Average Cooperativity Effect and Site Binding Constants in Homotropic Complexes

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Abstract

The equilibria between metals or receptors and ligands are described by the formation function

$\bar{n}$ = ligand bound/total receptor

From the experimental formation function, the binding polynomial

$$\Sigma_M = 1 + \beta_1[A] + \ldots + \beta_i[A]^i + \ldots + \beta_t[A]^t$$

or formation (gran canonical) partition function is obtained as function of the cumulative constants β_i. Σ_M can be related to the stepwise equilibrium constants by introducing a dissociation partition function and a saturation function $F_M^c = \Sigma_M/\Sigma_D$. The standard value, $F_M^{c\ominus}$ coincides with $\beta_t = K_1 K_2 \ldots K_i \ldots K_t$ of the completely saturated receptor or metal.

By calculating $K_{\bar{\gamma}} = (\beta_i^{1/i}/K_1)(1/k_{st(\bar{\gamma})})$ one obtains an average cooperativity effect between binding molecules. In nickel—ammonia system at 30 °C the cooperativity effect comes out to be $\Delta\mu^\circ_{\bar{\gamma}(i)} = -0.752 + 0.621(i - 1)$ kJ/mol and in the system of bovine serum albumin (BSA) with copper(II) at 25 °C is $\Delta\mu^\circ_{\bar{\gamma}(i)} = 0.034 + 0.123(i - 1)$ kJ/mol.

By comparing the experimental binding polynomial with a model partition function for cooperative equal binding, $\Sigma_{M,CE}$, e.g. for three site receptors or metals

$$\Sigma_{M,CE} = 1 + 3k[A] + 3\bar{\gamma}_2 k^2[A]^2 + \bar{\gamma}_3 k^3[A]^3$$

with k = equal intrinsic site constant, one obtains $\bar{\gamma}_2 = K_{\bar{\gamma}}^2$ and $\bar{\gamma}_3 = K_{\bar{\gamma}}^3$. The values of $\bar{\gamma}_2$, $\bar{\gamma}_3$ thus obtained are then introduced in a corrected formation function $\bar{n}_{corr}$ which gives very good linear correlations on the Scatchard plot. From these plots the values of the intrinsic binding constant k are obtained which are $k = 92.4$ for nickel—ammonia at 30 °C and $k = 1.3 \times 10^3$ for copper—BSA at 25 °C. These values correspond to values $\Delta\mu^\circ_k = -RT \ln k$ of -11.41 kJ/mol and -17.8 kJ/mol, respectively. Also the equilibrium constants of the nickel—ammonia system at other temperatures and ionic strengths as well those of the cobalt(II)—ammonia system, have been analysed following the same procedure. In the nickel—hydrazine system the cooperativity is almost null and in the cadmium—ammonia system two different sets of sites are put in evidence.

The strict parallelism and possible coupling of chemical and biochemical systems are discussed.

Introduction

The equilibria between receptor M (macromolecule or metal) and ligand A are described by the formation function $\bar{n}$ of Bjerrum [1] which can be

$\bar{n}$ = ligand bound/total receptor (1)

obtained directly from the experimental data. The experimental formation function $\bar{n}$ can be expressed as function of cumulative constants

$$\beta_i = [MA_i][M]^{-1}[A]^{-i}$$

from

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

being

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

the so called binding polynomial. The β_i's can be calculated, starting from initial guessed values, by optimizing the agreement between observed and calculated formation function $\bar{n}$; alternatively the agreement can be searched for by recalculating via (3) and (2) directly the experimental data such as mass balance equations of the reagents, electromotive forces, spectroscopic absorbances, etc. (*cf.* [2, 3] for good reviews and many examples of equilibrium constants calculation from potentiometric or spectrophotometric data). Being the binding atoms of the ligand all of the same homogeneous type, the complexes are said *homotropic.*

We have shown in a preceding paper [4] how the binding polynomial obtained from the experimental

formation function $\bar{n}$ can be related to either the stepwise equilibrium constants or to the site equilib-

$$K_i = \frac{[MA_i]}{[MA_{i-1}][A]}$$

rium constant k_i. The stepwise equilibrium constants K_i behave as factors of a convoluted function, F_M^c, which is the ratio between the formation partition function (1), Σ_M, and the dissociation partition function,

$$\Sigma_M^D = 1 + \frac{1}{K_t[A]} + \frac{1}{K_t K_{t-1}[A]^2} + \ldots$$

$$+ \frac{1}{K_t K_{t-1} \ldots K_1 [A]^t} \qquad (4)$$

The binding polynomial can be considered as a gran canonical partition function of the statistical thermodynamics, and more precisely a formation partition function.

