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Defining the blanks – Pharmacochaperoning of SLC6 transporters and ABC transporters

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ABSTRACT

SLC6 family members and ABC transporters represent two extremes: SLC6 transporters are confined to the membrane proper and only expose small segments to the hydrophilic milieu. In ABC transporters the hydrophobic core is connected to a large intracellular (eponymous) ATP binding domain that is comprised of two discontiguous repeats. Accordingly, their folding problem is fundamentally different. This can be gauged from mutations that impair the folding of the encoded protein and give rise to clinically relevant disease phenotypes: in SLC6 transporters, these cluster at the protein–lipid interface on the membrane exposed surface. Mutations in ABC-transporters map to the interface between nucleotide binding domains and the coupling helices, which provide the connection to the hydrophobic core. Folding of these mutated ABC-transporters can be corrected with ligands/substrates that bind to the hydrophobic core. This highlights a pivotal role of the coupling helices in the folding trajectory. In contrast, insights into pharmacochaperoning of SLC6 transporters are limited to monoamine transporters – in particular the serotonin transporter (SERT) – because of their rich pharmacology. Only ligands that stabilize the inward facing conformation act as effective pharmacochaperones. This indicates that the folding trajectory of SERT proceeds via the inward facing conformation. Mutations that impair folding of SLC6 family members can be transmitted as dominant or recessive alleles. The dominant phenotype of the mutation can be rationalized, because SLC6 transporters are exported in oligomeric form from the endoplasmic reticulum (ER). Recessive transmission requires shielding of the unaffected gene product from the mutated transporter in the ER. This can be accounted for by a chaperone-COPII (coatomer protein II) exchange model, where proteinaceous ER-resident chaperones engage various intermediates prior to formation of the oligomeric state and subsequent export from the ER. It is likely that the action of pharmacochaperones is contingent on and modulated by these chaperones.

1. Introduction

Many (monogenic) diseases result from point mutations that lead to aberrant folding of a protein. In fact, the term molecular medicine can be traced back to the efforts of Max Perutz to solve the structure of hemoglobin and of Linus Pauling and coworkers to understand the defect underlying sickle cell anemia [1]. Folding diseases may be rare by comparison to other widespread afflictions that figure prominently in health care budgets such as diabetes mellitus, obesity and coronary heart disease. However, as pointed out by Sir Archibald Garrod some 75 years ago, major scientific advances have come from the study of rare diseases [2]. Understanding protein folding remains a fascinating challenge, which is succinctly illustrated by the *Gedanken experiment* of Cyrus Levinthal [3]. Its insights can be recapitulated in a back-of-the-envelope calculation: (i) for the sake of simplicity, assume that the two bonds per amino acid, which define the peptide backbone of a protein, can exist in two stable states (e.g., the angles that define an α-helix and β-strand). (ii) New conformations can be visited in 0.1 ps, i.e. at a rate at which single bonds orient (10^{-13} s^{-1}). Hence, a protein can explore 10^{13} states in one second. (iii) Under these assumptions, an average protein of some 400 amino acids has to sample some 2^{400} ($\approx 10^{120}$) conformational states in the absence of any additional constraint. In other words, exploring these states requires 10^{107} s. It is evident that this is an implausible time frame, for the currently estimated age of the

Abbreviations: ABC transporters, ATP-binding cassette containing transporters; DAT, dopamine transporter; ER, endoplasmic reticulum; GAT, GABA transporter with 4 isoforms (GAT-1, GAT-2, GAT-3, GAT-4); NBD, nucleotide binding domains; NET, norepinephrine transporter; SLC6, solute carrier-6; SERT, serotonin transporter.

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known universe is 4.41×10^{17} s (1.4×10^{10} years). This discrepancy – referred to as the Levinthal paradoxon – proves that the folded structure of a protein is reached via a limited set of trajectories rather than a random, unbiased search of all possible conformations.

The folding trajectories of polytopic membrane proteins are subject to a number of constraints: (i) insertion of the nascent polypeptide chain into the SEC61 translocon channel restricts the movement of amino acid side chains and limits the search space. (ii) Transmembrane segments exit via a lateral gate of the translocon into lipid bilayer. This gate allows for passage of one or at most two transmembrane segments [4]. (iii) The milieu of the lipid bilayer is anisotropic. The presence of charged residues within the transmembrane segment, for instance, imposes a high energetic cost [5].

Transmembrane helices are typically 21–22 residues long [6]. The conformationally important backbone of a single transmembrane helix has 315–330 degrees of freedom and can – according to the concept outlined above – sample $\sim 10^6$ conformations. Folding of single transmembrane helices in the SEC61 translocon channel occurs on the time scale of peptide chain synthesis or faster. Once the transmembrane helix is inserted into the membrane, the biophysical properties of the membrane environment dramatically restrict this available search space: to a first approximation, (i) the number of accessible conformations is limited to only the all-helical state. (ii) The relevant degrees of freedom are reduced to 4, as the helix can only diffuse in the plane of the membrane (x and y), rotate along its main axis, and tilt relative to the plain of the membrane. Diffusion of helices that are connected by loops is a slow process. However, the search space is dramatically reduced by the steep drop in the number of degrees of freedom and in the number of accessible conformations.

