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Chelate Effect and Cooperativity Effect in Metal–Ligand and Macromolecule–Ligand Equilibria. I. Chemical Potential Changes and Cooperativity-Chelation Parameters

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Chelate and cooperativity effects both in the field of complexes formed in solution by metal ion with ligands and in the field of binding between protein and ligands were examined on the basis of thermodynamic arguments.

The analysis was carried out by means of the formation function $\bar{n} = \partial \ln \Sigma_M / \partial \ln[A]$ *where* Σ_M *is a partition function having free metal or macromolecule as basis reference level and A is a ligand. The chemical potential changes due to cooperativity and chelation are calculated from differences between areas of the diagram* $\bar{n} = f(\ln[A])$. *The chemical potentials are:* $\Delta\mu^\circ_\gamma = -RT \ln K_\gamma$ *(homotropic cooperativity),* $\Delta\mu^\circ_{\gamma'} = -RT \ln K_{\gamma'}$ *(heterotropic cooperativity),* $\Delta\mu^\circ_\epsilon = -RT \ln K_\epsilon$ *(homotropic chelation),* $\Delta\mu^\circ_{\epsilon'} = -RT \ln K_{\epsilon'}$ *(heterotropic chelation). The cooperativity and chelation parameters* K_γ, $K_{\gamma'}$, K_ϵ, $K_{\epsilon'}$ *are related to each other by other parameters* $K_\eta = K_\epsilon \cdot K_\gamma$ *and* $K_{\eta'} = K_{\epsilon'} \cdot K_{\gamma'}$. *All these dimensionless parameters are derived as ratios of experimental equilibrium constants. Therefore a corresponding consistent chemical potential scale can be obtained from experimental data for all these effects, leading to quantitative comparisons between cooperative and chelate effects, either homotropic or heterotropic.*

Thermodynamic function changes in metal–ligand complexes can also be compared on this same scale with the energetic changes in protein–ligand complexes.

Introduction

The thermodynamics of complexes formed in solution by a metal ion and one or more ligands presents close analogies with the thermodynamics of protein–ligand interactions. The physical-chemical models are strictly related, as well as the mathematical treatment. In order to achieve a unitary description of these two fields, we have examined meanings and connections of two important concepts, namely the chelate effect and the cooperativity effect. The former is particularly employed in the study of the thermodynamics of complexes, the latter in that of macromolecules.

The term chelate effect has been used by Schwarzenbach [1] to indicate that a polydentate ligand is able to form more stable complexes than the corresponding monodentate ligand. The molecular explanations of the chelate effect in terms of thermodynamic functions ΔG°, ΔH°, ΔS° are complex and rather controversial, probably because of the inadequate system of evaluation.

The possible factors which contribute to the chelate effect are, according to Myers [2]: the difference in free energy of solution from the gaseous state between chelate ($\mathcal{L}$) and monodentate ligand (A), the difference in steric and electrostatic repulsion between ligands in metal complexes $M\mathcal{L}$ and MA_n, respectively, the difference in crystal field effects, the number and type of chelate rings, the changes of solvation at chelation, the changes of degrees of freedom of the chelating ligand, *etc.* These factors contribute differently to the enthalpy and entropy of reaction.

The cooperativity effect on the other hand is a concept which is invoked in molecular biology [3] to explain how the attack of a first molecule on a multi-site macromolecule can influence further attacks by other molecules. The cooperativity can be either positive or negative. The possible causes of cooperativity at the molecular level are: the direct and short range interactions (hydrogen bonds, van der Waals forces) between two ligands competing for the same site of binding or adjacent sites, the long-range electrostatic interactions between two ligands at separate sites, the conformational changes of the macromolecule by which the affinity of the binding site for the ligand is changed, the variation of the solvent in the first solvation sphere with consequent changes in activity coefficient, *etc.* The causes of macromolecule–ligand, metal–ligand and ligand–ligand interactions are parallel, but sometimes the intensity of the effect is different. For instance the

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electrostatic interactions can be even more important in metal complexes than in macromolecules, the stereochemical variations of the complexes are parallel to the conformational changes of the macromolecules, but sharper than them.

