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Cooperativity Effect in Metal–Ligand and Macromolecule–Ligand Equilibria

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Abstract

An evaluation of the cooperativity effect in metal–ligand and protein–ligand complexes can be made by means of dimensionless parameters K_γ (homotropic), and $K_{\gamma'}$ (heterotropic). Starting from the relation $\bar{n} = \partial \ln \Sigma_M / \partial \ln[A]$ between formation function $\bar{n}$ and partition function $\Sigma_M = 1 + \beta_1[A] + \beta_2[A]^2 + \ldots \beta_i[A]^i \ldots + \beta_t[A]^t$ it is shown that on the Bjerrum plane $\bar{n} = f(\ln[A])$, the standard free-energy ΔG° and therefore the standard chemical potentials $\Delta\mu^\circ_\gamma = -RT \ln K_\gamma$ and $\Delta\mu^\circ_{\gamma'} = -RT \ln K_{\gamma'}$ can be obtained exactly from a convoluted or saturation function F^c_M, which has the property of being 1 when $\Delta\mu^\circ = 0$, as do the equilibrium constants.

The standard convoluted function $F^{c\,\circ}_M$ for multistep equilibria coincides with the maximum term, β_t, of Σ_M and can be calculated on the Bjerrum plane as balance between two integrals, one integral giving the contribution to free-energy of the 'association' partition function, Σ_M, the other giving the contribution to free-energy of the 'dissociation' partition function, Σ^D_M.

F^c_M can be calculated as the product of stepwise equilibrium constants $K_1, K_2, \ldots, K_t$. Differences between areas on the Bjerrum plane measure $\Delta\mu^\circ_\gamma/RT$ and $\Delta\mu^\circ_{\gamma'}/RT$. The statistical factors for homotropic and heterotropic complexes are equal.

The chemical potential changes due to the cooperativity effect can be better evaluated from $K_{\bar{\gamma}} = \beta_i^{1/i}/(K_1 k_{st(\bar{\gamma})})$ (average cooperativity effect) rather than by $K_\gamma = (\beta_i/\beta_{i-2})^{1/2}/(k_{st(\gamma)}\beta_{i-1}/\beta_{i-2})$ (stepwise cooperativity effect). The former is well correlated with the successive additions of ligands. The cooperativity effect is strongly influenced by changes in ionic strength, thus confirming the interpretation of the phenomenon as due to ligand–ligand external interactions. The cooperativity effect, although small ($0 < |\Delta\mu^\circ_\gamma| < 6$ kJ/mol), is significantly greater than the experimental error. The cooperativity effect in homotropic and heterotropic metal complexes is of the same order of magnitude as the cooperativity effect in macromolecule–ligand equilibria.

Introduction

It has been shown in a preceding paper [1] that the free-energy changes ΔG°_γ at pH = 7 of divalent metal–ligand complexes coupled with the deprotonation processes of the ligand fall in the same range of values as the free-energy changes $\Delta G^{\circ\prime}$ of biologically important reactions such as ATP hydrolysis, PGP hydrolysis, etc. Comparable free-energy changes are the necessary pre-requisite for coupling in metabolic reactions.

It is the purpose of the present research to see if by application of the partition function method [2–5] appropriate chemical potential changes can be obtained suited to measure ligand–ligand cooperative effects both in metal–ligand and macromolecule–ligand reactions.

The homotropic or heterotropic cooperativity effects obtained in both metal–ligand and protein–ligand equilibria come out to be of the same order of magnitude. All of these analogies suggest that comparative thermodynamic studies of metal–ligand and protein–ligand equilibria can be of some help to elucidate problems in receptor–ligand thermodynamics and in bioenergetics both from formal and experimental points of view.

The cooperativity effects can be measured on the same scale as the chelate effect [6] by means of parameters which correspond to changes in chemical potentials. The parameter K_γ indicates the extent of homotropic cooperativity, K_ϵ and K_η the extent of homotropic chelation, with and without interdonor cooperativity, respectively. The parameters are related by $K_\eta = K_\epsilon K_\gamma$.

Corresponding parameters $K_{\eta'}$, $K_{\epsilon'}$, $K_{\gamma'}$ are used for heterotropic cooperativity and chelation. The evaluation of these parameters can be made by a general expression

$$K_e = R_{obs(e)}(1/k_{st(e)})$$

where effect $e = \eta, \epsilon, \gamma$, and other indexes. $R_{obs(e)}$ is a ratio, variable with e of experimental equilibrium constants, for instance $R_{obs(\gamma)} = \beta_2^{1/2}/K_1$, and $k_{st(\gamma)}$

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is an appropriate statistical factor. When used as such $R_{obs(e)}$ indicates a chemical reaction, for instance a disproportionation reaction [7]

$$2MA = MA_2 + M$$

or

$$MA + MB = MAB + M$$

but if corrected for statistical factor $k_{st(e)}$, it becomes a thermodynamic parameter K_γ related to the intrinsic affinity of binding. At the same time the product of the experimental constant of the denominator of $R_{obs(e)}$ multiplied by $k_{st(e)}$ represents the reference (REF) state of the parameter K_e e.g.

$$K_\gamma = \beta_2^{1/2}/(K_1 k_{st(\gamma)}) = \beta_2^{1/2}/K_{REF(\gamma)}$$

We wish to show how these constants are related to the partition function Σ_M, how they can be correctly interpreted, and what kinds of information they offer.

Standard State and Convoluted Function

The formation function of Bjerrum

$$\bar{n} = \frac{[MA] + 2[MA_2] + \ldots i[MA_i] \ldots + t[MA_t]}{[M] + [MA] + [MA_2] + \ldots [MA_i] \ldots + [MA_t]} \quad (1)$$

is a stoichiometric ratio between moles of ligand A bound and total moles of receptor M (macromolecule or metal) present. For mononuclear complexes, it is related to the thermodynamic function 'free-energy' by means of the partition function of statistical thermodynamics [2–5]. In fact, under the condition of mononuclear complexes,

$$\bar{n} = \frac{\partial \ln \Sigma_M}{\partial \ln [A]} \quad (2)$$

where

$$\Sigma_M = 1 + \beta_1[A] + \beta_2[A]^2 + \ldots \beta_i[A]^i + \ldots + \beta_t[A]^t \quad (3)$$

is a partition function. The β_i parameters are cumulative formation constants. The statistical thermodynamic statement

$$\ln \Sigma_M = \frac{-\Delta G}{RT} \quad (4)$$

attributes to eqn. (2) the meaning of a partial derivative of a thermodynamic function and transforms it from an analytical to a thermodynamic relation.

