Microcalorimetry as a tool for the development of drug delivery system

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ABSTRACT *Small-molecule drug discovery involves the optimization of various physicochemical properties of a ligand, particularly its binding affinity for its target receptor (or receptors). In recent years, there has been growing interest in using calorimetric profiling of ligand–receptor interactions in order to select and optimize those ligands that might be most likely to become drug candidates with desirable physicochemical properties. Microcalorimetry is an wonderful analytical tool in the sense of measuring the property (heat) as ubiquitous. Any type of binding process is always open to microcalorimetric investigation, since change in heat content is always associated with that process. The thermodynamics of small molecule binding is influenced by multiple factors, including hydrogen bonding and hydrophobic interactions, desolvation, residual mobility, dynamics and the local water structure. This article discusses key issues in understanding the effects of these factors and applying this knowledge in drug discovery.*

Keywords: *Microcalorimetry, drug, Isothermal titration calorimetry, Differential scanning calorimetry.*

1. Introduction

New analytical tools are constantly being developed and there is a wide array of techniques with which to interrogate pharmaceuticals and biomolecules. Popular among these are those classified under 'Thermal Analysis'; of these, two distinct ultrasensitive microcalorimetric techniques, isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC) are very widely used because of their unique set of qualities they offer. Prime among these are the facts that the measured parameter (heat) is ubiquitous and the calorimeter is invariant to the physical form of the sample, which means that as well as (the traditionally studied) single compounds, even complex formulated medicines can be investigated [1-3]. Thermal analysis is considered as one of the most popular techniques in material sciences and engineering and is a highly sensitive technique to study the thermotropic properties of many different macromolecules. Thermal analysis has been applied to the pharmaceutical field for studying excipients, biomaterials, and drugs. Applications of this technique to lipid-based systems such as artificial biological membranes include the measurement of thermodynamic parameters and a detailed characterization of thermotropic and phase transition behaviour [4]. Furthermore, thermal analysis casts light in a total new scientific perspective by treating drugs as biomaterials and not as plain materials. In the pharmaceutical sciences, only a handful of techniques are commonly employed, but the information gained and phenomena, like aggregation, that can be explored are countless. As formulations become more and more complex and characterizing them becomes more difficult, manufacturers have done an excellent work in keeping pace with more precise and sensitive yet more durable instruments. Especially, advanced drug delivery technologies focus on the connection between physicochemical characteristics (polymorphism, fluidity, surface charge, thermotropic behaviour of lipidic membranes, etc.) of membranes with the alteration of pharmacokinetics and bioavailability of drugs.

The biophysical and the thermodynamic approaches for the design and development of nano systems and advanced drug delivery platforms can be correlated with their phase transitions, i.e., from gel (L_β) to liquid crystalline phase (L_α), possible through metastable phases (ripple phase, P_β) depending on the chemical and physicochemical properties of the building blocks of the system. Moreover, these metastable phase transitions are considered as 'thermodynamical vehicles' that could be correlated with the functionality of the nano system and the effectiveness of the nanoparticulate medicine (i.e., release and kinetics properties) [5].

Biology is based on the study of defined interactions between macromolecules such as proteins, DNA and lipids. Therefore, to understand biology, we must have a way of quantifying the driving forces which make such interactions occur. Thermodynamic parameterization provides one form of quantification. By assuming the interaction occurs under equilibrium conditions, we can provide a measure of the 'tightness' or 'affinity' of the interaction. A further level of information can be gleaned by determining the underlying changes in enthalpy (ΔH) and entropy (ΔS) for the interaction. These data can be determined directly

employing the technique of Isothermal Titration Calorimetry (ITC) or by indirect method using van't Hoff analysis thorough the measurement of fluorescence or absorbance at different temperatures.

2. Results and Discussion

2.1. Isothermal titration calorimetry

Isothermal titration calorimeters measure the heat change that occurs when two molecules interact. Heat is liberated or absorbed as a result of the redistribution of noncovalent bonds when the interacting molecules go from the free to the bound state. ITC monitors these heat changes by measuring the differential power required to maintain zero temperature difference between a reference and a sample cell as the binding partners are mixed. The reference cell usually contains water or buffer, while the sample cell contains one of the binding partners and a stirring syringe that holds the other binding partner (the ligand). The ligand is injected into the sample cell, typically in 0.5–2 μl aliquots, until the ligand concentration is two to threefold greater than the sample. Each ligand injection results in a heat pulse that is integrated with respect to time and normalized for concentration to generate a titration curve of kcal/mol versus molar ratio (ligand/sample). A binding model is fitted to the the resulting isotherm (data) to obtain the equilibrium binding constant (K_B), stoichiometry (N), and enthalpy of interaction (ΔH). The Gibbs free energy (ΔG) and the change in the entropy (ΔS) upon binding can then be calculated using the relationship

