Evidence for an allosteric mechanism of substrate release from membrane-transporter accessory binding proteins

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AUTHOR SUMMARY

Diverse membrane import systems in bacteria rely on water-soluble proteins that function to sequester compounds (called substrates) from the environment and to deliver them to a suitable transporter protein located on the cell membrane (1). The transporter subsequently enables the substrate to cross the membrane and enter the cell. Typically, the binding strength, or affinity, of the water-soluble proteins—referred to as substrate-binding proteins (SBP)—is several orders of magnitude greater than that of the corresponding importers. Therefore, in order for transport to proceed the importer must somehow capture the substrate from the SBP; that is, it must out-compete its accessory SBP for the substrate. In this study, we used a number of biophysical methods to investigate the molecular mechanism by which this process is accomplished. In particular, we assessed the hypothesis that substrate release from the SBP is induced by the membrane importer itself through a mechanism of conformational modulation (i.e., a change in the protein structure) of the SBP (Fig. P1). This hypothesis is an intuitive, widely assumed notion in the field (2, 3); however, direct evidence supporting it is lacking. Here, we demonstrate that an allosteric mechanism (a process in which the activity of a molecule is altered by bringing about a change in its structure or dynamics) of substrate release is indeed plausible from an energetic standpoint.

Our model system is TeaA, an SBP that specifically binds to the compound ectoine and is a component of the TeaABC import system from Halomonas elongata, a halophilic (“salt-loving”) bacterium (4). Halophilic bacteria use ectoine to withstand the osmotic stress induced by the high salinity of their environment. First, we determined the atomic structure of TeaA in the ectoine-free state and compared it with the structure of the bound state, which had also been solved previously at atomic resolution (5). TeaA features the prototypical SBP architecture, consisting of two globular protein domains, or lobes, and a deep binding cleft between them. When ectoine binds to the cleft, the two lobes come closer together and lock the substrate into the protein. A distinct feature of TeaA, however, is a long α-helix (a helical, rod-shaped type of chemical structure), termed α9, which runs along the protein, flanking both lobes but away from the binding cleft. Importantly, this helix becomes bent and partially unfolded upon ectoine binding.

On the basis of these experimental structures, we carried out a comprehensive computer-simulation study of the interplay between the binding of ectoine and the changes in the protein structure. Two initial key insights from this analysis were that the protein naturally exists in a dynamic exchange between open and closed conformations, and that this dynamic state is very much influenced by the conformation of α9; crucially, however, α9 has no direct influence on the ability of TeaA to recognize ectoine, because this event precedes the full closure of the binding cleft.

These findings enabled us to assess the general allosteric mechanism of substrate release from TeaA. By altering the structural properties of the α-helix α9, we reasoned that we...
should be able to modulate the conformational dynamics of TeaA without directly interfering with the binding site and thus mimic the influence of the membrane importer. More specifically, this approach would enable us to examine the hypothesis that the induced opening of the SBP is sufficient to diminish the high affinity of the SBP to the degree necessary for substrate hand-over to the low-affinity transporter (Fig. P1). As mentioned, to our knowledge, this basic mechanistic principle has not been directly demonstrated to date, structurally or otherwise.

Thus, additional simulations were employed to identify a mutation (a change in the amino-acid sequence) in helix α9 that precludes its bending by stabilizing its α-helical fold and therefore favors semi-open conformations of TeaA. If the allosteric mechanism outlined above is realistic, this mutation should diminish the affinity of TeaA for ectoine. Indeed, additional experimental measurements demonstrated that the affinity of the mutant was reduced by two orders of magnitude, despite the fact that the binding cleft was not directly perturbed. Therefore, in this conformationally biased state, the TeaA mutant is effectively a low-affinity binding protein.

By analogy, membrane importers can out-compete their high-affinity SBP by shifting the conformational equilibrium of the SBP toward open-like states, because the affinity of these is markedly diminished. Of course, the reshaping of the SBP conformational landscape costs energy—in ATP-driven transporters, for example, this cost is likely compensated by the gain upon dimerization of nucleotide-binding domains at the other side of the membrane. However, whether driven by ATP or ion gradients, we conclude that the proposed allosteric mechanism of substrate release from the SBP on to the membrane importer is viable both structurally and energetically.

The emergence of multidrug resistance in pathogenic bacteria is a threat to public health on a global scale. This study contributes to advance our understanding of a class of highly efficient bacterial import systems, which could be potentially exploited to deliver novel antibiotics.