Role of the Conserved Quartets of Residues Located in the N- and C-Terminal Halves of the Transposon Tn10-Encoded Metal–Tetracycline/H^+ Antiporter of *Escherichia coli*†

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ABSTRACT: In the putative secondary structure of the transposon Tn10-encoded metal–tetracycline/H^+ antiporter [Yamaguchi, et al. (1992) *J. Biol. Chem. 267*, 7490–7498], Tyr50-XXX-Gln54 in transmembrane helix 2 and Gly80-XXX-Asp84 in helix 3 are thought to face each other in the N-terminal region. At the corresponding positions in the C-terminal region, a similar quartet of residues, His257-XXX-Gln261 in helix 8 and Gly281-XXX-Asp285 in helix 9, is also located. The quartets involve the residues Asp84 and Asp285, which have been revealed to be essential for the tetracycline transport function. When Gln54 and Gln261 were replaced with Ala by site-directed mutagenesis, the active tetracycline transport activity decreased to about 10% and 40% of the wild-type level, respectively. The K_m values of the Q54A and Q261A mutants for tetracycline were 140 and 160 μM, respectively, which are about 8-fold higher than that of the wild type. Thus, the two Gln residues may contribute to the substrate recognition. On the other hand, the replacement of Gly80 and Gly281 with Leu caused a complete loss of the transport activity, whereas the G80A and G281A mutants retained about 10% and 30% of the activity of the wild-type, respectively, suggesting that a bulky side chain at positions 80 and 281 causes steric hindrance of the transport. The mutation of Tyr50 to Ala or His caused a decrease in activity by a factor of about 3 without a significant change in the K_m value, whereas the Y50C mutant showed no transport activity at all. The H257Y and H257A mutants showed 30% and 8% of the wild-type activity, respectively. Surprisingly, the double mutants as to Tyr50 and His257 showed intermediate activities between those of the respective two single mutants or similar activity to one of the single mutants, suggesting that the two residues may act as a functional pair.

The transposon Tn10-encoded metal–tetracycline/H^+ antiporter (Yamaguchi et al., 1990c) is one of the tetracycline efflux proteins (TetA)[1] which confer bacterial resistance to tetracycline (McMurry et al., 1980). It mediates the 1:1 antiport (Yamaguchi et al., 1990c, 1991b) of a chelation complex of tetracycline and a divalent cation with a proton. The DNA sequence was determined by Nguyen et al. (1983) and Hillen and Schollmeier (1983). The amino acid sequence deduced from the DNA sequence comprises 401 amino acid residues. The sequence of Tn10-TetA showed about 48% identity to those of TetA proteins encoded by plasmid pBR322 and transposon Tn1721 (Waters et al., 1983). On the basis of the hydropathy profile, and the results of protease digestion (Eckert & Beck, 1989) and antibody binding (Yamaguchi et al., 1990a), a secondary structure model of Tn10-TetA including 12 membrane-spanning α-helices connected by 11 hydrophilic interhelix loops was proposed (Eckert & Beck, 1989; Yamaguchi et al., 1992a). The NH_2 and COOH termini both face the cytoplasmic side (Eckert & Beck, 1989; Yamaguchi et al., 1990b). The similar secondary structure model was also confirmed in the pBR322-TetA protein on the basis of the experiments using the *tet*A-*pho*A fusion genes (Allard & Bertrand, 1992). Such a putative structure is common not only in bacterial drug efflux proteins like NorA (Yoshida et al., 1990) and Bmr (Neyfakh et al., 1991) but also in many secondary transporters (Henderson, 1990) including *lac* permease (Kaback, 1987).

In addition, the secondary structure of Tn10-TetA comprises two structurally symmetrical halves containing six transmembrane segments, which exhibit functional intracistronic complementarity (Curiale et al., 1984). Rubin et al. (1990) proposed the hypothesis of gene duplication in the evolution of the bacterial tetracycline efflux proteins. We pointed out the duplication of conserved sequence motifs, GXXXX(R/K)XG(R/E)(R/K), located in hydrophilic $loop_{2-3}$ in the N-terminal half and $loop_{8-9}$ in the C-terminal half, which showed significant resemblance in the structural roles in Tn10-TetA (Yamaguchi et al., 1993). Further significant symmetry of the functional residues is observed in the transmembrane region (Yamaguchi et al., 1992a). Essential Asp residues, Asp84 and Asp285, are located at corresponding positions in the N- and C-terminal halves, respectively. In the vicinity of these Asp residues, conserved residues are symmetrically arranged to form quartets of residues (Yamaguchi et al., 1992a). That is, Gly80-XXX-Asp84 in the N-terminal region corresponds to Gly281-XXX-Asp285 in the C-terminal region, and Tyr50-XXX-Gln54 corresponds to His257-XXX-Gln261. The pairs Gly80-Asp84 and Tyr50-Gln54 possibly spatially face each other in the secondary structure model (Figure 1), and other pairs, Gly281-Asp285 and His257-Gln261, may be arranged in a similar manner. These conserved quartets of residues are located in helices 2 and 3 and helices 8 and 9. Interhelix loops 2–3 and 8–9 had been revealed to be important for the transport function (Yamaguchi et al., 1992c). Therefore, there is a possibility that these quartets of residues may form a substrate translocation channel and/or a substrate

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[1] Abbreviations: TetA, tetracycline-resistant protein; MOPS, 3-(*N*-morpholino)propanesulfonic acid; SDS, sodium dodecyl sulfate.