$$\Delta G = -RT \ln K_B = \Delta H - T\Delta S \qquad (1)$$

where R is the gas constant and T is the absolute temperature in Kelvin. In addition to these parameters, it is possible to determine the change in heat capacity of an interaction (ΔC_P) by determining the change in enthalpy at different temperatures (T) and using the relationship

$$\Delta \boldsymbol{C_p} = \frac{\partial \Delta H}{\partial T} \qquad (2)$$

It should be noted that the calorimetric experiment does not just include measurement of the heat associated with the formation of a complex, but also the heat associated with other potential accompanying events such as solvent rearrangement, protonation/deprotonation and conformational changes in the interacting molecules [6-9].

Two further derivative forms of isothermal calorimetry are popular; isothermal titration calorimetry (ITC) and solution calorimetry (SC). In ITC small aliquots of a titrant solution (held in a reservoir external to the instrument) are added in sequential aliquots to a solution held within the calorimetric vessel and the heat change per injection is recorded. In SC the heat change when a solute (usually a solid but liquids may also be used) is dispersed in a large volume of solvent (to ensure complete dissolution) is measured. Usually this is achieved using 'ampoule breaking' instrumentation (wherein the solute is held in an ampoule which is mechanically broken into the solvent reservoir). Two types of solution calorimeter design are commercially available; instruments that operate on a semi-adiabatic principle (i.e. that record a temperature change upon reaction) and instruments that operate on a heat-conduction principle (i.e. that record a power change directly upon reaction). Further details of the experimental advantages and disadvantages of the techniques have been reported elsewhere [10].

2.2. Van't Hoff analysis

It is possible to access enthalpy and entropy values without the need for calorimetric experiments. These thermodynamic parameters may be estimated using indirect methods, which make use of the temperature dependence of the binding affinity, by employing the van't Hoff equation. This allows estimates of entropy and enthalpy to be made using any technique that allows the determination of the binding affinity at a range of temperatures. Equation 3 is an integrated form of the van't Hoff equation, and it is clear from inspection that the enthalpy can be derived from changes in binding affinity as long as the constant pressure heat capacity change upon ligand binding (ΔCp) is known or can be fitted. The binding entropy can then be determined from the Gibbs–Helmholtz equation in the usual way. Thus, a number of alternative methods to measure K_B, including SPR, microscale thermophoresis (MST), fluorescence, and radioligand binding assays can be used to determine van't Hoff enthalpies. The experimental design should be such that binding affinities are determined over a wide temperature range (within which the protein retains its native fold) so that the enthalpy change associated with binding can then be calculated using the van't Hoff relationship shown in Equation 3.

$$\boldsymbol{ln}\frac{\boldsymbol{K_1}}{\boldsymbol{K_2}} = \frac{(\Delta H - T_1 \Delta C_p)}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) + \frac{\Delta C_p}{R}\,\boldsymbol{ln}\frac{T_2}{T_1} \qquad (3)$$

where the values for K_1 and K_2 are the dissociation constants at different temperatures, T_1 and T_2.

The use of the indirect van't Hoff approach is not without potential difficulties. Firstly, binding enthalpy is itself temperature dependent, and so the inclusion of the ΔC_p term is required. Estimating ΔC_p in the absence of calorimetric data is often difficult, as deriving ΔC_p from Equation 3 requires true curvature in the van't Hoff plot to be distinguishable from apparent curvature due to errors in the affinity measurement. Hence, this indirect approach requires accurate and precise K_B values. Secondly, the temperature dependent change in ΔG often is relatively small, which makes deriving the two correlated parameters from this data quite challenging, which may result in relatively large uncertainty in the derived enthalpy compared with the direct calorimetric approach.

2.3. Thermodynamic parameters of biological system

The thermodynamic parameters from the ITC experiment interpret biologically relevant interactions and as input to drug development. One major reason for quantification of biomolecular interactions is to ascertain how tightly they interact. The K_B and hence the derived ΔG give a direct evaluation of this. A good example of the use of the affinity measurement in understanding biology is in understanding the issue of specificity and selectivity in protein interactions in tyrosine kinase-mediated signal transduction [9, 11-13].

