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Thermodynamic linkage of large-scale ligand aggregation with receptor binding

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ABSTRACT

There are many examples in the literature that deal explicitly with the coupling of ligand oligomerization with receptor binding. For example, many transcription factors dimerize and this plays a fundamental role in sequence specific DNA recognition. However, many biological macromolecules undergo reversible, large scale aggregation processes, some of which are indefinite. The thermodynamic coupling of these aggregation processes to other processes, such as protein–protein and protein–DNA interactions, has not been explored in depth. Here we consider the thermodynamic consequences of large scale ligand aggregation on the determination of fundamental thermodynamic parameters, such as equilibrium binding constants and ligand–receptor stoichiometries. We find that a fundamental consequence of an aggregating ligand is that the free ligand concentration (ligand that is not found in aggregates) is buffered over a wide total ligand concentration range. In general, the larger the size of the aggregates, the wider the range over which the free ligand concentration is buffered. An additional consequence of this observation is that an upper limit is set on the fractional occupancy of the ligand's receptor, such that even if the ligand is over-expressed to very high levels in the cell, this will not necessarily ensure that 100% of the ligand's receptors will be occupied. The implications of these results for sequence specific DNA binding proteins will be discussed.

1. Introduction

Many proteins are difficult to study *in vitro* due to their propensity to self aggregate (1). In most cases, investigators attempt to limit this aggregation experimentally, typically by studying truncated proteins. The potential role that reversible protein aggregation may play in the mechanisms of action of these proteins is often ignored, or only studied when the protein forms relatively small, well defined aggregates, such as dimers [2,3]. For example, several DNA binding proteins have been shown to reversibly assemble into large aggregates [4–8], and many transcription factors have been shown to posses one of the most common protein domains observed in nature, Sterile Alpha Motif (SAM) [9], which has been shown in many cases to confer the ability to polymerize in a head to tail fashion [5,6,10–12].

A fundamental consequence of large scale protein aggregation is that the free concentration of the smallest polymerizing unit (e.g. the monomer), will be buffered over a wide total protein concentration range [8]. This observation has significant consequences when considering a wide range of biological processes, since the fractional occupancy of the receptor is determined solely by the free ligand concentration [13], where the free ligand is defined as the smallest polymerizing protein unit. Thus, cells could conceivably overexpress a self-assembling protein ligand to high levels, while simultaneously maintaining a constant free ligand concentration, and guarantee a fixed fractional saturation of the ligand's receptor. Importantly, the fractional saturation may be set at a value less than 100%, even when a large excess of total ligand is produced. Previous seminal works that consider the linkage of large scale ligand aggregation with receptor binding have not explicitly dealt with this phenomena [4,14]. We show here that if a ligand aggregates reversibly, the linkage of ligand aggregation with receptor binding must be accounted for to deduce the equilibrium binding mechanism. Failure to do so in model development will result in incorrect measurements of equilibrium binding constants, cooperativities and ligand–receptor stoichiometries. Furthermore, consideration of this linkage suggests novel regulation mechanisms for control of cellular processes.

2. Theory and results

2.1. Large aggregation processes buffer the free monomer concentration

Our objective here is to study the dependence of the free monomer concentration, or equivalently, the free concentration of the smallest polymerizing unit, on the total monomer concentration for reversible, large scale aggregation processes. To begin this investigation, we have used an isodesmic assembly model (Scheme 1), where the affinity for addition of each monomer to the growing aggregate is described by the same equilibrium constant, L [15,16].

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$$(N\text{-}1)A + A \underset{L}{\rightleftharpoons} (N\text{-}2)A + A_2 \underset{L}{\rightleftharpoons} (N\text{-}3)A + A_3 \underset{L}{\rightleftharpoons} \cdots \underset{L}{\rightleftharpoons} A_N$$

Scheme 1. Isodesmic assembly which terminates at a stoichiometry of *N*.

We use the term "large scale aggregation processes" to describe the formation of aggregates with stoichiometries larger than around 30. Using the isodesmic model, we can obtain an expression that relates the total monomer concentration, $[A_T]$, to the free monomer concentration, $[A_1]$. For an aggregation process that terminates at a stoichiometry of N, the total monomer concentration (in mole/L) is given by:

$$[A_T] = [A_1] + 2[A_2] + 3[A_3] + \cdots + N[A_N] \tag{1}$$

The equilibrium constant for the formation of each stoichiometric species is given by:

$$L = \frac{[A_j]}{[A_{j-1}][A_1]} \tag{2}$$

Substitution of Eq. (2) into Eq. (1) yields:

$$[A_T] = [A_1] + 2L[A_1]^2 + 3L^2[A_1]^3 + \cdots + NL^{N-1}[A_1]^N \tag{3}$$

Multiplying Eq. (3) by L, and defining $L[A_1] \equiv x$ yields:

$$L[A_T] = x + 2x^2 + 3x^3 + \cdots + Nx^N \tag{4}$$

Dividing Eq. (4) by x yields:

