Intrinsic Binding Parameters as a Necessity to Correlate Energetics with Structure
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Introduction
One of the major obstacles in the structure-based drug design field remains the poor correlation between structure and energy of lead compound binding. In order to draw a reasonable energy and structure correlation one has to determine the intrinsic binding thermodynamic parameters. The intrinsic binding parameters of inhibitor-protein complex usually differ from the observed parameters obtained directly by isothermal titration calorimetry (ITC) or other affinity experiments. ITC is a well-established method for determining the association constant and other thermodynamic parameters (such as equilibrium binding enthalpy, entropy, and the Gibbs free energy) of intermolecular interactions in aqueous solutions. This method is also very powerful for determination of intrinsic binding parameters that could be used in structure-energy correlations. Moreover, the limitations of ITC when determining extremely weak and tight ligands binding can be partially overcome by supplementing the ITC results with data from thermal shift assay (TSA). Thus, TSA helps to determine or confirm affinities obtained by ITC. Here we present the determination of the intrinsic binding parameters for two inhibitor-target protein systems: radicicol–Hsp90 and ethoxzolamide–carbonic anhydrase (CA II). Both proteins are widely used as drug targets and the improved inhibitors are being sought.

ITC and TSA raw data and ligand dosing curves

Determination of the intrinsic enthalpy
The relationship between observed (ΔbHobs) and intrinsic (ΔbHint) enthalpies is

\[ \Delta_{bH}^{obs} = \Delta_{bH}^{intr} + n \Delta_{bH}^{complex} + n \Delta_{bH}^{surface} \]

where n is the number of linked protonation events and ΔbHcomplex, ΔbHsurface is the enthalpy of protein and/or ligand binding-linked protonation.

Three steps are necessary to determine the intrinsic binding enthalpy:

a. the binding ITC experiment must be conducted in several buffers (left panel);
   b. if there is difference in observed enthalpies, then it is desirable to repeat ITC experiments in several buffers at multiple pHs (top right panel);
   c. the numbers of protons transferred are plotted as a function of pH, determining the pKa of the linked protonation reaction (bottom right panel).

Comparison of binding and inhibition constants

References

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