Biophysical Chemistry, 45 (1992) 181–191
Elsevier Science Publishers B.V., Amsterdam

BIOCHE 01708

Thermodynamic model of cooperativity in a dimeric protein: Unique and independent parameters formulation

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(Received 18 September 1991; accepted in revised form 22 July 1992)

Abstract

A model of the cooperative interaction of ligand binding to a dimeric protein is presented based upon the unique and independent parameters (UIP) thermodynamic formulation (Gutheil and McKenna, Biophys. Chem. 45 (1992) 171–179). The analysis is developed from an initial model which includes coupled conformational and ligand binding equilibria. This completely general model is then restricted to focus on conformationally mediated cooperative interactions between the ligands and the expressions for the apparent ligand binding constant and the apparent ligand–ligand interaction constant are derived. The conditions under which there is no cooperative interaction between the ligands are found as roots to a polynomial equation. Consideration of the distribution of species among the various conformational states in this general model leads to a set of inequalities which can be represented as a two dimensional plot of boundaries. By superimposing a contour plot of the value of the apparent ligand–ligand interaction constant over the plot of boundaries a complete graphical representation of this system is achieved similar to a phase diagram. It is found that the parameter space homologous to a Koshland–Nemethy–Filmer type of model is most consistent with both positive and negative cooperativity in this model. The maximal amount of positive and negative cooperativity are found to be simple functions of K_c, the equilibrium constant associated with the change of a subunit and ligand from the unligated to ligated conformation. It is shown that under certain limiting conditions the apparent allosteric interaction between ligands is equal to the conformational interaction between subunits. The methods presented are generally applicable to the theoretical analysis of thermodynamic interactions in complex systems.

Keywords: Cooperativity; Protein–ligand binding; Thermodynamics

1. Introduction

Protein mediated cooperative interactions are well recognized in biochemistry and allow for the regulation of a number of diverse processes including oxygen transport, metabolic pathways, and gene expression [1]. The coupling of ligand binding energy with the lowering of the transition state energy for a given reaction is an important component of enzymatic catalysis [2]. Several authors have proposed models to explain the observation of cooperativity in multimeric proteins, e.g. the Monod–Wyman–Changeux [3] (MWC) and

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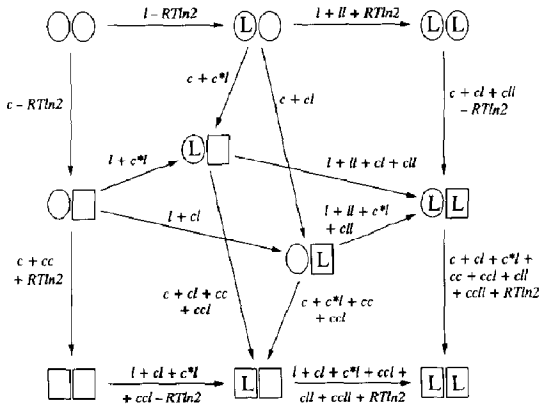

Fig. 1. Complete thermodynamic formulation for a dimeric two ligand binding protein including conformational states using the UIP thermodynamic reformulation [6]. Lower case italicized letters represent UIP formulation ΔGs as defined in the text. RT ln(x) terms represent statistical correction terms which relate intrinsic ΔG of a single site to the macroscopically observable ΔG of multiple binding sites and can be considered as the entropic contribution of multiple binding sites.

the Koshland–Nemethy–Filmer [4] (KNF) models. The hallmark of these models is to apply simplifying assumptions, based upon mechanistic arguments, to the model in order to simplify the algebraic analysis. The simplifying assumptions typically have the effect of removing various states from the model. It has long been recognized that these mechanistic models are in turn submodels of the complete thermodynamic model for these systems in which all of the conformational and ligand bound states are included [5]. However, a theoretical treatment based upon such a complete model has not previously been described due to the limitations of current thermodynamic formulations.

In the preceding article the unique and independent parameters (UIP) reformulation of thermodynamic models was presented [6]. The advantage of the UIP formulation for theoretical applications is that the higher order terms represent the modulation of lower order terms and the potential variability of a complex system can often be suitably approximated by a subset of lower order terms. This paper reports the theoretical analysis of conformationally mediated ligand–ligand interaction in a dimeric protein, including all ligand bound and conformational states, based upon the UIP reformulation as presented in the preceding paper [6].

2. Theory

2.1 Complete formulation of ligand binding to a two site dimeric protein

A model for ligand binding to a dimeric protein, including conformational and ligand bound states, was previously proposed by Eigen [5]. Using the principles outlined previously for the reformulation of complex thermodynamic schemes [6] the model in Fig. 1 can be derived. The terms in this model are as follows [1];

- c – microscopic ΔG of conformational change of one subunit and ligand from ligand unbound to ligand bound conformation.
- l – microscopic ΔG of ligand binding to a site in the absence of conformational change.
- cc – ΔG of interaction representing the effect of the first subunits conformational change on the second subunits conformational change.
- ll – ΔG of interaction representing the effect of the binding of the first ligand on the binding on the second ligand (in the absence of conformational changes).