By integration of eqn. (2) with respect to $\ln[A]$

$$\int_{[A]=0}^{[A]=1} \bar{n}\, d \ln[A] = -\frac{\Delta G^\circ_F}{RT} = \ln \Sigma^\circ_M \quad (5)$$

we obtain the statistical thermodynamic free-energy change, $\Delta G^\circ_F/RT$ for all the chemical associations taking place between M and A, for concentrations $0 < [A] < 1$.

In classical chemical thermodynamics, the standard free-energy change for the whole process, $\Delta G^\circ/RT$ can be subdivided into partial chemical potential changes, each corresponding to the addition of one ligand A to the metal or macromolecule M:

$$\frac{\Delta G^\circ}{RT} = \frac{\Delta \mu_1^\circ}{RT} + \frac{\Delta \mu_2^\circ}{RT} + \ldots + \frac{\Delta \mu_i^\circ}{RT} + \ldots + \frac{\Delta \mu_t^\circ}{RT} \quad (6)$$

By assuming that $\Delta \mu_i^\circ$ corresponds to the addition to M of the i-th ligand, we state implicitly that the partition function is factorable as a product of stepwise partition functions. Σ_M, however, for example for the three-site receptor, can be factored in terms of site microconstants k_1, k_2, k_3 as a product of site partition functions

$$\Sigma_M = (k_1[A] + 1)(k_2[A] + 1)(k_3[A] + 1) \quad (7)$$

but we do not know if factoring of Σ_M according to eqn. (7) is satisfying the requirements of eqn. (6), of being $\Delta G^\circ/RT$ sum of stepwise chemical potentials. We can note that the explicit form of eqn. (7) is equal to eqn. (2) if $\beta_1 = k_1 + k_2 + k_3$, $\beta_2 = k_1k_2 + k_1k_3 + k_2k_3$, $\beta_3 = k_1k_2k_3$. On the other hand if we define the constants K_1, K_2, K_3 as apparent stoichiometric macroconstants interrelated by $\beta_1 = K_1$, $\beta_2 = K_1K_2$, $\beta_3 = K_1K_2K_3$, we apparently assume a factoring of partition function different from eqn. (7) and subsequently a particular subdivision, possibly satisfying eqn. (6) of $\Delta G^\circ/RT$.

For simplicity, but without loss of generality, we start with the case of a one-site complex, *i.e.* with $\beta_1 = k_1 = K_1$. The formation function of this simple complex is

$$\bar{n} = \frac{K_1[A]}{1 + K_1[A]} \quad (8)$$

and it is equal to the mole fraction of MA, α_1.

The value of Σ°_M is related to the mole fraction of MA by

$$\ln \Sigma_M = \int_{[A]=0}^{[A]=1} \alpha_1\, d \ln[A] \quad (9)$$

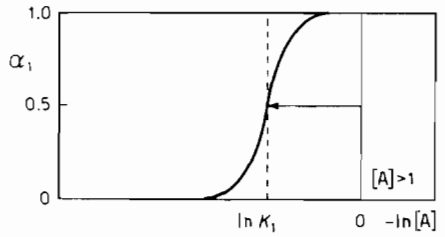

Fig. 1. Diagram of $\alpha_1 = K_1[A]/(1 + K_1[A])$ as a function of ln[A] (logistic curve). The areas between the curve and the axis at ln[A] = 0 are proportional to standard free-energies.

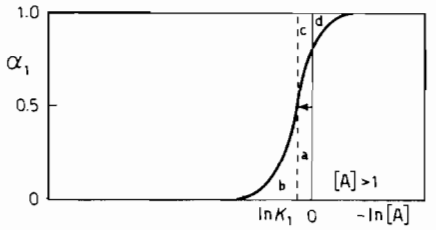

Fig. 2. Mole fraction α_1 as function of ln[A] for cases when the approximation $K_1 + 1 \approx K_1$ is no longer valid. a, b, c are areas corresponding to free-energy changes. $a + b$ is less than the standard chemical potential ($a + b < \Delta\mu^\circ_1/RT = a + c$). For symmetry conditions $b = c + d$.

with

$$\ln \Sigma_M^\circ = \ln(1 + K_1) \tag{10}$$

However, only if $K \gg 1$ is the result of eqn. (10) completely in accord with the classic thermodynamic equality

$$\frac{-\Delta\mu_1^\circ}{RT} = \ln K_1 \tag{11}$$

which could satisfy eqn. (6).

The curve (Fig. 1) representing $\alpha_1 = K_1[A]/(1 + K_1[A])$ as a function of ln[A] is perfectly symmetrical with respect to $\alpha_1 = 0.5$. In fact

$$\frac{\partial \alpha_1}{\partial \ln[A]} = [A]\frac{\partial\{K_1[A]/(1 + K_1[A])\}}{\partial[A]}$$

$$= \alpha_1(1 - \alpha_1) \tag{12}$$

and the standard chemical potential is represented by the area between the curve and the vertical axis at [A] = 1. This area is equal to the area of the rectangle having $\alpha_1 = 1$ as base and ln K_1 as height. When K_1 is small (Fig. 2), the value of the integral and the value of the area of the rectangle of height ln K_1 do not coincide any more: the value of the integral is

$$\left| \ln \Sigma_M \right|_{[A]=0}^{[A]=1} = a + b \tag{13}$$

and the area of the rectangle amounts to

$$\ln K_1 = a + c \tag{14}$$

with difference, for the symmetry condition, eqn. (12),

$$(a + b) - (a + c) = d \tag{15}$$

between the two areas.