The formation partition function (3) indicates the probability, relative to free M, of finding every complex MA_i formed by association starting from free M, whereas the dissociation partition function (4), indicates the probability, relative to MA_t, of finding every complex MA_i formed by dissociation from the completely saturated complex MA_t.

The standard convoluted function coincides with the cumulative formation constant $\beta_t = K_1 K_2 \ldots K_i \ldots K_t$ of the saturated complex MA_t.

We have also shown that by using the stepwise equilibrium constants $K_i = \beta_i/\beta_{i-1}$, we can calculate stepwise cooperativity parameters

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

$$(5)$$

whereas by using the cumulative equilibrium constants we can calculate average cooperativity parameters

$$K_{\bar{\gamma}(i)} = \beta_i^{1/i}/(K_1 k_{st(\bar{\gamma})}) \qquad (6)$$

The average cooperativity $K_{\bar{\gamma}}$ takes into account the effect of the rearrangement of the bonds upon the free energy of binding.

It is the purpose of the present paper to show how, by means of the average cooperativity constants, the site binding constant, k, in homotropic complexes can be calculated.

Cooperativity

The experimental binding polynomial (3) can be compared with a model partition function $\Sigma_{M.CU}$, for cooperative unequal (CU) binding sites, e.g. for a three site receptor, M

$$\Sigma_{M.CU} = 1 + (k_1 + k_2 + k_3)[A] + (k_1 k_2 + k_1 k_3 + k_2 k_3)\bar{\gamma}_2 [A]^2 + k_1 k_2 k_3 \bar{\gamma}_3 [A]^3 \qquad (7)$$

where $\bar{\gamma}_2$, $\bar{\gamma}_3$ are coefficients of average interaction between pairs and triples of ligands A contemporarily bound to M. For homotropic ligands ($k_1 = k_2 = k_3 = k$) the expression (7) simplifies to a cooperative equal (CE) model,

$$\Sigma_{M.CE} = 1 + 3k[A] + 3k^2\bar{\gamma}_2 [A]^2 + k^3\bar{\gamma}_3 [A]^3 \qquad (8)$$

From (8) we can calculate the formation function

$$\bar{n} = \frac{\partial \ln \Sigma_{MCE}}{\partial \ln[A]}$$

$$= \frac{3k[A] + 6k^2\bar{\gamma}_2 [A]^2 + 3k^3\bar{\gamma}_3 [A]^3}{1 + 3k[A] + 3k^2\bar{\gamma}_2 [A]^2 + k^3\bar{\gamma}_3 [A]^3} \qquad (9)$$

and from this the Scatchard function

$$\frac{\bar{n}}{[A]} = \frac{3k + 6k^2\bar{\gamma}_2 [A] + 3k^3\bar{\gamma}_3 [A]^2}{1 + 3k[A] + 3k^2\bar{\gamma}_2 [A]^2 + k^3\bar{\gamma}_3 [A]^3} \qquad (10)$$

This function plotted against $\bar{n}$ is a curve convex upward or downward depending on the value of $\bar{\gamma}_2$, $\bar{\gamma}_3$ [5, 6].

The values of $\bar{\gamma}_2$ and $\bar{\gamma}_3$ can be obtained by comparison of (3) with (8), from which we identify $\beta_1 = 3k$, $\beta_2 = 3k^2\bar{\gamma}_2$, $\beta_3 = k^3\bar{\gamma}_3$. Then if we calculate

$$K_{\bar{\gamma}(2)} = \frac{\beta_2^{1/2}}{\beta_1} \frac{1}{k_{st(\bar{\gamma})}} \qquad (11)$$

with $k_{st(\bar{\gamma})} = 3^{1/2}/3$ we obtain $K_{\bar{\gamma}(2)} = \bar{\gamma}_2^{1/2}$ and analogously from

$$K_{\bar{\gamma}(3)} = \frac{\beta_3^{1/3}}{\beta_1} \frac{1}{k_{st(\bar{\gamma})}} \qquad (12)$$