Analytical and Thermodynamic Evaluation of the Chelate Effect

The chelate effect is evaluated in analytical chemistry by the displacement to the right of the equilibrium $M + \mathcal{L} \rightleftharpoons M\mathcal{L}$ with respect to the equilibrium $M + A \rightleftharpoons MA$, starting from equal concentrations of the reactants. This displacement can be calculated by the equilibrium constants of the two reactions

$$K_{M\mathcal{L}} = [M\mathcal{L}]/[M][\mathcal{L}], \quad K_{MA} = [MA]/[M][A] \qquad (1)$$

Following this idea Jameson [4] has shown how the chelate effect can be revealed by distribution diagrams (Fig. 1) by comparing the curves obtained for solutions of the same metal with different ligands.

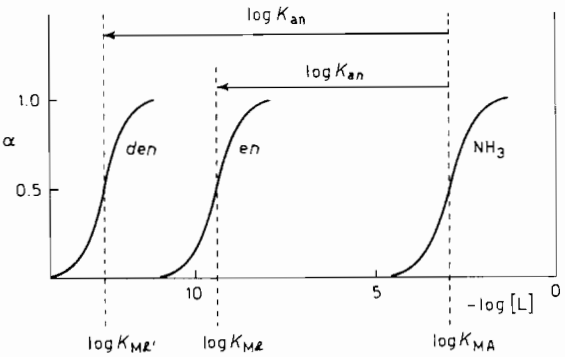

Fig. 1. Phenomenological description of the chelate effect on the distribution diagram. M = Ni(II); L = NH_3, $\mathcal{L}$ = en, $\mathcal{L}'$ = den. en = ethylenediamine, den = diethylenetriamine.

The diagrams for the reactions of Ni(II) with a) ammonia (A), (unidentate), b) ethylenediamine ($\mathcal{L}$), (bidentate), and c) diethylenetriamine ($\mathcal{L}'$), (tridentate) plotted as functions of log[A] or log[$\mathcal{L}$] or log[$\mathcal{L}'$] show how the complexes reach the same fraction α at three concentrations of complexing agents considerably different from one another. The concentration ratio of the ligands at $\alpha = 0.5$ equals the inverse ratio of the formation constants K_{MA}, $K_{M\mathcal{L}}$, $K_{M\mathcal{L}'}$, respectively. An analytical chelation constant $K_{an} = K_{M\mathcal{L}}/K_{MA}$ can represent straightforwardly the increase in concentration of chelate complex $M\mathcal{L}$ with respect to that of MA. These equilibrium constants are taken as operational analytical constants with dimensions depending on the concentration units; although this seems to be questionable [5], it is still very useful in order to check the correctness of the procedures.

The thermodynamic evaluation of the chelate effect is usually done according to Schwarzenbach [1] by chel = $\log K_{M\mathcal{L}} - \log \beta_{MA_2}$ which corresponds to a comparison of the reactions:

$$M + \mathcal{L} \rightleftharpoons M\mathcal{L}, \quad \Delta G^\circ = -RT \ln K_{M\mathcal{L}} \qquad (2)$$

$$M + 2A \rightleftharpoons MA_2, \quad \Delta G^\circ = -RT \ln \beta_{MA_2} \qquad (3)$$

This comparison should give the difference between the formation of two metal–donor atom bonds in different situations. These operative constants however are not dimensionally homogeneous because β_{MA_2} depends on [conc unit]$^{-2}$ whereas $K_{M\mathcal{L}}$ depends on [conc unit]$^{-1}$.

The transformation of the operative analytical constants into thermodynamic quantities can be done either by considering the concentrations as numbers [5] indicating the ratios between equilibrium concentrations and standard state concentrations, or by multiplying the concentrations by activity coefficients with dimensions [conc]$^{-1}$, or by means of the partition function, where dimensional equilibrium constants are multiplied by the appropriate powers of concentrations. We prefer to adopt the latter two points of view which preserve traces of the units. In the free-energy scale the comparison between $\Delta G^\circ_{MA_2}$ and $\Delta G^\circ_{M\mathcal{L}}$, derived from equilibrium constants with different dimensions, proves to be between capacitative functions of different capacity *i.e.* different numbers of moles of reactants.