The areas a and b can be calculated from the definite integrals

$$b = \left| \ln \Sigma_M \right|_{[A]=0}^{[A]=1/K_1} = \ln 2 \tag{16}$$

and

$$a = \left| \ln \Sigma_M \right|_{[A]=1/K_1}^{[A]=1} = \ln(1 + K_1) - \ln 2 \tag{17}$$

In order to evaluate area c we introduce a reciprocal partition function

$$\Sigma_M^R = 1/\{1 + (1/K_1[A])\} \tag{18}$$

which is $0 < \Sigma_M^R < 1$, instead of $1 \leqslant \Sigma_M < \infty$. Observe that $\Sigma_M^R = \alpha_1$, the fraction of M present as species MA. Then the area c is

$$c = \left| \ln \Sigma_M^R \right|_{[A]=1/K_1}^{[A]=1} = \ln \frac{K_1}{1 + K_1} + \ln 2 \tag{19}$$

Therefore

$$(a + c) = \ln(1 + K_1) - \ln 2 + \ln K_1 - \ln(1 + K_1) + \ln 2$$

$$= \ln K_1 \tag{20}$$

in perfect agreement with eqn. (11). This means that the general relationship stated by statistical thermodynamics between partition function and equilibrium constant is more correctly expressed by

$$-\Delta\mu^\circ/RT = \ln \Sigma_M^\circ + \ln \Sigma_M^{R\circ} = \ln F_M^{c\circ} \tag{21}$$

F_M^c is a convoluted function which represents the joint probability of existence of complexes with $K > 1$ (associating with respect to the standard state) and those with $K < 1$ (dissociating with respect to the standard state). In other words it covers both fields of concentrations above and below unitary concentrations. It has the property that its value is unity when $-\Delta\mu^\circ/RT = 0$.

Factoring of the Convoluted Function

The convoluted function is also suited to be factored

$$F_M^c = F_{M(1)}^c F_{M(2)}^c \ldots = \Pi_{i=1}^t F_{M(i)}^c \tag{22}$$

so to make the capacitative properties of $-\Delta G^\circ/RT$ correspond to a sum of stepwise chemical potentials $-\Delta\mu_i^\circ/RT$, as required by eqn. (6).

Consider the formation function $\bar{n}$ for complexes MA and MA_2. The curve $\bar{n} = f(\ln[A])$ is represented

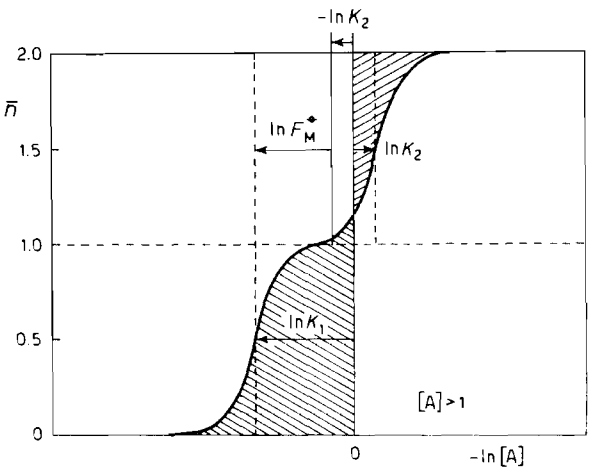

Fig. 3. Formation function and standard free-energy ($-\Delta G^\circ/RT = \ln F^\circ_M$) of a complex MA_2, with step MA + A = MA_2 displaced to the left ($K < 1$). Hatched areas correspond to the two integrals of eqn. (28). $\ln F^\circ_M$ can be obtained as difference between hatched areas or between rectangular areas as well.

in Fig. 3 and it has been sketched in such a way as to show the case where the equilibrium MA + A $\rightleftharpoons$ MA_2 is displaced to the left. For this case also, we need a convoluted function to represent the behaviour of the system with respect to the reference state.

In order to find the convoluted function for a general case of multistep equilibria, we introduce a dissociation partition function, Σ^D_M.

This function can be derived from α_t the molar fraction of the maximum term MA_t contained in Σ_M.

For example with $t = 2$ we have

$$\frac{1}{\alpha_2} = \frac{1 + \beta_1[A] + \beta_2[A]^2}{\beta_2[A]^2} = 1 + \frac{1}{K_2[A]} + \frac{1}{K_2K_1[A]^2} \tag{23}$$

that has clearly the structure of a dissociation partition function in which the ground state is MA_2 and the single species are obtained from it as stepwise dissociation reactions.

Therefore in general

$$\Sigma^D_M = \frac{1}{\alpha_t} = 1 + \frac{1}{K_t[A]} + \frac{1}{K_tK_{t-1}[A]^2} + \ldots + \frac{1}{K_tK_{t-1}\ldots K_1[A]^t} \tag{24}$$

Note that the stepwise equilibrium constants K_i are necessary to represent the dissociation process and they must be equal in both association and dissociation reactions.

The standard convoluted function is obtained from eqn. (21) as

$$\Sigma^\circ_M \Sigma^{R\circ}_M = F^{c\circ}_M \tag{25}$$

Because of eqn. (24) we can also write

$$\Sigma_M/\Sigma^D_M = F^c_M \tag{26}$$

In analogy with eqns. (2), (4) and (5) we can write

$$\frac{\partial \ln \Sigma^D_M}{\partial \ln [A]} \, d \ln[A] = \frac{\partial \ln \alpha}{\partial \ln[A]} \, d \ln[A] = \frac{\partial(-\Delta G_D/RT)}{\partial \ln[A]} \, d \ln[A] \tag{27}$$

Recalling eqn. (5) and remembering that eqn. (21) is sum of two integrals, we can also write

$$-\frac{\Delta G^\circ}{RT} = -\frac{\Delta G^\circ_M}{RT} + \frac{\Delta G^\circ_D}{RT} = \int_{[A]=0}^{[A]=1} \bar{n} \, d \ln[A] + \int_{[A]=\infty}^{[A]=1} \frac{\partial \ln \alpha_t}{\partial \ln[A]} \, d \ln[A] = \left| \ln \Sigma_M \right|_{[A]=0}^{[A]=1} + \left| \ln \frac{\beta_t[A]^t}{\Sigma_M} \right|_{[A]=\infty}^{[A]=1} \tag{28}$$

The two component integrals have been obtained by rearranging the integrals of eqn. (21) by means of eqns. (13), (14) and (15). They correspond to the hatched areas in Fig. 3.