with $k_{st(\bar{\gamma})} = 1/3$ we obtain $K_{\bar{\gamma}(3)} = \bar{\gamma}_3^{1/2}$. It is possible therefore to obtain a corrected formation function

$$\bar{n}_{corr} = \frac{\beta_1[A] + 2(\beta_2/\bar{\gamma}_2)[A]^2 + 3(\beta_3/\bar{\gamma}_3)[A]^3}{1 + \beta_1[A] + (\beta_2/\bar{\gamma}_2)[A]^2 + (\beta_3/\bar{\gamma}_3)[A]^3}$$

$$= \frac{\partial \ln \Sigma_{M.IE}}{\partial \ln [A]} \qquad (13)$$

where $\Sigma_{M.IE}$ is the partition function of a model with independent and equal (IE) sites. The function $\bar{n}_{corr}/[A] = f(\bar{n})$ should be a straight line provided that $\bar{\gamma}_2$ and $\bar{\gamma}_3$ had been properly calculated.

TABLE I. Octahedral Receptors: Statistical Factors for Stepwise ($k_{st(\gamma)}$) and Average ($k_{st(\bar\gamma)}$) Cooperativity with Monodentate and Bidentate Ligands[a]

Monodentate ligand				Bidentate ligand	
$K_{\gamma(i)}$		$K_{\bar\gamma(i)}$		$K_{\gamma(i)}$	
$R_{\gamma(i)}$	$k_{st(\gamma(i))}$	$R_{\bar\gamma(i)}$	$k_{st(\bar\gamma(i))}$	$R_{\gamma(i)}$	$k_{st(\gamma(i))}$
$\beta_2^{1/2}/\beta_1$	$15^{1/2}/6 = 0.645$		$15^{1/2}/6 = 0.645$	$\beta_2^{1/2}/\beta_1$	$12^{1/2}/12 = 0.289$
$(\beta_3\beta_5)^{1/2}/\beta_4$	$(6 \times 20)^{1/2}/15 = 0.730$	$\beta_5^{1/5}/\beta_1$	$20^{1/3}/6 = 0.452$	$(\beta_3\beta_1)^{1/2}/\beta_2$	$(1/12)^{1/2}/12 = 0.289$
$(\beta_2\beta_4)^{1/2}/\beta_3$	$(15 \times 15)^{1/2}/20 = 0.750$	$\beta_6^{1/6}/\beta_1$	$15^{1/4}/6 = 0.328$	$K_{\bar\gamma(i)}$	
				$R_{\bar\gamma(i)}$	$k_{st(\bar\gamma(i))}$
$(\beta_3\beta_5)^{1/2}/\beta_4$	$(6 \times 20)^{1/2}/15 = 0.730$	$\beta_5^{1/5}/\beta_1$	$6^{1/5}/6 = 0.238$	$\beta_3^{1/3}/\beta_1$	$1/12 = 0.083$
$(\beta_4\beta_6)^{1/2}/\beta_5$	$(1 \times 15)^{1/2}/6 = 0.645$	$\beta_6^{1/6}/\beta_1$	$1/6 = 0.167$		

[a]$R_{\gamma(i)}$ or $R_{\bar\gamma(i)}$ is the ratio, R_{obs} [4] between roots of the experimental equilibrium constants used in the evaluation of the cooperativity effect $K_{\gamma(i)}$ and $K_{\bar\gamma(i)}$, respectively.

Statistical Factors

The statistical factors k_{st} derive from the statistical occupancy of sites. For instance in a receptor with three sites at the second level of binding (β_2) the occupancy fractions are in an IU model

$$x_{12} = k_1k_2/(k_1k_2 + k_1k_3 + k_2k_3)$$
$$x_{13} = k_1k_3/(k_1k_2 + k_1k_3 + k_2k_3) \quad (14)$$
$$x_{23} = k_2k_3/(k_1k_2 + k_1k_3 + k_2k_3)$$

which reduce to $x_2 = 1/3$ in an IE model. x_i is the inverse of the multiplicity coefficient m_i [4].

For a receptor with four sites at the same level of binding (β_2) we have

$$x_{12} = k_1k_2/(k_1k_2 + k_1k_3 + k_1k_4 + k_2k_3 + k_2k_4$$
$$+ k_3k_4) \quad (15)$$

etc., which become $x_2 = 1/6$ in an IE model.

