MASS SPECTROMETRY OF A TRANSMEMBRANE PUMP REVEALS ITS SPECIFIC DRUG AND LIPID BINDING PROPERTIES

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Introduction

Multidrug resistance is a serious barrier to successful treatment of many human cancers. P-glycoprotein is over-expressed in cancer tissues, which then export the chemotherapeutic agents and profoundly affect drug therapies. Understanding these molecular mechanisms is therefore of paramount importance. While structures of inward facing conformations have been solved[1], the drug binding mechanism is still poorly understood. Furthermore P-gp, is known to bind lipids with a broad specificity[2]. However, the role of lipid binding and the competition between lipid and drug binding is poorly understood. We show here that it is possible to observe both lipid and drug binding to P-gp using electrospray MS of the protein complex from detergent micelle. We propose structural models based on these MS results.

Results

Drug binding:

Following incubation of P-gp with cyclosporine A (CsA), up to three additional charge state series were observed, corresponding to binding of up to three CsA adducts. The binding of more than one molecule of CsA per transporter is not surprising, given the existence of up to four different binding sites within the binding pocket.

ATP/ATPγS binding and concomitant binding:

Whether or not P-gp can bind concomitantly two molecules of ATP is still subject to great debate. Our data confirms that P-gp can bind two ATP/ATPγS at the same time. The concomitant binding of lipids and ATP, CsA and ATP and CsA and cardiolipin is clearly visible using this technique.

Lipid binding:

Upon addition of 7 different phospholipids to P-gp, we could observe the sequential binding of up to 8 molecules of lipid per transporter. We also investigated the binding of cardiolipins of different size. The time scale observed for the binding of lipids to P-gp is in good accordance with previous fluorescence quenching experiments. Further analysis of the data enabled us to get Kd for each one of these lipids and each one of the binding sites. We could relate the specificity of binding to the net charge and the molecular volume of the lipids.

Modeling:

Using a shape based approached we docked the drugs/lipids onto the structure of P-gp available and minimized the energy with Firedock. Our data confirms the fact that P-gp can bind up to 3 CsA or 2 cardiolipins in the binding pockets. Molecular dynamics simulations are under progress.

Novel aspect

First study of drug and lipid binding to a membrane complex introduced into the gas phase in a detergent micelle.

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[1] *Aller, S. G. et al. Structure of P-Glycoprotein Reveals a Molecular Basis for Poly-Specific Drug Binding. Science 323, 1718-1722, doi:10.1126/science.1168750 (2009).*

[2] *Van Helvoort, A. et al. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 87, 507-517, doi:S0092-8674(00)81370-7 [pii] (1996).*