Minireview

The sodium/substrate symporter family: structural and functional features

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Abstract Members of the sodium/substrate symporter family (SSSF, TC 2.A.21) catalyze the uptake of a wide variety of solutes including sugars, proline, pantothenate, and iodide into cells of pro- and eukaryotic origin. Extensive analyses of the topology of different SSSF proteins suggest an arrangement of 13 transmembrane domains as a common topological motif. Regions involved in sodium and/or substrate binding were identified. Furthermore, protein chemical and spectroscopic studies reveal ligand-induced structural alterations which are consistent with close interactions between the sites of sodium and substrate binding, thereby supporting an ordered binding mechanism for transport. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Secondary transport; Sodium/solute symport; PutP

1. Introduction

Sodium/substrate symport (or cotransport) is a widespread mechanism of solute transport across cytoplasmic membranes of pro- and eukaryotic cells. Thereby the energy stored in an inwardly directed electrochemical sodium gradient (sodium motive force, SMF) is used to drive solute accumulation against a concentration gradient. The SMF is generated by primary sodium pumps (e.g. sodium/potassium ATPases, sodium translocating respiratory chain complexes) or via the action of sodium/proton antiporters. Sodium/substrate transporters are grouped in different families based on sequence similarities [1]. However, it should be noted that assignment of a family membership does not automatically provide information on the mechanism of energy coupling. In fact, there are mixed families of transporters whose members differ in the choice of the coupling ion or catalyze symport or antiport processes. Furthermore, in individual transporters the choice of the coupling ion can be influenced by the nature of the substrate [2,3].

We are investigating the sodium/proline transporter (PutP) of *Escherichia coli* as a model system to obtain new insights into the molecular mechanism of sodium/substrate transport. PutP is a member of the sodium/substrate symporter family (SSSF, TC 2.A.21) [1,4,5]. Known members of this family use predominantly sodium as a coupling ion. This minireview discusses recent achievements with PutP and highlights common structural and functional features of members of the SSSF.

2. Functional properties of members of the SSSF

The SSSF contains far over hundred members of pro- and eukaryotic origin (a selection is shown in Fig. 1). Proteins of this family utilize a SMF to drive uphill transport of substrates like sugars, amino acids, vitamins, ions, *myo*-inositol, phenyl acetate, urea, and water [1,6]. Most of the transporters are part of catabolic pathways and are used for the acquisition of the corresponding substrate by the cells as source of nitrogen and/or carbon [7]. In addition, there are transporters (e.g. OpuE, a Na^+/proline transporter of *Bacillus subtilis*) which are involved in cell adaptation to osmotic stress [8]. PutP of *Staphylococcus aureus* contributes to in vivo survival of this causative organism of a wide variety of human infections, thereby representing a target for the development of new drugs against pathogens [9]. Besides transport proteins, members of the SSSF show a distant similarity to the N-terminal domains of some sensor kinases of bacterial two component signal transduction pathways [5,10] (Fig. 1). Information is not available yet whether the sensor proteins can also function as transporters.

Among the best characterized members of the SSSF are the human sodium/glucose transporter (hSGLT1), the sodium/iodide transporter (NIS), and PutP of *E. coli* (see [6,7,11,12] for recent reviews). SGLT1 and NIS catalyze uptake with a 2:1 sodium/substrate stoichiometry while the stoichiometry value of sodium/proline transport by PutP is 1:1 [11–13]. In addition, SGLT1 couples the uptake of two sodium and one sugar with the transport of 264 water molecules [14]. In the rabbit isoform of SGLT1 (rSGLT1), sodium can be substituted by protons or lithium ions whereby the apparent sugar affinity (apparent $K_{m(sugar)}$) decreases by two orders of magnitude (from 0.2 to 30 mM) [15]. In PutP of *E. coli*, lithium can substitute for sodium; however, despite earlier assumptions proton-driven proline uptake by PutP could not be demonstrated [13]. Kinetic analyses of sodium/substrate transport catalyzed by different members of the SSSF including PutP suggest that transport occurs according to an ordered binding mechanism [13,16,17].