The occupancy fractions are directly related to the entropy of mixing of the species at the same i level of binding. For the first example above the entropy of mixing is

$$\Delta S_{mix} = -x_{12}R \ln x_{12} - x_{13}R \ln x_{13} - x_{23}R \ln x_{23} \quad (16)$$

and for equal sites

$$\Delta S_{mix} = -R \ln \tfrac{1}{3} \quad (17)$$

For a linear receptor with t equal sites and a monodentate ligand the multiplicity coefficient $m_i = 1/x_i$, and hence the entropy of mixing can be calculated from

$$m_i = t!/\{(t-1)! \, i!\} \quad (18)$$

In order to make meaningful comparisons between intrinsic affinities of binding a correction for such

entropic terms must be introduced at both numerator and denominator of $K_{\gamma(i)}$ or $K_{\bar\gamma(i)}$. Therefore $k_{st(\gamma(i))}$ or $k_{st(\bar\gamma(i))}$ is a ratio between appropriate roots of multiplicity coefficients.

The multiplicity coefficients depend on the geometry and faults of the receptor and on the denticity of the ligand. A list of statistical factors for different geometries of the receptor and different denticities of the ligand are reported in Table I and in Table II. The multiplicity coefficients for receptors with faults or excluded volumes deserve a special treatment and will be presented separately.

Applications

The experimental data have been obtained from the literature. The system nickel—ammonia has been studied at 30 °C by Bjerrum [1] and then by Nagypal, Gergely and Jekel [7]. The system can be considered representative of an inorganic reaction.

The biochemical example has been taken from Klotz and Curme [8]. They refer to the equilibria between copper(II) ion as ligand and bovine serum albumin (BSA) as receptor measured by dialysis. The data have been interpreted on the basis of sixteen stepwise equilibrium constants. We have retained this model although in a successive paper [9] it has been interpreted on the basis of fourteen steps, with introduction of imaginary roots for the binding polynomial. The imaginary roots for the solution of the binding curves, have been invoked also by Fletcher and Spector [10], and recently by Pedersen and Pedersen [11]. The imaginary roots however should be considered as mathematical tricks rather than physical concepts compatible with the interpretation of the partition function terms as probabilities. The

TABLE II. Tetrahedral Receptors: Statistical Factors for Stepwise ($k_{st(\gamma)}$) and Average ($k_{st(\bar{\gamma})}$) Cooperativity with Monodentate and Bidentate Ligands[a]

Monodentate ligand				Bidentate ligand	
$K_{\gamma(i)}$		$K_{\bar{\gamma}(i)}$		$K_{\gamma(i)} = K_{\bar{\gamma}(i)}$	
$R_{\gamma(i)}$	$k_{st(\gamma(i))}$	$R_{\bar{\gamma}(i)}$	$k_{st(\bar{\gamma}(i))}$	$R_{\gamma(i)}$	$k_{st(\gamma(i))}$
$\beta_2{}^{1/2}/\beta_1$	$6^{1/2}/4 = 0.612$		$6^{1/2}/4 = 0.612$	$\beta_2{}^{1/2}/\beta_1$	$1/6 = 0.167$
$(\beta_1\beta_3)^{1/2}/\beta_2$	$(4 \times 4)^{1/2}/6 = 0.667$	$(\beta_3)^{1/3}/\beta_1$	$4^{1/3}/4 = 0.397$		
$(\beta_2\beta_4)^{1/2}/\beta_3$	$(1 \times 6)^{1/2}/4 = 0.612$	$(\beta_4)^{1/4}/\beta_1$	$1/4 = 0.250$		

[a]$R_{\gamma(i)}$ or $R_{\bar{\gamma}(i)}$ is the ratio, R_{obs} [4] between roots of the experimental equilibrium constants used in the evaluation of the cooperativity effect $K_{\gamma(i)}$ and $K_{\bar{\gamma}(i)}$, respectively.