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Table I: Mutagenic Primers Used for Site-Directed Mutagenesis and Codons Changed in the Mutant Plasmids[a]

plasmid	primer sequence	substituting residue: position	codon	amino acid
pQ54A	5'-CTTTACGCGTTAATGGCGGTTATC-3' (*MluI*)	54	CAG → GCG	Gln → Ala
pQ261A	5'-TCTAGGCCTTTTACACTCAGTATTCGCAGC-3' (*StuI*)	261	CAA → GCA	Gln → Ala
pG80L	5'-TCATTAATACTCGCGAGCCTGGAT-3' (*NruI*)	80	GGC → CTC	Gly → Leu
pG281L	5'-CTGCTCCTCTTTATTGCAGACTCGAGTGCA-3' (*XhoI*)	281	GGA → CTC	Gly → Leu
pG80A	5'-TCATTAATAGCCGCTAGCCTGGAT-3' (*NheI*)	80	GGC → GCC	Gly → Ala
pG281A	5'-CTGCTAGCCTTTATTGCAGACTCGAGTG-3' (*NheI*, *XhoI*)	281	GGA → GCC	Gly → Ala
pY50A	5'-TATTGCTAGCGCTTGCTGCGTTAA-3' (*Eco*47III, *NheI*)	50	TAT → GCT	Tyr → Ala
pY50H	5'-TATTGCTAGCGCTTCACGCGTTAA-3' (*Eco*47III, *MluI*, *NheI*)	50	TAT → CAC	Tyr → His
pY50C	5'-TATTGCTAGCGCTTTGTGCGTTAA-3' (*Eco*47III, *NheI*)	50	TAT → TGT	Tyr → Cys
pH257Y	5'-TTAAGCGGGTCTAGGCCTTTTATATTCAGTA-3' (*StuI*)	257	CAC → TAT	His → Tyr
pH257A	5'-GGGTCTAGGCCTTTTAGCCTCAGT-3' (*StuI*)	257	CAC → GCC	His → Ala

[a] Asterisks indicate the mismatches. Underlines indicate the new restriction sites.

recognition site. In the current study, site-directed mutagenesis of the quartet residues was performed.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids. Escherichia coli MV1184 (Vieira & Messing, 1987), TG1 (Taylor et al., 1985) and W3104 (Yamamoto et al., 1981) were used for single-stranded DNA preparation, transformation, and the preparation of inverted membrane vesicles, respectively. The N-terminal *Eco*RV–*Eco*RI fragment (485 bp) and C-terminal *Eco*RI–*Bam*HI fragment (868 bp) of the *tet*A gene of transposon Tn10 were separately subcloned into pER and pTB2 using pUC118 as a vector (Yamaguchi et al., 1992a) in order to perform site-directed mutagenesis. The 2.45-kb *Bgl*II–*Bam*HI fragment containing the *tet*A and *tet*R genes of transposon Tn10 was cloned into a low copy-number plasmid, pLGT2, using pLG339 (Stoker et al., 1982) as a vector for the expression of the TetA protein.

Site-Directed Mutagenesis. Mutagenesis was performed by the method of Taylor et al. (1985) with an oligonucleotide-directed in vitro mutagenesis system (Version 2; Amersham), using pER or pTB2 as a template. The mutagenic primers used are listed in Table I and were synthesized with a Cyclone Plus DNA/RNA synthesizer (MilliGen Biosearch Co.). These primers contain silent DNA mismatches to create a new restriction site in addition to mismatches to cause a codon change. Mutations were at first detected as the appearance of a new restriction site and then confirmed by DNA sequencing. The *Eco*RV–*Eco*RI fragment of pER or the *Eco*RI–*Bam*HI fragment of pTB2 containing these mutations was then transferred to pLGT2 by means of fragment exchange. The reconstitution of the mutant *tet*A gene was verified by restriction analysis.

