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TrkH and Its Homolog, TrkG, Determine the Specificity and Kinetics of Cation Transport by the Trk System of *Escherichia coli*

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The corrected sequence of the *trkH* gene of *Escherichia coli* predicts that the TrkH protein is a hydrophobic membrane protein of 483 amino acid residues, of which 41% are identical to those of the homologous and functionally analogous TrkG protein. These two proteins form the transmembrane component of the Trk system for the uptake of K^+. Each protein alone is sufficient for high-level Trk activity. When Trk is assembled with the TrkG protein, Rb^+ and K^+ are transported with a K_m near or below 1 mM; however, the V_{max} for Rb^+ is only about 7% of that for K^+. When Trk is formed with TrkH, the affinities for both for K^+ and Rb^+ are somewhat lower, and the V_{max} for Rb^+ is only 1% of that for K^+ transport. The kinetics of transport in strains with wild-type alleles at *trkG* and at *trkH* suggest that both products participate in transport.

Four proteins (TrkA, TrkE, TrkG, and TrkH), which are the products of widely separated genes, have been implicated as key components of Trk, the major constitutive K^+ transport system of *Escherichia coli* (for a review, see reference 2). Of these four proteins, only TrkA, a 50.4-kDa peripheral membrane protein that binds NAD and is devoid of predicted membrane-spanning regions (19), is absolutely required for Trk activity. Either the TrkG or the TrkH product is necessary for activity; only when both are mutated is Trk activity abolished. The TrkE protein, which is encoded by the *E. coli* homolog of the *sapD* gene of *Salmonella typhimurium* (12, 13), is required when only TrkH is present. When TrkG is present, loss of TrkE alters the kinetics of transport, with a significant reduction in transport rate (3, 7).

The fact that either TrkG or TrkH is required for Trk activity suggests that these two proteins perform similar functions in Trk and should therefore resemble each other. This expectation is confirmed by the DNA sequence of the *trkH* gene and the predicted structure of its product. Here, we report that these two proteins are homologous and that over 40% of their amino acid residues are identical.

We were alerted to the initial report of the sequence of the *trkH* gene by Kenneth Rudd (17), who noticed that an open reading frame of 421 amino acids (11) was similar to TrkG. A subsequent report of DNA sequence data for this region indicated a slightly longer open reading frame of 431 amino acids (5). We found that the location of this open reading frame agreed perfectly with our transductional mapping of *trkH* (7) and that the sequence agreed with those of the restriction sites we had mapped in plasmid pWE211, which carries *trkH* (6, 7). However, it was obvious that the gene must be larger than that predicted from the published sequences, since the homology of TrkH with TrkG would end in the middle of a predicted membrane-spanning region. Further, plasmids that we constructed that ended at amino acid residue 458 did not complement *trkH* mutations, whereas plasmids carrying an additional 180 bp of distal sequence did complement *trkH* mutations. It seemed most likely that the published sequences had missing or extra bases toward the end of the gene, resulting in an erroneous frameshift.

We have resequenced the distal part of the gene as well as other selected regions using the T7 sequencing system (Sequenase; U.S. Biochemicals) with double-stranded DNA. We used dITP in place of dGTP when band compression was a problem. Artifactual bands due to nonspecific termination were eliminated by treatment with terminal transferase (8).

Our sequence data reveal the protein to have 483 residues with homology to *trkG* extending over the full lengths of the two proteins (Fig. 1). In comparison with the most recent sequence (5), we find two differences. When bases in the corrected sequence are counted, such that A in the *trkH* start codon is 1, there are three extra G residues immediately after the G at base 204, and there is an extra C after base 1249 (TTATCGCCACGGG [extra base underlined]). The last base was apparently missed in previous studies, because there is severe band compression in this part of the sequence. We have reported the corrections of the published DNA sequence to GenBank accession number M87049 (5).

Homology is extensive: 41% of the residues of TrkH are identical to those in TrkG, and an additional 18% of the residues represent conservative replacements. TrkH, like TrkG, is predicted to have 10 membrane-spanning regions, which are similarly placed in the two proteins (Fig. 1) and which lead to a very similar predicted structure (20).