The problem of homogeneity of dimensions has raised a long controversy. Adamson [6] suggested that one should employ the molar fractions to represent the solute and solvent concentrations and concludes that the chelate effect disappears with this choice of standard states. Beech [7] argues along the same line and states that the chelate effect is non-existent, while Agterdenbos [8] thinks that an antichelate effect arises when some concentration units are used. Jameson [4] calls attention to the observation that the complexes formed by polydentate ligands reach high concentrations even at very low concentration of the ligand. Munro [9] argues that the different numbers expressing the chelate effect when the units are changed are due mainly to an entropy effect which is different at different concentrations. On the other hand Hancock [10, 11] expresses the opinion that the chelate effect can be explained not only by the asymmetry of the standard states of solute and solvent, but also by the diminution of steric hindrance and of mutual electrostatic repulsion in the chelates with respect to complexes with monodentate ligands. The discussion of the influence of the choice of units on the so called 'cratic' term has also involved biophysical chemists [3].

The Cooperativity Effect and Its Evaluation

The cooperativity effect in macromolecule–ligand equilibria can be distinguished as homotropic and heterotropic cooperativity depending on the fact that the ligands are either equal, A (homotropic) or different, A, B,... (heterotropic). In the field of metal

complexes homotropic cooperativity is reflected in relative variations of stability of the species MA, MA_2, MA_3,... The evaluation of this homotropic cooperativity is usually done by comparison of the stepwise formation constants:

$$K_{MA} = [MA]/[M][A], \quad K_{MA_2} = [MA_2]/[MA][A],... \tag{4}$$

Such comparisons do not raise problems of homogeneity of units, because the ratios of these constants are all dimensionless.

The evaluation of the heterotropic cooperativity effect is usually done by comparison between the formation constants of homotropic and heterotropic complexes [12, 13]. For instance for a complex MAB one can consider the reaction

$$MA + MB \rightleftharpoons MAB + M$$
with constant $K_\Delta = \beta_{MAB}/\beta_{MA} \cdot \beta_{MB}$ (5)

or the reaction

$$MA_2 + MB_2 \rightleftharpoons 2MAB$$
with constant $K_M = \beta_{MAB}/\beta^{1/2}_{MA_2} \cdot \beta^{1/2}_{MB_2}$ (6)

or sometimes [14] with the constant $\chi_M = K^2_M$.

These constants are dimensionless but their thermodynamic meaning is obscure [15, 16]. More clearly biochemists [17] state that K_Δ is a measure of cooperativity. The values of K_Δ, K_M, χ_M, whatever is chosen, give different free energy changes ΔG° if the constants are raised to different powers (*e.g.* $-RT \ln \chi_M = -2\ RT \ln K_M$).

We have therefore searched for measures of the chelate and cooperativity effects that are, on the stability constant scale, independent of the concentration units and on the free energy scale are independent of the number of moles [18].

Results

Formation Function, Partial Molar Quantities and Homotropic Cooperativity

The algorithms and relationships used in metal–ligand [19] and protein–ligand thermodynamics [18] are interchangeable, although they have been developed independently by different Authors.

The Bjerrum formation function, $\bar{n}$ indicates the average number of ligands bound per mole of metal or macromolecule. We limit ourselves to the treatment of systems with mononuclear complexes, *i.e.* with one metal or one macromolecule per unit; in such systems $\bar{n}$ is independent of the total metal or macromolecule concentration. This limitation means that we treat the changes in affinity between binding site and ligand, together with allosteric or stereochemical variations but exclude the polysteric processes. The latter are those where more macromolecular units associate under the influence of the ligand. The analogous process with metal complexes is the formation of homo- and hetero-nuclear complexes.

The formation function of mononuclear complexes is

$$\bar{n} = \partial \ln \Sigma_M / \partial \ln [A] \tag{7}$$

where

$$\Sigma_M = 1 + \beta_{MA}[A] + \beta_{MA_2}[A]^2 + \ldots = \sum_i \beta_i [A]^i \tag{8}$$

and $\beta_{i=0} = 1$ refers to free metal or macromolecule. Σ_M has the character of a partition function [20] for a macrocanonical ensemble of statistical mechanics. The partition function is referred to a molecular distribution having the concentration of the free metal or of the free macromolecule in solution as basis reference level. Therefore one can write

$$\Sigma_M = e^{-\Delta G/RT} \tag{9}$$

and hence $\ln \Sigma_M = -\Delta G/RT$.