TABLE III. Calculation of the Average Cooperativity Effect in the System $Ni^{2+}-NH_3$ at 30 °C, $I = 2.0$ M[a]

i	$\log \beta_i$	$\frac{1}{i} \log \beta_i$	$-\log k_{st(\bar{\gamma})}$	$\log \dfrac{\beta_i{}^{1/i}}{k_{st(\bar{\gamma})}}$	$\log K_{\bar{\gamma}(i)}$	$\log K_{\bar{\gamma}(i)}{}^{calc}$ [b]	$\log \dfrac{\beta_i}{(K_{\bar{\gamma}(i)}{}^{calc})^i}$
1	2.78	2.78	0	2.78	0	0	2.78
2	5.05	2.525	0.190	2.715	−0.065	−0.068	5.186
3	6.70	2.233	0.345	2.578	−0.202	−0.187	7.261
4	8.01	2.003	0.484	2.487	−0.293	−0.305	9.230
5	8.66	1.732	0.622	2.354	−0.426	−0.424	10.780
6	8.74	1.457	0.778	2.235	−0.545	−0.542	11.992

[a]Data from ref. 7.   [b]Calculated from equation of Table V.

TABLE IV. Calculation of the Average Cooperativity Effect in the System $Cu^{2+}-BSA$ at 25 °C and pH = 4.83[a]

i	$\log \beta_i$	$\frac{1}{i} \log \beta_i$ [b]	$-\log k_{st(\bar{\gamma})}$	$\log \dfrac{\beta_i{}^{1/i}}{k_{st(\bar{\gamma})}}$	$\log K_{\bar{\gamma}(i)}$	$\log K_{\bar{\gamma}(i)}{}^{calc}$ [c]	$\log \dfrac{\beta_i}{(K_{\bar{\gamma}(i)}{}^{calc})^i}$
1	4.331	4.331	0	4.331	0	0	4.331
2	8.288	4.144	0.165	4.309	−0.022	−0.027	8.342
3	11.996	3.999	0.288	4.287	−0.044	−0.049	12.139
4	15.502	3.876	0.389	4.265	−0.066	−0.070	15.781
5	18.833	3.767	0.476	4.243	−0.088	−0.092	19.229
6	22.003	3.667	0.554	4.221	−0.109	−0.113	22.689
7	25.021	3.574	0.624	4.198	−0.132	−0.135	25.974
8	27.891	3.486	0.690	4.176	−0.155	−0.156	29.150
9	30.614	3.402	0.753	4.155	−0.176	−0.178	32.218
10	33.189	3.319	0.814	4.133	−0.198	−0.199	35.209
11	35.611	3.237	0.874	4.111	−0.220	−0.221	38.071
12	37.873	3.156	0.932	4.088	−0.243	−0.242	40.825
13	39.960	3.074	0.993	4.067	−0.264	−0.264	43.449
14	41.845	2.989	1.056	4.045	−0.286	−0.285	45.850
15	43.480	2.899	1.124	4.023	−0.309	−0.306	48.156
16	44.742	2.796	1.204	4.000	−0.331	−0.328	50.082

[a]Data from ref. 8.   [b]$(1/i) \log \beta_i = 4.249 - 0.09149\, i$, $r = 0.9973$.   [c]$\log K_{\bar{\gamma}(i)}{}^{calc} = -0.006 - 0.02147(i - 1)$, $r = 0.9987$. $\Delta\mu°_{\bar{\gamma}(i)} = 0.034 + 0.123(i - 1)$.

details of the calculation of the average cooperativity constants are reported in Tables III and IV for nickel—ammonia and copper(II)—BSA systems, respectively.

After having found the experimental β_i values and introduced the corrections for the statistical effect, the values of the average cooperativity chemical
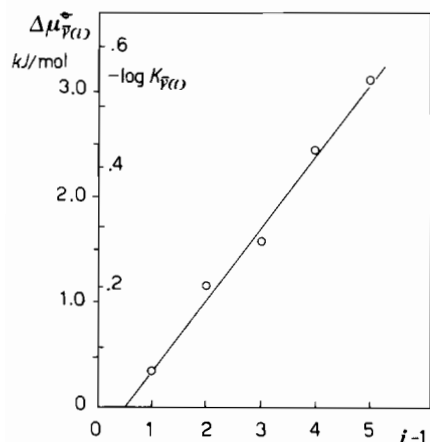
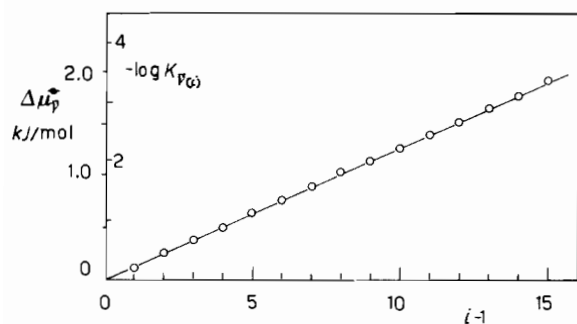Fig. 1. Average cooperativity effect for nickel(II)–ammonia system at 30 °C, $I = 2.0$ M.

