha$ -glucoside did not inhibit the lactose transport. In this study, it has been also shown that melibiose and galactose transport activities reduced in the presence of intravesicular  $\text{IIA}^{\text{Glc}}$ .

Further evidence for the involvement of  $\text{IIA}^{\text{Glc}}$  in the transport regulation of non-PTS sugar has been obtained in the lactose transport system by Osumi and Saier (1982; 1982). They used *lacY* gene cloned into a multicopy plasmid, overproduced the lactose transporter and studied the binding of purified  $\text{IIA}^{\text{Glc}}$  to the membrane fragment from this strain. They demonstrated that  $\text{IIA}^{\text{Glc}}$  directly bound to the membrane containing the overproduced lactose transporter, and the binding activity was enhanced

by the presence of substrate of the lactose transporter. The results of Osumi and Saier have been confirmed and further quantified by Nelson *et al.* (1983; 1984). Since phosphorylated  $\text{IIA}^{\text{Glc}}$  did not bind to the lactose transporter, it was concluded that only non-phosphorylated  $\text{IIA}^{\text{Glc}}$  could bind to the transporter and inhibit the activity. It has been estimated that there were 1 to 1.5 molecules of  $\text{IIA}^{\text{Glc}}$  bound per a molecule of the lactose transporter.

Among four non-PTS sugar transport systems, direct binding of  $\text{IIA}^{\text{Glc}}$  to the transporter has been shown except in the melibiose system. However, since it has been shown that inclusion of  $\text{IIA}^{\text{Glc}}$  in membrane vesicles inhibited the melibiose transporter, interaction between melibiose transport protein and  $\text{IIA}^{\text{Glc}}$  is expected to be similar to that in the other three systems.

### Identification of Mutations Which Tolerate the Regulation by $\text{IIA}^{\text{Glc}}$

Although inducer exclusion is observed in wild-type *ptsI*<sup>+</sup>, *ptsH*<sup>+</sup> strains, the inhibition is much stronger in *ptsI*-leaky strain. Using such inducer exclusion-hypersensitive strain, Saier and coworkers isolated mutants of *E. coli* and *S. typhimurium* in which individual non-PTS sugar transport system was no longer sensitive to inducer exclusion (Saier *et al.*, 1978). These mutations were mapped in or closely linked to the gene(s) responsible for the metabolism of each non-PTS sugar. This report touched off the extensive studies on the domains or amino acid residues of the transporters that interact with  $\text{IIA}^{\text{Glc}}$ .

With respect to the lactose transport system, Nelson *et al.* (1983) first reported that a mutant lactose carrier with triple amino acid substitutions in N-terminal region exhibited no binding to  $\text{IIA}^{\text{Glc}}$ . They concluded that the N-terminal cytoplasmic portion of the LacY was probably a binding site of  $\text{IIA}^{\text{Glc}}$ . Wilson *et al.* reported the amino acid substitutions in lactose transport mutants of *E. coli* resistant to inducer exclusion (Wilson *et al.*, 1990). It was demonstrated that Ala-198 and Ser-209 were replaced in these mutants. The results suggested that cytoplasmic central loop of the lactose carrier protein was also a binding site of  $\text{IIA}^{\text{Glc}}$ . Hoischen *et al.* (1996) later confirmed the results of Wilson *et al.* by using direct binding assay. Hoischen *et al.* reported that Pro-192 which is located in cytoplasmic central loop of the protein was also important for the regulation by the PTS. In contrast to the results of Nelson *et al.* some deletion mutations in N-terminal region had little effect on the binding. It might be due to the difference between point mutation and deletion.

Seok *et al.* (1997) examined the binding activity of LacY mutants in which 6 contiguous His residues were inserted into each loop or N-terminus, or C-terminal deletion to  $\text{IIA}^{\text{Glc}}$ . Insertion mutations of cytoplasmic loops IV/V and VI/VII (which is called cytoplasmic central loop as indicated above) resulted in retention of sugar transport (i.e. binding) activity, but loss of  $\text{IIA}^{\text{Glc}}$  binding. Interestingly, a mutant with insertion in periplasmic loop VII/VIII also exhibited similar properties. Since  $\text{IIA}^{\text{Glc}}$  is a cytoplasmic soluble protein and binds to the LacY from cytoplasmic side, involvement of this periplasmic loop in  $\text{IIA}^{\text{Glc}}$  binding remains to be examined. Sondej *et al.* (1999) further examined the cytoplasmic loops IV/V and VI/VII by Cys-

scanning mutagenesis. It has been shown that replacement of either Val-132, Arg-135, or Arg-142 in or adjacent to loop IV/V by Cys resulted in decreased binding with no effect on the transport activity. In loop VI/VII, mutation of either Thr-196, Val-197, Asn-199, Gly-202, Asn-204 or Ser-206 had similar effects.