Preparation of Inverted Membrane Vesicles. E. coli W3104 cells harboring pLGT2 or mutant plasmids were grown on the minimal medium supplemented with 0.2% glucose and 0.1% casamino acids. At the middle of the logarithmic phase, *tet*A gene expression was induced for 2 h by incubation with 0.25 μg/mL heat-inactivated chlorotetracycline. Inverted vesicles were prepared by cell disruption with a French press in 50 mM MOPS–KOH buffer (pH 6.6) containing 0.1 M KCl and 10 mM EDTA, as described previously (Yamaguchi et al., 1990c). The vesicles were washed with 50 mM MOPS–KOH (pH 7.0) containing 0.1 M KCl and then stored at −80 °C.

Immunoblot Analysis. Immunoblot analysis of inverted membrane vesicles was performed as described previously (Yamaguchi et al., 1992a), using a site-directed antiserum specific to the C-terminal 14 amino acid sequence (Yamaguchi et al., 1990a).

Transport Assays. [^{3}H]Tetracycline (Du Pont-New England Nuclear) uptake by inverted membrane vesicles, driven by the addition of 2.5 mM NADH, was assayed as described previously (Yamaguchi et al., 1990c) in the presence of 10 μM [^{3}H]tetracycline and 50 μM $CoCl_2$ in 50 mM MOPS–KOH buffer (pH 7.0) and 0.1 M KCl.

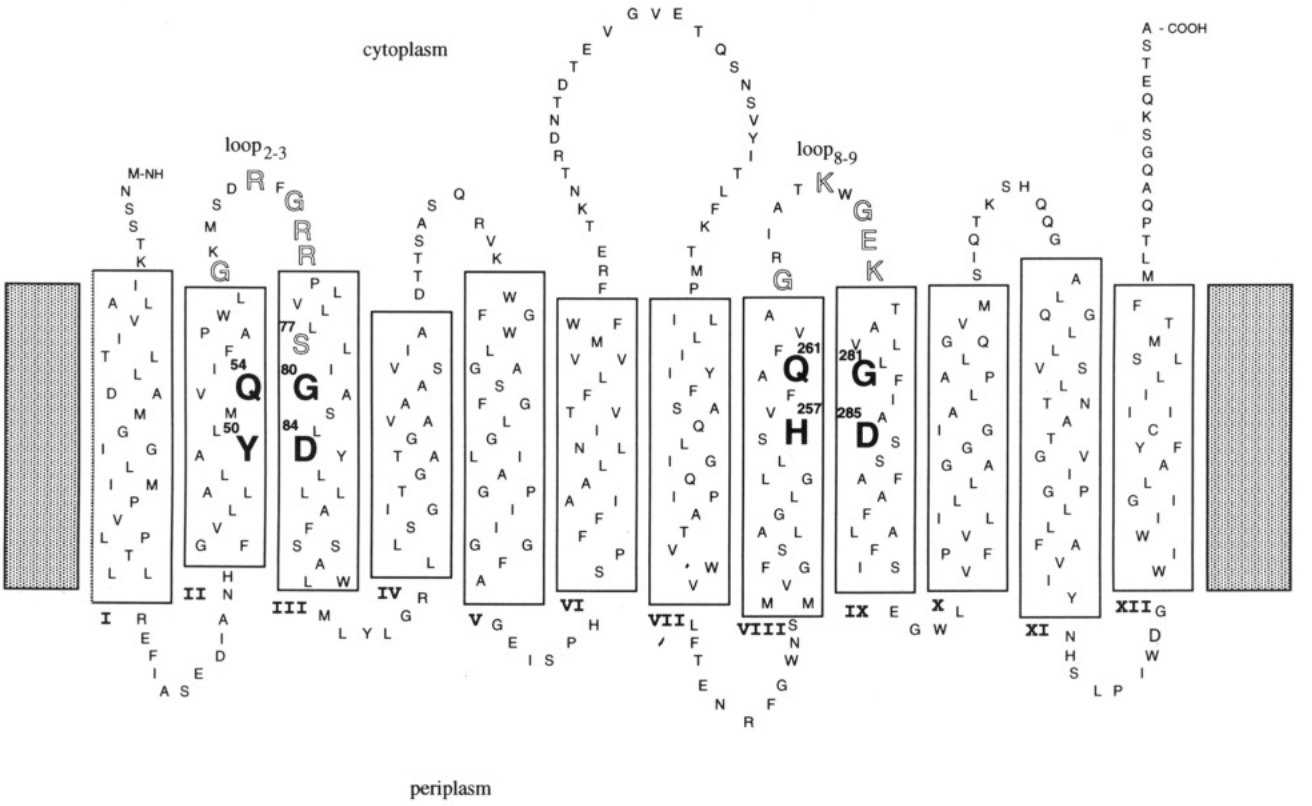

FIGURE 1: Putative secondary structure of the transposon Tn10-encoded metal–tetracycline/H^+ antiporter (Yamaguchi et al., 1992a). Hydrophobic α-helical segments are enclosed by boxes. The bold letters indicate the conserved quartets of residues studied in this work. The outlined letters indicate the conserved sequence motifs in $loop_{2-3}$ and $loop_{8-9}$ and the important serine-77 in helix 3 (Yamaguchi et al., 1992b).

