Determination of intrinsic enthalpy of CA-ligand binding and attempts to correlate it with the crystallographic structures of the protein-ligand complexes

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Over 700 primary sulfonamides were synthesized with the goal to determine the chemical structure correlations with the thermodynamics of binding to the family of 12 human carbonic anhydrase (CA) isoforms. Human CA isoform catalytic domains were affinity-purified in large quantities, sufficient for isothermal titration calorimetry (ITC) and crystallography. In addition to ITC, the binding affinities were determined by the thermal shift assay (FTSA or differential scanning fluorimetry, DSF), inhibition of enzymatic activity, and SPR. The enthalpy and entropy changes upon binding were determined by ITC for a selection of compounds and CA isoforms. A correlation map between the compound chemical structure and the binding Gibbs energies (ΔG) and enthalpies (ΔH) was drawn. The map showed which structural features of the compounds yielded the highest increments in affinity and exothermicity of compound binding. Furthermore, only some structural features were useful in generating compounds that selectively bind to cancer-expressing CA isoforms, but would not bind to other essential-for-life human CA isoforms. Over 70 X-ray crystal structures showed the position of compounds bound in the enzyme active center.

Target-based drug design is based on the discovery and selection of a most-strongly binding compound to a target protein. However, the binding affinity and the binding mechanism is a result of highly compensating enthalpic and entropic contributions. Homologous compounds having similar affinities often exhibited significantly different enthalpies and entropies of binding. It was essential to dissect the contributions from binding-linked reactions such as buffer, ligand or protein protonation. After the subtraction of pH-dependent buffer contribution to the enthalpy of binding, the *intrinsic* Gibbs energies and enthalpies of binding were obtained. It was important to calculate the intrinsic parameters and use only them in the structure-thermodynamics correlation maps when designing compounds of higher affinity as drug candidates.

The intrinsic enthalpies of binding spanned a region from -90 to +10 kJ/mol for relatively similar structures and affinities of binding. The entropy largely compensated the enthalpy resulting in the well-known phenomenon of enthalpy-entropy compensation that could not arise due to an error of ITC measurements. Based on high-resolution crystallographic structures of unliganded and liganded proteins, there were no observable movements of the main protein chain upon ligand binding. Therefore, it seems that the only possible explanation for large variation of the enthalpies must be hidden in the water molecule behavior at the hydrating layers of the protein and ligand.