Eqn. (28) is an important correction to the conclusion of Schellman [4] concerning the relationship between binding free-energy and partition function. Because

$$\lim_{[A]\to\infty} \frac{\beta_t[A]^t}{\Sigma_M} = \lim_{[A]\to\infty} \alpha_t = 1 \tag{29}$$

we have

$$-\frac{\Delta G^\circ}{RT} = \ln \beta_t \tag{30}$$

where β_t is the 'saturation' function of Gill [5]. It is evident that eqn. (30) corresponds to a new statistical thermodynamic statement (standard state)

$$-\frac{\Delta G^\circ}{RT} = \ln F^{c\circ}_M \tag{31}$$

different from eqn. (5). The convoluted function $F^c_M = \beta_t[A]^t$ is a pure number giving the product of activities. Its value in the standard state ([A] = 1) coincides with the maximum term β_t of Σ_M [5].

The convoluted function can be factored to satisfy eqn. (22). In Fig. 3, the area under the curve measures the free-energy change for the formation of the

complex MA_2. The area corresponding to $\ln F_M^{c\circ}$ can be subdivided into two contributions

$$-\frac{\Delta G^\circ}{RT} = -\frac{\Delta\mu_1^\circ}{RT} - \frac{\Delta\mu_2^\circ}{RT} = \ln K_1 + \ln K_2 \qquad (32)$$

where for this example $-\Delta\mu_2^\circ/RT < 0$.

Each contributing area can be thought as delimited by a symmetrical sigmoidal curve. In fact the partial formation functions corresponding to each step have mid-point symmetry as shown by Nagypal *et al.* [8]. These authors, while discussing the possible asymmetry of the Bjerrum formation function, treated the problem of successive steps in metal–ligand complexes in terms of reduced formation functions and the Fronaeus [9] equation. They conclude that the formation function as a whole can be asymmetric but can be represented as a combination of symmetrical reduced steps. Each symmetrical portion delimits an area which is equivalent to a rectangular area as already shown in Fig. 2.

The introduction of the dissociation partition function Σ_M^D, and of the convoluted or saturation function F_M^c presents important advantages. First of all the statistical thermodynamic function F_M^c results as a balance at the standard state between dissociation free-energy and association free-energy. It behaves as the equilibrium constants in classical thermodynamics. The contradiction between the statistical thermodynamic statement (eqn. (10)) and the classical thermodynamic equality (eqn. (11)) is removed. The second advantage of the convoluted function is that it can be factored in terms of stepwise partition functions ($K_1[A], K_2[A]$, etc.), each of which is an activity and gives the probability of finding that species in the solution at concentration [A]. These probabilities are experimentally determinable as equilibrium constants.

One characteristic of the dissociation partition function and of the convoluted function is that for eqn. (29) to be valid one has to prove experimentally by increasing [A] that the saturation of sites has been achieved. This is perfectly equivalent to determining t in the Scatchard plot by extrapolating to $\bar{n}/[A] \to 0$.

Statistical Factors

The stepwise equilibrium constants $K_1, K_2, \ldots, K_t$ and the cumulative formation constants β_i are related to the site microconstants (k_i). Each K_i is associated with multiplicity coefficients m_{iK} and each β_i with multiplicity coefficients m_i.

In general for t possible binding sites the i-th partial constant is related to the site affinity k (equal for every site) by [10]

$$K_i = (t - i + 1)k/i \qquad (33)$$

The term $(t - i + 1)/i = m_{iK}$ represents the statistical factor for the i-th constant. In order to calculate ratios or geometrical averages of equilibrium constants, ratios and geometrical averages of the multiplicity coefficients will be used.

In case the intrinsic affinities are different, as a first approximation the same m_i holds. For heterotropic complexes we choose as a comparison term the affinity of an average ligand $\bar{L}$,

$$K_{M\bar{L}} = (K_{MA}K_{MB})^{1/2} \qquad (34)$$

and the algebra of the hypothetical microconstants is the same as that for homotropic complexes of the ligand $\bar{L}$.

Different stoichiometries can engender uncertainties in the choice of t, the maximum coordination number.

Stepwise and Average Cooperativity

The factoring of the convoluted function into stepwise partial functions $K_1[A]$, $K_2[A]$, etc. corresponds, in accordance with Nagypal *et al.* [8], to represent the stepwise additions of ligand to metal or macromolecule by means of a family of curves (Fig. 4) as in an ordinary distribution diagram. Each curve delimits an area of height $\ln K_i$ proportional to $-\Delta\mu_i^\circ/RT$, *i.e.* the chemical potential change for addition of the i-th ligand.

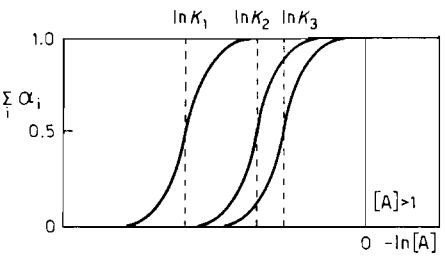

Fig. 4. Chemical potential changes for successive additions of ligand.

The cooperativity effects are measured in Fig. 4 by areas which are differences between the areas of rectangles of height $\ln K_i$. We can distinguish two types of differences. The difference between $\ln K_i$ and $\ln K_{i-1}$ divided by two, measures the stepwise cooperativity effect, whereas $\ln K_{\bar{\gamma}} = (1/i)\Sigma \ln K_i - \ln K_1$, gives the average cooperativity. In the calculation of these averages and differences, values of k_{st} must be taken into account. The product of the appropriate statistical factor by the constant at the denominator (K_{i-1} or K_1) defines the reference (REF) compound. For example, for a 4-site complex, we have the following stepwise cooperativity parameters

$$K_\gamma = \beta_2^{1/2}/K_1 k_{st(\gamma)} \qquad \text{with } K_{REF} = K_1 k_{st(\gamma)}$$

$$K = [\beta_3/\beta_1]^{1/2}/K_2 k_{st(\gamma)} \qquad \text{with } K_{REF} = K_2 k_{st(\gamma)}$$

$$K = [\beta_4/\beta_2]^{1/2}/K_3 k_{st(\gamma)} \qquad \text{with } K_{REF} = K_3 k_{st(\gamma)}$$

and the average cooperativity parameters

$$K_{\bar{\gamma}} = \beta_2^{1/2}/K_1 k_{st(\bar{\gamma})} \qquad \text{with } K_{REF} = K_1 k_{st(\bar{\gamma})}$$

$$K_{\bar{\gamma}} = \beta_3^{1/3}/K_1 k_{st(\bar{\gamma})} \qquad \text{with } K_{REF} = K_1 k_{st(\bar{\gamma})}$$

$$K_{\bar{\gamma}} = \beta_4^{1/4}/K_1 k_{st(\bar{\gamma})} \qquad \text{with } K_{REF} = K_1 k_{st(\bar{\gamma})}$$

The values of k_{st} are obtained as averages and ratios of the coefficient m_i of single constants.