RESULTS

Effect of the Replacement of Gln54 and Gln261 with Ala. Gln54 and Gln261 are located in the third putative helices in the N- and C-terminal regions, respectively, and are conserved in the class A, B, and C TetA proteins of Gram-negative bacteria (Waters et al., 1983). Gln261 is four residues apart from the important residue, His257 (Yamaguchi et al., 1991a), the distance corresponding to one turn of the α-helix, and thus the two residues are adjacent to each other in the putative helix 8. A similar relationship exists between the corresponding conserved residues, Gln54 and Tyr50, in the putative helix 2 (Figure 1). Gln54 and Gln261 were replaced with Ala by site-directed mutagenesis. Immunoblot analysis of the inverted membrane vesicles prepared from cells expressing these mutant *tet* genes showed no significant alteration in the amounts of TetA proteins (Figure 2), indicating that the mutations affected neither the expression of the *tet* genes nor the insertion and stability of TetA proteins in the membrane.

As shown in Figure 3, the tetracycline transport activity decreased substantially on the replacement of Gln54 or Gln261 with Ala, but a significant amount remained. The initial rates of [^{3}H]tetracycline uptake by Q54A (Gln54 → Ala) and Q261A (Gln261 → Ala) vesicles were 10% and 40% of the wild type level, respectively, in the presence of 10 μM tetracycline and 50 μM $CoCl_2$. This observation indicates that neither Gln54 nor Gln261 is essential for the tetracycline transport function but that the two residues contribute to the efficiency of the transport. The K_m values for the tetracycline transport were measured in the presence of 1 mM $CoCl_2$ (Table II). The value for the vesicles containing wild-type TetA was 18 μM, which was similar to the value reported previously (Yamaguchi et al., 1990b). On the other hand, the K_m values of Q54A and Q261A mutant vesicles were 142 and 156 μM, respectively, which are 8–9-fold larger than the value of the wild type, indicating that these Gln residues help maintain the high affinity of the TetA protein to tetracycline, although it is not clear whether they directly play a role in the substrate recognition or whether the reduction in the affinity is merely due to a remote conformational effect of the replacement of these residues.

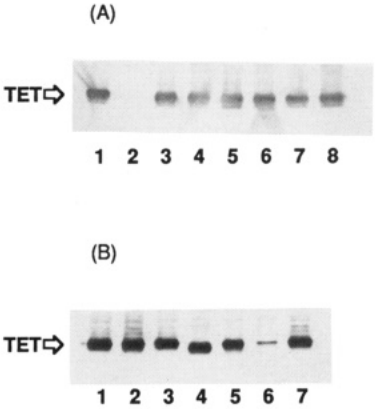

FIGURE 2: Immunoblot analysis of inverted membrane vesicles prepared from *E. coli* W3104 cells harboring the wild-type or a mutant plasmid. Each lane contained about 5 μg of total protein. (A) Lane 1, pLGT2 (wild-type); lane 2, no plasmid; lane 3, Y50H (Tyr50 → His); lane 4, Y50A (Tyr50 → Ala); lane 5, Y50C (Tyr50 → Cys); lane 6, Q54A (Gln54 → Ala); lane 7, G80L (Gly80 → Leu); lane 8, G80A (Gly80 → Ala). (B) Lane 1, H257Y (His257 → Tyr); lane 2, H257A (His257 → Ala); lane 3, Q261A (Gln261 → Ala); lane 4, G281L [Gly281 → Leu (CTC)]; lane 5, G281A (Gly281 → Ala); lane 6, G281L2 [Gly281 → Leu (CTA)]; lane 7, pLGT2 (wild type).

Effect of the Replacement of Gly80 and Gly281. Gly80 and Gly281 are conserved residues occupying corresponding positions in the N- and C-terminal halves, respectively. The two glycines are located at four residues before the essential

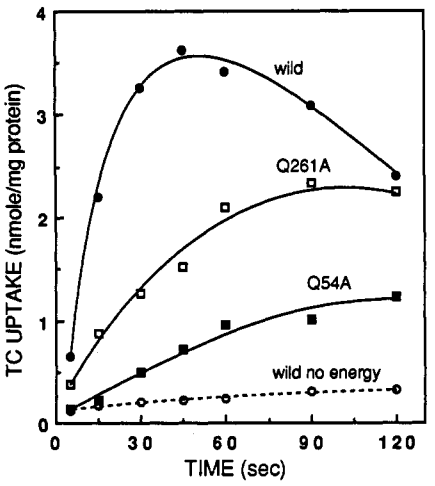

FIGURE 3: Tetracycline (TC) uptake by inverted membrane vesicles prepared from *E. coli* W3104 cells containing the wild-type or a mutant TetA protein with substitution of Gln54 or Gln261. The uptake was measured after the addition of 2.5 mM NADH in the presence of 10 μM [^{3}H]tetracycline, 50 μM $CoCl_2$, 100 mM KCl, and 50 mM MOPS–KOH (pH 7.0). The broken line indicates the uptake by wild-type vesicles in the absence of NADH.