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Abbreviations: SMF, sodium motive force; SSSF, sodium/substrate symporter family (TC 2.A.21); TM, putative transmembrane domain

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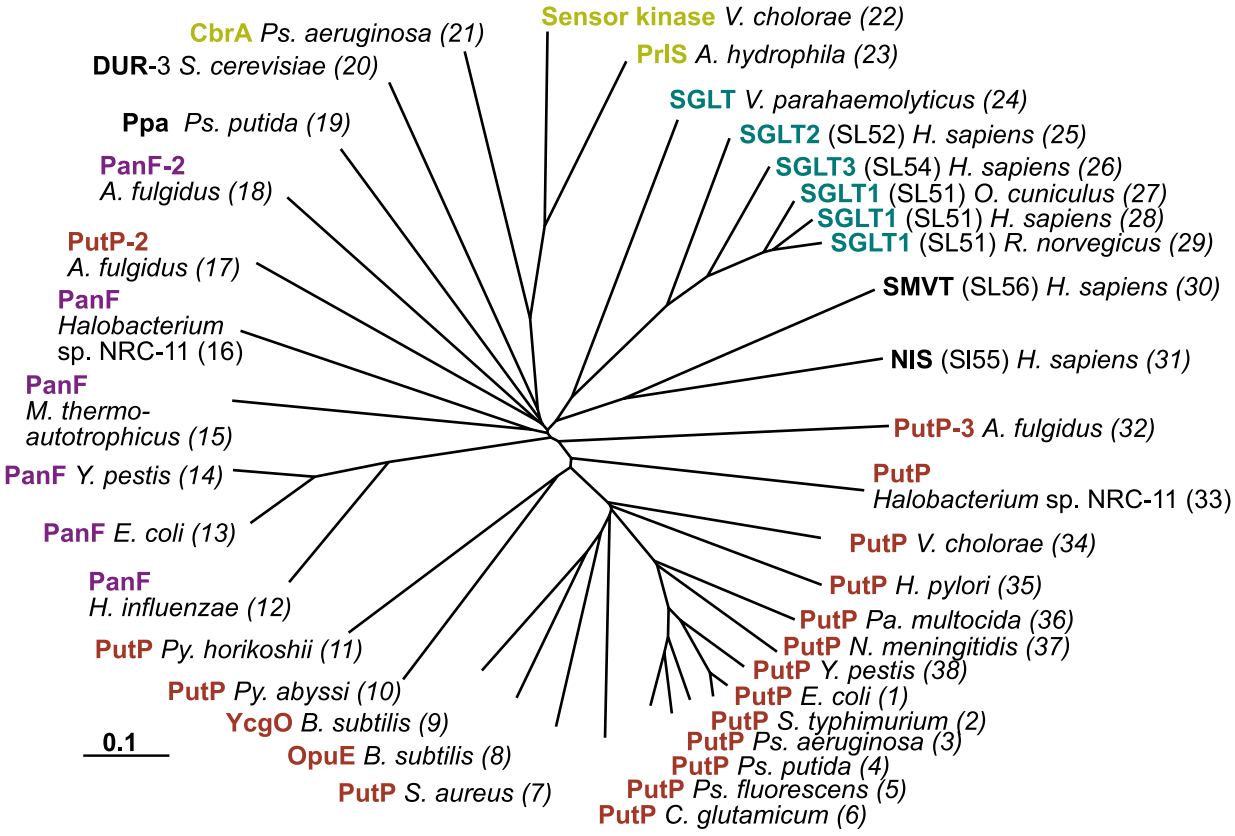

Fig. 1. Unrooted phylogenetic tree of members of the SSSF. Phylogenetic relationships were analyzed with the CLUSTAL W multiple sequence alignment algorithm [37]. The accession numbers refer to the proteins shown in the tree by arbitrary numbers. (1) P07117; (2) P10502; (3) Q9I5F5; (4) Q9R9T6; (5) Q57023; (6) O32355; (7) O30986; (8) O06493; (9) P94392; (10) Q9V2P3; (11) O59086; (12) P44963; (13) P16256; (14) Q8ZAX5; (15) O27884; (16) Q9HP63; (17) O29293; (18) O29063; (19) O50471; (20) P33413; (21) Q9HV74; (22) Q9KV54; (23) Q93N33; (24) P96169; (25) P31639; (26) Q9NY91; (27) P11170; (28) P13866; (29) P53790; (30) Q9Y289; (31) Q92911; (32) O29046; (33) gi|10580468; (34) Q9I5W6; (35) O24896; (36) Q9CN55; (37) Q9JR87; (38) Q8ZF65. Red, (putative) sodium/proline transporters; green, sodium/sugar transporters; blue, (putative) sodium/pantothenate transporters; yellow, sensor domains of putative sensor kinases.