[1] The following conventions are used throughout this article. Lower case italicized letters are used for UIP formulated ΔGs [6]. Uppercase italicized letters denote the ΔG of formation of the complex from the standard state of free protein and ligands. For example ABC denotes the ΔG of the formation of the protein complex containing the ligands A, B, and C from free protein and ligands. The corresponding association equilibrium constants are denoted by a K followed by the corresponding lowercase letters for UIP formulated equilibrium constants or uppercase letters for equilibrium constants of formation. The symbols used in the text for species depicted in figures by circles for the unligated conformation and squares for the ligated conformation are abbreviated with a P to denote the protein, subscripted o and c to denote the unligated or ligated conformational states of the subunits, respectively, and L or LL to denote one or two ligands, respectively. The two potentially ambiguous cases of $P_{oc}L$ and $P_{co}L$ denote protein with the ligand bound to the subunit in the ligated conformation or unligated conformation, respectively.

cl – ΔG of interaction representing the effect of the conformational change of a subunit and ligand on ligand binding to that subunit and vice versa.

*c*l* – ΔG of interaction representing the effect of conformational change of a subunit on ligand binding to the other subunit and vice versa.

ccl – third order ΔG of interaction representing the effect of ligand binding on *cc*, the effect of the conformational state of the subunit to which the ligand is bound on *c*l*, and the effect of the conformational state of the subunit to which the ligand is not bound on *cl*.

cll – third order ΔG of interaction representing the effect of one subunits conformational state on *ll* etc. as above.

ccll – fourth order ΔG of interaction representing the effect of a subunits (and ligands) conformational state on *cll* and of bound ligand on *ccl*.

Note that the total ΔG between states in this model is path independent and that the number of terms necessary to describe this system, nine, is equal to the number of states in this model other than the standard state of free protein and ligand and therefore represents the minimal number necessary to uniquely relate each state to the standard state.

2.2 Simplification of the complete formulation for the analysis of conformationally mediated coupling

The complete formulation presented in Fig. 1 includes terms both for conformationally mediated ligand–ligand interaction and for direct ligand–ligand interaction. In order to focus on conformationally mediated interaction the above formulation will be simplified by eliminating terms associated with direct ligand–ligand interaction and with the direct influence of a ligand bound to one subunit on the other subunit. The scheme shown in Fig. 2 results. Note that terms associated with direct ligand–ligand interaction, *ll*, *cll*, and *ccll*, and of a ligand binding to one subunits interaction with the other subunit, *c*l*, *ccl*, and *ccll* (already mentioned), are implied to be zero

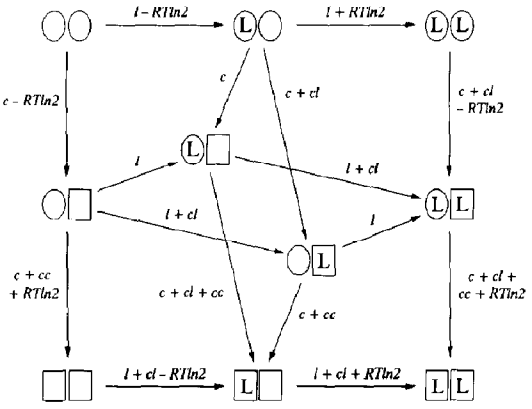

Fig. 2. Formulation for model where ligand–ligand interaction is mediated solely via subunit subunit interaction.

based on the premise that interaction between ligands is modulated solely via the conformational states of the subunits.

2.3 Algebraic compression of the microscopically defined model into the macroscopically observed model

The next step in this analysis is to relate what is observed macroscopically to the parameters defining the microscopic model. In order to do this it is first necessary to convert the ΔG formulated model into the equilibrium constant formu-

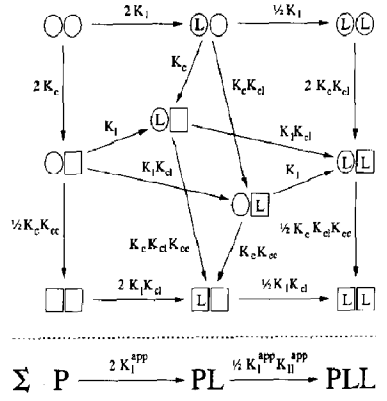

Fig. 3. Equilibrium constant equivalent formulation for Fig. 2 and illustration of the relationship between the apparent intrinsic ligand binding constant, K_l^{app}, and the apparent ligand–ligand interaction constant, K_{ll}^{app}, and the microscopically defined model. The expressions for K_l^{app} and K_{ll}^{app} are derived in the text.

lated model as shown in Fig. 3. The microscopically defined model can be "compressed" into the macroscopically observed model via the equilibrium constant formulation. Note that every state in this model is referenced to the standard state of P_{oo} and free ligand. Using the equilibrium relationships the concentrations of the unligated species are

$$[P_{oo}] = [P_{oo}] \tag{1}$$

$$[P_{co}] = 2K_c[P_{oo}] \tag{2}$$

$$[P_{cc}] = 2K_c\tfrac{1}{2}K_cK_{cc}[P_{oo}] = K_c^2K_{cc}[P_{oo}] \tag{3}$$

The total concentration of unligated species is therefore

$$\begin{aligned}[P] &= [P_{oo}] + [P_{co}] + [P_{cc}] \\ &= [P_{oo}](1 + 2K_c + K_c^2K_{cc})\end{aligned} \tag{4}$$

