Biophysical Chemistry 241 (2018) 38–49

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

Derivation and numerical profile analysis of a hierarchically formulated microscopic model of hemoglobin oxygen binding

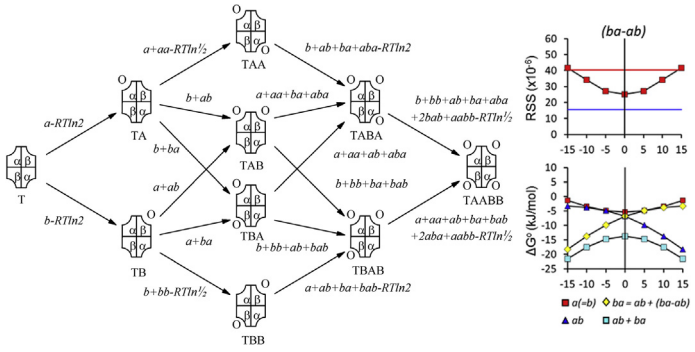
William G. Gutheil

School of Pharmacy, Division of Pharmaceutical Sciences, University of Missouri Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, USA

HIGHLIGHTS

- The hierarchical interaction concept is used to incorporate NMR-based observations into a microscopic model of hemoglobin oxygen binding.
- This provides a simple model (two independent ΔG°s) consistent with both NMR and UV–vis monitored hemoglobin oxygen binding data.
- A numerical-graphical profiling approach is then used to assess confidence intervals and parameter correlations for parameters within this model.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Protein-ligand binding
Thermodynamics
Hierarchical interaction
Cooperativity
Allosterism
Statistical analysis

ABSTRACT

To address complex thermodynamic systems with multiple interacting events, we have developed the concept of hierarchical thermodynamic interactions. In this study, this concept is extended to protein-ligand systems with similar but not identical protein subunits, and applied to the analysis of previously published NMR and UV–vis monitored hemoglobin oxygen binding data. Non–linear regression provided estimated errors for statistically significant parameters, but not for null (zero) valued parameters. A numerical/graphical profiling approach was therefore used to assess confidence intervals and correlations for both the statistically significant and nulled valued parameters in this model. Individual parameters were set to fixed values around their best–fit value, and the subset of statistically significant parameters re–minimized against hemoglobin oxygen binding data. Plots provide a graphical representation of parameter confidence intervals and correlations, and demonstrate how the two different data types – UV–vis and NMR – constrain the range of values for each parameter. This analysis further illustrates the value of hierarchically formulated models for the analysis of complex state systems, and illuminates the complexity of parameter space around the derived minimum microscopic model of hemoglobin oxygen binding.

1. Introduction

Hemoglobin remains the paradigm example of cooperative ligand binding in a multimeric protein, and provides the conceptual framework for understanding cooperative and allosteric regulation in biological systems [1–4]. Despite > 150 years of study, a complete understanding of hemoglobin's oxygen binding properties remains elusive. Understanding hemoglobin requires knowledge of the microscopic equilibrium constants defining all of the intermediates during oxygenation. UV–vis monitored hemoglobin oxygen binding data can

E-mail address: gutheilw@umkc.edu.

https://doi.org/10.1016/j.bpc.2018.07.008
Received 6 June 2018; Received in revised form 25 July 2018; Accepted 28 July 2018
Available online 04 August 2018
0301-4622/

be analyzed with macroscopic equilibrium models (reviewed in [5]), but the resulting macroscopic constants cannot readily be related to the microscopic constants defining the oxygen binding properties of hemoglobin. NMR monitored hemoglobin oxygen titrations have been informative concerning the relative proportion of oxygenated α and β subunits in hemoglobin, and on the conformational state and interactions of subunits [6–11]. However, NMR titrations have so far not been interpretable in terms of the microscopic equilibrium constants governing hemoglobin oxygen binding.

The difficulty in extracting values for the microscopic equilibrium constants from studies of native human hemoglobin is due in part to the dynamic nature of oxygenated hemoglobin intermediates, and in part to the cooperative nature of hemoglobin, which substantially reduces the concentration of oxygenated intermediates over the oxygen-binding isotherm. One approach to avoiding these problems is based on the use of ligation inert hemoglobin variants, and detailed studies of these variants have provided a consensus set of microscopic binding constants [12–17]. However, this approach has been questioned due to experimental problems [18–21], and also as to the validity of constants determined from such modified hemoglobin variants for native hemoglobin [22]. Therefore, despite efforts using a wide variety of experimental approaches, there is still a need for a microscopic thermodynamic model for hemoglobin oxygen binding which is derived from native hemoglobin oxygen binding data. Such a model would be of high interpretive value, and a reference point for comparison with the results obtained from other approaches. The aim of this study is to provide such a model.