Fig. 2. Average cooperativity effect for copper(II)–bovine serum albumin (BSA) at 25 °C, pH = 4.83.

Fig. 3. Nickel–ammonia system at 30 °C, $I = 2.0$ M: Scatchard plots; ○, uncorrected; □, corrected for cooperative equal (CE) model.

Fig. 4. Copper(II)–bovine serum albumin (BSA) system at 25 °C, pH = 4.83: Scatchard plots; ○, uncorrected; □, corrected for cooperative equal (CE) model.

Fig. 5. Nickel–ammonia system at 30 °C, $I = 2.0$ M: average enthalpy, $(1/i)/\Delta H_i^\circ$. Data from ref. 23.

potentials, $\Delta\mu^\circ_{\bar{\gamma}(i)} = -RT \ln K_{\bar{\gamma}(i)}$, are plotted against $(i - 1)$ (Figs. 1 and 2). The linearity and monotonicity of the dependence of the average cooperativity potentials upon $(i - 1)$ indicates that the binding sites belong to a unique class. The average cooperativity potential change results to be $\Delta\mu^\circ_{\bar{\gamma}(i)} = -0.752 + 0.621(i - 1)$ kJ/mol for nickel–ammonia at 30 °C and $\Delta\mu^\circ_{\bar{\gamma}(i)} = 0.034 + 0.123(i - 1)$ kJ/mol for copper–BSA, respectively. The values of log $K_{\bar{\gamma}(i)}{}^{calc}$ obtained from these relationships are then used to obtain the values of $\beta_i/\bar{\gamma}(i)$ to introduce into the corrected formation function (13). Observe that the relationships $\Delta\mu^\circ_{\bar{\gamma}(i)}$ and log $K_{\bar{\gamma}(i)}$ are explicit expressions for the function $\varphi(\bar{n})$ used by Tanford [5] and Cantor and Schimmel [6].

The data uncorrected and corrected for the two systems are presented as Scatchard plots in Figs. 3 and 4, respectively. The corrected points produce very nice linear dependences of $\bar{n}_{corr}/[A]$ upon $\bar{n}_{corr}$. The two straight lines give as values of the intrinsic binding constant for nickel–ammonia at 30 °C $k = 92.4$ corresponding to $\Delta\mu_k^\circ = -11.41$ kJ/mol and for copper–BSA at 25 °C $k = 1.3 \times 10^3$ corresponding to $\Delta\mu_k^\circ = -17.8$ kJ/mol, respectively.
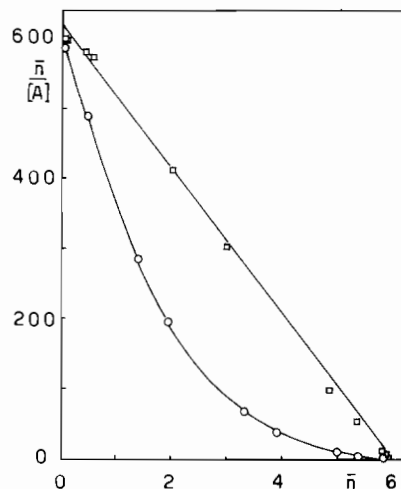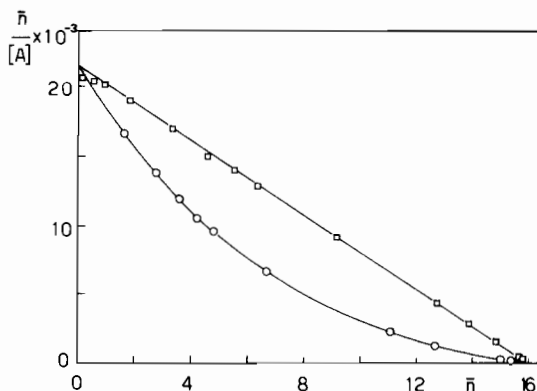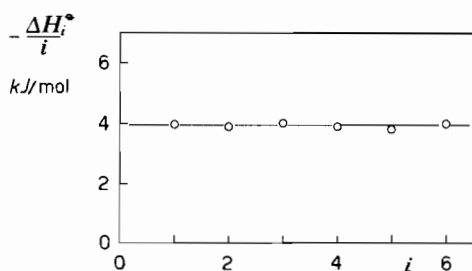
Further insight into the origin of the cooperativity effect can be offered by the examination of the enthalpic contribution to the cooperativity effect. Values of $(1/i)\Delta H_i^\circ$ when plotted against i for Ni–NH$_3$ system are practically constant (Fig. 5).