Similarly for the singly and doubly ligated species

$$\begin{aligned}[PL] &= [P_{oo}L] + [P_{oc}L] + [P_{co}L] + [P_{cc}L] \\ &= [P_{oo}][L]2K_l(1 + K_c + K_cK_{cl} + K_c^2K_{cl}K_{cc})\end{aligned} \tag{5}$$

$$\begin{aligned}[PLL] &= [P_{oo}LL] + [P_{oc}LL] + [P_{cc}LL] \\ &= [P_{oo}][L]^2K_l^2(1 + 2K_cK_{cl} + K_c^2K_{cl}^2K_{cc})\end{aligned} \tag{6}$$

Expressions can now be derived for the macroscopically apparent equilibrium constant, K_l^{app}, and for the apparent ligand–ligand interaction equilibrium constant, K_{ll}^{app}, by their definitions using the equilibrium constant equivalent expressions derived previously for a homotropic two site proteins (cf. [6], eqs. 21 and 22). Note that we are going from a UIP formulated microscopic model to a UIP formulated macroscopic model.

$$\begin{aligned}K_l^{app} &= \frac{1}{2}\frac{[PL]}{[P][L]} \\ &= \frac{1}{2}\frac{[P_{oo}][L]2K_l(1 + K_c + K_cK_{cl} + K_c^2K_{cl})}{[P_{oo}][L](1 + 2K_c + K_c^2K_{cl}K_{cc})} \\ &= K_l\frac{(1 + K_c + K_cK_{cl} + K_c^2K_{cl}K_{cc})}{(1 + 2K_c + K_c^2K_{cc})}\end{aligned} \tag{7}$$

$$\begin{aligned}K_{ll}^{app} &= 4\frac{[P][PLL]}{[PL]^2} \\ &= 4\{[P_{oo}](1 + 2K_c + K_c^2K_{cc}) \\ &\quad \times [P_{oo}]L^2K_l^2(1 + 2K_cK_{cl} + K_c^2K_{cl}^2K_{cc})\} \\ &\quad \times \{([P_{oo}]L2K_l \\ &\quad \times (1 + K_c + K_cK_{cl} + K_c^2K_{cl}))^2\}^{-1} \\ &= \{(1 + 2K_c + K_c^2K_{cc}) \\ &\quad \times (1 + 2K_cK_{cl} + K_c^2K_{cl}^2K_{cc})\} \\ &\quad \times \{(1 + K_c + K_cK_{cl} + K_c^2K_{cl}K_{cc})^2\}^{-1}\end{aligned} \tag{8}$$

2.4 Algebraic analysis of K_l^{app} and K_{ll}^{app}

K_l^{app} in this model is a function of K_l as modulated by the other parameters appearing in the expression above. Several limiting cases serve to illustrate its behavior.

$$\text{As } K_c \to 0 \text{ (i.e. as } c \to \infty);\ K_l^{app} \to K_l. \tag{9}$$

As $K_c \to \infty$ (i.e. as $c \to -\infty$);

$$K_l^{app} \to K_l\frac{K_c^2K_{cl}K_{cc}}{K_c^2K_{cc}} = K_lK_{cl}. \tag{10}$$

These two extreme cases represent the top and bottom ligand binding pathways in Fig. 3, respectively.

For $K_{cl} = 1$ (i.e. for $cl = 0$);

$$K_l^{app} = K_l\frac{(1 + 2K_c + K_c^2K_{cc})}{(1 + 2K_c + K_c^2K_{cc})} = K_l \tag{11}$$

For $K_{cc} = 1$ (i.e. for $cc = 0$);

$$\begin{aligned}K_l^{app} &= K_l\frac{(1 + K_c + K_cK_{cl} + K_c^2K_{cl})}{(1 + 2K_c + K_c^2)} \\ &= K_l\frac{(1 + K_c)(1 + K_cK_{cl})}{(1 + K_c)^2} \\ &= K_l\frac{(1 + K_cK_{cl})}{(1 + K_c)}\end{aligned} \tag{12}$$

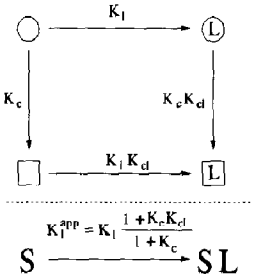

Fig. 4. Equilibrium constant formulation for a one site protein including ligand binding and conformational equilibria. Below is the macroscopically observable ligand binding to a site with the apparent equilibrium constant given as a function of the microscopic parameters from above.

The result for $K_{cl} = 1$ is consistent with what would be expected from inspecting Fig. 3, since K_{cl} is the only way for the conformational state of a subunit to exert an effect on ligand binding. The result for $K_{cc} = 1$ is a bit more complicated but can be explained quite easily if one considers the nature of the assumption which led to it. The assumption that $K_{cc} = 1$ is, within the context of the other assumptions implicit in this model, equivalent to stating that the two subunits of the protein are completely independent from one other and are in a sense separate entities. If we construct a simple scheme for a one binding site protein it will be seen that the apparent binding constant is in fact that obtained above when $K_{cc} = 1$, as shown in Fig. 4.