3. Transporter structure

The average hydropathy plot for SSSF proteins predicts 11 to 15 putative transmembrane domains (TMs) in α-helical conformation [4]. For PutP of *E. coli*, gene fusion analyses, Cys accessibility studies, site-specific proteolysis, and site-directed spin-labeling studies were performed to obtain information on the topological arrangement of the transporter [18,19]. The studies suggest a secondary structure model according to which PutP contains 13 TMs with the N-terminus located on the periplasmic side of the membrane and the C-terminus facing the cytoplasm (Fig. 2). The results are in good agreement with studies on SGLT1 and NIS, thereby supporting the idea of a common topological motif for members of the SSSF. Transporters with a C-terminal extension (e.g. hSGLT1) are proposed to have an additional 14th TM [4]. As far as investigated, the 13-helix motif appears to be a special feature of the SSSF.

Information on tertiary interactions within members of the SSSF has recently been gained by chemical cross-linking of splits of the sodium/galactose transporter of *Vibrio parahaemolyticus* (vSGLT) [20]. The studies suggest that the hydrophilic loops between TMs IV and V and between X and XI are within 8 Å of each other.

Analysis of the cross-sectional area of freeze-fracture particles of SGLT1 indicates that the transporter is an asymmetrical monomer [21]. Little is known about the oligomeric state of other members of the SSSF.

4. Structure–function relationships

4.1. Sites of sodium binding

Labeling experiments, random and site-directed mutagenesis have been employed to identify functionally important sites in PutP of *E. coli* and *Salmonella typhimurium*. Site-directed mutagenesis studies established that amino acids of TM II of PutP are of particular functional importance. Thus, the carboxylate of Asp55 (TM II) proved to be essential for transport, and significant albeit highly reduced activity was detected only with Glu in place of Asp55 [22] (see Fig. 2 for location of residues). Kinetic analysis of active transport catalyzed by the latter PutP derivative revealed a 50-fold decrease of the apparent affinity of the protein for sodium ions compared to the wild-type transporter. On the other hand, only a relatively small alteration of the apparent affinity for proline was observed [22]. These findings make Asp55 a good candidate for a residue directly involved in binding of the coupling ion. Also in the well-studied melibiose permease of *E. coli* (MelB), which is not homologous to PutP, amino acids located in the N-terminal part of the protein (Asp55, Asp58, Asp124) are implicated in sodium binding [3]. However, PutP clearly differs from the latter sugar transporter by the fact that