Pharmacochaperoning is – by definition – linked to the problem of protein folding. The question arises: why should it be of interest to study pharmacochaperoning of ABC-transporters[3] and SLC6 transporters? From a medical perspective, these two classes of transporters are highly relevant to pharmacotherapy either as drug targets (in particular the SLC6 family members SERT, NET, DAT, GAT-1) or as efflux pumps (ABC-transporters). In addition, mutations in ABC transporters are the cause of monogenic diseases other than the paradigmatic folding disease cystic fibrosis (e.g., cholestasis, gout). In addition, these two classes illustrate the folding problem of dynamic polytopic membrane proteins. They have 12 or more transmembrane segments. They are thought to undergo major conformational changes and thus domain motions during the transport cycle. This raises the question of which conformations are visited by the folding trajectory. Finally, SLC6 transporters and ABC-transporters are representative two extremes: in SLC6 family members, the hydrophobic core comprises the bulk of the protein, intracellular and extracellular segments are small by comparison. This confines the folding problem to the membrane. In contrast, the folding trajectory of ABC-transporters must accommodate both and the folding of the large intracellular domains must be coupled to that of the transmembrane segment. In fact, mutations associated with folding defects of ABC transporters cluster at the interface between the transmembrane domain and the NBDs (see below). This highlights the Achilles heel of ABC transporters in the folding trajectory. Here we argue that experimental approaches to pharmacochaperoning these transporters may provide insights into the folding process or at the very least allow for defining the problem. We also examine the conjecture that commonly used drugs may also act as pharmacochaperones when administered for therapeutic or recreational purposes [7]. Finally, in the long run, pharmacochaperoning may be used to correct folding diseases.

1.1. Folding-deficient versions of SLC6 transporters

The human genome encodes 19 SLC6 transporters. SLC6A10 is thought to be a pseudogene. Hence the numbering extends from SLC6A1 (i.e., GAT-1) to SLC6A20 (i.e., the renal imino acid transporter). Human diseases appear to be rarely associated with mutations in these genes. This can be illustrated with the gene encoding GAT1: the NCBI database lists more than 1000 single nucleotide polymorphisms in the human SLCA1 gene. Seventeen of these result in non-synonymous substitutions and thus give rise to coding variants of GAT-1. It is at present not known, if one of these SNPs are related to a clinically relevant phenotype. However, there are some examples of (monogenic) diseases and clinically relevant phenotypes that can be linked to mutations in SLC6-family genes (Table 1); there are also less well-defined phenotypes that may eventually be found to arise from defective folding; pertinent examples are also listed in Table 1.

It is worth noting that mutations in SLC6 family members can be transmitted in both, an autosomal dominant and a recessive manner. It is trivial to understand why recessive alleles only cause symptoms in homozygous (or compound homozygous) individuals: clinical symptoms only appear in the total absence of a transporter.

A dominant negative phenotype can be envisaged, if the mutated transporter forms a complex with the product of the unaffected allele and precludes its surface expression. This is for instance evident in NET and GLYT2, where the mutated versions NET-A457P [8] and GLYT2-S510R [21] retain the corresponding wild type transporter within the cell (see Table 1). SLC6 family members form oligomers [44]. Hence, dominant negative effects can be readily rationalized. However, this model does not account for recessive transmission, which is observed for instance in DAT mutations associated with infantile dystonia/parkinsonism [15,16].

We mapped the residues that cause folding deficiency onto a common structural scaffold, i.e., a recently published hDAT structure [45]. This shows that the mutations are not randomly distributed (Fig. 2, color coded by transporter). The largest group clusters at the protein–lipid interface on the membrane exposed surface of the transporter. Most of these mutations are conservative in amino acid substitution, retaining the hydrophobic property of the residue. Polarity changes are therefore not expected to add a significant destabilizing energy to the folding process. Interactions with other proteins within the membrane are not expected to be severely affected, because dispersive hydrophobic interactions are rather unspecific and because hydrophobicity driven complexes have large interaction surfaces stabilized by cumulative contributions. What could then be at the origin of their effect? Almost all of these disease causing mutations change the helical properties of the mutated residue. It is therefore conceivable that helical stability is affected, which links these mutations directly to the protein folding and stability.

A second group of mutations clusters at helix crossing motifs or affect helix packing, thereby destabilizing the global structure of the transporter. Some mutations linked to diseases are found in water exposed loops. These are all found at critical position of structural motifs. Their mutation is expected to reduce protein stability. One mutation is located close to the Sec24 interaction motif (residue K605N of SERT]). It may affect recognition of SERT by Sec24C.

A small number of mutations are expected to have a strong effect on the conformational equilibrium. This has for example been shown for the completely conserved arginine in the outer vestibule on transmembrane helix 1. In the inward facing state, this arginine forms a salt bridge across the outer vestibule with an aspartate or glutamate in transmembrane helix 10. Hartnup disease that results in dermatitis and seizures is caused by a mutation of this

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