The expression describing K_{ll}^{app} (eq. 8) is considerably more complicated than that for K_l^{app}. If we set this expression equal to 1 (i.e. no cooperative interaction) and rearrange we obtain

$$\left(1 + 2K_c + K_c^2K_{cc}\right)\left(1 + 2K_cK_{cl} + K_c^2K_{cl}^2K_{cc}\right) = \left(1 + K_c + K_cK_{cl} + K_c^2K_{cl}K_{cc}\right)^2 \quad (13)$$

Multiplying out both sides gives

$$\begin{aligned} &1 + 2K_c + K_c^2K_{cc} + 2K_cK_{cl} + 4K_c^2K_{cl} \\ &\quad + 2K_c^3K_{cc}K_{cl} + K_c^2K_{cl}^2K_{cc} + 2K_c^3K_{cl}^2K_{cc} \\ &\quad + K_c^4K_{cc}^2K_{cl}^2 \\ &= 1 + 2K_c + 2K_cK_{cl} + 2K_c^2K_{cl}K_{cc} + K_c^2 \\ &\quad + 2K_c^2K_{cl} + 2K_c^3K_{cl}K_{cc} + K_c^2K_{cl}^2 \\ &\quad + 2K_c^3K_{cl}^2K_{cc} + K_c^4K_{cl}^2K_{cc}^2 \end{aligned} \quad (14)$$

Canceling common terms and dividing out K_c^2 from both sides gives

$$K_c^2\left(K_{cl}^2K_{cc} + 2K_{cl} + K_{cc}\right) = K_c^2\left(1 + 2K_{cl}K_{cc} + K_{cl}^2\right) \quad (15)$$

After setting equal to zero this expression can be factored to give

$$K_c^2(1 - K_{cl})^2(1 - K_{cc}) = 0 \quad (16)$$

which has roots of $K_{cl} = 1$, $K_{cc} = 1$, and $K_c = 0$.

This result indicates that no cooperative interaction will be apparent in this model if $K_{cl} = 1$, if $K_{cc} = 1$, or if $K_c = 0$. If $K_{cc} = 1$ then the two subunits conformational states do not influence one another in going from the ligand unbound to the ligand bound conformation. If $K_{cl} = 1$ then a given subunit has the same affinity for ligand in its unbound conformation as it does in its ligand bound conformation which would render any conformational coupling between subunits ineffective in producing apparent coupling in ligand binding. For this to occur would imply that a subunit and ligand either do not change their conformation at all upon ligand binding or that the conformational change does not require the input of energy and therefore the distribution between ligand bound and unbound conformations is unchanged upon ligand binding. Closer inspection (below) reveals that this term cannot under most conceivable situations be equal to 1. The result for $K_c = 0$ is not physically realistic since a finite amount of energy must be required to change a subunit from the ligand unbound to ligand bound conformation. A result of $K_{ll}^{app} = 1$ can also be obtained for $K_c = \infty$ which is also physically unrealistic.

2.5 Specific models as a function of parameter values

The model presented above is a construction based upon thermodynamic cycles segregating the observed binding energy into several components; K_c, K_l, and K_{cl}. K_c as used in this model reflects the conformational energy change of both the protein and the ligand. For the purposes of this

analysis it is not necessary to break this parameter down further. In the analysis presented above it was not necessary to consider the magnitudes of the parameters in the model. At this point it is informative to discuss the range of values consistent with various submodels.

Consider the equilibria among the unligated species in the model shown in Fig. 3. Three possibilities exist. Case A; the proteins preferred conformation is P_{oo} which is the standard state in this model. Case B; the proteins preferred conformation is P_{oc}. Case C; the proteins preferred conformation is P_{cc}. Each case imposes a set of conditions on the parameter values for K_c and K_{cc}. Case A requires $2K_c < 1$ and $K_c^2 K_{cc} < 1$. Similarly Case B requires $2K_c > 1$ and $2K_c > K_c^2 K_{cc}$. Case C requires that $K_c^2 K_{cc} > 1$ and $K_c^2 K_{cc} > 2K_c$. For the purpose of this discussion we will limit ourselves to realistic subsets of models. Case A is certainly realistic. Case B is not untenable but implies that the work necessary to change the conformation of the first subunit has been provided by the process of dimerization and is outside of the scope of the model as presented here. Case C can be omitted from consideration since it is analogous to Case A by symmetry and runs counter to the definition of this state as representing the diligated conformation. Only Case A will be considered further below. Given Case A the following constraints apply;

$$2K_c < 1 \text{ or } K_c < \tfrac{1}{2} \text{ and}$$

$$K_c^2 K_{cc} < 1 \text{ or } K_{cc} < 1/K_c^2 \qquad (17)$$

Next consider the equilibria among the diligated species. It is expected that the diligated protein would adopt the symmetrically diligated conformation (i.e. $P_{cc}LL$) just as the unligated protein would adopt the symmetrically unligated conformation. This imposes the following constraints;