In the last past decades a series of guidelines have emerged that have been broadly used to interpret thermodynamic data and have been proposed as key drivers for lead optimization programs. At the simplest level they can be summarized as [14,15] :

a) Hydrogen bonds have a favourable enthalpy.

b) Hydrophobic interactions have a favourable entropy.

c) Conformational changes are entropically unfavourable.

By applying these guidelines the medicinal chemist can, in theory, test the success or failure of their optimization strategies. For example, if an effective hydrogen bond was successfully introduced, then one would expect to see an increase in the affinity of the interaction and a more negative enthalpy. If so, further iterations could be tested, and if not, determination of the complex structure may reveal some interesting and unexpected SAR. Equally, the success or failure of strategies to rigidify a ligand scaffold can be assessed by monitoring any reduction in unfavourable entropy of an interaction.

Either coincidentally or because of the emergence of ITC as a convenient assay to determine the quality of a hydrogen bond, there have been a number of articles promoting enthalpy driven lead optimization strategies [16, 17]. It is clearly an attractive prospect to be able to quickly develop a drug with high efficacy using a combination of ITC,X-ray crystallography, molecular modeling, and medicinal chemistry. However, more recently, and perhaps not surprisingly, examples[1] have emerged suggesting that thermodynamic lead optimization is more complex than originally thought. Here we outline a number of additional factors that need to be considered when attempting thermodynamic lead optimization.

2.4. Thermodynamic-structure correlation

Our understanding of the enthalpic and entropic contributions to binding is one of the major challenges in molecular biophysics to be improved. The major hurdle in this endeavour involves the correlation of thermodynamic data with structural detail. Provided with a high-resolution structure which enables precise definition of the positions and lengths of non-covalent bonds and its variation, it would be a major achievement to be able to predict the affinity of an interaction and the underlying thermodynamic parameters associated with binding. This would be of potentially huge financial and temporal benefit to the pharmaceutical industry, since it could circumvent the synthesis and assaying of numerous compounds designed to inhibit a specific binding site. Alternatively, it also would be of great value to be able to make some estimate of the structural nature of a binding site (or protein conformational change) simply from ascertaining the thermodynamic binding data on going from the free to the complexed form of a protein and ligand. The emphasis of this effort has to be in providing a tool capable of making simple correlations that can be determined easily, as opposed to more sophisticated tools such as complex energy calculations from molecular dynamics simulations. With this in mind, there have been numerous attempts to find correlations between binding data and changes in structural detail on going from the free to the bound state [14–16]. In many of these investigations, the structural component of the correlation has been represented by molecular surface area burial. This is easily measured using standard techniques (such as that described by Lee and Richards[17] based on the change in water-accessible surface on going from the free to the bound state.

2.5. Development of drug delivery system

In spite of the clearance of ITC for compatibility assessment, the benefits for formulated products are perhaps even greater. This is because of the stability of the technique to monitor the sample over time. The only issue facing the operator is to select a fraction of the sample for investigation that is representative of

the whole. ITC can also be useful in the development of drug delivery systems; for example, investigating the swelling of polymers often used in modified release systems. The observed rate of drug release from a polymeric drug delivery system is governed by a combination of diffusion, swelling and erosion so it is not a simple task to determine the effects of the polymer on the observed drug release rate[18]. They dispersed powdered polymer samples in water or buffer in a calorimeter and the heat associated with the swelling phenomena was recorded.

Dissolution occurred immediately following hydration of the polymer. Properties of the polymer blends were different from those of either constituent and correlated with those seen for polymer tablets during dissolution experiments. The data implied that solution calorimetry could be used to construct quantitative structure-activity relationships (QSARs) and hence to optimise selection of polymer blends for specific applications.

3. Concluding remarks

Calorimetric data are a vital complement to structural data in drug development and for the optimization of lead compounds. A complete thermodynamic profile for a binding interaction includes the binding free energy enthalpy entropy and heat capacity change. The thermodynamic profile reveals the predominant forces that drive the binding interaction. Favorable enthalpy contributions arise from favorable hydrogen bonding and van der Waals interactions, and favorable entropic contributions arise primarily from hydrophobic interactions and desolvation. Thermal denaturation methods provide valuable tools for rapid screening of the binding compound libraries and for quantitative measurement of ultratight binding interactions. High specificity does not demand high affinity. Full integration of calorimetry and thermodynamics into the drug discovery process requires development of high-throughput calorimeters to facilitate the acquisition of quantitative data [19].

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review

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