Recalling (7) and integrating we get

$$\int_{[A_1]}^{[A_2]} \bar{n}\ d \ln [A] = \int_{[A_1]}^{[A_2]} \frac{\partial(-\Delta G/RT)}{\partial \ln [A]}\ d \ln [A] \tag{10}$$

and we conclude (Fig. 2) that in the Bjerrum plane, where $\bar{n} = f(\ln [A])$, any area below the formation curve between two values of $\ln [A]$ represents the free energy change in $-\Delta G/RT$ units for the formation of the corresponding complex. The area is assigned a negative sign if in the integral (10) $[A_2] > [A_1]$ and a positive sign if the integration path is reversed. The sign of the integral depends on the concentration $[A_2]$ which is chosen as reference standard state. By this assignment the sign of the integrated area coincides with the sign of the corresponding ΔG.

Schellman [20] and Fronaeus [21] have shown that for any value of [A] the integral (10) holds

$$\Delta G/RT = -\int_0^{[A]} \bar{n}\ d \ln [A] = -\ln \Sigma_M \tag{11}$$

The area under the curve can be obtained by integration of the differential with respect to the variable $\bar{n}$ as well. Wyman [22] has shown in this way that for $\bar{n}_{max} = m$ (m coordinating sites)

$$\Delta G/RT = -m \ln [A]_{\bar{m}} \tag{12}$$

where $[A]_{\bar{m}}$ is the concentration of the ligand at the middle of the curve. In case the affinity of the ligands for the sites is distinctly different it is convenient to subdivide the curve into subsets each of which can be treated independently.

With reference to Fig. 3 the central point is the inflection at $\bar{n} = 1$ with $[A]_{\bar{m}} = \beta^{-1/2}_{MA_2}$ and therefore

$$\Delta G^\circ/RT = -2 \ln \beta_{MA_2}^{1/2} = -\ln \beta_{MA_2} \quad (13)$$

If we divide (13) by $\bar{n}_{max} = 2$ we get the change of chemical potential $\Delta\mu_{M\bar{L}}$ referred to the ligand A binding to M forming MA_2. The chemical potential corresponds therefore on the Bjerrum plane (Fig. 3) to the area $-a$, negative because obtained by integration from the standard state $[A_2] = 1$,

$$\Delta\mu^\circ_{M\bar{L}}/RT = -\frac{1}{2} \ln \beta_{MA_2} = -\frac{1}{2} (\ln K_{MA_2} + \ln K_{MA}) \quad (14)$$

being $\beta_{MA_2} = K_{MA} \cdot K_{MA_2}$.

The area $-a$ in Fig. 3 can be interpreted as the variation of chemical potential $\Delta\mu^\circ_{M\bar{L}}$ for addition to M of one mole of a hypothetical ligand, $\bar{L}$ with average affinity. The average is taken between the first and the second molecule of ligand A when both are binding together to the same metal or macromolecule, *i.e.* when cooperativity is operating:

$$\Delta\mu^\circ_{M\bar{L}} = \frac{1}{2} (\Delta\mu^\circ_{MA} + \Delta\mu^\circ_{MA_2}) \quad (15)$$

In order to calculate the value of the area c which is a measure of the cooperativity we consider the equation by Schellman [20] with limits of integration reversed, in the interval $0 \leqslant \bar{n} \leqslant 1$ and $0 \leqslant [A] \leqslant \beta_{MA_2}^{-1/2}$ (Fig. 4). This gives a positive value of the chemical potential

$$\Delta\mu^{\circ *}_{coop}/RT = \ln(1 + \beta_{MA}/\beta_{MA_2}^{1/2}) \simeq \ln \beta_{MA} - \ln \beta_{MA_2}^{1/2} = \frac{1}{2} (\ln K_{MA} - \ln K_{MA_2}) \quad (16)$$

The approximation holds for $\beta_{MA} \gg \beta_{MA_2}^{1/2}$ but the final result is valid whenever (*cf.* Fig. 4) the area c is symmetrical with respect to $\bar{n} = 0.5$ or reducible to that symmetrical shape. This value can therefore be obtained as the difference between two chemical potentials

$$\Delta\mu^{\circ *}_{coop} = \Delta\mu^\circ_{M\bar{L}} - \Delta\mu^\circ_{MA} \quad (17)$$

$\Delta\mu^\circ_{MA}$ being the reference state with which $\Delta\mu^\circ_{M\bar{L}}$ (which also contains the information concerning cooperativity) is compared. The area $\Delta\mu^{\circ *}_{coop}/RT$ (area c of Fig. 3) is starred to indicate that it is due both to cooperativity and a statistical factor.