Table II: K_m Values of Inverted Membrane Vesicles Containing Wild Type or Gln54-, Gln261-, Tyr50-, and His257-Substitution Mutant TetA Proteins[a]

Tet protein	K_m (μM)
wild type	18
Q54A (Gln54 → Ala)	142
Q261A (Gln261 → Ala)	156
Y50A (Tyr50 → Ala)	13
Y50H (Tyr50 → His)	22
H257Y (His257 → Tyr)	32

[a] The initial uptake (30 s) of [^{3}H]tetracycline was measured in the presence of 1 mM $CoCl_2$ and various concentrations of [^{3}H]tetracycline. K_m values were calculated from Lineweaver–Burk plots.

Asp residues, Asp84 and Asp285. This distance corresponds to one turn of the α-helix, and thus the Gly and Asp pairs should be vertically adjacent in the same putative membrane-spanning helices (Figure 1). Gly80 is positioned in the middle between Asp84 and another important residue, Ser77 (Yamaguchi et al., 1992b). It can be estimated that Ser77-Gly80-Asp84 form a vertical stripe of functional residues on the wall of the hypothetical substrate translocation channel (Yamaguchi et al., 1992b). The replacement of the codons GGC for Gly80 and GGA for Gly281 with CTC for Leu caused no significant difference in the amount of TetA proteins in inverted vesicles (Figure 2). However, the replacement of the codon GGA for Gly281 with CTA for Leu caused significant decrease in the amount of TetA protein (Figure 2), probably due to the difference in the codon usage. In the following experiments, the G281L mutant having the CTC codon for Leu was used.

As shown in Figure 4, the replacement of Gly80 or Gly281 with Leu caused a complete loss of the tetracycline transport activity. The G281L2 mutant vesicles which had the CTA codon also showed no transport activity (data not shown). There is a possibility that the bulky side chain of Leu caused steric hindrance or a conformational change resulting in inactivation of the TetA protein. Thus, the two glycines were further replaced with Ala. The resultant mutations also did not affect the amounts of the TetA proteins (Figure 2). The G80A (Gly80 → Ala) and G281A (Gly281 → Ala) vesicles showed about 10% and 30% of the initial rate of [^{3}H]tetracycline uptake by the wild-type vesicles (Figure 4). These observations indicate that there is a relationship between the TetA function and the volume of the side chains at positions 80 and 281. It is possible that these glycines may help maintain sufficient room for the substrate binding site.

Effect of the Replacement of Tyr50 and His257. We reported in our previous paper (Yamaguchi et al., 1991a) that His257 is important for the proton translocation function of the TetA protein since the H257E (His257 → Glu) and H257D (His257 → Asp) mutants had lost the tetracycline-coupled proton translocation activity, despite that tetracycline transport activity remained. In the current study, His257 was replaced by Tyr or Ala. The position in the N-terminal region corresponding to His257 in the C-terminal region is occupied by Tyr50, which is also conserved in the class A, B, and C TetA proteins of Gram-negative bacteria (Waters et al., 1983). Tyr50 was replaced by His, Ala, and Cys. The resultant mutants were also normally produced (Figure 2).

When His257 was replaced by Tyr and Ala, about 30% and 8%, respectively, of the wild-type tetracycline transport activity