An analysis of the base composition and codon preferences of TrkH indicates that *trkH* is typical of genes of *E. coli*, while *trkG* seems to originate from some other species. The G+C content of *trkH* is 51%, matching the 50% typical of the *E. coli* genome, while *trkG* has a G+C content of only 37% (21). The average codon preference of *trkH* is 0.74 and thus is similar to that of other transport proteins in *E. coli* (9); the average preference for TrkG is only 0.41. These data further extend the idea that *trkG* is a foreign gene that probably originated in some other organism and was inserted with the *rac* prophage, within which *trkG* is located, into strains of *E. coli* that have *rac* (21).

We have cloned *trkH* under the control of the T7 promoter (22, 23) and find that the product has the electrophoretic mobility in sodium dodecyl sulfate gels containing urea of a

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H- M---HFRAITRIVGLLVILFSGTMIIPGLVALIYRDGAGRAFTQTFFVALAIGSMLWWPNR -58
G- MNTSHVRVVTHMCGFLVWLYSLSMLPPMVVALFYKEKSLFVFFITFVIFFCIGGGAWYTTK -61
          α-helix 1                        α-helix 2

H- KEKGELKSREGFLIVVLFWTVLGSVGALPFIFSESPNLTITDAFFESFSGLTTTGATTLVG -119
G- KSGIQLRTRDGFIIIVMFWILFSVISAFPLWIDSELNLTFIDALFEGVSGITTTGATVIDD -122
       α-helix 3

H- LDSLPHAILFYRQMLQWFGGMGIIVLAVAILPILGVGGMQLYRAEMPGPLKDNKMRPRIAE -180
G- VSSLPRAYLYYRSQLNFIGGLGVIVLAVAVLPLLGIGGAKLYQSEMPGPFKDDKLTPRLAD -183
                 α-helix 4

H- TAKTLWLIYVLLTVACALALWFAGMDAFDAIGHSFATIAIGGFSTHDASIGYFDSPTINTI -241
G- TSRTLWITYSLLGIACIVCYRLAGMPLFDAICHGISTVSLGGFSTHSESIGYFNNYLVELV -244
       α-helix 5

H- IAIFLLISGCNYGLHFSLLSGRSLKVYWRDPEFRMFIGVQFTLVVICTLVLWFHNVYSSAL -302
G- AGSFSLLSAFNFTLWYIVISRKTIKPLIRDIELRFFLLIALGVIIVTSFQVWHIGMYDLHG -305
     α-helix 6                          α-helix 7

H- MTINQAFFQVVSMATTAGFTTDSIARWPLFLPVLLLCSAFIGGCAGSTGGGLKVIRILLLF -363
G- -SFIHSFFLASSMLTDNGLATQDYASWPTHTIVFLLLSSFFGGCIGSTCGGIKSLRFLILF -365
                                              α-helix 8

H- KQGNRELKRLVHPNAVYSIKLGNRALPERILEAVWGFFSAYALVFIVSMLAIIATGVDDFS -424
G- KQSKHEINQLSHPRALLSVNVGGKIVTDRVMRSVWSFFFLYTLFTVFFILVLNGMGYDFLT -426
                                                α-helix 9

H- AFASVVATLNNLGPGLGVVADNFTSMNPVAKWILIANMLFGRLEVFTLLVLFTPTFWRE   -483
G- SFATVAACINNMGLGFGATASSFGVLNDIAKCLMCIAMILGRLEIYPVIILFSGFFWRS   -485
                                              α-helix 10
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FIG. 1. Homology of the TrkG and TrkH proteins. The predicted amino acid sequence of TrkG is from Schlösser et al. (21), while the TrkH sequence is from Daniels et al. (5) and Nakahigashi and Inokuchi (11), as corrected in this paper (see text). :, identical residues; ·, conservative replacements in which the change is within one of the following groups: A-G, D-E, D-N, E-Q, F-Y, I-L-V, K-R, N-Q, and S-T. Membrane-spanning segments, which are predicted on the basis of hydrophobicity, are underlined.