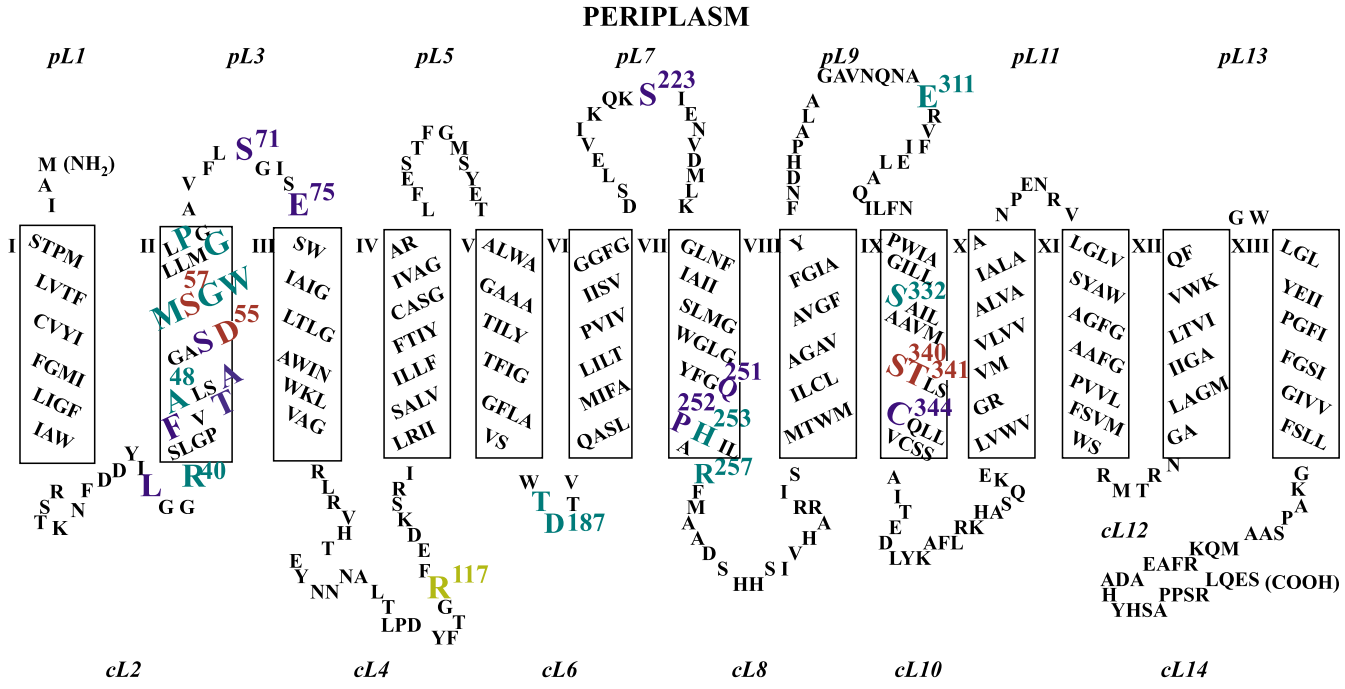

Fig. 2. Secondary structure model of PutP of *E. coli* highlighting functional important residues. The model is based on a gene fusion approach, Cys accessibility analyses, site-directed spin-labeling, and site-specific proteolysis [18]. TMs are represented as rectangles and numbered with roman numerals; loops are numbered with arabic numerals starting from the N-terminus. Amino acids proposed to be directly involved in ligand binding are shown in red. Amino acids proposed to be involved in ligand-induced conformational alterations based on Cys accessibility analyses, fluorescence and electron paramagnetic resonance (EPR) measurements are highlighted in purple. Other residues of structural and/or functional importance are represented in green. Substitution of Arg117 leads to highly reduced amounts of PutP in the membrane [24].

in PutP the carboxylate at only one position (Asp55) is essential for substrate transport while other acidic residues in the N-terminal domain proved to be dispensable for function [22].

Besides Asp55 in TM II, also residues in the central part of PutP are crucial for ion binding. In particular, removal of the hydroxyl group at the positions of either Ser340 or Thr341 led to highly decreased apparent affinities for sodium and lithium ions. Furthermore, analysis of the accessibility of Cys individually placed at different positions in TM IX indicates a participation of the TM in the formation of a hydrophilic pore or cleft (M. Böhm, M. Nietschke, and H. Jung, unpublished information). The data support the idea that amino acids of TM IX of PutP contribute to the formation of an ion translocation pathway of PutP. This conclusion is further supported by the fact that Ser340 as well as Thr341 are highly conserved within the SSSF. Replacement of the corresponding residues in NIS revealed that the hydroxyl groups are essential for NIS activity [12]. To date there is no direct evidence for an involvement of amino acid residues in ion binding in other members of the SSSF [11].

A variety of residues proved to be crucial for efficient coupling of sodium and substrate transport. In PutP, this involves, e.g., Arg40 at the cytoplasmic end of TM II and Asp187 in the cytoplasmic loop between TMs V and VI [23,24]. Interestingly, neutralization of Asp at the position of hSGLT1 corresponding to Asp187 of PutP converts the sugar transporter into a ligand-gated proton channel [25]. Furthermore, evidence has been presented suggesting a participation of Ala166 of hSGLT1 in the coupling mechanism [26].

4.2. Regions important for proline binding

Besides the discussed role of amino acids of TM II of PutP in sodium liganding, some residues in this segment are important for proline binding. Substitution of Ser57 by Ala, Cys, Gly, or Thr results in a dramatic reduction of the apparent proline and cation affinities [27,28]. Interestingly, upon increasing the proline concentration the apparent sodium affinity of PutP bearing replacements for Ser57 converges towards the wild-type value, indicating a close co-operativity between cation and substrate site(s). This notion is supported by the fact that sodium-stimulated site-specific fluorescence labeling of a single Cys at position 57 is completely reversed by the addition of proline [27]. In conclusion, it is very likely that Ser57 participates in the transmission of a sodium-induced conformational alteration leading to high affinity proline binding to a site that is at least close to this position in the tertiary structure of the transporter.