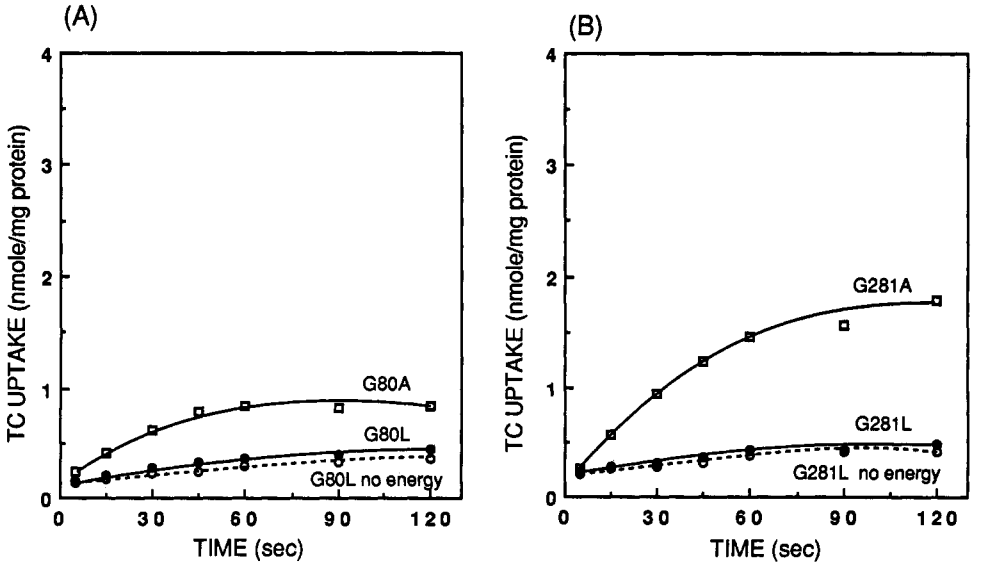

FIGURE 4: Tetracycline uptake by inverted membrane vesicles prepared from *E. coli* W3104 cells harboring Gly80- or Gly281-substitution mutant plasmids. [^{3}H]Tetracycline uptake was measured under the same conditions as in Figure 3. (A) Gly80-substitution mutants; (B) Gly281-substitution mutants. The G281L mutant had a CTC codon for Leu281. No energy indicates the absence of NADH.

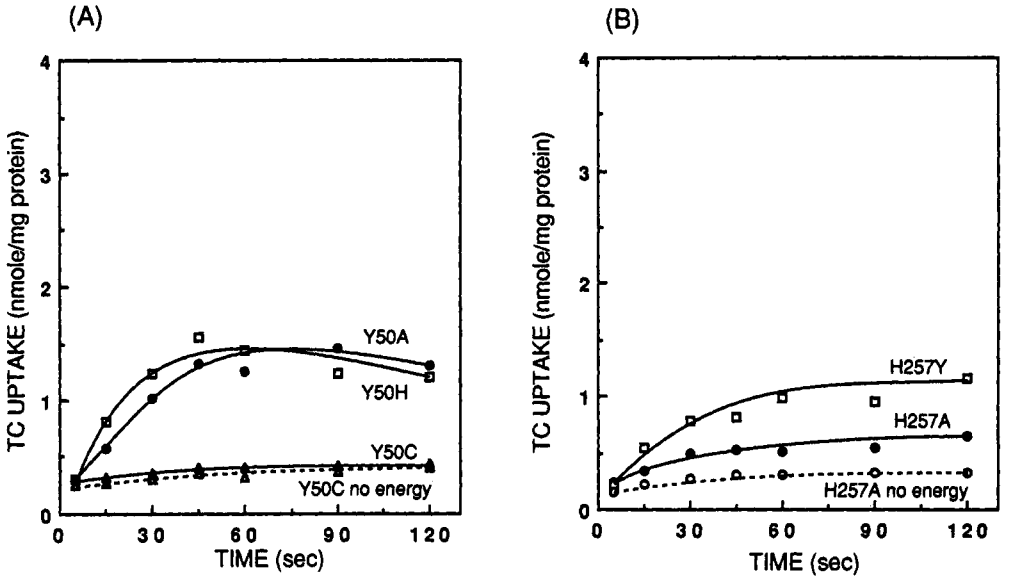

FIGURE 5: Tetracycline uptake by inverted membrane vesicles prepared from *E. coli* W3104 cells harboring Tyr50- or His257-substitution mutant plasmids. The assay conditions were the same as in Figure 3. (A) Tyr50-substitution mutants; (B) His257-substitution mutants.

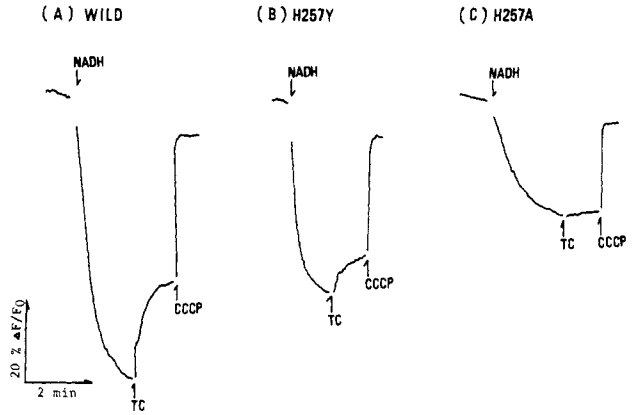

FIGURE 6: Proton translocation across inverted membrane vesicles of the wild-type and H257Y (His257 → Tyr) and H257A (His257 → Ala) mutants. Quinacrine fluorescence was measured as an indicator of proton translocation in the presence of 10 mM $MgSO_4$, 100 mM KCl, and 50 mM MOPS–KOH (pH 7.0), as described in our previous paper (Yamaguchi et al., 1991a). (A) Wild-type; (B) H257Y mutant; (C) H257A mutant. Arrows indicate the times of addition of reagents. The final concentrations of NADH, tetracycline (TC), and CCCP were 0.63 mM, 10 μM, and 25 μM, respectively.