TABLE V. Average Cooperativity Effect for Ni^{2+}–NH_3 System at Different Conditions

i	$T = 30\,°C, I = 2.0\ M$[a]			$T = 25\,°C, I = 0.0\ M$[b]			$T = 25\,°C, I = 2.0\ M$[b]		
	$\log \beta_i$	$\frac{1}{i}\log \beta_i$	$\log K_{\bar{\gamma}(i)}$	$\log \beta_i$	$\frac{1}{i}\log \beta_i$	$\log K_{\bar{\gamma}(i)}$	$\log \beta_i$	$\frac{1}{i}\log \beta_i$	$\log K_{\bar{\gamma}(i)}$
1	2.78	2.78	0.0	2.72	2.72	0.0	2.81	2.81	0.0
2	5.05	2.525	−0.065	4.89	2.445	−0.085	5.08	2.540	−0.080
3	6.70	2.233	−0.202	6.55	2.183	−0.197	6.85	2.283	−0.187
4	8.01	2.003	−0.286	7.67	1.918	−0.318	8.12	2.033	−0.296
5	8.66	1.732	−0.426	8.34	1.668	−0.430	8.93	1.786	−0.402
6	8.74	1.457	−0.545	8.31	1.385	−0.557	9.08	1.513	−0.522

$(1/i)\log \beta_i^{\mathbf{calc}} = a + bi$

a	3.044 ± 0.0096			2.980 ± 0.0057			3.060 ± 0.0059		
b	−0.264 ± 0.003			−0.2649 ± 0.0019			−0.2571 ± 0.0019		
r	0.9997			0.9999			0.9999		

$\log K_{\bar{\gamma}(i)}^{\mathbf{calc}} = a + b(i - 1)$

a	0.050 ± 0.015			0.036 ± 0.0047			0.032 ± 0.0047		
b	−0.1184 ± 0.0046			−0.1177 ± 0.0014			−0.1099 ± 0.0014		
r	0.9977			0.9998			0.9998		

$\Delta \mu_k^° \ (kJ/mol)$

	−11.41 ± 0.28			−10.54 ± 0.42			−11.04 ± 0.34		

[a]Ref. 1.   [b]Ref. 23, p. 40.

TABLE VI. Average Cooperativity Effect for Various Systems

i	Co^{2+}–NH_3[a] $T = 30\,°C, I = 2.0\ M$			Ni^{2+}–N_2H_4[b] $T = 20\,°C, I = 0.5\ M$			Cd^{2+}–NH_3[c] $T = 25\,°C, I = 2.0\ M$		
	$\log \beta_i$	$\frac{1}{i}\log \beta_i$	$\log K_{\bar{\gamma}(i)}$	$\log \beta_i$	$\frac{1}{i}\log \beta_i$	$\log K_{\bar{\gamma}(i)}$	$\log \beta_i$	$\frac{1}{i}\log \beta_i$	$\log K_{\bar{\gamma}(i)}$
1	2.10	2.10	0.0	2.76	2.76	0.0	2.72	2.72	0.0
2	3.67	1.835	−0.075	5.20	2.600	−0.070	4.90	2.450	−0.057
3	4.78	1.593	−0.170	7.35	2.450	−0.070	6.32	2.107	−0.212
4	5.53	1.383	−0.233	9.20	2.300	−0.064	7.38	1.845	−0.273
5	5.75	1.150	−0.328	10.75	2.150	−0.052	7.02	1.404	0.0
6	5.14	0.857	−0.465	11.99	1.998	−0.068	5.41	0.902	−0.324

$(1/i)\log \beta_i^{\mathbf{calc}} = a + bi$

a	2.334 ± 0.0165			2.907 ± 0.0024			3.154 ± 0.0669		
b	−0.2436 ± 0.0055			−0.1517 ± 0.0008			−0.3569 ± 0.022		
r	0.9990			0.9999			0.9924		