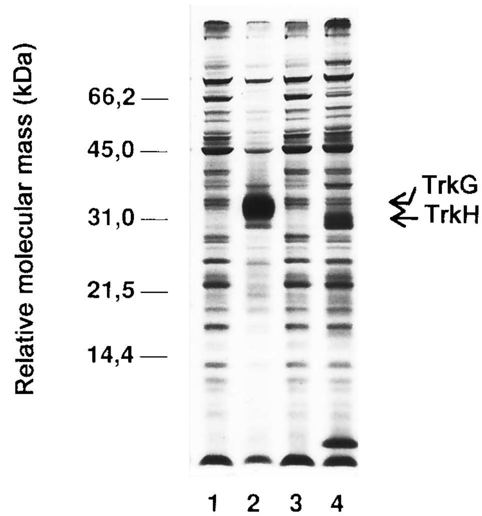

FIG. 2. An autoradiogram showing preferential expression of the TrkG and the TrkH proteins under the control of a T7 promoter. Strain BL21(DE3) (pLysS) was used as described elsewhere (22) with the T7 promoter plasmids described by Tabor and Richardson (24). The plasmids used to express the TrkG and TrkH proteins were shown by restriction enzyme analysis to have the N terminus of the gene adjacent to the T7 promoter of the plasmid. T7 polymerase expression was induced by addition of 1 mM isopropyl-β-D-thiogalactoside to a culture at a density of about 5×10^8 cells per ml. Proteins were labeled for 5 min with 10 μCi of [^{35}S]methionine. Samples were separated by electrophoresis as described by Schägger and von Jagow (18) in a gel containing 10% acrylamide. Lanes: 1, control plasmid pT7-5; 2, plasmid pAS8, which has a 1.8-kb *Dra*I-*Sca*I fragment including the *trkG* gene inserted into the *Sma*I site of pT7-5; 3, control plasmid pT7-7; 4, plasmid pAS10, in which a 1.9-kb *Eco*RI-*Hpa*I fragment including *trkH* was inserted in pT7-6 that had been cut with *Eco*RI and *Sma*I.

31-kDa soluble protein (Fig. 2), which is much more rapid than the mobility expected from its predicted size of 52,945 Da. In similar studies performed with a different T7 expression system (24), in which analysis was performed with gels not containing urea, TrkH had the mobility of a 39-kDa protein (data not shown). Membrane proteins usually have electrophoretic mobilities greater than those expected from their sizes (4).

We constructed isogenic strains in which one or both of the genes were deleted. Transport data for these strains are shown in Table 1. The results for K^+ are similar to those reported earlier for point mutations in these genes (3, 7). When only TrkG is present, uptake occurs with a relatively low K_m as well as a low V_{max}, while both parameters are significantly higher for the system with only TrkH present. The K_m of the wild type is intermediate between those of the strains lacking one of the two proteins, suggesting that when both TrkG and TrkH are present, both are active. As in the earlier studies with point mutations, we did not find the V_{max} values to be additive.

Rb^+ uptake is at a much lower rate but with somewhat better affinity than that for K^+ (Table 1). Most of the Rb^+ uptake in the wild type appears to be via TrkG, since loss of TrkG resulted in a much larger drop in rate than did loss of TrkH. The difference in the ways in which the two proteins handle Rb^+ is seen dramatically when the V_{max} of Rb^+ uptake is expressed as a percentage of that for K^+. The result is 4 to 8% for the wild type and for the strain in which TrkG alone is present, but only about 1% for the strain in which only TrkH is present.

In strains lacking all saturable K^+ uptake systems, the rate of uptake is a linear function of external cation concentration and exhibits little discrimination between K^+ and Rb^+ (15, 16). We confirmed this result for strain TK2450, finding that uptake of Rb^+ was linearly dependent on its concentration; the rate was 2 μmol g^{-1} min^{-1} at an Rb^+ concentration of 30 mM.

