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Porter domain opening and closing motions in the multi-drug efflux transporter AcrB

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ABSTRACT

Acriflavine resistance protein B acts as the active transporter in the multi-drug efflux pump Acriflavine resistance proteins A / B - Tolerance to colicins protein in *Escherichia coli*. Within the same reaction cycle intermediate all Acriflavine resistance protein B X-ray structures display highly similar conformations of the substrate-recruiting and transporting porter domain. To assess if this structural homogeneity is an intrinsic feature of Acriflavine resistance protein B or stems from other causes we performed a series of six independent, unbiased 100 ns molecular dynamics simulations of membrane-embedded, asymmetric, substrate-free wild type Acriflavine resistance protein B in a 150 mM NaCl solution. We find the porter domain more flexible than previously assumed displaying clear opening and closing motions of the proximal binding pocket (L and T-state) and the exit of the drug transport channels (O-intermediate). Concurrently the hydrophobic binding pocket favors a closed conformation in all three protomers. Our findings suggest that the conformational homogeneity seen in the crystal structures is likely an effect of bound but structurally unresolved substrate. Our simulations further imply that each of the known three reaction cycle intermediates occurs in at least two variants, the Thr676 loop independently regulates porter domain access and likely plays a key role in substrate transport. On a 100 ns time scale we find no evidence supporting the proposed LLL resting state in the absence of substrate. If the proximal binding pocket dynamics have an inhibiting effect on Acriflavine resistance protein B pump activity lowering the life time of substrate-accessible conformations, the observed dynamics could provide a structural explanation for the Acriflavine resistance protein B activity-enhancing effect of the adaptor protein Acriflavine resistance protein A stabilizing PC1 and PC2 subdomain orientations.

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1. Introduction

Preventing drug access to the target molecule is one of the main ways by which bacteria achieve multi-drug resistance [1,2]. In Gram-negative bacteria a prominent example for this mechanism of action is an overproduction of multi-drug efflux pumps of the resistance nodulation division (RND) protein super family such as AcrAB-TolC [3] (Fig. 1a). Combining three different protein components, AcrAB-TolC comprises the outer membrane efflux duct TolC [4], the inner membrane-anchored adaptor protein AcrA [5] and the inner membrane transporter Acriflavine resistance protein B (AcrB) acting as engine of the assembled pump [6–8]. Converting the energy of proton gradient over the inner membrane into a cyclic sequence of conformational changes [9–12], AcrB transports a broad variety of substrates from periplasmic space out of the cell.

Whereas proton conduction takes place in the AcrB trans-membrane domain (TMD), substrate recruitment and transport occur in the porter domain (PD) (Fig. 1b) [13] where the "Phe617"/"switch loop" divides the transport channels into an outer "access"/"proximal binding pocket" and an inner "deep"/"distal"/"hydrophobic binding pocket" (HBP) [9,14–16] from where substrates are transported towards the central funnel formed by the AcrB docking domain (DD) (Fig. 1). Entrance (PDe) and exit (PDx) of the porter domain substrate transport channels have been found trapped in monomer-specific states of substrate accessibility in recent AcrB crystal structures (Fig. 1c,d) [9,14,17]. Whereas in monomers A and B – proposed as "*L*oose/access" and "*T*ight/binding" intermediates in the AcrB reaction cycle [9,14,17] – the transport channels exhibit open PDe/proximal binding pocket but closed PDx conformations, in monomer C – the "*O*pen/extrusion" reaction cycle intermediate – PDe is closed but PDx is open. Whether other conformational states besides the known X-ray intermediates occur in the AcrB reaction cycle is currently unknown. However, combined mutagenesis and mass spectrometry experiments introducing engineered disulfide bonds reported that while conformational transitions between L (monomer A) and T (monomer B) protomers occur in vivo, there is never more than one monomer displaying the O (monomer C) conformation [11].

