Determination of effector binding affinities using PAC

by David Butcher | Jovany J Betancourt | Jaroslava Miksovska

Abstract Id: ***219*** *Submitted:* ***March 8, 2017*** *Event:* ***Conference for Undergraduate Research at FIU 2017***
Topic: ***Biochemistry***

Determination of binding affinity and thermodynamic parameters of interactions between two molecules is a common procedure in biochemical contexts. Steady-state fluorescence titration is one of the most commonly used techniques to determine the binding affinity of small effector compounds and ligands to large biomolecules such as proteins. This method, however requires 1) the ligand to be fluorescent and exhibit a measureable change in fluorescence intensity between the bound and free states or 2) a fluorescent residue (e.g. tryptophan) near the ligand binding site whose fluorescence changes as a result of ligand binding. The new technique presented here utilizes photoacoustic calorimetry (PAC) in conjunction with conventional titration techniques to determine the binding affinity and thermodynamic parameters of a ligand binding to a protein. This technique does not require a fluorescent ligand or amino acid residue to be present and instead utilizes the change in the PAC signal as a function of ligand concentration to determine these parameters. So far, we have used this technique to determine the dissociation constant (K_D) and enthalpy change (?H) for binding of inositol hexakisphosphate (IHP) (K_D = 73.5 mM at 20 °C, ?H = 9.8 kJ mol^{-1}) to human adult hemoglobin (HbA). Future trials will look at other common ligands for HbA as well as other heme proteins. Being able to determine the binding affinity and thermodynamic parameters for ligand binding to proteins is extremely useful for understanding the environment of the ligand binding site potentially allowing us to create new and more effective drugs. The use of PAC may provide a possible method of evaluating these characteristics quickly.

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