$$\frac{L[A_T]}{x} = 1 + 2x + 3x^2 + \cdots + Nx^{N-1} \tag{5}$$

Subtracting Eq. (4) from Eq. (5) yields:

$$\frac{L[A_T]}{x} - L[A_T] + Nx^N = 1 + x + x^2 + \cdots + x^{N-1} \tag{6}$$

Now we multiply the right hand side (RHS) of Eq. (6) by x, and then subtract this result from the RHS of Eq. (6):

$$\text{RHS} - \text{RHS}\cdot x = 1 - x^N \tag{7}$$

Solving Eq. (7) for RHS yields:

$$\text{RHS} = \frac{1-x^N}{1-x} \tag{8}$$

Substituting Eq. (8) into the RHS of Eq. (6), inserting $L[A_1]$ in for x, and finally solving for $[A_T]$ yields:

$$[A_T] = \frac{[A_1]\left(1-(L[A_1])^N\right)}{(1-L[A_1])^2} - \frac{N(L[A_1])^{N+1}}{L(1-L[A_1])} \tag{9}$$

Taking the limit of Eq. (9), when N approaches infinity and $L[A_1] < 1$ yields:

$$[A_T] = \frac{[A_1]}{(1-L[A_1])^2} \tag{10}$$

which is the well known result for the indefinite, isodesmic aggregation case [15,16], i.e. when there is no upper limit on the stoichiometry of the highest aggregation state (and Scheme 1 represents an infinite series). For the indefinite case, it can be seen that the product $L[A_1]$ must be less than one; otherwise the infinite sum shown in Eq. (4) will be guaranteed to diverge. For the definite case, i.e. when N is equal to a finite value (the aggregation terminates at a stoichiometry of N), there is no such requirement. However, inspection of Eq. (9) reveals that it is undefined when $L[A_1] = 1$, or equivalently, when $[A_1] = 1/L$. To determine the value of $[A_T]$ when $L[A_1] = 1$, we evaluated Eq. (4) when $x = 1$ (i.e. $L[A_1] = 1$), which yields:

$$[A_T] = \frac{N(N+1)}{2L} \tag{11}$$

Thus, the function $[A_T]$ is correctly defined as piecewise continuous, where it takes the form of Eq. (9) for the domain $0 < [A_1] < 1/L$ and $[A_1] > 1/L$, and it takes on the form of Eq. (11) for $[A_1] = 1/L$.

Fig. 1A shows simulations using Eqs. 9–11. In these simulations, L was fixed at 100 μM^{-1}, and $[A_T]$ was calculated over a wide range of $[A_1]$. In Fig. 1, we have plotted $[A_1]$ as a function of $[A_T]$ to illustrate the "buffering capacity" of the large aggregates with respect to the free monomer concentration. Fig. 1A shows that for an isodesmic aggregation process that terminates at a stoichiometry of 30 or greater, the free monomer concentration changes very little between 0.5 and 10 μM total monomer. Even for smaller aggregation processes, e.g. when $N = 10$, we see that $[A_1]$ only increases from ~10 nM to ~14 nM upon increasing the total monomer concentration, $[A_T]$ from 0.5 μM to 10 μM. In other words, a 20 fold increase in the total monomer concentration only results in a 1.4 fold increase in the free monomer concentration. Fig. 1B shows the same simulations plotted on a log scale. For aggregation processes that terminate at $N = 30$ or greater, we see that the dependence of $[A_1]$ on $[A_T]$ is weak over a very wide total monomer concentration range. For example, for $N = 30$, it takes a four order of magnitude increase in $[A_T]$ to increase $[A_1]$ from ~9.2 nM to 14 nM. Thus, for most practical considerations, we see that large aggregation processes behave similarly to indefinite aggregation processes ($N \to \infty$) in terms of placing an upper limit on the free monomer concentration that is practically achievable in solution. For the isodesmic case, this upper limit is given to a very good approximation by $1/L$. We conclude that over physiologically relevant concentration ranges, the free monomer concentration can be buffered at around $1/L$, if the monomer participates in a reversible, isodesmic large scale aggregation process.

2.2. Linkage of ligand aggregation with receptor binding for large aggregation processes

To investigate the effect of free ligand buffering on the fractional saturation of a ligand's receptor, we have performed a series of simulations according to Scheme 2. In this scheme, a ligand monomer can bind reversibly to its receptor, or it can reversibly aggregate up to a maximal stoichiometry of N. The total ligand monomer concentration, for Scheme 2, is given by:

$$[A_T] = [AR] + [A_1] + 2[A_2] + 3[A_3] + \cdots + N[A_N] \tag{12}$$

The equilibrium association constant for free monomer binding to receptor is given by:

$$K = \frac{[AR]}{[A_1][R_f]} \tag{13}$$

where $[R_f]$ is the free receptor concentration. The total receptor concentration is given by:

$$[R_T] = [R_f] + [AR] = [R_f] + K[A_1][R_f] = [R_f](1 + K[A_1]) \tag{14}$$