At the time of writing a total of 33 different AcrB crystal structures have been published. Of these 19 structures are in a three-fold symmetric form showing identical monomer conformations representing the L state [18–25], whereas in 14 structures each monomer was trapped in a different conformation representing the LTO reaction cycle intermediates [9,14–17]. Remarkably, when comparing the PD conformation of these structures using Cα root mean square displacement after least squares fitting to the asymmetric and ligand-free 2GIF AcrB X-ray structure [9] – which we used as starting structure for our molecular dynamics (MD) simulations – all structures are very similar, displaying Cα-RMSDs of less than 0.1 nm for each

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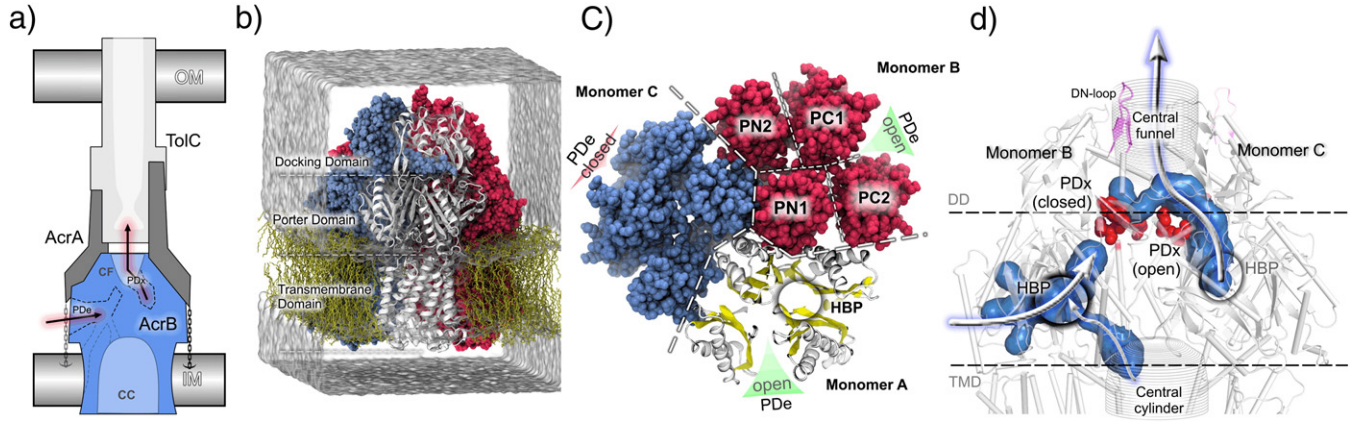
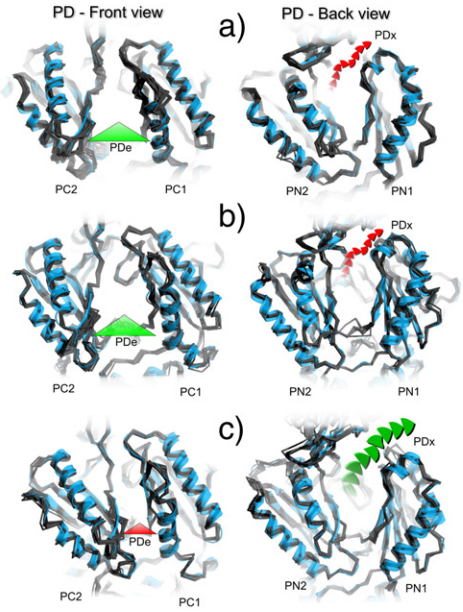


