Role of Solvation and effect of cosolvents/crowders in the molecular recognition of hydrophobic drugs.

INTRODUCTION

The conformation, stability, binding, and biological function of a protein are governed by its Gibbs energy *G* (including enthalpy and entropy). The protein Gibbs energy is itself the results of two contributions, one coming from the protein atoms and the other one from the water molecules hydrating the protein (i.e. water molecules which thermodynamic properties have been altered by the presence of the protein). From the variation in Gibbs energy (ΔG) associated with a binding event, we can derive its binding constant K_B, knowing that $\Delta G = -RT \times \ln(K_B)$. Since a protein-ligand binding is generally accompanied by dehydration, *i.e.* a release of water molecules from the binding site, dehydration represents an energetics player[1]. In fact, it has been shown that (de)hydration account for up to 50% of affinity and specificity in molecular recognition mechanisms. This feature is preferentially observed when the binding sites are dominantly hydrophobic[2]. The process of (de)hydration is closely related to water activity, in particular to the difference in activity between water molecules hydrating the protein and those in the bulk. Many events can modify water activity and thus alter binding properties. First, it has been established that a controlled crowding/confinement level modulates water activity[3]. This fact is fundamental as therapeutic targeted proteins are located *in vivo*, within a crowded or/and confined biological micro-environment (where up to 20 – 30% of a cell is occupied[4]). Unfortunately, most studies on drug-protein bindings are performed *in vitro* in a diluted sample, hence a drug that has been demonstrated to be efficient *in vitro* may become useless *in vivo*. Secondly, the study of insoluble hydrophobic drugs requires the presence of a cosolvent (dimethyl sulfoxide (DMSO) and ethanol are the most common) that helps its solubilization. Since most studies of protein binding are performed in a diluted sample and/or in the presence of a co-solvent, they do not reflect the binding event properties occuring *in vivo.* More generally, little is known on these binding properties *in vivo*.

All these facts have an important impact in the pharmaceutical field, and more specifically on drug design. Elaborating hydrodrophobic drugs that possess a high and specific affinity for a given therapeutic target (*e.g.* protein or DNA) is generally difficult because only a few techniques allow direct measurements of hydration changes. It remains very unpractical to obtain viable information about the entropic contribution due to hydration changes, although it plays a major role on the affinity of hydrophobic ligands.

In this project, we propose an original solution to this problem by mixing volumetric experiments as well as molecular simulations. In the long term, our strategy should help the development of hydrophobic drugs.

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OBJECTIVES

First, we aim to provide at the molecular level an understanding of the role of hydration in ligand binding events when available space is restricted, as in a crowded cell. Secondly, we wish to establish simple rules, based on surface coefficients, that will help accurately evaluate the hydration contribution to entropy and binding constant. Finally, we wish to characterize the modifications induced to hydration by co-solvents commonly used in the study of insoluble drugs.

PROJECT DESCRIPTION

Systems under studies. To reach our objectives, we will focus our investigation on a few proteins exhibiting binding sites that are mainly hydrophobic and exhibiting little conformation changes during the binding event. Indeed, in this case the binding event is driven by the dehydration process. Our project will start with two pharmaceutical drug targets: the enzymes Quinone Reductase 2 (QR2) and Glucokinase (GK). Several recent studies suggest that QR2 occupies a central part in neurodegenerative diseases and is thus considered as a novel therapeutic strategy. Whereas GK is the main glucose phosphorylating enzyme expressed in pancreatic β-cells and hepatocytes of most mammals, where it plays a central role in glucose homeostasis. In β-cells, GK is a critical component of the glucose sensor mechanism controlling insulin secretion. Both enzymes exist as dimers where each monomer possesses one hydrophobic binding site available for small hydrophobic drugs. In addition, the two enzymes first have to bind to their cofactors (flavin adenine dinucleotide and adénosine-5'-triphosphateATP, respectively) before being able to bind drugs. These cofactors rigidify the enzymes and suppress potential conformation changes induced by drug binding.

