Chapter 7  Thermodynamic Data for Protein-Ligand Interaction

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Symbols

ΔG₀: standard Gibbs energy change
ΔH₀: standard enthalpy change
ΔS₀: standard entropy change
ΔC₀: standard molar heat capacity change
ΔH^[s]: apparent molar enthalpy change
ΔG^[s]: apparent molar Gibbs energy change
ΔS^[s]: apparent molar entropy change
ΔC^[s]: apparent molar heat capacity change

1 Introduction

Knowledge of the magnitude of the energy, entropy, and heat capacity changes involved in ligand binding equilibria of biological compounds is essential for a prediction of the thermodynamic properties of the systems under different environmental conditions. Quantitative thermodynamic data will provide the ultimate test for the quality of ab initio theoretical stability calculations of binding phenomena if such treatments become possible in the future. Heat capacity changes are an excellent qualitative indicator of the structural changes (including the hydration changes) that many proteins and ligands undergo on complex formation [1, 2]. Since heat capacity is related to the number of degrees of freedom in the distribution of enthalpy states [3], changes in heat capacity resulting from ligand binding may reflect changes in the static and the dynamic properties of the macromolecule.

The structural changes associated with the energy changes on complex formation can result in permanent shifts of the relative positions of the atoms of the macromolecule large enough to be detectable by X-ray analysis. This is understandable if one realizes that the forces operative between proteins and ligands are of the same order of magnitude as the forces stabilizing the native structure of proteins [4–6]. However, X-ray analysis will only detect the more spectacular structural alterations, since, depending on the characteristic internal macromolecular interactions, energy changes can be distributed among a large number of weak interactions, each of which need not produce sizable changes in the static structure of the respective groups. Therefore changes in heat capacity will be a more sensitive but less uniquely interpretable diagnostic tool for the detection of “structural” changes particularly, since changes in heat capacity may also reflect
changes in the soft internal vibrational and rotational modes of the macromolecule [1], as well as reactions with third components, such as water or buffer ions, concomitant with ligand binding. Thus, when properly interpreted, the energy and entropy data can provide more than just thermodynamic insight into the reaction.

2 Thermodynamic Quantities and Their Measurements

The thermodynamic quantities useful for characterization of a ligand binding equilibrium are the standard Gibbs energy change, $\Delta G^0$, the standard enthalpy change, $\Delta H^0$, the standard entropy change, $\Delta S^0$, and the heat capacity change at constant pressure, $\Delta C_p^0$.

2.1 Gibbs Energy Changes

For biochemical ligand binding equilibria $\Delta G^0$ is usually obtained from the equilibrium constant, $K_{eq}$, employing the relationship

$$\Delta G^0 = -RT \cdot \ln K_{eq},$$

where $R$ is the gas constant and $T$ the absolute temperature. $\Delta G^0$ will be given in J mol$^{-1}$, if $R$ is expressed in J mol$^{-1}$ K$^{-1}$. The value used for $R = 8.314$ J mol$^{-1}$ K$^{-1}$. In general the numerical value of $\Delta G^0$ will depend on the concentration units employed in calculating the equilibrium constant, unless $\Sigma n_i = 0$. For clarification, see the excellent chapters in [3] and [7]. While for reactions between inorganic or organic compounds mole fractions are often appropriate concentration units, the most widely used measure of concentration in biochemical studies is the molarity, i.e., the number of moles of the solute per dm$^3$ of the solution.

Although generally formulation of the equilibrium constant would involve the activities of the reacting species, lack of knowledge of activity coefficients for most reactions involving biological macromolecules necessitates assuming the activity coefficients to be unity and using the equilibrium concentrations. Therefore the value of the equilibrium constant and concomitantly that of $\Delta G^0$ may vary with composition. Since protein ligand equilibria often depend on many factors such as pH, salts, reducing agents, and buffering compounds, and since their influence on $K_{eq}$ is seldom known, it is appropriate to use apparent equilibrium constants $K_{eq}'$ and apparent Gibbs energies $\Delta G^0'$. It should be mentioned that this definition is not identical to the convention often used in biochemical research, where a standard Gibbs energy change referring to pH 7 is specified by a prime.

2.2 Enthalpy Changes

Apparent standard enthalpy changes of ligand binding reactions can be indirectly obtained from the dependence on temperature of the apparent equilibrium constant $K_{eq}'$ according to the van’t Hoff equation

$$\frac{d\ln K_{eq}'}{d(1/T)} = -(\Delta H^0'/R),$$

(2)