The proposed participation of TM II in proline binding apparently contradicts results obtained with SGLT1, suggesting that the C-terminal domain is responsible for substrate binding and translocation. So, phlorizin, a competitive inhibitor of sugar transport by SGLT1, is suggested to interact with a region between amino acids 604 and 610 of the sugar transporter [29]. Furthermore, a SGLT1 fragment comprising only the last five TMs of the transporter is able to catalyze sodium-independent facilitated diffusion of sugar [30]. In addition, Gln457 at the extracellular end of TM XI is proposed to directly interact with hydroxyl groups of the pyranose ring [31]. However, it has to be taken into account that the C-terminal SGLT1 fragment catalyzes sodium-independent fa-

cilitated diffusion of sugar with a highly reduced sugar affinity. In conclusion, the consensus idea might be that multiple regions in individual members of the SSSF contribute to efficient substrate binding. For PutP the idea is also supported by the fact that deletion mapping of *putP* mutants with an altered substrate specificity revealed a clustering in three distinct regions of the *putP* gene [32]. Furthermore, the proposed close proximity of ion and organic solute binding sites in the tertiary structure of this class of membrane proteins – thereby ensuring strong co-operativity – might be the key for the molecular mechanism of the coupled translocation of cation and substrate.

4.3. Conformational alterations related to transporter function

A series of protein chemical and spectroscopic studies have been employed to obtain information on functional dynamics of SSSF proteins. In the case of PutP of *E. coli* it was shown, e.g., that binding of sodium and/or proline alters the mobility of a nitroxide group attached to positions 37 (cytoplasmic loop preceding TM II) or 45 (TM II) [19]. Furthermore, fluorescence of PutP site-specifically labeled with a fluorescence group at position 223 (periplasmic loop between TMs VI and VII) is altered by addition of proline if sodium is present. In addition, the accessibility of Cys individually placed at a variety of positions (e.g. in TMs II and VII) is altered by addition of sodium and/or proline. Since substitution of the native amino acids at most of these positions has no or only very little effect on PutP transport kinetics, the altered Cys accessibility is attributed to conformational alteration of the protein upon ligand binding (T. Pirch and H. Jung, unpublished information).

In the absence of sugar, stepwise changes of membrane voltage leads to charge movements within SGLT1 which are attributed to conformational changes of the transporter [33]. Recent studies demonstrate that sodium, sugar and voltage alter the local environment of a fluorophore attached to position 457 of hSGLT1 [34]. Conformational changes have also been reported for vSGLT [35,36]. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy of vSGLT revealed that substrate binding also affects the secondary structure of the protein. So a stepwise increase in α-helicity is observed which is accompanied by a decrease in β-structural elements [36].

The observed conformational alterations correlate well with the ordered binding model of sodium/substrate transport [13,17]. In this scheme, sodium binds to the empty transporter first, thereby inducing a conformational alteration which increases the affinity of the transporter for the solute. The formation of the ternary complex induces another structural change that exposes sodium and substrate to the other site of the membrane. Substrate and sodium are released and the empty transporter re-orientates in the membrane allowing the cycle to start again.

5. Conclusions

It is obvious that many results discussed above, particularly those related to the molecular mechanism of energy coupling, remain speculative without information on the tertiary structure. As demonstrated by the pioneering work on lactose permease, analysis of second site revertants, Cys cross-linking, and a variety of spectroscopic approaches can yield valuable information on helix packing and tilts. These techniques are currently applied to the investigation of members of the SSSF. Notwithstanding the power of these studies, a high-resolution structure obtained, e.g., by crystallographic means is indispensable for the understanding of interactions between amino acid side-chains and between side-chains, coupling ion, and substrates. Therefore, the development of strategies allowing crystallization of the polytopic membrane transport proteins is currently one of the major challenges in the field of secondary transport. Moreover, the results obtained with SGLT1 and PutP indicate that these proteins require a high degree of conformational flexibility in order to function, making it imperative to obtain dynamic information in order to fully understand function. Here, site-directed labeling in combination with spectroscopic measurements such as EPR and FTIR spectroscopy are very powerful techniques to obtain information on the nature and the extent of the changes in high time resolution.

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