The different molecular interpretations of stepwise and average cooperativity parameters can be better explained with reference to diagrams.

The diagram in Fig. 5 represents the addition of ligand without rearrangement of bonds. Here the affinity is symbolically represented by the length of the M–A bond, the dotted line representing the ligand–ligand interactions and the full arrows the complex–solvent interactions. Were this the actual situation, then the appropriate evaluation of cooperativity would have been the stepwise cooperativity parameter

$$K_\gamma = \frac{(\beta_i/\beta_{i-2})^{1/2}}{(\beta_{i-1}/\beta_{i-2})k_{st(\gamma)}} = \frac{(K_i K_{i-1})^{1/2}}{(K_{i-1}k_{st(\gamma)})} \qquad (35)$$

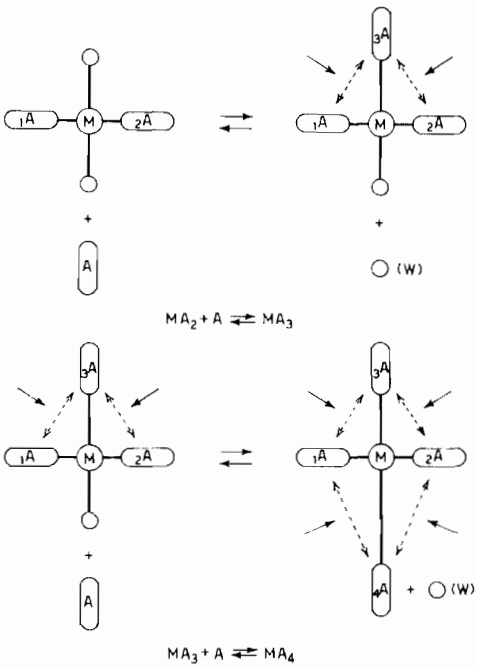

Fig. 5. Stepwise addition of ligand without rearrangement of bonds. The dotted arrows represent ligand–ligand interactions and the full arrows the interactions with the solvent.

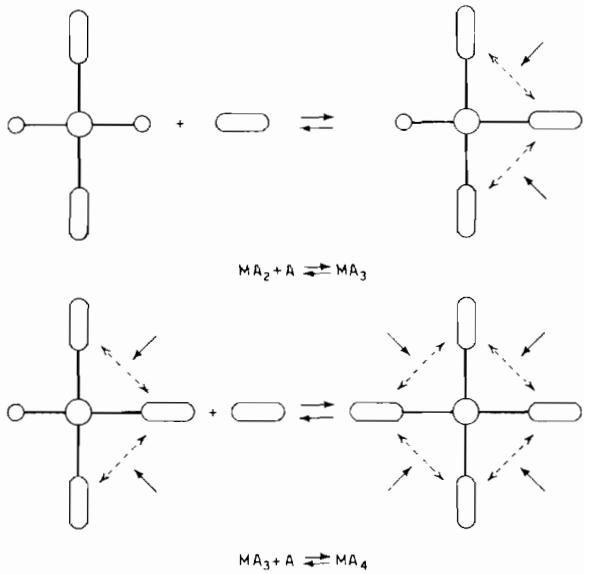

Fig. 6. Stepwise addition of ligand with rearrangement of bonds. The dotted arrows represent ligand–ligand interactions and the full arrows the interactions with the solvent.

This, however, is not the general case because the crystal structure determinations have shown how the general trend is towards equal average bond distances M–A.

The situation with averaging of the bonds is depicted in Fig. 6, where the extreme case with perfectly equal bonds has been drawn. Reality in both metal–ligand and macromolecule–ligand complexes is nearer to these schemes rather than to that of the preceding diagram.

The average cooperativity constant

$$K_{\bar{\gamma}} = \frac{\beta_i^{1/i}}{K_1 k_{st(\bar{\gamma})}} = \frac{\beta_i^{1/i}}{K_{REF}} \qquad (36)$$

measures the difference between the average free-energy change on formation, e.g. of MA and MA_2, and the affinity of A for M on formation of one single bond M–A. The average cooperativity parameter $K_{\bar{\gamma}}$ is the most appropriate indicator of the real molecular process. This parameter measures the external ligand–ligand interactions, those which are highly exposed to the action of the solvent and therefore to the changes in ionic strength.

Cooperativity Effect and Experimental Error

The calculation of the cooperativity free-energy implies the calculation of differences between standard chemical potentials and hence between logarithms of equilibrium constants. It is therefore very important to assess the significance of the differences calculated with respect to the experimental error in

TABLE I. Average and Stepwise Cooperativity Effects in Nickel(II)–Ammonia System in Aqueous Solution at 30 °C[a]

i	$\log \beta_i$	$\Delta\mu^\circ_\gamma = -RT \ln K_\gamma$ (kJ/mol)			
		Average		Stepwise	
		Uncorrected	Corrected	Uncorrected	Corrected
1	2.78	0	0	0	0
2	5.05	1.46	0.36	1.46	0.36
3	6.70	3.12	1.13	1.77	0.98
4	8.01	4.43	1.62	0.86	0.13
5	8.66	5.98	2.37	0.33	−0.46
6	8.74	7.55	3.04	1.63	0.53
$y = ai + b$					
a		1.50	0.62	0.11	−0.02
b		−1.51	−0.75	0.62	0.32
r		1.00	0.99	0.28	0.07
σ_y		0.1	0.2	0.7	0.5

[a]Data from ref. 8.