remained (Figure 5B). The tetracycline transport activity of the H257Y mutant was greater than those of the H257E and H257D mutants previously reported (Yamaguchi et al., 1991a). It should be noted that the H257Y mutant showed significant tetracycline-coupled proton translocation activity (Figure 6), whereas the proton transport activity of the H257A mutant was not significant. Thus, it is clear that an imidazole ring at position 257 is essential for neither the proton translocation function nor proton/tetracycline coupling. Since the role of His257 is one of the keys for elucidating the tetracycline/H^+ antiport function, more detailed studies on the characteristics of all 19 different kinds of His257 mutants will be reported in our next paper.

When Tyr50 was replaced by His or Ala, the two mutants showed similar remaining activity, i.e., about 30% of the wild-type level (Figure 5A). In contrast, replacement with Cys caused a complete loss of the activity (Figure 5A). Surprisingly, the K_m value was not significantly altered on replacement of Tyr50 with His or Ala (Table II). Thus, it is not likely that Tyr50 plays a direct role in tetracycline binding. Since the replacement of His257 with Tyr also did not significantly affect the K_m value, there seems to be some functional resemblance between Tyr50 and His257.

In order to examine the functional complementarity between Tyr50 and His257, three double mutants, Y50HH257Y (Tyr50 → His, His257 → Tyr), Y50AH257Y (Tyr50 → Ala, His257 → Tyr), and Y50HH257A (Tyr50 → His, His257 → Ala), were prepared. The double mutations resulted in neither an additive loss nor restoration of the activity. The activities of the double mutants were unexpectedly similar to or intermediate between the respective two single mutants (Figure 7). The Y50HH257Y and Y50AH257Y mutants showed activities similar to one of the respective parent mutants, H257Y and Y50A. On the other hand, the activity of the Y50HH257A mutant was 13%, which was intermediate between those of Y50H (30%) and H257A (8%). This result suggested that Tyr50 and His257 contribute to the transport function as a pair. Since the exchange of Tyr50 and His257 caused no restoration of the activity, a histidine residue at position 50 is not likely to replace the function of His257.

DISCUSSION

There are two symmetrically conserved quartets of residues in the transmembrane region of the putative secondary structure of Tn10-TetA. One is composed of Tyr50, Gln54, Gly80, and Asp84, which are located in the N-terminal half, and the other is composed of His257, Gln261, Gly281, and Asp285, which are located in the C-terminal half (Figure 1). In this study, site-specific mutants of six of these eight residues were prepared, the exceptions being Asp84 and Asp285, which had been revealed to be essential for the transport function in our previous study (Yamaguchi et al., 1992a). As a result, all six residues were found to be important or significantly related to the transport function, although none of them is essential.

The most impressive observation was the striking resemblance of corresponding residues in the two quartets in their behavior on mutation. The replacement of either of the two glycines with Leu had the same result, that is, a complete loss of the activity, whereas the replacement with Ala caused a decrease, but activity remained. The replacement of one of the glutamines caused a moderate decrease in activity and a drastic increase in the K_m value. In addition, the double