The presence of two genes that encode products with virtually identical functions is not common in bacteria. Except for multiple copies of rRNA genes in cases in which copy number seems to be used to attain a sufficient synthetic rate, most genes are unique. In some cases, there are multiple enzymes for one step to allow for specific control by different end

TABLE 1. Kinetic parameters of transport by TrkG and TrkH[a]

Strain[b]	Kinetics of K^+ uptake		Kinetics of Rb^+ uptake	
	K_m (mM)	V_{max} (μmol g^{-1} min^{-1})	K_m (mM)	V_{max} (μmol g^{-1} min^{-1})
FRAG90	1.7[c]	510[c]	0.5, 0.7	29, 18
TK2447 (Δ*trkG*)	6 ± 1 (3)	800 ± 180 (6)	2, 4	10, 8
TK2448 (Δ*trkH*)	1.0, 1.1	300, 310	0.4, 0.4	17, 24
TK2450			—[d]	—[d]

[a] Initial rates of uptake were measured at 30°C in cells depleted of K^+ by treatment with 2,4-dinitrophenol (15). Values are duplicates or averages ± standard deviations. Numbers of determinations are given in parentheses. Rates are expressed per gram (dry weight).

[b] FRAG90 is F^- *thi rha lacZ gal* Δ(*kdpFABC*)*5 trkD1*. The other strains were constructed from it by transduction and carry in addition a *trkG* deletion (TK2447), a *trkH* deletion (TK2448), or both deletions (TK2450). The deletions were made in vitro by replacing most or all of the respective genes. A Kan^r cassette replaced a 1.9-kb *Sca*I-*Dra*I fragment of *trkG*; this mutation was inserted into the chromosome in a *polA* mutant (10). The construct in which a Cam^r cassette replaced a 1.2-kb *Nsi*I fragment of *trkH* was inserted into the chromosome by transformation of the linearized plasmid into strain JC7623 (26).

[c] Results of a single determination. Other studies of wild-type Trk strains yield K_ms ranging from 1 to 2 mM, and V_{max}s of 300 to 600 μmol g^{-1} min^{-1} (3, 15).

[d] —, see text.

products of the pathway, such as the three 3-deoxy-D-arabino-heptulosonate-7-phosphate synthetases (14). There are multiple transport systems for some substrates; however, they are usually markedly different in affinity and/or energy coupling, with the result that the system with higher affinity and higher energy consumption is reserved for conditions in which the substrate is present at low concentrations. This pattern of multiple transport systems is true for K^+ transport, among others (2, 25).

Wild-type strains of *E. coli* probably express both TrkG and TrkH, since the kinetics observed are different from those when only one of the two is present (Table 1). Rb^+ uptake by the wild type is consistent with both systems being present and additive, although the contribution of TrkH is small. The K_m for K^+ of the wild type is consistent with additivity, since it lies between the K_ms of the individual systems. However, not only is the V_{max} not additive, but the *trkG* mutant has rates consistently higher than those of all of the other strains. In a slightly different genetic background in which UV light-induced mutations, presumably point mutations, were used, the *trkG* mutant had a V_{max} similar to that of the wild type (7). The high rates of Trk may not be additive for reasons independent of Trk itself. Proton excretion, which is needed to maintain charge balance during K^+ uptake, may have a lower maximum rate than does the Trk system.

The kinetic data imply that both gene products are expressed and active; however, the way in which they act in the wild type is not known. If the Trk complex has only one subunit of the TrkG-TrkH type, then the wild type has only two types of Trk, each of which is seen alone in the *trkG* and the *trkH* mutants, respectively. However, if this type of subunit is present in two or more copies per Trk complex, then heteromeric forms whose properties may be different from those of each of the homomeric forms might exist.

The suggestion has been made that *trkG* is a foreign gene, since it has an unusually low G+C content, has a codon usage different from those of most genes of *E. coli*, and is located in the cryptic *rac* prophage sequence (6, 21). Our studies provide one additional argument in support thereof. The other saturable K^+ transport systems of *E. coli* discriminate strongly against Rb^+ (1, 25). The Trk system resembles the other K^+ transport systems more closely when it is assembled with the TrkH protein than it does with TrkG.

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