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Evolution of Antiparallel Two-Domain Membrane Proteins. Swapping Domains in the Glutamate Transporter GltS

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Supporting information to

Evolution of two-domain membrane proteins. Swapping domains in the glutamate transporter GltS

by Adam Dobrowolski and Juke S. Lolkema.

Methods

Table T1. Sequences of loops linking the N and C domain in wild type GltS and GltS^{swap} versions

Table T2. Sequences of the primers used in this study

Figure S1. Reconstructed evolutionary states of GltS of *Escherichia coli*.

Methods

Escherichia coli strain DH5 α harboring the *gltS* constructs described here was grown in Luria Bertani Broth (LB) medium with 50 μ g/ml ampicillin at 37 °C under continuous shaking at 150 rpm. Production of the proteins was induced by addition of 0.01% arabinose when the optical density of the culture measured at 660 nm reached a value of 0.6. Preparation of RSO membranes and uptake studies of radiolabeled glutamate were done as described before (Kaback, 1974; Konings *et al*, 1971; Dobrowolski and Lolkema, 2009). His-tagged proteins were purified by Ni²⁺-NTA affinity chromatography using a small scale purification procedure described before (Dobrowolski and Lolkema, 2009).

Construction of *gltS*^{split} and *gltS*^{swap} carrying plasmids. Construction of the plasmids encoding the GltS variants used in this study were based on plasmid pBADHN-GltS described before (Dobrowolski *et al*, 2007). All genetic manipulations were done in *E. coli* DH5 α cells. Plasmid pGltS-hNC was constructed by PCR insertion mutagenesis. Synthetic primers GltS_RBS_F and GltS_RBS_R (see Supplementary Table T2) were designed to insert a sequence containing a termination codon, a ribosomal binding site (RBS) and a start codon into the *gltS* gene in plasmid pBADHN-GltS between the codons encoding T201 and A202 in GltS. Plasmid pGltS-hNC encodes a protein containing the N-terminal 201 amino acid residues of the GltS protein (N domain) extended with a N-terminal His-tag and a protein consisting of the C terminal 200 amino acid residues of GltS with an additional Met residue on N-terminus (C domain). Plasmid pGltS-hC was constructed by deleting a *NcoI*-*NcoI* fragment from pGltS-hNC which results in a single gene encoding the C domain extended with His-tag at the N-terminus. Plasmid pGltS-hNhC was constructed by ligating a *EcoRI*-*EcoRI* fragment

excised from pGltS-hNC into pGltS-hC digested with the same restriction enzyme. Plasmid pGltS-hNhC encodes a protein containing the N-terminal 201 amino acid residues of the GltS protein (N domain) extended with a N-terminal His-tag and a protein consisting of the C terminal 200 amino acid residues of GltS (C domain) and an additional Met residue at N-terminus, and, also, extended with a N-terminal His-tag (GltS^{split}). Plasmid pGltS-hChN was constructed by ligating a DNA fragment containing the N domain of GltS, produced by PCR with the use of primers GltS_hN_F and GltS_N_R and pGltS-hNC as template, and digested with *HpaI/SphI* restriction enzymes into pGltS-hC digested with the same enzymes. Plasmid pGltS-hChN encodes the N and C domain as in pGltS-hNhC but in the reverse order (GltS^{split}). Plasmid pGltS-hC2N was constructed by ligating a DNA fragment, produced by PCR with the use of primers GltS_N_F and GltS_N_R and pGltS-hNC as template, and digested with *StuI/SphI* into pGltS-hC digested with *HpaI/SphI* restriction enzymes. Plasmid pGltS-hC2N encodes a single protein containing 404 amino acid residues (a Met residue followed by the C domain (200 residues) linked with 2 Ser residues linked to the N domain (201 residues)) extended with a N-terminal His-tag (GltS^{swap}). Plasmid pGltS-hC12N was constructed by ligating a DNA fragment, produced by PCR with the use of primers GltS_Nlnk12_F and GltS_N_R and pGltS-hNC as template, and digested with *StuI/SphI* into pGltS-hC digested with *HpaI/SphI* restriction enzymes. Plasmid pGltS-hC12N encodes a single protein containing 414 amino acid residues (like pGltS-hC2N, but with 12 residues in the linker) extended with a N-terminal His-tag (GltS^{swap}). Plasmid pGltS-hC19N was constructed by ligating a DNA fragment, produced by PCR with the use of primers GltS_Nlnk19_F and GltS_N_R and pGltS-hC12N as template, and digested with *StuI/SphI* into pGltS-hC digested with *HpaI/SphI* restriction enzymes. Plasmid pGltS-hC19N encodes a single protein containing the 421 amino acid (like pGltS-hC2N, but with 19 residues in the linker) extended with a N-terminal His-tag. Plasmids pGltS-hNC and pGltS-hC were not used during this study to produce proteins. All plasmids were sequenced and shown to contain the designed inserts (ServiceXS, Leiden, The Netherlands).