The difference between two potentials is equivalent to assuming an equlibirum contant:

$$e^{-\Delta\mu^{\circ *}_{coop}/RT} = K^*_{coop} \quad (18)$$

with $K^*_{coop} = \beta_{MA_2}^{1/2}/\beta_{MA}$.

Such a constant is dimensionless and does not depend on the concentration units. At the same time $\Delta\mu^{\circ *}_{coop}$ being an intensive variable does not depend on the stoichiometry of the complex.

Actually we have to take into account that this quantity, $\Delta\mu^{\circ *}_{coop}$ contains not only the change of

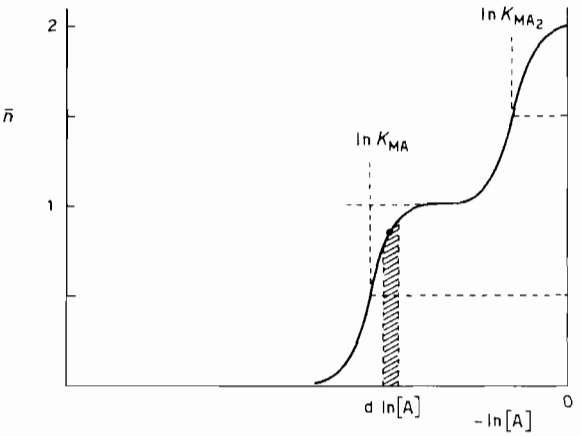

Fig. 2. Bjerrum diagram $\bar{n} = f(-\ln[A])$ for successive homotropic complexes MA and MA_2. The whole area under the curve represents the free energy change $\Delta G^\circ/RT$ of the reaction $M + 2\,A = MA_2$. The curve is the phenomenological evidence of cooperativity (negative, $\ln K_{MA_2} < \ln K_{MA}$).

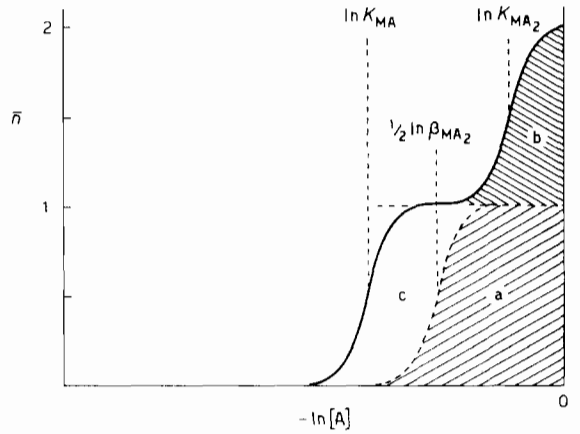

Fig. 3. Cooperativity effect: chemical potentials, $\Delta\mu^\circ$ (areas a, b, c of heights $\Delta\bar{n} = 1$). $-a = \Delta\mu^\circ_{M\bar{L}}$ for average $\bar{L}$; $-b = \Delta\mu^\circ$ for reaction $MA + A = MA_2$; $c = \Delta\mu^\circ_\gamma + \Delta\mu^\circ_{st}$ = cooperativity A··A. Note that $\ln K_{MA} = \ln \beta_{MA}$.

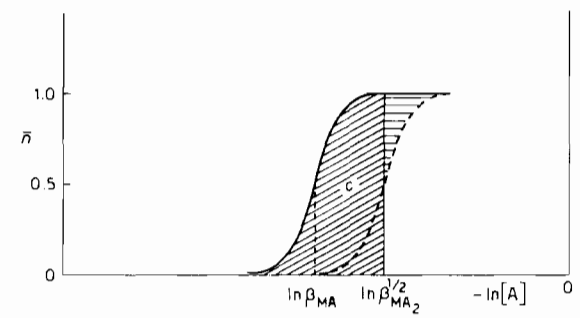

Fig. 4. Area c corresponding to cooperativity and statistical factor calculated by integration between limits $[A] = 0$ and $[A] = \beta_{MA_2}^{-1/2}$. Area c is symmetrical with respect to the line at $\bar{n} = 0.5$.

affinity due to the reciprocal interactions between the two ligands but also the statistical factors depending on the probability of occupancy of the binding sites. Therefore we make the correction, on the chemical potential scale

$$\Delta\mu^{\circ *}_{coop} = \Delta\mu^{\circ}_{\gamma} + \Delta\mu^{\circ}_{st} \quad (19)$$

from which one gets a pure cooperativity constant

$$K_{\gamma} = (K_{MA_2}/K_{MA})^{1/2} \cdot k_{st}^{-1/2} \quad (20)$$

The dimensionless constant, K_{γ}, corrected for the statistical factor, is independent even of the number of ligands and can be used for comparisons with other complexes whatever the number of ligands.