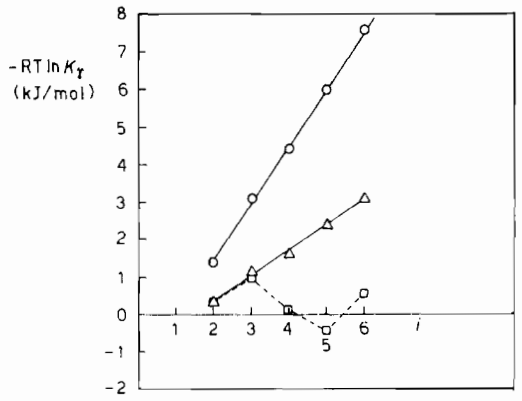

Fig. 7. Stepwise and average cooperativity effects for nickel(II)–ammonia system. ○, $\Delta\mu^\circ_{\bar\gamma} - \Delta\mu^\circ_{\bar K_{st(\bar\gamma)}}$ (average, uncorrected); △, $\Delta\mu^\circ_{\bar\gamma}$ (average); □, $\Delta\mu^\circ_\gamma$ (stepwise).

the determination of the equilibrium constants. The analysis of precision and accuracy [11, 12] has shown:

(i) that the main source of error in $\log K_i$ stems from differences between titrations and ranges from 0.005 to 0.5 logarithmic units, which corresponds to 0.02–0.20 kJ/mol.

(ii) that the error in $\log K_i$ is the same as that in the corresponding $\log \beta_i$.

The significance of the assessment can be better explained with reference to real cases. The data of stepwise and average cooperativity for the nickel–ammonia system in aqueous solutions at 30 °C are reported in Table I and represented in Fig. 7. The stepwise cooperativity shows a jumping behaviour. The stepwise uncorrected data interpolated by the line $y = 0.62 + 0.11\,i$ with $\sigma_y = 0.7$ kJ/mol come out to be significantly spread, much more than the estimated experimental error which is $\sigma_y = 0.09$ kJ/mol. The interpolation line does not represent the actual behaviour and the data must be joined by a discontinuous line (Fig. 7) similar to that drawn by Klotz and Hunston [10]. The interpretation of this trend in terms of molecular processes is rather difficult.

The average cooperativity, on the other hand, shows strikingly regular behaviour. The data of the uncorrected average cooperativity are interpolated by the line $y = -1.51 + 1.50\,i$ with $\sigma_y = 0.1$ kJ/mol, practically coincident with the experimental error. The correlation is highly significant and indicates that the rearrangement of the bonds takes place and that the ligand–ligand interaction is 1.5 kJ/mol per ligand added. The corrected stepwise values are again uncorrelated whereas the corrected average values are interpolated by the line $y = -0.75 + 0.62\,i$ with error $\sigma_y = 0.2$ kJ/mol. The statistical correction increases the error slightly, remaining however close to the experimental error. The corrected cooperativity effect is 0.62 kJ/mol per ligand added and this is the actual difference in intrinsic affinity due to cooperativity effects in this system. They are not very high but distinctly different from the experimental error.

Another example (Table II) shows the incidence of the ionic strength on the cooperativity effect. The data refer to bismuth–thiocyanato system and are represented in Fig. 8, where the stepwise cooperativity data are not drawn because they show the same unpredictable zig-zag behaviour of the nickel–ammonia system. On the other hand the average cooperativity data at $I = 3$ M ($LiClO_4$ are interpolated by the line $y = 0.32 + 0.70\,i$ with $\sigma_y = 0.3$. The trend is not so smooth as for nickel–ammonia system, with −0.7 kJ/mol per ligand added. The waving behaviour could indicate that the rearrangement of the bonds is not completely regular. By extrapolation of the data at different ionic strengths, Fedorov *et al.* [13] obtained the data reported in Table II for $I = 0$. The trend shows a striking similarity with the behaviour of the nickel–ammonia system. The average cooperativity free-energy per ligand added is 1.43 kJ/mol for uncorrected values and 0.56 kJ/mol for the corrected ones. The change of the cooperativity effect from a favourable value ($\Delta\mu^\circ_i < 0$) at high ionic strength to an anticooperativity effect at null ionic strength clearly shows the strong interference between the external ligand–ligand interaction and the electrostatic forces acting in the solvent.

A third example of cooperativity effect is offered by complexes with mixed ligands or heterotropic complexes. A set of compounds [14] formed by copper(II) with dicarboxylates in conjunction with other ligands, has been chosen.

The calculation of the statistical factor can be done by considering that the maximum number of

TABLE II. Average and Stepwise Cooperativity Effects in Bismuth(III)–Thiocyanato System at 25 °C in Aqueous Solutions with Different Ionic Strengths[a]

i	$\log \beta_i$	$\Delta\mu^\circ_\gamma = -RT \ln K_\gamma$ (kJ/mol) I = 3 M ($LiClO_4$)				$\log \beta_i$	$\Delta\mu^\circ_\gamma = -RT \ln K_\gamma$ (kJ/mol) I = 0 M			
		Average		Stepwise			Average		Stepwise	
		Uncorrected	Corrected	Uncorrected	Corrected		Uncorrected	Corrected	Uncorrected	Corrected
1	1.28	0	0	0	0	2.3	0	0	0	0
2	2.67	−0.31	−1.39	−0.31	−1.39	3.7	2.05	0.97	2.05	0.97
3	3.74	0.19	−1.78	0.91	−1.05	4.4	4.24	2.28	2.25	1.47
4	5.2	−0.11	−2.87	−1.11	−3.87	5.2	5.19	2.43	−0.29	−1.00
5	5.9	0.57	−2.98	2.17	−1.38	5.8	5.99	2.44	0.57	−0.21
6	6.9	0.74	−3.70	0.86	−3.58	5.4	7.47	3.03	2.85	−0.23
$y = ai + b$										
a		0.17	−0.70	0.28	−0.59		1.43	0.56	0.21	−0.66
b		−0.42	0.32	−0.55	0.19		−0.86	−0.11	0.31	0.55
r		0.80	0.97	0.45	0.73		0.98	0.93	0.30	0.13
σ_y		0.2	0.3	1.0	1.0		0.5	0.4	1.3	2.4

[a]Data from ref. 13.

