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Review

A biophysical glance at the outer surface of the membrane transporter SGLT1 ☆

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ABSTRACT

Proteins mediating the transport of solutes across the cell membrane control the intracellular conditions in which life can occur. Because of the particular arrangement of spanning a lipid bilayer and the many conformations required for their function, transport proteins pose significant obstacles for the investigation of their structure–function relation. Crystallographic studies, if available, define the transmembrane segments in a "frozen" state and do not provide information on the dynamics of the extramembranous loops, which are similarly evolutionary conserved and thus as functionally important as the other parts of the protein. The current review presents biophysical methods that can shed light on the dynamics of transporters in the membrane. The techniques that are presented in some detail are single-molecule recognition atomic force microscopy and tryptophan scanning, which can report on the positioning of the loops and on conformational changes at the outer surface. Studies on a variety of symporters are discussed, which use gradients of sodium or protons as energy source to translocate (mainly organic) solutes against their concentration gradients into or out of the cells. Primarily, investigations of the sodium–glucose cotransporter SGLT1 are used as examples for this biophysical approach to understand transporter function.

Contents

☆ Dedicated in loving memory to Prof. Dr. Evamaria Kinne-Saffran.

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1. Introduction

Transport proteins play an essential role in biology. As soon as the first unicellular organisms enwrapped in a cell membrane emerged, the control of solute traffic across the cell barrier became of utmost importance. This importance is reflected in the fact that about 25% of the genome is predicted to code for proteins involved in transmembrane transport [1]. The transport of a solute can either be active, when it occurs against an electrochemical gradient, or passive following the gradient. In turn, active transport can be achieved by so-called primary active transport where, as in the case of the various ATPases, chemical energy is used directly to energize the translocation of ions. Secondary active transport systems use the ion gradients established by the primary active transport systems via cotransport (symport and antiport) for the accumulation of other solutes.

One of these symport systems is the sodium-D-glucose cotransporter (SGLT) found as early as in *Vibrio* and essential for nutrient uptake in mammals [2]. There it serves as the mechanism by which D-glucose is taken up into the body in the small intestine; in the kidney two different SGLTs recover D-glucose filtered in the glomerulus from the primary urine and prevent loss of the carbohydrate [3]. Major breakthroughs in the understanding of the mechanisms of transport and in the molecular basis of transport of SGLT are the expression cloning by the group of Ernest Wright [4] and, most recently, the crystallization and the structural analysis of the *Vibrio* transporter by Faham et al. [5].

Membrane transporters are peculiar in their function in that during the translocation of a solute they undergo a cycle of conformational changes. In the outside orientation in the case of the SGLT binding of sodium followed by the binding of glucose occurs, then an occluded state is postulated. This step is followed by a conformation where the solute is now accessible from the cytoplasmic space, and sodium and the sugar are released into the cell. The transporter finally assumes the outside orientation again. Recently, several transporters have been crystallized and possible detailed mechanisms of cotransport have been proposed [5–10].

Recently, Claxton et al. [11] identified ion/substrate-dependent conformational changes in bacterial homolog of neurotransmitter: sodium symporter LeuT by using site-directed spin labelling and electron paramagnetic resonance. Their results outline the Na^+-dependent formation of a dynamic outward-facing intermediate that exposes the primary substrate-binding site and the conformational changes that occlude this binding site upon subsequent binding of the leucine substrate. Furthermore, their studies demonstrate that the binding of the transport inhibitors induce structural changes that distinguish the resulting inhibited conformation from the Na^+/leucine-bound state. Applying the same technique on lactose permease (LacY), Smirnova et al. [12] identified sugar-binding-induced outward-facing conformation of the symporter; conformational cycle of ABC transporter MsbA [13] in liposome was also identified using double electron–electron resonance spectroscopy.

Another widespread appraoch to identify functionally important parts in membrane cotransporter proteins is the subsituted cysteine accessibility method (SCAM) [14]. Frillingos et al. [15] have extensively used the SCAM technique to identify different aspects of Lac permease cotransporter. SCAM can be used to identify functionally important sites as well as reveal and/or confirm secondary structural details, including the peri- or cytoplasmic orientation of acccessible sites. A functionally important region in the putative third extracellular linker (ECL-3) of the renal Na^+/Pi cotransporter (NaPi-IIa) was probed using SCAM [16], and results of this study provide a valuable structure–function insights into cotransport meachnism. SCAM is also widely used for the identification of functional aspects of differnet membrane proteins, namely, cyclic nucleotide-gated channel [17], acetylcholine receptor [18,19], *Plasmodium falciparum* equilibrative nucleoside transporter 1 (PfENT1) [20], and the serotonin transporter [21]. Using the Cys residue accessibility toward MTS in the putative sugar translocation pathway of SGLT1 in combination with electrophysiology and fluorescence measurements, Loo et al. [22,23] elegantly measured real-time conformational changes and charge movements. Their finding provides strong support for an alternating access mechanism for Na^+-driven cotransporters. They also successfully identified two different conformational states of transporter, Na^+-bound state (CNa_2), and Na^+/sugar-bound state (CNa_2S).

Electrophysiology is another importnat biophysical tool to elucidate dynamics of membrane transporters. By measuring the electrophysiological properties of hSGLT1 in the cut-open oocyte technique, Chen et al. [24] identified two time constants, tau 1 and tau 2, which correspond to two steps in the conformational change of the free carrier. Na^+-binding/debinding modulates the slow time constant (tau 1), and a voltage-independent slow conformational change of the free carrier accounts for the observed plateau value of 10 ms. In an electrophysiology study on rabbit Na^+/glucose cotransporter 1, Hazama et al. [25] have shown that SGLT1 exhibits a presteady-state current after step changes in membrane voltage in the absence of sugar. These currents reflect voltage-dependent processes involved in cotransport and provide insight on the partial reactions of the transport cycle. Simulations of a 6-state ordered kinetic model for rabbit Na^+/glucose cotransport indicate that charge movements are due to Na^+-binding/dissociation and a conformational change of the empty cotransporter. In a recent electrophysiology study on rabbit SGLT1 [26], Na^+ and voltage dependence of transient SGLT1 kinetics was reported. Using step changes in membrane potential, in the absence of glucose but with 100 or 10 mM Na^+, transient currents were measured, corresponding to binding/debinding of Na^+ and to conformational changes of the protein.

There is evidence from other transporters, e.g., γ-aminobutyric acid transporters and citrate/malate transporter CimH, showing that exposed surface loops can act as a binding region for organic substrates [27] or can form a reentrant pore-loop-like structure with the accessibility depending on the conformation of the transporter [28–30]. During recent years, therefore, our group and others have concentrated on methods that are able to report on the conformational changes of those areas not resolved in the crystallography studies to determine their contribution to transmembrane transport.

One of these methods is atomic force microscopy, which, in its basic form, can resolve surface structures at an atomic level. Such