Mass spectrometry analysis. Partially purified GltS variants were separated by SDS-PAGE using a 12 % gel and stained by Coomassie Brilliant Blue. Selected bands were cut from the gel. The pieces of gel were fragmented in smaller pieces, destained in 50 mM ammonium bicarbonate in 40% ethanol, dehydrated by a three times repeated treatment with 100 µl acetonitril, and dried completely using a SpeedVac centrifuge. The pieces of gel were reswollen by adding 20 µl of a 10 ng/µl trypsin solution and the samples were incubated overnight at 37°C. The peptides were extracted from the fluid by shaking for 20 min with 30 µl of a mixture of 60% acetonitril and 1% trifluoroacetic acid (TFA) in water. The extracted peptides were dried in a SpeedVac centrifuge and dissolved in 10 µl of 0.1% TFA in water. Aliquots of 0.75 µl of the peptide suspension were spotted on the MALDI target and mixed on the target in a 1:1 ratio with the matrix solution consisting of 10 mg/ml α -cyano-4-hydroxycinnamic acid (dissolved in 70% acetonitril and 0.1% TFA). The spots were allowed to dry completely before the MALDI-TOF experiment was performed on the Applied Biosystems 4700 Proteomics Analyzer.

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Supplementary tables.

Table T1. Sequences of loops linking the N and C domain in wild type GltS and GltS^{swap} versions

protein	Linker	Loop sequence ^a
GltS ^{wt}	-	ARYLVKHSTTPNGIPDDQEVPTAFEKPDVGRMI
GltS ^{swap}	2	IKLYLMLPIFAG SS SMVH
GltS ^{swap}	12	IKLYLMLPIFAG SS SGSGSGSGSGSMVH
GltS ^{swap}	19	IKLYLMLPIFAG SS SGSGSGSGSGSGSGSGSGSMVH

^a the linker is given in bold

Table T2. Sequences of the primers used in this study

Primer name	Primer sequence ^a	Introduced restriction sites
GltS_RBS_F	5'-CCAGGAAGTCCCGACGTAAGCTAGCAGGAGGAATTCACCATGGCGTTTGAAAAGCCGG ^{b,c}	<i>NheI, NcoI, EcoRI</i>
GltS_RBS_R	5'-CCGGCTTTTCAACGCGCATGGTGAATTCCTCCTGCTAGCTTACGTCGGGACTTCCTGG ^{b,c}	<i>NheI, NcoI, EcoRI</i>
GltS_hN_F	5'-CGCGGTAAACGCTAGCAGGAGGAATTC	<i>HpaI</i>
GltS_N_R	5'-CGCGCGCATGCTTCCTCCTGCTAGCTTAC	<i>SphI</i>
GltS_N_F	5'-CGCGAGGCCTCAATGGTTCATCTCGATAC ^c	<i>StuI</i>
GltS_Nlnk12_F	5'-CGCGAGGCCTCAGGATCCGGGAGCGGTTTCGGGAAGTGGCTCAATGGTTCATCTCGATAC ^c	<i>StuI</i>
GltS_N_lnk19_F	5'-CGCGAGGCCTCAGGATCCGGGAGCGGATCAGGTGGATCCGGGAGCGGTTTC	<i>StuI</i>

^a indicated in bold are introduced restriction sites,

^b indicated by underline are introduced termination codon, a ribosomal binding site (RBS) and a start codon,

^c indicated in italic are bases, which correspond to sequences in the *gltS* gene.

Supplementary figure

Figure S1. Reconstructed evolutionary states of GltS of *Escherichia coli*. Left, genetic constructs. The N and C domain encoding part of the genes were indicated in yellow and red, respectively. From top to bottom, wild type *gltS* gene, *gltS^{split}* operons containing the genes encoding the N and C domain in both orders, *gltS^{swap}* gene with swapped N and C domains. Right, topology models of the encoded GltS, GltS^{split} and GltS^{swap} proteins. Cylinders represent transmembrane segments. The re-entrant loops between the 4th and 5th TMS were indicated. The periplasm is above, the cytoplasm below the models.