$$K_c^2 K_{cl}^2 K_{cc} > 1 \text{ and } K_c^2 K_{cl}^2 K_{cc} > 2K_c K_{cl} \qquad (18)$$

which can be rearranged to give

$$K_{cl}^2 K_{cc} > 1/K_c^2 \text{ and } K_{cl} K_{cc}/2 > 1/K_c \qquad (19)$$

Since the terms in these inequalities are always

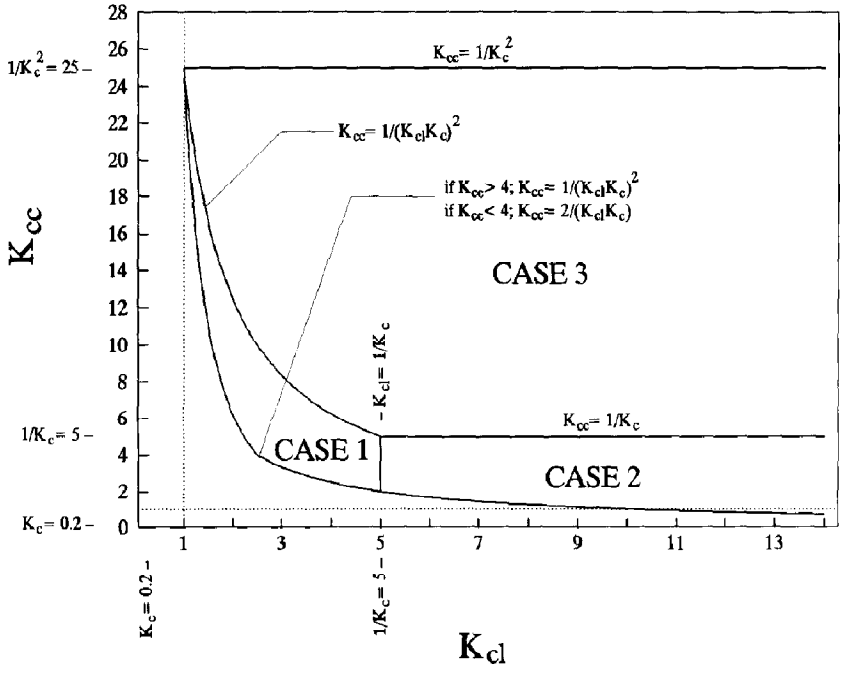

Fig. 5. Graphical depiction of the parameter space available to K_{cc} and K_{cl} at a fixed value of $K_c = 0.2$ with the boundaries imposed by the submodels as discussed in the text.

positive the second of the above inequalities can be squared to give

$$K_{cl}^2 K_{cc}^2/4 > 1/K_c^2 \tag{20}$$

These inequalities require that both $K_{cl}^2 K_{cc}$ and $K_{cl}^2 K_{cc}^2/4$ be greater than $1/K_c^2$ so that the lesser of these two must be greater than $1/K_c^2$. Therefore

$$\text{if} \quad K_{cl}^2 K_{cc} < K_{cl}^2 K_{cc}^2/4, \quad \text{i.e.}$$

$$\text{if} \quad K_{cc} > 4 \quad \text{then} \quad K_{cl}^2 K_{cc} > 1/K_c^2 \tag{21}$$

and conversely

$$\text{if} \quad K_{cc} < 4 \quad \text{then} \quad K_{cl}^2 K_{cc}^2/4 > 1/K_c^2 \tag{22}$$

Now consider the equilibria among the monoligated species. There are four species indicated in the model for the monoligated species. One of these, $P_{co}L$, can be excluded as the major species based on the previously determined constraint of $K_c < \frac{1}{2}$. Three possibilities exist as to which of the other species will predominate and each case imposes an additional set of constraints on the parameter values as follows.

Case 1: $P_{oo}L$ predominant;

$$1 > K_c K_{cl}, \quad \text{i.e. } K_{cl} < 1/K_c \tag{23}$$

$$\text{and} \quad 1 > K_c^2 K_{cl} K_{cc},$$

$$\text{i.e. } K_{cl} K_{cc} < 1/K_c^2 \tag{24}$$

Case 2: $P_{oc}L$ predominant;

$$K_c K_{cl} > 1, \quad \text{i.e. } K_{cl} > 1/K_c \tag{25}$$

$$\text{and} \quad K_c K_{cl} > K_c^2 K_{cl} K_{cc},$$

$$\text{i.e. } K_{cc} < 1/K_c \tag{26}$$

Case 3: $P_{cc}L$ predominant;

$$K_c^2 K_{cl} K_{cc} > 1,$$

$$\text{i.e. } K_{cl} K_{cc} > 1/K_c^2 \tag{27}$$

$$\text{and} \quad K_c^2 K_{cl} K_{cc} > K_c K_{cc},$$

$$\text{i.e. } K_{cc} > 1/K_c \tag{28}$$

The inequalities above have been written to show their dependance on the value of K_c. This provides a means of graphically depicting the complex parameter boundaries resulting from