Fig. 1. Simulation system and porter domain accessibility. Embedded in the inner cell membrane (IM) AcrB acts as active transporter in the tripartite AcrAB–TolC multidrug efflux pump transferring a broad range of substrates towards TolC via the central funnel (CF) in AcrB docking domain (a). Here we report molecular dynamics simulation of asymmetric 2GIF AcrB in a phospholipid membrane/water environment at a 150 mM NaCl concentration (b). Monomer asymmetry is mainly based on different conformations of the porter domain (PD). Monomers A and B exhibit a large periplasmic cleft between PC1 and PC2 subdomains marking the main entrance PDe of the substrate transport channel, which is closed in monomer C (b). Conversely, the exit of the transport channel PDx is open only in monomer C. Resultant different accessibilities of the porter domain are highlighted by monomer-internal tunnel-like cavities as detected by Caver 2.0 [55] (c).

monomer (Fig. 2). Whether this high conformational similarity represents an intrinsic feature of AcrB or originates from other causes is currently not known.

So far computational investigations of AcrB have focused on assessing conformational flexibility via normal mode analyses [26], coarse-grained molecular dynamics (MD) studies of isolated protein sections [27], simulating conformational transitions using targeted MD techniques [28–30], as well as predicting water distribution and dynamics in the energy-converting trans-membrane domain based on which three possible proton conduction pathways were derived [31]. Here we report molecular dynamics simulations of asymmetric AcrB addressing the question why all available crystal structures show very similar, monomer-characteristic PD conformations. To provide evidence whether this high level of conformational homogeneity represents an intrinsic feature of the protein or could be related to AcrB crystallization conditions, we simulated wild-type, substrate-free AcrB [9] in a close-to-native, phospholipid membrane/water environment at 150 mM NaCl concentration to obtain samples of unrestrained wild type AcrB dynamics outside a crystal environment in a series of six unbiased and independent MD runs each 100 ns long. As in our previous work [31] standard protonation states were assumed for titratable except for the known key residues of proton conductance Asp407, Asp408, Lys940 and Arg971 which were protonated monomer-specifically according to [10].

Using distance, cross-sectional area and radius of gyration analyses to monitor the PDe, PDx and HBP opening state in each monomer, we find that the porter domain is more flexible than previously assumed displaying clear opening and closing motions of the proximal binding pocket in the L and T states as well as in the exit region of the drug transport channels in the O intermediate supporting the hypothesis of Gln125 and Tyr758 acting as gating residues [17]. Concurrently in all simulations the hydrophobic binding pocket collapses in the T monomer resulting in predominantly closed HBP conformations in all three protomers. Comparing our protein conformations to AcrB X-ray structures our findings



Symmetric AcrB		Asymmetric AcrB			
PDB-ID	RMSD (Å)	PDB-ID	A	B	C
1IWG	0.642	2DHH	0.491	0.498	0.560
1OY6	0.651	2DR6	0.536	0.572	0.570
1OY8	0.661	2DRD	0.461	0.506	0.559
1OY9	0.686	2J8S	0.680	0.514	0.423
1OYD	0.710	3AOA	0.462	0.448	0.564
1OYE	0.686	3AOB	0.412	0.392	0.532
1T9T	0.697	3AOC	0.464	0.442	0.516
1T9U	0.713	3AOD	0.393	0.372	0.506
1T9V	0.707	3NOC	0.440	0.667	0.410
1T9W	0.748	3NOG	0.816	0.779	0.817
1T9X	0.727	4DX5	0.350	0.427	0.385
1T9Y	0.728	4DX6	0.482	0.504	0.430
2HQC	0.680	4DX8	0.404	0.407	0.366
2HQD	0.703	2HRT	0.244	0.238	0.237
2HQF	0.654				
2HQG	0.602				
2I6W	0.638				
2W1B	0.735				
2RDD	0.680				
3D9B	0.537				
Av.	0.683	Av.	0.488	0.474	0.491

Fig. 2. Comparison of AcrB crystal structures. Superimposing the available 33 X-ray structures using the porter domain (PD) α-carbons of the 2GIF X-ray as reference (blue) it becomes evident, that all crystal structures display nearly identical PD conformations in the same reaction cycle intermediate with Ca root mean square displacements below 0.1 nm. Green and red arrows mark open and closed PDe and PDx conformations.

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