In general for n equal ligands one obtains

$$K_{\gamma} = (K_n \cdot K_{n-1} \cdot \ldots K_2/K_1^{n-1})^{1/n} \cdot k_{st}^{-1/n} \quad (21)$$

These constants are related to the interaction constants of Klotz [23] and Weber [17].

The equilibrium constants used to calculate K_{γ} are all homogeneous, being expressed in $[conc]^{-1}$. This is the same scale which is used to represent the equilibrium constants on the Scatchard diagrams [24] $\bar{n}/[A] = f(\bar{n})$, where the equilibrium constants are given by the slopes of the curves (measured in units of $[conc]^{-1}$), and the cooperativity constant, K_{γ} is given as the ratio of the slopes.

Chemical Potential and Homotropic Chelation

Using the same arguments as for the cooperativity effect we can now define the variation of chemical potential for the formation of a homotropic chelate, *i.e.* with equal donor atoms. Such variation, $\Delta\mu_{\epsilon}^{\circ}$ can be obtained by putting on the Bjerrum plane (Fig. 5) the formation function of the chelate $M\mathcal{L}$ which is centered at $\bar{n} = 0.5$ around $\ln K_{M\mathcal{L}}$. We consider the area proportional to the chemical potential of formation of the chelate $M\mathcal{L}$,

$$\Delta\mu^{\circ}_{M\mathcal{L}}/RT = -\ln K_{M\mathcal{L}} \quad (22)$$

which is the sum of the areas *a*, *c*, *s*, and *d*. The area *a* measures the affinity of the average ligand $\bar{L}$, the area *c* measures the cooperativity A...A and the area *s* measures the statistical contribution. The area *d* corresponds to $\Delta\mu^{\circ}_{an}/RT = \ln K_{M\mathcal{L}} - \ln K_{MA}$ and the area $(d + c + s)$ to the actual increment of affinity due to chelation. Therefore

$$\Delta\mu^{\circ}_{\epsilon}/RT = \Delta\mu^{\circ}_{an}/RT - \Delta\mu^{\circ *}_{coop}/RT \quad (23)$$

and the change of chemical potential on chelation results as the difference between the potentials of complexes MA and $M\mathcal{L}$ (area *d*) plus the energy employed to overcome the negative cooperativity between ligands (area $c + s$), hence

$$\Delta\mu^{\circ}_{\epsilon} = -RT \ln K_{M\mathcal{L}} + RT \ln \beta^{1/2}_{MA_2} \quad (24)$$

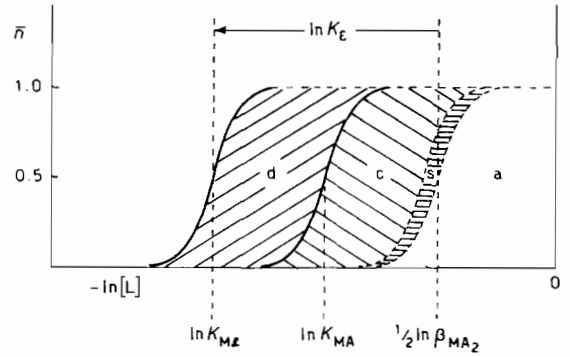

Fig. 5. Chelate effect: chemical potentials, $\Delta\mu^{\circ}$ (areas *a*, *c*, *d*, *s* of heights $\Delta\bar{n} = 1$). $-a = \Delta\mu^{\circ}_{M\bar{L}}$ for average ligand $\bar{L}$; $-(d + c + s) = \delta\mu^{\circ}_{\epsilon}$ = chelate effect; $c = \Delta\mu^{\circ}_{\gamma}$ = cooperativity; $s = \Delta\mu^{\circ}_{st}$ = statistical factor; $-d = \Delta\mu^{\circ}_{an}$ = analytical chelate effect.

The correction for the statistical contribution (area *s*) is not subtracted here because it should hold both for cooperativity and chelation.