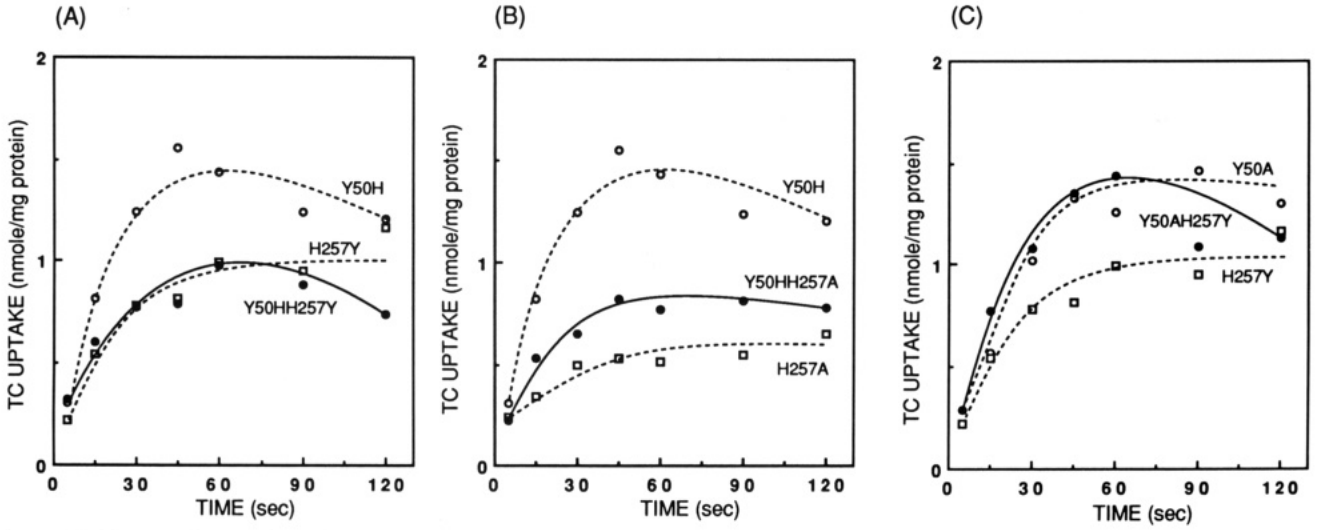

FIGURE 7: Tetracycline uptake by inverted membrane vesicles prepared from *E. coli* W3104 cells harboring Tyr50-His257 double-substitution mutant plasmids. The assay conditions were the same as in Figure 3. (A) Y50HH257Y (Tyr50 → His, His257 → Tyr) mutant; (B) Y50HH257A (Tyr50 → His, His257 → Ala) mutant; (C) Y50AH257Y (Tyr50 → Ala, His257 → Tyr) mutant. The broken lines indicate the uptake by the respective single mutants.

mutants as to Tyr50 and His257 caused no additive decrease in the activity but resulted in intermediate activity between those of the two single mutants, suggesting that the two residues act as a pair in the transport function; that is, for example, the activity of the His50-Ala257 pair in the double mutant might be intermediate between those of the pairs of Tyr50-Ala257 and His50-His257 in the single mutants as a substitute for the Tyr50-His257 pair in the wild-type TetA protein. The two quartets are far apart in the secondary structure; however, if they are spatially apart from each other in the folded structure of TetA, the results described above are difficult to explain. One possible explanation is that the two quartets constitute the two symmetrical halves of one active site, which may be a substrate recognition site or a part of a substrate translocation channel. Of these residues, the pair of Tyr50 and His257 may occupy a unique position, which may not directly interact with tetracycline and act as a functional unit, although the precise function of the unit remains unclear.

It should be noted that the eight residues constituting the two quartets are all located on the hydrophilic sides of amphiphilic helices (Figure 8). If helices 2, 3, 8, and 9 are bundled in such a manner that their hydrophilic sides face each other, with some other helices, and form an aqueous substrate translocation channel (Figure 8), the residues must be located on the wall of the channel. Of course, the existence of such a channel-like structure in the TetA protein will not be established until determination of the 3-D structure of the protein. However, there does appear to be some structural symmetry between the helix 2–$loop_{2-3}$–helix 3 and helix 8–$loop_{8-9}$–helix 9 regions [this work and Yamaguchi et al. (1993)].

One of the controversial subjects is the role of His257. Kaback proposed that His322, which is located in the transmembrane region of *lac* permease, plays a role in proton relay (Kaback, 1987). On the other hand, King and Wilson (1989) claimed that, on the replacement of His322 with Tyr or Phe, the substrate-dependent proton transport activity remained. The striking similarity between His257 in TetA and His322 in *lac* permease in their behavior on mutation is very interesting; that is, on the replacement of His257 with Tyr, tetracycline-dependent proton translocation remained. Establishment of the precise role of His257 requires detailed analysis of many more His257 mutants. We replaced His257 with 19 other amino acids and are currently examining the properties, which will be reported in our next paper.

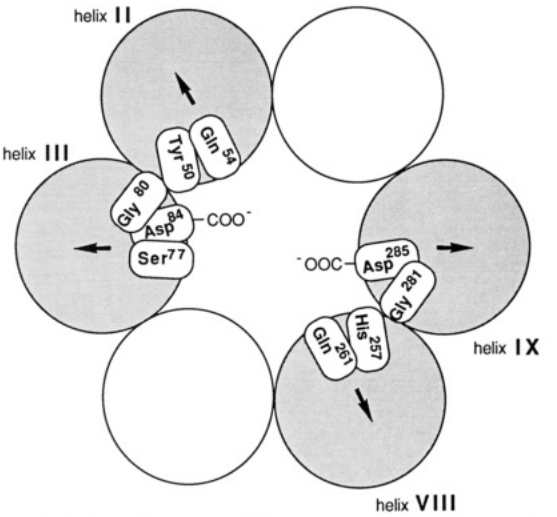

FIGURE 8: A hypothetical model of a tetracycline transport channel of the Tn10-TetA protein. Transmembrane helices are shown as helical-wheel projections, as seen from the cytoplasmic side. Each helix is arranged in the manner in which its hydrophobic moment is outwardly directed. Arrows indicate the directions of the hydrophobic moments. The hydrophobic moments were calculated on the basis of the hydropathy index of Kyte & Doolittle (1982).

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