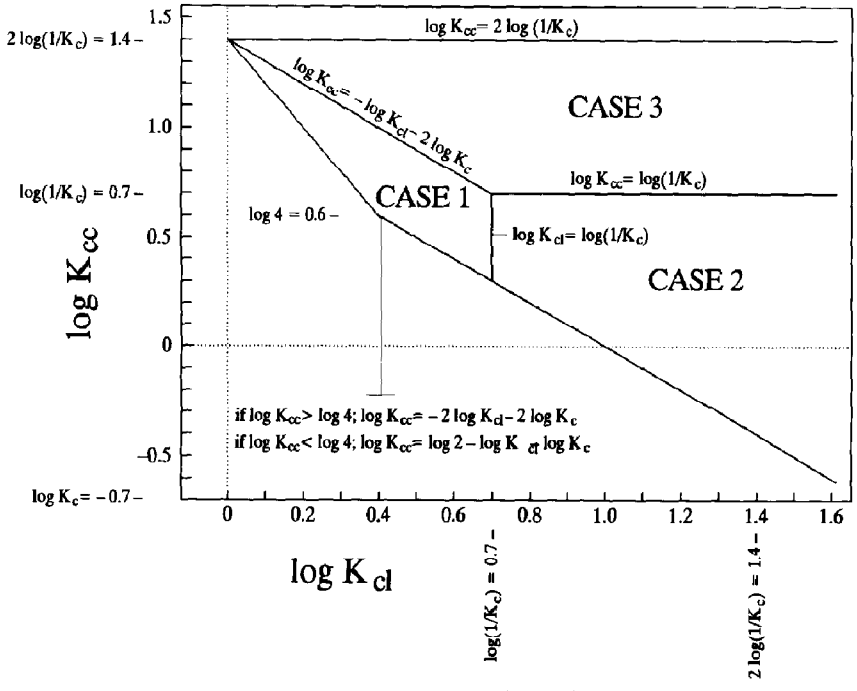

Fig. 6. Same as Fig. 5 but in log (base 10) form.

these inequalities as shown for a value of $K_c = 0.2$ in Fig. 5 and the logarithmic equivalent in Fig. 6. From Figs. 5 and 6 it is apparent that K_{cl} cannot be less than 1 given the constraints imposed above. That K_{cl} be greater than 1 is also consistent with the physical interpretation for this parameter which implies that *cl* always be less than 0. The parameter space below the Case 1 and 2 area is where $P_{cc}LL$ is not the dominant species among the diligated species. The region above Case 3 is where P_{oo} is not the dominant species among the unligated species. Case 1 and 3 correspond to the classical MWC model [3] which requires conservation of subunit symmetry. Case 2 corresponds to the classical KNF type of model [4]. The boundaries between the three cases represent the parameter values at which the predominant species associated with each case are present at equal concentrations. Note that the concept of degrees of freedom, which is useful in the interpretation of phase diagrams, is also applicable here. At the "triple point" between the three cases, apparent at the parameter values of $K_{cc} = 1/K_c$ and $K_{cl} = 1/K_c$, the three complexes $P_{oo}L$, $P_{oc}L$, and $P_{cc}L$ will be present in equal concentration. The logarithmic form of such a diagram (Fig. 6) is more useful than the original form (Fig. 5), since it linearizes the boundaries, represents in better proportion those regions at the extremes of the parameter values, and is proportional to the $-\Delta G$ of the corresponding K.

2.6 *Magnitude of K_{II}^{app} as a function of K_c, K_{cc}, and K_{cl}*

Consider the possible magnitude of the apparent cooperative interaction, K_{II}^{app}. Using the equation for K_{II}^{app} derived above (eq. 8) it is possible to calculate a value for K_{II}^{app} as a function of K_c, K_{cc}, and K_{cl}. With K_c set to a value of 0.01 the predicted values for log K_{II}^{app} as a function of log K_{cc} and log K_{cl} are diagramed as a contour plot superimposed on a boundary condition plot in Fig. 7. K_c in this case is set consid-