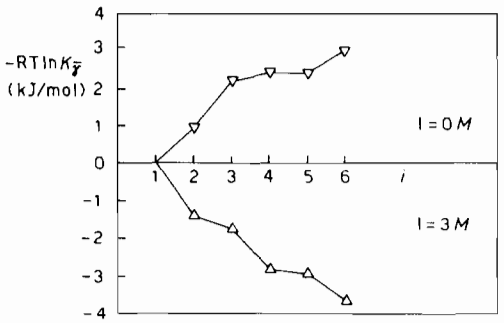

Fig. 8. Average cooperativity effect in the system bismuth(III)–thiocyanato in aqueous solution at 25 °C: ▽, ionic strength I = 0 M; △, ionic strength I = 3 M (Li)ClO_4.

sites around the metal is 6, in octahedral arrangement. The data are reported in Table III and show how the cooperativity effect in these heterotropic complexes is favourable ($\Delta\mu^\circ_{\gamma'} < 0$). The values range from 0.3 to 5.7 kJ/mol and indicate that the energy involved is generally not very high. These values, however, as demonstrated by Weber [15], are enough to produce dramatic changes in the distribution of the complexes. 8 kJ/mol displace the equilibrium, e.g. MA + MB = MAB + M, toward the right or the left up to 90%. In the same Table the values of $\log K_\Delta$ [7] are reported. They indicate the displacement of the disproportionation reaction but do not put in evidence how the ligand–ligand interaction free-energy is cooperative. Values of cooperativity enthalpy, $\Delta h^\circ_{\gamma'}$, and entropy, $\Delta s^\circ_{\gamma'}$, can be calculated (Table IV).

On the other hand the homotropic complexes formed by the same carboxylato ligands with copper(II) (Table V) show an anticooperativity effect ($\Delta\mu^\circ_i > 0$), but the positive values are of the same order of magnitude as the heterotropic complexes. The enthalpy contribution to cooperativity, Δh°_γ, in these cases is favourable ($\Delta h^\circ_\gamma < 0$), whereas

TABLE III. Heterotropic Cooperativity Effect in Metal Complexes[a] at 25 °C and I = 0.1 M

Compound	$\log K_\Delta$	$\log K_{\gamma'}$	$\Delta\mu^\circ_{\gamma'}$ (kJ/mol)
Cu(bpy)(mal)	0.25	0.664	−3.79
Cu(bpy)(succ)	0.24	0.659	−3.76
Cu(bpy)(male)	0.36	0.719	−4.10
Cu(bpy)(pht)	0.49	0.812	−4.63
Cu(bpy)(ox)	0.70	0.889	−5.07
Cu(bpy)(CPRD)	1.38	1.229	−7.01
Cu(bpy)(CBUD)	1.11	1.094	−6.24
Cu(bpy)(CPED)	0.26	0.669	−3.82
Cu(bpy)(CHED)	0.29	0.684	−3.90
Cu(OPDA)(mal)	−0.36	0.359	−2.05
Cu(OPDA)(succ)	−0.39	0.344	−1.96
Cu(OPDA)(male)	−0.29	0.394	−2.25
Cu(OPDA)(ox)	−0.37	0.354	−2.02
Cu(hm)(ox)	−0.22	0.429	−2.45
Cu(hm)(mal)	−0.49	0.294	−1.68
Cu(hm)(succ)	−0.31	0.384	−2.19
Cu(hm)(ita)	−0.35	0.364	−2.08
Cu(hm)(male)	−0.29	0.394	−2.25
Cu(hm)(CBUD)	−0.50	0.289	−1.65

[a]Ligand A: bpy = 2,2′-bipyridyl, OPDA = ortho-phenylendiamine, hm = histamine. Ligand B: mal = malonato, succ = succinato, male = maleato, pht = phtalato, ox = oxalato, ita = itaconato, CPRD = cyclopropane-1,1-dicarboxylato, CBUD = cyclobutane-1,1-dicarboxylato, CPED = cyclopentane-1,1-dicarboxylato, CHED = cyclohexane-1,1-dicarboxylato. $\log K_\Delta = \log K(\mathrm{MA} + \mathrm{MB} = \mathrm{MAB} + \mathrm{M})$. $\log K_{\gamma'} = \log\{\beta_{\mathrm{MAB}}{}^{1/2}/[(K_{\mathrm{MA}}K_{\mathrm{MB}})^{1/2}k_{\mathrm{st}(\gamma')}]\}$. Data from ref. 14.

TABLE IV. Heterotropic Cooperativity Effect: Chemical Potential, $\Delta\mu^\circ_{\gamma'}$, Enthalpy, $\Delta h^\circ_{\gamma'}$, and Entropy, $\Delta s^\circ_{\gamma'}$, at 25 °C and I = 0.1 M

Metal	Ligand		ΔH°_{MA} (kJ/mol)	$\Delta H^\circ_{M\bar{L}}$ [c] (kJ/mol)	ΔH°_{MAB} (kJ/mol)	$\Delta h^\circ_{\gamma'}$ [d] (kJ/mol)	$\Delta\mu^\circ_{\gamma'}$ (kJ/mol)	$\Delta s^\circ_{\gamma'}$ [e] (J/mol K)
	A[a]	B[b]						
Cu	CPRD	bpy	3.766	−20.397	−45.982	−2.59	−7.01	14.8
Cu	CBUD	bpy	10.879	−16.840	−36.400	−1.36	−6.24	16.4
Cu	CPED	bpy	13.598	−15.481	−35.980	−2.51	−3.82	0.4
Cu	CHED	bpy	14.184	−15.189	−35.606	−2.61	−3.90	0.4
Cu	mal	bpy	5.899	−19.330	−42.676	−2.01	−3.79	0.6
Cu	pht	bpy	10.293	−17.130	−33.860	0.20	−4.63	1.6
Cu	male	bpy	14.477	−15.040	−34.685	−2.30	−4.10	0.6
Cu	oda	bpy	14.895	−14.832	−29.874	−0.10	−1.22	3.8
Cu	thda	bpy	4.058	−20.251	−40.711	−0.10	−2.65	8.6
Cu	seda	bpy	10.083	−17.238	−35.941	−0.66	−4.30	12.2
Cu	thdp	bpy	16.778	−13.891	−38.451	−5.34	−1.82	−11.8
Cu	sedp	bpy	15.104	−14.728	−40.543	−5.54	−3.44	−7.1
Cu	succ	bpy	11.464	−16.548	−42.676	−4.79	−3.76	−3.5

[a]CPRD, CBUD, CPED, CHED, mal, pht, male, see Table III. oda = oxydiacetato, thda = thiodiacetato, succ = succinato, seda = selenodiacetato, thdp = thiodipropionato, sedp = selenodipropionato. [b]ΔH°_{MB} = −44.560 kJ/mol. [c]$\Delta H^\circ_{M\bar{L}} = (\Delta H^\circ_{MA} + \Delta H^\circ_{MB})/2$. [d]$\Delta h^\circ_{\gamma'} = \Delta H^\circ_{MAB}/2 - \Delta H^\circ_{M\bar{L}}$. [e]$\Delta s^\circ_{\gamma'} = (\Delta h^\circ_{\gamma'} - \Delta\mu^\circ_{\gamma'}) \times 1000/T$; T = 298.12 K. Data from ref. 14.

