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On the link between conformational changes, ligand binding and heat capacity☆

S. Vega [a], O. Abian [a,c,d,e], A. Velazquez-Campoy [a,b,c,f,*]

[a] Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Unit IQFR-CSIC-BIFI, Universidad de Zaragoza, Zaragoza, Spain
[b] Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza, Zaragoza, Spain
[c] IIS Aragón, Zaragoza, Spain
[d] Instituto Aragonés de Ciencias de la Salud (IACS), Zaragoza, Spain
[e] Centro de Investigación Biomédica en Red en el Área Temática de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain
[f] Fundacion ARAID, Government of Aragon, Spain

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ABSTRACT

Background: Conformational changes coupled to ligand binding constitute the structural and energetics basis underlying cooperativity, allostery and, in general, protein regulation. These conformational rearrangements are associated with heat capacity changes. ITC is a unique technique for studying binding interactions because of the simultaneous determination of the binding affinity and enthalpy, and for providing the best estimates of binding heat capacity changes.
Scope of review: Still controversial issues in ligand binding are the discrimination between the "conformational selection model" and the "induced fit model", and whether or not conformational changes lead to temperature dependent apparent binding heat capacities. The assessment of conformational changes associated with ligand binding by ITC is discussed. In addition, the "conformational selection" and "induced fit" models are reconciled, and discussed within the context of intrinsically (partially) unstructured proteins.
Major conclusions: Conformational equilibrium is a major contribution to binding heat capacity changes. A simple model may explain both conformational selection and induced fit scenarios. A temperature-independent binding heat capacity does not necessarily indicate absence of conformational changes upon ligand binding. ITC provides information on the energetics of conformational changes associated with ligand binding (and other possible additional coupled equilibria).
General significance: Preferential ligand binding to certain protein states leads to an equilibrium shift that is reflected in the coupling between ligand binding and additional equilibria. This represents the structural/energetic basis of the widespread dependence of ligand binding parameters on temperature, as well as pH, ionic strength and the concentration of other chemical species. This article is part of a Special Issue entitled Microcalorimetry in the BioSciences — Principles and Applications, edited by Fadi Bou-Abdallah.

1. Introduction

The conformational landscape of a protein is constituted by a collection of states with populations depending on their conformational Gibbs energy. The conformational Gibbs energy can be modulated by extrinsic factors such as temperature, pressure, pH, ionic strength and ligands [1–4]. Different conformational states of a protein interact with a given ligand with different binding affinities, leading to a reduction in their overall Gibbs energy in a different extent. The conformational equilibrium is then redistributed towards those conformational states able to bind that ligand. These changes in the population of certain conformational states are observed macroscopically as an "apparent" global conformational change induced by the interaction with the ligand. However, the ligand does not elicit a conformational change, but it provides the Gibbs energy required for the redistribution of the populations and shifts the equilibrium towards particular conformational states within a pre-existing conformational equilibrium. This scenario is consistent with the broad definition of allostery: allosterism is the modulation of the protein conformational equilibrium by ligand binding [5–9].

This definition can be reconciled with the traditional, more restrictive, definition of allostery, that is, the cooperative phenomenon in which the binding of a given ligand to a macromolecule is influenced by the binding of another ligand. The different conformational states possess different ability to interact with other biological partners (i.e., binding affinity), leading to increased or decreased biological

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* Corresponding author.
E-mail address: adrianvc@unizar.es (A. Velazquez-Campoy).

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activity. Therefore, if the interaction of a protein with a ligand shifts the populations of the different conformational states, that interaction will determine its overall biological activity by influencing its ability to interact with other ligands. Hence, ligand binding interactions and coupled conformational changes constitute the basic mechanism for protein function regulation and control. It is important to point out that allostery does not imply cooperativity, but cooperativity usually implies allostery. A protein with a single ligand binding site displaying a simple conformational equilibrium with two conformational states having different ligand binding affinities is an allosteric system, but there is no cooperative behavior.

Conformational changes in a protein are accompanied by changes in the solvent accessible (polar and apolar) surface, which are in turn associated with a heat capacity change. In fact, solvent accessible surface burial is the main responsible for the heat capacity change upon ligand binding [10], and prediction algorithms for estimating binding heat capacities based on this notion have been developed [11–17]. Protein conformational changes upon ligand binding can be modeled applying the "conformational selection" and "induced fit" models. Although some controversy still remains related to the applicability and mechanistic relevance of these models, they will be compared and reconciled, as well as discussed within the context of intrinsically (partially) unstructured proteins. In fact, both models represent two different scenarios that are fully compatible with real situations. In addition, a temperature independent binding heat capacity is very often incorrectly considered as an indication of negligible conformational changes occurring upon ligand binding. However, conformational changes may be associated with either temperature dependent or independent binding heat capacity changes. As it will be explained, a simple general model is able to solve these controversies.