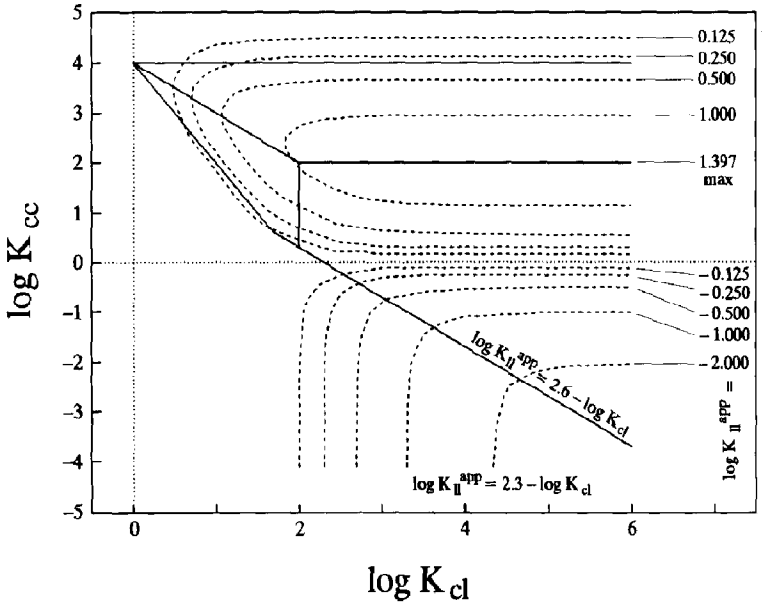

Fig. 7. Contour plot of the value of log K_{II}^{app} superimposed on a boundary condition plot for a value of log $K_c = -2$. The numbers along the right hand side label the contours for the value of log K_{II}^{app}. The maximum value for log K_{II}^{app}, which occurs along the boundary between the Case 2 and Case 3 regions at values of $K_{cl} \gg 1/K_c$, is also indicated (at $K_{cl} \gg 1/K_c$; log K_{II}^{app} = log$(1/4K_c) = 1.397$). The minimum value for log K_{II}^{app} (i.e. maximal of negative cooperativity) shows more complex behavior since it is dependent on the value for log K_{cl}. The expressions shown for the value of log K_{II}^{app} with log $K_c = -2$, and given $K_{cl} \gg 1/K_c$, are from expressions derived in the text.

erably lower than in Figs. 5 and 6, since the maximum and minimum values of K_{II}^{app} depend on the value for K_c as discussed further below. A value of K_c of 0.01 allows physically significant values for apparent positive and negative interaction.

Notice that when $\log K_{cc} = 0$ (i.e. $K_{cc} = 1$); $\log K_{II}^{app} = 0$ (i.e. $K_{II}^{app} = 1$) as derived above (eq. 16) which represents a situation of no cooperative interaction. With $\log K_{cc} < 0$ represents the region of negative cooperativity and with $\log K_{cc} > 0$ the region of positive cooperativity. At a value of $\log K_{cl}$ suitably high to allow for significant negative cooperativity within the allowed boundaries, for example $\log K_{cl} = 5$, as $\log K_{cc}$ is decreased below 0, $\log K_{II}^{app}$ becomes increasingly negative down to some minimal value. The leveling off transition occurs in the vicinity of the boundary as indicated in Fig. 7. Therefore a substantial amount of the possible negative cooperativity can be realized (exactly $\frac{1}{2}$ as derived below) without going outside of the boundaries imposed above. Note that the maximal amount of negative cooperativity apparent in this case is well in excess of a $-\log K_{II}^{app}$ of 2. This corresponds to a greater than 100 fold decrease in the apparent equilibrium association constant between the binding of the first and second ligand.

Now consider the region of positive cooperativity in Fig. 7. At a sufficiently high value for $\log K_{cl}$ (i.e. $\log K_{cl} = 5$), as $\log K_{cc}$ is increased above 0, $\log K_{II}^{app}$ initially increases but reaches a maximum and then decreases. The maximum occurs along the boundary between the Case 2 and 3 regions which represents the transition between where $P_{oc}L$ is the dominant monoligated species (KNF type of model) and where $P_{cc}L$ is the dominant monoligated species (MWC type of model). When $\log K_{cl} < 2$ the point of maximum $\log K_{II}^{app}$ begins shifting upwards following the boundary line between Case 1 and 3.

2.7 Probable model for both positive and negative cooperativity and consequences for K_{II}^{app}

It is apparent from this analysis that the parameter values encompassing Case 2 in these diagrams constitutes nearly the full range for positive and negative cooperativity available at a given value for K_c. Note that, as is most easily seen in Fig. 4, just as one would expect P_o to be the predominant unligated species one would expect P_cL to be the predominant monoligated species and that therefore K_{cl} would be greater than $1/K_c$. In a dimeric protein it has been demonstrated above that there is no overriding requirement to modify this expected behavior in order to account for significant positive or negative cooperativity. In fact negative cooperativity requires $K_{cl} > 1/K_c$. Case 2 therefore represents the parameter space most consistent with the observation of both positive and negative cooperativity.

In Fig. 7 the contour lines for the value of $\log K_{II}^{app}$ become parallel at increasing $\log K_{cl}$. This reflects the fact that beyond a point increasing K_{cl} has no discernable effect on the value of K_{II}^{app} nor on the effect of K_{cc} on K_{II}^{app}. This observation can be used to obtain simple relationships between K_{II}^{app} and the other parameters. In the vicinity of $K_{cc} = 1$ (i.e. $\log K_{cc} = 0$), when $K_c \ll 1$ and $K_{cl} > 1/K_c$ (i.e. to the right and close to the line $\log K_{cc} = 0$ in Fig. 7), the expression for K_{II}^{app} can be reduced to

$$K_{II}^{app} \approx \frac{(1)(K_c^2 K_{cl}^2 K_{cc})}{(K_c K_{cl})^2} = K_{cc} \tag{29}$$

This is an important result demonstrating that, given the conditions imposed in deriving this relation, the observed cooperative interaction will be approximately equal to K_{cc}; the conformational effect of one subunit upon the other.