TABLE V. Homotropic Cooperativity Effect: Chemical Potential, $\Delta\mu^\circ_{\gamma}$, Enthalpy, Δh°_{γ}, and Entropy, Δs°_{γ}, at 25 °C and I = 0.1 M

Metal	Ligand[a]	ΔH°_{MA} [b] (kJ/mol)	$\Delta H^\circ_{MA_2}$ [c] (kJ/mol)	Δh°_{γ} [d] (kJ/mol)	$\Delta\mu^\circ_{\gamma}$ [e] (kJ/mol)	Δs°_{γ} [f] (J/mol K)
Cu	CPRD	3.766	4.059	−1.74	3.73	−18.3
Cu	CBUD	10.878	13.765	−4.00	1.38	−18.1
Cu	CPED	13.598	20.056	−3.56	2.89	−21.6
Cu	CHED	14.184	21.297	−3.54	2.68	−20.9
Cu	mal	5.899	5.104	−3.35	3.60	−23.3
Cu	pht	10.293	15.690	−2.45	1.05	−11.7
Cu	male	14.477	17.364	−5.80	1.21	−23.5

[a]CPRD, CBUD, etc. see Table III. [b]$\Delta H^\circ_{MA} = \Delta H^\circ(M + A)$. [c]$\Delta H^\circ_{MA_2} = \Delta H^\circ(M + 2A)$. [d]$\Delta h^\circ_{\gamma} = \Delta H^\circ_{MA_2}/2 - \Delta H^\circ_{MA}$. [e]$\Delta\mu^\circ_{\gamma} = \Delta G^\circ_{MA_2}/2 - \Delta G^\circ_{MA} + \Delta\mu^\circ_{st(\gamma)}$, with $\Delta\mu^\circ_{st(\gamma)}$ = 3.074 kJ/mol. [f]$\Delta s^\circ_{\gamma} = (\Delta h^\circ_{\gamma} - \Delta\mu^\circ_{\gamma}) \times 1000/T$, T = 298.12 K. Data from ref. 14.

the entropy contribution is unfavourable ($\Delta s^\circ_{\bar{\gamma}} < 0$).

It is important to emphasize the fact that the cooperativity effect exceeds the experimental error.

A fourth example (Table VI) shows how the cooperativity effect in macromolecule–ligand equilibria is of the same order of magnitude as the effects in metal–ligand complexes. Because small values of $\Delta\mu^\circ_{\gamma}$ and $\Delta\mu^\circ_{\gamma'}$ are sufficient to displace the equilibria and because the cooperativity effects are closely connected with changes in the ionic medium, it is easy to explain why many examples are found of denaturation of proteins caused by changes of the ionic strength and many other examples in which coupling between inorganic, organic and biological reactions takes place.

Conclusions

The parameters $K_{\bar{\gamma}}$ (homotropic) and $K_{\bar{\gamma}'}$ (heterotropic) and the thermodynamic quantities related to them are useful to evaluate the cooperativity effect.

In multiligand complexes the best way to evaluate the cooperativity effect is to calculate the average cooperativity by

$$K_{\bar{\gamma}} = \frac{\beta_i^{1/i}}{K_1 k_{st(\bar{\gamma})}} \tag{37}$$

The corresponding chemical potential change $\Delta\mu^\circ_{\bar{\gamma}}$ can in fact be correlated with the number of occupied sites. On the contrary the stepwise cooperativity parameter

TABLE VI. Heterotropic Cooperativity Effect in Biochemical Systems[a]

Protein	Ligand[b]		$\Delta\mu^{\circ}_{\gamma'}$[c] (kJ/mol)
	A	B	
Haemoglobin	O_2	2,3-DPG	2.72
Haemoglobin	O_2	IHP	4.81
Serum albumin, bovine	ANS	3,5-dbh	3.14
Pyruvatokinase	PEP	K	−2.51
Pyruvate kinase	K	Mn(II)	−2.93
Pyruvate kinase	Phenylal.	Mn(II)	1.67
Aspartatetransacarbamoylase	CTP	succ	1.05
Lactate dehydrogenase, chicken	NADH	ox	−3.14

[a]Data from ref. 15. [b]Ligand A: ANS = 1-anilinonaphthalene 8-sulphonato, PEP = phosphoenolpyruvato, CTP = cytidine triphosphato, NADH = hydrogennicotinamide-adenindinucleotide. Ligand B: 2,3-DPG = 2,3-diphosphoglycerato, IHP = inositol hexaphosphato, 3,5-dhb = 3,5-dihydroxobenzoato, succ = succinato, ox = oxalato. [c]Not corrected for statistical factor.

$$K_{\gamma} = \frac{(K_i K_{i-1})^{1/2}}{K_{i-1} k_{st(\gamma)}} \qquad (38)$$

is not clearly interpretable.

The values of $\Delta\mu^{\circ}_{\bar{\gamma}}$ and $\Delta\mu^{\circ}_{\bar{\gamma}'}$ are significantly greater than the experimental error, at least in the cases examined. This circumstance should be verified whenever possible.

The interference of the cooperativity effect with effects due to changes of the ionic strength confirms that the cooperativity effect should be mainly attributed to external ligand–ligand interactions, exposed to the action of the solvent. The free-energy changes due to ligand–ligand interactions in both homotropic and heterotropic metal–ligand equilibria are of the same order of magnitude as those in macromolecule–ligand equilibria and the corresponding reactions could be therefore coupled in real biological systems.

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