With respect to the melibiose transport system, we isolated inducer exclusion resistant mutants in MelB of *S. typhimurium* and identified the following substitutions of amino acid residues: Asp-438 with Tyr, Arg-441 with Ser, and Ile-445 with Asn (Kuroda *et al.*, 1992). Recently, we have identified different amino acid substitution (Arg-441 with Gly) in another mutant (unpublished result). The replacement of Ile-445 by Asn was identified in more than half of independently isolated mutants, suggesting that Ile-445 was the most important residue for interaction with the IIA<sup>Glc</sup>. Amino acid residues substituted in the mutants were located in carboxyl-terminal cytoplasmic region and the region containing these residues was predicted to form an  $\alpha$ -helix structure. According to the helical wheel analysis, these residues were on the same side of the  $\alpha$ -helix (Figure 2) To further evaluate the role of the C-terminal region of the melibiose transporter, site-directed mutants were constructed and characterized. Mutants of Asp-449 and Arg-452, which are on the same side of the  $\alpha$ -helix, exhibited the transport activity fairly resistant to inducer exclusion (unpublished result). Surprisingly, any amino acid replacements of Ile-445 (including replacements by Val or Leu residues) tested resulted in resistance to the PTS regulation. Since prediction of the secondary structure by the method of Chou and Fasman revealed that these substitutions did not significantly affect the secondary structure of the C-terminal region, this result suggests that side chain of Ile-445 is critical for the binding of IIA<sup>Glc</sup> (unpublished result).

We introduced a series of C-terminal truncations in the *E. coli* MelB (each mutants lacked 16, 19, 20, 21, 22, 23, or 24 amino acid residues from the C-terminus) and examined regulation by the PTS. The inhibition was 65, 65, 63, 46, and 19% in the MelB mutants lacking 16, 19, 20, 21, 22 amino acid residues, respectively, whereas inhibition in wild type was 84 %. Mutants lacking 23 residues and lacking 24 residues exhibited very little transport activity. These results suggest that the C-terminal region of MelB is essential not only for the regulation of activity by the IIA<sup>Glc</sup> but also for the transport activity itself (unpublished result).

#### Deduction of Interaction Sites in Target Protein of IIA<sup>Glc</sup>

In the maltose transport system, analysis of inducer exclusion resistant mutants has identified amino acid residues in MalK, which were involved in IIA<sup>Glc</sup> interaction (Dean *et al.*, 1990; Kühnau *et al.*, 1991). Additionally, analysis of three-dimensional structure of *E. coli* glycerol kinase-IIA<sup>Glc</sup> complex revealed amino acid residues at the contact sites (Hurley *et al.*, 1993).

Based on studies summarized above, some reports postulated the consensus sequences of proteins that interact with IIA<sup>Glc</sup> (Dean *et al.*, 1990; Titgemeyer *et al.*, 1994; Sondej *et al.*, 1999). Especially, REGION I and II proposed by Sondej *et al.* are also present in HPr, which

contacts with IIA<sup>Glc</sup> in phospho-transfer reaction of the PTS. Among four proteins (LacY, MelB, MalK, and GlpK) that interact with IIA<sup>Glc</sup>, MelB is considered to be a little different one, since some of the conserved residues in REGION II are absent. In fact, our recent experiments demonstrated that deletion of Arg-215 to Leu-220, which corresponds to the latter half of the REGION II, did not affect the PTS regulation and transport activity in the melibiose carrier of *S. typhimurium*. In agreement with this result, REGION II is not conserved well in four MelBs of enteric bacteria, sequences of which have been reported so far. Furthermore, *E. coli* PTS can regulate MelBs from *S. typhimurium* and *K. pneumoniae* (unpublished result). Therefore, we proposed that C-terminal region of the MelB protein is at least one of the interaction sites with IIA<sup>Glc</sup>, instead of the REGION II.

#### Discussion

Although several reports revealed amino acid residues that were involved in IIA<sup>Glc</sup> binding, consensus sequences with complete match seems not to be completely deduced so far. As shown in these studies, several portions of target proteins are considered to be required to form regulatory complex with IIA<sup>Glc</sup>. To solve the contact sites, three-dimensional structure of target protein-IIA<sup>Glc</sup> complex remains to be studied. Through these studies, it has been demonstrated that IIA<sup>Glc</sup> is one of the most important proteins that regulates various events occurring in Enterobacteriae.

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