Similarly the maximum for K_{II}^{app} for a given K_c occurs when $K_{cc} = 1/K_c$ at the boundary between Case 2 and 3. It can be shown that along this boundary

$$K_{II}^{app} \approx \frac{(1)(K_c^2 K_{cl}^2 K_{cc})}{(K_c K_{cl} + K_c^2 K_{cl} K_{cc})^2} = \frac{K_{cc}}{(1 + K_c K_{cc})^2} = \frac{1/K_c}{4} \tag{30}$$

and therefore the maximum possible positive cooperative interaction is one-fourth $1/K_c$ at a

value of $K_{cc} = 1/K_c$ as long as K_{cl} is sufficiently greater than $1/K_c$.

The maximal amount of negative cooperativity (minimum value for K_{II}^{app}), at a given K_c within the boundaries imposed above, is found along the lower boundary of the Case 2 region. This boundary is defined by $K_{cc} = 2/(K_{cl}K_c)$ (Figs. 5 and 6). Along this boundary the expression for K_{II}^{app} is

$$K_{II}^{app} \approx \frac{(2K_cK_{cl} + K_c^2K_{cl}^2K_{cc})}{(K_cK_{cl})^2}$$

$$= \frac{(2K_cK_{cl} + 2K_cK_{cl})}{(K_cK_{cl})^2} = \frac{4}{K_cK_{cl}} \qquad (31)$$

If no boundaries are imposed on the lower value of K_{cc} it can be shown that

$$\text{as } K_{cc} \to 0;\ K_{II}^{app} \to 2/K_cK_{cl} \qquad (32)$$

which is $\frac{1}{2}$ of the value along the lower boundary derived above.

3. Summary and conclusions

The analysis of conformationally mediated cooperative interaction in a dimeric protein presented above differs from previous analyses of cooperativity in several important respects. The simplest possible system, a dimeric protein, was used for this analysis. The complete thermodynamic model for this system contains 10 combinations of conformational and ligand bound states and requires the derivation of terms for two equilibrium constants. Previous analyses have commonly used hemoglobin as the model [3,4] which would require a minimum 44 states for the complete thermodynamic model [5] and the derivation of terms for each of the four equilibrium constants. To avoid the inherent complexity in the theoretical analysis of models of hemoglobin previous analyses have simplified either by the elimination of states, or by assuming certain conformational states do not interact with the ligand, or both.

With the UIP formulated model for a dimeric protein it is not necessary to invoke any of these assumptions. The only simplifying assumption necessary was that the ligand–ligand interaction be mediated solely via conformational interactions between the subunits, an assumption implicit in essentially all previous analyses of cooperativity in multimeric proteins. This assumption has the effect of eliminating all the terms associated with direct ligand–ligand interaction, or of the effect of a ligand binding to one subunits effect on the other subunit. This simplification is mechanistic in origin, but is implemented by removing the thermodynamic terms not associated with conformationally mediated ligand–ligand interaction, and not by removing states from the model. This has the effect of maintaining the distribution of states given such a model and thereby allows the relationships between the distribution of states and the model parameters to be addressed quantitatively.

Once the model is formulated and simplified algebraic analysis proceeds much as in other models of cooperativity but rather than deriving the algebraic expression for the saturation function as in the MWC analysis [3] or the Hill constant as in the KNF analysis [4] the algebraic expressions for K_I^{app} and K_{II}^{app} are derived since these parameters have a fundamental thermodynamic significance. Setting up a system of inequalities dependent on what the dominant species at each step of ligation might be, and integrating these inequalities with calculated values for K_{II}^{app}, results in the complete graphical representation shown in Fig. 7. This graphical representation is similar to a phase diagram for this system. This analysis establishes unequivocally that the Case 2 parameter space is the most consistent with the observation of both positive and negative cooperativity in this system. Conservation of subunit symmetry is not necessary for the observation of positive nor negative cooperativity and is at odds with the fact that both symmetric and unsymmetric states must exist together in equilibrium, an observation embodied in the classical KNF model [4]. The upper and lower limits for the value of K_{II}^{app} as a function of K_c and K_{cl} serve to set certain boundaries on these parameters given a value of K_{II}^{app} but these limits are at the Case 2 boundaries. It is in fact quite likely that the true values of K_c and K_{cl} for

many protein ligand interactions are at least several orders of magnitude beyond the values used in the foregoing analysis (i.e. $K_c \ll 1$, $K_{cl} \gg 1/K_c$). If this were the case the observed cooperative interaction (K_{II}^{app}) would be approximately equal to K_{cc} as demonstrated above. That K_{II}^{app} in such a simple system might relate directly to a fundamental parameter such as K_{cc} has important implications. From the point of view of interpreting an observed ligand–ligand interaction for a dimeric protein it would be a reasonable first approximation to assume that this observed ligand–ligand interaction term is in fact representative of K_{cc}. Such a hypothesis is probably testable and this concept extended to more complex systems such a hemoglobin.

There are a wide variety of physical phenomena amenable to analysis using the methods presented above including enzymatic catalysis and energy coupling in chemical and biochemical systems. The UIP reformulation upon which the preceding analysis is dependent is also applicable to the analysis and interpretation of equilibrium binding data pertaining to complex systems [7] and the numerical/statistical methods necessary for such an analysis will be the subject of a forthcoming article.

Acknowledgement

This work was supported by NIH postdoctoral fellowship 1F32HL08314-01.

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