Nowadays ITC is commonly used in molecular and structural biology labs, becoming the preferred technique for characterizing binding interactions. The practical use of ITC ranges from the study of the interaction between two natural binding partners or the elucidation of binding cooperativity phenomena underlying allosteric regulation, to the optimization of lead compounds in drug discovery and development. ITC is unique among the many tools intended to study binding in that it allows the simultaneous determination of the association equilibrium constant, the binding enthalpy and the binding stoichiometry. Because of this, ITC provides the best estimates for binding heat capacity changes and evaluate their potential temperature dependency. Thus, ITC is very appropriate for obtaining energetic information on conformational changes associated with ligand binding, as well as assessing conformational changes associated with ligand binding through heat capacity measurements.

Here we intend to stress the connection between binding heat capacity changes and conformational changes upon ligand binding to a protein from an experimental point of view in isothermal titration calorimetry. The main points are: 1) describing different scenarios that a researcher might find regarding the experimental determination of binding heat capacities when studying a particular protein–ligand interaction; 2) explaining those different scenarios by a single general model and connect them to special cases of interaction models (lock-and-key, induced-fit, conformational selection), which can be considered special cases of the general model; and 3) providing a set of guidelines for interpreting possible experimental results.

2. A simple allosteric model: conformational change coupled to ligand binding

The simplest allosteric system consists in a protein P that may populate two conformational states, A and B, exhibiting different binding affinities for a ligand L (see Fig. 1) [18]. The binding polynomial for this system is given by [19–21]:

$$Z = 1 + K_{conf} + K_A[L] + K_{conf}K_B[L] \tag{1}$$

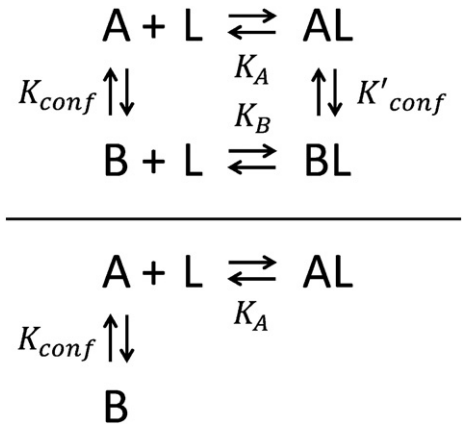

Fig. 1. Scheme depicting the coupling between the conformational equilibrium between two structurally distinguishable conformations for a protein and the ligand binding equilibrium. In the upper panel, both conformations are able to interact with the ligand with different binding affinities, K_A and K_B. In addition, both conformations A and B are related through a conformational equilibrium constant, K_{conf}. The additional equilibrium constant, K'_{conf}, connecting the complexes AL and BL, is not independent from the other constants and can be calculated from them (as a consequence of the energy conservation principle): $K'_{conf} = K_{conf}K_B/K_A$. For a molecule in conformation A forming the complex AL the equilibrium constant will be K_A; however, for a molecule in conformation B forming the complex AL the equilibrium constant will be either K_A/K_{conf} or K_B/K'_{conf}, depending on the route in the scheme. However, because there will be a mix of states (A, B, AL, BL), the apparent association constant is the population-weighted average of the intrinsic association constants (Eq. (4)). In the lower panel, conformation B is not able to interact with the ligand ($K_B = 0$). This assumption greatly simplifies the model and does not represent a loss in applicability, because the key point is the binding affinity difference and not the absolute values of the binding affinities for the two conformational states. The general case ($K_B \neq 0$) can be developed in a straightforward way and the conclusions drawn are equivalent.

where K_{conf} is the equilibrium constant for the conformational equilibrium, and K_A and K_B are the intrinsic association constants for the ligand binding to each conformational state:

$$\begin{aligned} K_{conf} &= \frac{[B]}{[A]} \\ K_A &= \frac{[AL]}{[A][L]} \\ K_B &= \frac{[BL]}{[B][L]} \end{aligned} \tag{2}$$

The two conformations may differ structurally in a variable extent and the conformational change connecting both structures may be small or large. Thus, conformations A and B may be two structured conformations, or conformation B may be (partially) unstructured. Each term in the binding polynomial reflects the relative statistical weight of the populations of the different protein states in the ensemble, taking the ligand-free conformation A as the reference state. From the point of view of the ligand binding it is convenient to renormalize the binding polynomial by regrouping all ligand-free terms (equivalent to consider as a reference the subensemble of ligand-free conformational states):

$$Z = 1 + \frac{K_A[L]}{1 + K_{conf}} + \frac{K_{conf}K_B[L]}{1 + K_{conf}} \tag{3}$$

This expression can be simplified by introducing an apparent association constant, K_{app}:

$$Z = 1 + \frac{K_A + K_{conf}K_B}{1 + K_{conf}}[L] = 1 + K_{app}[L] \tag{4}$$