The relationship between potentials implies that we measure the intensity of chelation by a chelation constant

$$K_{\epsilon} = K_{M\mathcal{L}}/\beta^{1/2}_{MA_2} \quad (25)$$

which is dimensionless and suited to express on a unique scale the chelate effect of any bidentate chelating agent with equal donor atoms. For a chelate with n equal donor atoms, this constant becomes

$$K_{\epsilon} = K_{M\mathcal{L}(n)}/\beta^{1/n}_{MA_n} \quad (26)$$

This constant makes it possible to compare on a unique scale the affinity of every homotropic chelating agent whatever its denticity.

Chemical Potential and Heterotropic Cooperativity or Chelate Effect

When the complexes are heterotropic because the binding atoms are different from one another, then the cooperativity effect can be evaluated if reference is made to mixed complexes. There is a variety of equilibrium constants employed to establish the thermodynamic stability, namely K_{Δ}, K_M, and χ_M but none of them gives satisfactory results and all have obscure or controversial interpretations [15, 16].

We can find a coherent definition of $\Delta\mu^{\circ}_{\gamma}$, the change of the chemical potential with heterotropic cooperativity if we draw on the Bjerrum plane (Fig. 6) the curve for $\Delta\mu^{\circ}_{M\bar{L}}/RT$ obtained from the average between the chemical potential change for the attack of both ligands A and B to M

$$\Delta\mu^{\circ}_{M\bar{L}} = -\frac{1}{2}\ln \beta_{MAB} \quad (27)$$

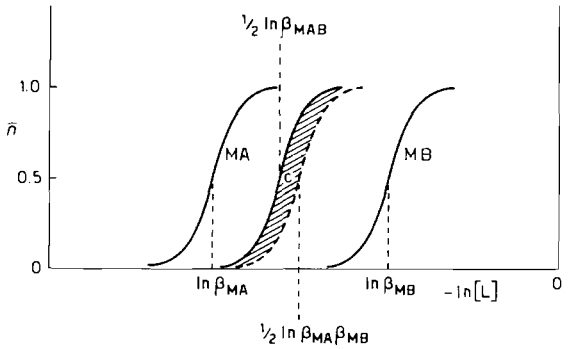

Fig. 6. Heterotropic cooperativity: chemical potential $\Delta\mu^{\circ}_{coop}/RT$ as measured by area c.

The area defined by this curve is now compared with the area obtained for the change of chemical potential for the formation of $M\bar{L}$, when A and B are bound to M separately, *i.e.* without any cooperativity effect,

$$\Delta\mu^{\circ}_{M\bar{L}}/RT = -(\Delta\mu^{\circ}_{MA} + \Delta\mu^{\circ}_{MB})/2RT = \\ = -\frac{1}{2}(\ln \beta_{MA} + \ln \beta_{MB}) \qquad (28)$$

This is the reference state. By comparison (*i.e.* subtraction) with the reference state one gets $\Delta\mu^{\circ}_{\gamma}'$,

$$\Delta\mu^{\circ}_{\gamma}' = -\frac{1}{2}\ln \beta_{MAB} + \frac{1}{2}(\ln \beta_{MA} + \ln \beta_{MB}) \qquad (29)$$

This evaluation $\Delta\mu^{\circ}_{\gamma}'/RT$ corresponds to using, when considering equilibrium constants, the dimensionless quantity

$$K_{\gamma}' = [\beta^{1/2}_{MAB}/(\beta_{MA}\cdot\beta_{MB})^{1/2}]\cdot k_{st}^{-1/2} \qquad (30)$$

where k_{st} is introduced to amend β_{MAB} by the required statistical factor. The equilibrium constant thus obtained is related to K_{Δ}, which would have been a correct parameter of cooperativity if one had taken its square root and corrected for the statistical factor. With the same arguments one can demonstrate that K_M and χ_M are meaningless.

For mixed complexes MA_2B, K_{γ}' will be given by:

$$K_{\gamma}' = [\beta^{1/3}_{MA_2B}/(\beta^{1/2}_{MA_2}\cdot\beta_{MB})^{1/2}]\cdot k_{st}^{-1/2} \qquad (31)$$

with parallel results on the scale of chemical potentials.