A major problem in analysing complex ligand binding systems such as hemoglobin has been the inadequacy of conceptual frameworks available for defining such systems. Two extreme approaches are to either define the system in terms of constants of assembly (i.e. the ΔG°s for assembly of a complex from free components), or to define the system in terms of state-to-state constants (Fig. A1–1). When viewed in terms of constants of assembly, many ligand interactions are lumped together. When a complex system is viewed in terms of state-to-state constants, with a constant for each state-to-state transition, there are many more constants than states in the system, with the result that the system is over-determined. We have developed a conceptual approach where complex thermodynamic systems are described in terms of hierarchical interactions between ligands [23–29] (summarized in Appendix A1). The hallmark of the hierarchical interaction concept is to take a system described by N ΔG°s of assembly and to recast them into N ΔG°s describing the hierarchy of potential interactions in the system [24, 25] (Fig. A1–2). Higher order hierarchical interaction terms (e.g. *abc* in Fig. A1–2) reflect the mutual interaction of multiple ligands, and for these higher order terms to exist physically (or statistically) requires all lower order terms which they modulate to exist physically (e.g. *ab*, *ac*, and *bc* in Fig. A1–2). This concept has significant advantages over alternative formulations (summarized in Appendix A1). It is extended and applied here to provide a minimal set of parameters defining the microscopic model (Fig. 1) for human hemoglobin oxygen binding.

In a previous study, the utility of hierarchically formulated thermodynamic models for the statistical analysis of hemoglobin oxygen binding data was demonstrated [26, 27]. This analysis was based upon the spectroscopically (UV–vis) monitored hemoglobin oxygen binding data of Imai [30], which was analyzed in terms of a homotropic square model where all of the subunits were considered identical (Fig. 2). Simple pairwise interactions (thermodynamic and spectroscopic) between directly adjacent subunits were demonstrated to be both necessary and sufficient to account for the UV–vis monitored hemoglobin oxygen binding data of Imai [30]. The use of a homotropic model in this analysis was justified since UV–vis data does not provide direct information on individual interactions with and between the α and β and subunits of hemoglobin. Hemoglobin is however an $\alpha_2\beta_2$ tetramer with two different but evolutionarily related subunits (α and β), as well as two different intersubunit contacts (αβ and βα, aka $\alpha_1\beta_1$ and $\alpha_1\beta_2$) (reviewed in [31]). In the present study, the results from the square model based analysis (Fig. 2) are extended to the microscopic level (Fig. 1) using a combination of the UV–vis oxygen binding data of Imai [30] and the NMR observations of Viggiano and Ho [7].

The derivation part of this analysis is performed in four steps; A, B, C, and D. Step A describes the use the NMR observation that α and β hemoglobin subunits are equally oxygenated over the oxygen binding isotherm [7, 9, 10] to derive simple algebraic constraints on terms in the microscopic model. Step B describes the derivation of the algebraic relationships between the microscopic model (Fig. 1) and the homotropic square model (Fig. 2) [26, 27]. One thermodynamic relationship remained incompletely defined at this point, that between the *ab* and *ba* terms in the microscopic model. This relationship is analyzed in Step C through statistical analysis against the oxygen binding data of Imai [30]. To perform this analysis, the concept of hierarchically formulated difference terms is introduced. This concept is then used to demonstrates that $ab = ba$. Given this final constraint, it is possible to define all of the parameters in the minimum microscopic model for hemoglobin oxygen binding in terms of the previously determine values for the minimum square model for hemoglobin oxygen binding (Step D). This provides the simplest microscopic model for hemoglobin oxygen binding consistent with both the UV–vis based observations of Imai [30] and NMR observations of Viggiano and Ho [7] on the oxygen binding properties of native human hemoglobin.

The parameter estimates obtained in the derivation portion of this analysis are comprised of a subset of statistically significant lower order terms with values other than zero, and remaining higher order terms with null (zero) values. For the statistically significant lower order terms, confidence intervals are available from the fitting procedure. However, for higher order terms with null (zero) values, confidence intervals are not available from the fitting procedure. It is desirable to have a means to visualize how individual parameter(s) – both statistically significant and null – can affect the overall fit to the oxygen binding data for hemoglobin (confidence intervals). It would also be desirable to know how each parameter can influence the best-fit values for the other statistically significant parameters in the model (parameter correlation). To address these needs, the analysis presented here also implements a numerical/statistical/graphical profiling approach for the analysis of parameters values obtained by non-linear regression [32–37]. In this profile analysis, the value of a parameter is set to fixed values around its best-fit value, and all other *statistically significant* (i.e. non-null valued) parameters are refit to the data used to derive this model. Graphical presentation of the results provides an effective means to visualize the data constrained confidence intervals and parameter correlations in a complex system. The results from this study provide a necessary reference point for analysis and comparison with data and models derived from studies of ligation modified hemoglobin variants [12, 17, 18, 38–41].

2. Methods

2.1. Theoretical background

The theory of hierarchical formulation and its applications has been presented previously [23–29], and is reviewed in Appendix 1 (A1). Lowercase italicized letters are used to denote hierarchical ΔG°s.

2.2. Data used in the analysis

Two sets of observations were used in this analysis. The first is the UV–vis hemoglobin oxygen binding data collected in the absence of organic phosphates by Imai [30] (Bis-Tris data set). This data was collected at 620 nm, at high hemoglobin concentration (1.2 mM) to minimize the effect of dimer-tetramer equilibria, and with catalase and superoxide dismutase added to minimize the formation of methemoglobin. The second observation upon which this analysis is based is