$\log K_{\bar{\gamma}(i)}^{\mathbf{calc}} = a + b(i - 1)$

a	−0.0272 ± 0.0238			−0.0714 ± 0.0081			(−0.035 ± 0.059)[d]		
b	−0.0938 ± 0.0072			−0.0022 ± 0.0025			(0.108 ± 0.027)[d]		
r	0.991			0.460			(0.9699)[d]		

$\Delta \mu_k^° \ (kJ/mol)$

	−7.41 ± 0.24			−11.41 ± 0.025			(−11.86 ± 0.19)[d] (0.66)[e]		

[a]Ref. 23, p. 40.   [b]Ref. 23, p. 43.   [c]Ref. 23, p. 41.   [d]Correlation over sites from $i = 1$ to $i = 4$.   [e]Sites $i = 5, 6$.

Therefore there is no enthalpic component of the cooperativity effect which should be due to entropic factors. This point is under study in this laboratory.

The same kinds of calculations have been applied to other sets of data (Tables V and VI). The values of $(1/i)\log \beta_i$ lie on straight lines as function of i except in

the system $Cd-NH_3$, where evidently the six sites are not all equivalent. Therefore by simple inspection of the plot $(1/i) \log \beta_i = f(i)$ or from the linear regression reported in Table IV, one can infer if the complexes are homogeneous or not. This can be confirmed by inspection of the Scatchard plot. The slopes of the plot $\log K_{\overline{\gamma}(i)} = f(i)$ do not change appreciably with change of ionic strength or temperature (Table V), either with change of metal from nickel(II) to cobalt-(II) (Table VI). Interesting enough the complexes of nickel with hydrazine at 20 °C present a site constant ($\log k = 2.00$, $\Delta\mu_k^\circ = -11.22$ kJ/mol) which is practically equal to that of ammonia; the cooperativity however in hydrazine complexes is very small and constant throughout.

Conclusions

The application of a common algebra to inorganic, bioinorganic, organic and biochemical equilibria as developed in preceding papers [4, 12, 13] is very promising because it makes easier the comparison between experiments and results obtained in different fields.

The free-energy change observed in many biological processes like the phosphate hydrolysis at pH = 7 of adenosin-5'-triphosphate (~ -30 kJ/mol) and of 3-phosphoglycerate and glycerol-3-phosphate (~ -10 kJ/mol) are of the same order of magnitude as the coupled reaction of deprotonation and complexation [12] of aminoacids with copper(II) (-30 kJ/mol) and oligopeptides with nickel ($-10/20$ kJ/mol), respectively. This makes plausible the coupling of the complexation reactions with biochemical reactions.

On the other hand the evaluation of the cooperativity effect [4] has shown how this effect corresponds to free-energy changes of comparable magnitude both in small and large molecules and subject to the influence of the solvent. This suggests the possibility of evaluating the coupling of the denaturation of proteins with the changes in the ionic strength of the medium.

The results of the present study confirm the general point of view that the complexation and protonation reactions of ligands are useful models for the interpretation of the behaviour of the macromolecules and that the vast selections of equilibrium constants [14–24] can be exploited to obtain useful informations for biochemists. The change in average cooperativity in nickel–ammonia complexes is $\Delta\mu_{\overline{\gamma}}^\circ = 0.621$ kJ/mol per step whereas the change in average cooperativity in binding of copper to bovine serum albumin is $\Delta\mu_{\overline{\gamma}}^\circ = 0.123$ kJ/mol per step. The values of $\Delta\mu_k^\circ$ (-11.41 kJ/mol for Ni–ammonia and -17.8 kJ/mol for Cu–BSA) are very similar. The

type of interaction is therefore very likely the same. The affinity of transition metal ions for tetrahedral nitrogen is higher than the affinity for oxygen. Therefore even the interaction between copper and BSA could imply copper–nitrogen interactions and very likely formation of chelate rings with improvement of affinity. It cannot be excluded therefore that the receptor BSA only as a first approximation can be considered as homotropic, each binding site being actually a function of the product of the two coupled binding atoms.

The solution of this problem could be given only if we will be able to find sets of reliable site constants k, obtained from ligand or acceptor surely homotropic.

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