The same arguments as for mixed complexes can be applied to heterotropic chelation, provided that the potential $\Delta\mu^{\circ}_{M\mathcal{L}}/RT = -\ln K_{M\mathcal{L}}$ is taken instead of $1/2 \ln \beta_{MAB}$ as the quantity to be calculated and compared. The term with which it is compared can be chosen in two ways. If one can dispose of the constant of the mixed complex MAB with the same donor atoms as the chelate $\mathcal{L}$, then the comparison can be done by

$$\Delta\mu^{\circ}_{\epsilon}/RT = -\ln K_{M\mathcal{L}} + \frac{1}{2}\ln \beta_{MAB} \qquad (32)$$

which is perfectly parallel to $\Delta\mu^{\circ}_{\epsilon}/RT$ of (23). This means that we can evaluate a dimensionless constant

$$K_{\epsilon}' = K_{M\mathcal{L}}/\beta^{1/2}_{MAB} \qquad (33)$$

This constant does not need a statistical correction factor because this operates equally on $K_{M\mathcal{L}}$ and $\beta^{1/2}_{MAB}$.

If the mixed complex cannot be formed or its constant is not known we can do the comparison

$$\Delta\mu^{\circ}_{\eta}'/RT = -\ln K_{M\mathcal{L}} + \frac{1}{2}(\ln \beta_{MA} + \ln \beta_{MB}) \qquad (34)$$

which corresponds to a constant

$$K_{\eta}' = [K_{M\mathcal{L}}/(\beta_{MA}\cdot\beta_{MB})^{1/2}]\cdot k_{st}^{-1/2} \qquad (35)$$

This constant must be corrected for a statistical factor which does not operate when A and B are bound to M separately. This constant K_{η}' includes however the cooperativity effect in the chelate effect or, say, does not add to the phenomenological increment of stability the further energy spent to overcome negative cooperativity (or does not subtract it if cooperativity is positive). The constant K_{η}' is perfectly analogous to K_{an} for homotropic chelates. Therefore for homotropic chelates an equilibrium constant

$$K_{\epsilon} = K_{an}\cdot k_{st}^{-1/2} \qquad (36)$$

can be adopted.

Conclusions

Cooperativity–Chelation Parameters

Using the arguments laid out in the preceding paragraphs we can draw a general picture of the equilibrium constants that can legitimately be used as parameters to compare the cooperativity and chelate effects, both homotropic and heterotropic on consistent and general scales (Table I.).

From an analysis of Table I some conclusions can be drawn: the constants which are here proposed as cooperativity–chelation parameters form a very compact and consistent set, and show clearly that the cooperativity and chelate effects are strictly related to one another. They can be compared on the same common scale. The relationship between the two effects, both in the homotropic and heterotropic case, can be calculated by

$$K_{\gamma}\cdot K_{\epsilon} = K_{\eta} \quad \text{and} \quad K_{\gamma}'\cdot K_{\epsilon}' = K_{\eta}' \qquad (37)$$

respectively.

Each indicator K_{γ}, K_{ϵ}, K_{η}, K_{γ}', K_{ϵ}', K_{η}' is obtained as a ratio between two equilibrium constants each of which depends on [conc unit]$^{-1}$.

TABLE I. Cooperativity–Chelation Parameters; Cooperativity: A····A, A····B; Chelation: Bidentate £.

Effect	Parameter
Cooperativity	
homotr.	$K_\gamma = [\beta_{MA_2}^{1/2}/\beta_{MA}] \cdot k_{st}^{-1/2}$
heterotr.	$K_{\gamma'} = [\beta_{MAB}^{1/2}/(\beta_{MA} \cdot \beta_{MB})^{1/2}] \cdot k_{st}^{-1/2}$
Chelation	
homotr.	$K_\epsilon = K_{ML}/\beta_{MA_2}^{1/2}$
heterotr.	$K_{\epsilon'} = K_{ML}/\beta_{MAB}^{1/2}$
Chelation with cooperativity	
homotr.	$K_\eta = [K_{ML}/\beta_{MA}] \cdot k_{st}^{-1/2}$
heterotr.	$K_{\eta'} = [K_{ML}/(\beta_{MA} \cdot \beta_{MB})^{1/2}] \cdot k_{st}^{-1/2}$
General relationships	
$K_\gamma \cdot K_\epsilon = K_\eta$	$K_{\gamma'} \cdot K_{\epsilon'} = K_{\eta'}$
$K_\eta = K_{an} \cdot k_{st}^{-1/2}$	

The latter constants are therefore homogeneous with the scale of the activity coefficients and comparable with them on the chemical potential scale (kJ mol^{-1}). This means that both effects, those explained by Arrhenius theory and those explained by Debye–Hückel theory, can be evaluated on a general common scale.

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