Strategy to be followed. The binding process will be studied by coupling measurements and simulations. Indeed, the strategy consists in studying the variations of volumetric parameters (*i.e.* volume and its derivatives like compressibility, expansibility...) that are known to be highly sensitive to any change in hydration. Among these parameters, the ultrasound sound velocity (related to adiabatic compressibility through Laplace-Newton equation) appears to be the most sensitive [ref]. If volumetric measurements give a macroscopic insight of the drug binding event, they are not sufficient to provide an understanding at the molecular level. Thus, molecular dynamics simulations coupled to experimental data will provide the molecular details of the binding event, with all the details concerning hydration. Simulations will be possible since the crystal structure of the enzymes (as well as drug-enzyme complexes) have already been determined by the industrial partner.

The binding of these enzymes will be studied with several hydrophobic drugs such as melatonin, revesterol, 5-methoxycarbonylamino-N-acetyltryptamine (MCA-NAT)... that are drugs known to binds QR2 and GK. As these drugs are not soluble in water, they will be solubilized either in the presence of a co-solvent (as done in most *in vitro* studies), or with the help of a specific known drug carriers such as cyclodextrin[1] or human serum albumin[2]. In order to mimic a crowded cell in a simplified manner, biological crowders such as amino acids (*e.g.* glycine) and short polypeptide chains (*e.g.* poly-glycine) will be added to the sample.

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For each drug-enzyme complex, the following task will be performed :

- First titration measurements will be performed in which aliquots of drug solution will be added to the enzyme solution. These measurements consist in measuring the density and sound velocity changes induced during the titration. The partial volume, partial expansibility, and partial adiabatic compressibility will be derived from these experimental data. These values contain information about conformational and hydration changes. We will also extract the thermodynamics properties of the drug-to-enzyme binding (i.e. Gibbs energy, enthalpy, and entropy).

- Second, molecular dynamics simulations will be performed on the same systems. The partial volume, expansibility, and adiabatic compressibility will be computed and compared to the experimental values to validate the simulation. If experimental and numerical values do not match, force-field optimization of the ligands may be considered.

Once validated, the simulations will be analyzed in order to derive a microscopic interpretation of hydration changes:

- Radial distribution functions (RDFs) between the solute (enzyme) and the cosolvent molecules (and/or between solute and crowders/co-solvent) will be calculated and used for evaluating Kirkwood Buff integrals (these latter are easily obtained by integrating the RDFs). We will relate these integrals to the thermodynamic properties (volume, compressibility, expansibility...) of the binding.

- Using Voronoï tesselations, we will correlate the changes in hydration to the surface of the binding sites. This result will give surface coefficients which should allow a rapid evaluation of the binding constant from simple surface area calculation.

This approach will be performed for:

- Several levels of crowding (*i.e.* quantity of added osmolyte). This will permit to understand how crowded spaces act on a binding event *in vivo* compared to a diluted sample in a test tube.

- For drugs solubilized in a co-solvent. This will provide an understanding of the change induced by a co-solvent in drug binding, knowing that the co-solvent may change the bulk water activity and alter the hydration shell of both enzyme and drug.

- For drug solubilized by a drug carrier. In such a case, the drug carrier does not alter the hydration shell of the enzyme as there is no direct interaction between enzyme and carriers. This system provides thus a “true” value for the binding constant in contrast to values measured in the presence of a co-solvent that modifies the activities of waters in bulk and in hydration shell.

Context. The experimental part will be performed under the supervision of N. Taulier. He is a physicist who masters and uses high precision ultrasound techniques to study hydration of numerous biomolecules. He possesses at LIB its own experimental set-up that have been specifically build for this purpose. The molecular dynamics simulations part will be supervised by P. Fuchs who is collaborating with the developers of GROMOS (a popular biomolecular force-field which is likely to be used in this project). This interdisciplinary project at the physics/biology interface will be performed in collaboration with “Institut de Recherches Servier”. The industrial partner will provide all the enzymes in sufficient quantity as well as for the studied drugs.