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COOPERATIVITY FUNCTIONS AND SITE BINDING CONSTANTS IN POLYPROTIC ACIDS

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ABSTRACT

The partition function $\Sigma_A = 1 + \beta_1[H] + \beta_2[H]^2 + \ldots + \beta_i[H]^i + \ldots + \beta_t[H]^t$ is used to interpret the variation in the stepwise formation constants for the equilibria between base A and proton H. The cumulative protonation constant of the saturated complex H_tA, $\beta_t = [H_tA]/([H]^t[A])$, is equal to the ratio between the formation partition function Σ_A and the dissociation partition function Σ_A^D, the former giving the probability of finding compounds H_iA of the ligand H binding to A and the latter giving the probability of finding species H_tA by dissociation of protons from H_tA.

The saturation function $\beta_t = \Sigma_A / \Sigma_A^D$ can be factorized as a product of stepwise constants $\beta_t = K_1 \cdot K_2 \ldots K_i \ldots K_t$. Comparisons between K_1 and the geometric means $\beta_i^{1/i}$ enable a calculation to be made of the average cooperativity constants $K_{\gamma(i)}$ which are explicit functions of i. The cooperativity functions $\gamma(i)$ require that the equilibria are described by means of model partition functions depending on the site affinity constant k and the coefficients of the cooperativity function $\gamma(i) = \exp\{2.302[a + b(i-1)]\}$.

By analysing the cumulative protonation constants of polysite receptors it is possible to determine if there actually are separate classes of sites each with site constant k_j and class cooperativity function $\gamma(i_j)$.

The analysis of the equilibrium constants of some polyprotic acids shows how both the site affinity constants k and the slope b of $\log \gamma(i)$ depend on the charge density of the base. The paramount importance of the electrostatic effect in the binding of the proton to the base is clearly apparent. The analysis of the contribution of enthalpy to the cooperativity effect for the same compounds shows varying behaviour. This needs to be investigated further.

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INTRODUCTION

We have shown in a preceding paper [1] that from the average chemical potentials $\Delta\mu_i^{\ominus} = -(1/i)RT \ln \beta_i$ in homotropic complexes formed by a receptor M and i moles of a ligand A, up to a maximum of $i = t$ sites, the average cooperativity parameters $\log K_{\gamma(i)}$ ($\log = \log_{10}$ throughout this paper) can be calculated. The cooperativity functions can then be obtained

$$\log \gamma(i) = a + b(i-1) \tag{1}$$

where $\gamma(i)$ is the value of this function at step i ($\gamma(i) \equiv K_{\gamma(i)}$).

Therefore the cumulative experimental constants β_i can be corrected for the cooperativity effect [2]. The correction leads to a linear Scatchard plot, whose slope gives the site constant k.

In heterosite bases there are different classes of binding sites for protons; each class j has a different site affinity constant k and a different cooperativity function $\gamma(i)$. In this paper, a group of polyprotic acids is analysed to show the procedure that can be followed, starting from the cumulative protonation constants β_i, to obtain both specific site affinity constants and specific class cooperativity functions, if the sites belong to different classes.

This treatment allows the use of thermodynamic data [3–5] measured in systems with small molecules to interpret the behaviour of macromolecular systems. This is the procedure reported by Franks [6] which exploits "small" systems as models for "large" molecules.

With the new method proposed here, it is possible to extract information concerning the physical factors which affect binding and cooperativity. Analysis of the contribution of enthalpy to the cooperativity effect may give further support to the validity of the method and may indicate future lines of research, e.g. using calorimetric techniques.

PARTITION FUNCTION AND FREE ENERGY

The cumulative protonation constants $\beta_i = [H_iA]/([H]^i[A])$ are the coefficients of a grand partition function

$$\Sigma_A = 1 + \beta_1[H] + \beta_2[H]^2 + \ldots + \beta_i[H]^i + \ldots + \beta_t[H]^t \tag{2}$$

which is related to experimentally determined quantities, e.g. absorbance, concentration of free proton [H], heat evolved q, etc., which, in turn, depend on the experimental method employed. A typical function which can be derived directly from the experimental data is the formation function $\bar{n}$ which measures the average number of sites occupied by the proton on the base. It has also been shown [1,2] that

$$\bar{n} = \partial \ln \Sigma_A / \partial \ln[H] \tag{3}$$

and, because from statistical thermodynamics

$$\Sigma_A = \exp(-\Delta G/RT) \tag{4}$$

we have

$$\bar{n} = \partial(-\Delta G/RT)/\partial \ln[H] \tag{5}$$

From the plot of $\bar{n}$ vs. ln[H] we can obtain the free energy of formation ΔG_F

$$-\Delta G_F/RT = \int_{[H]_1}^{[H]_2} \bar{n} \, d \ln[H] \tag{6}$$

In other words areas on the Bjerrum plot $\bar{n} = f(\ln[H])$ are proportional to free energies.

The stepwise equilibrium constants $K_i = [H_iA]/([H_{i-1}A][H])$ can be related to the partition function Σ_A by introducing a dissociation partition function

$$\Sigma_A^D = 1 + 1/K_t[H] + 1/K_t \cdot K_{t-1}[H]^2 + \ldots + 1/K_t \cdot K_{t-1} \ldots K_1[H]^t \tag{7}$$

The ratio $\Sigma_A/\Sigma_A^D = F_A^C$ is called the "convoluted" or "saturation" function because

$$F_A^C \equiv \beta_t[H]^t \tag{8}$$

where β_t is the cumulative constant of the completely protonated base H_tA. For the standard state [H] = 1, $F_A^{C\ominus} = \beta_t$.

We can consider a dissociation function $\bar{d}$, related to the dissociation partition function, that represents the mean number of dissociated protons

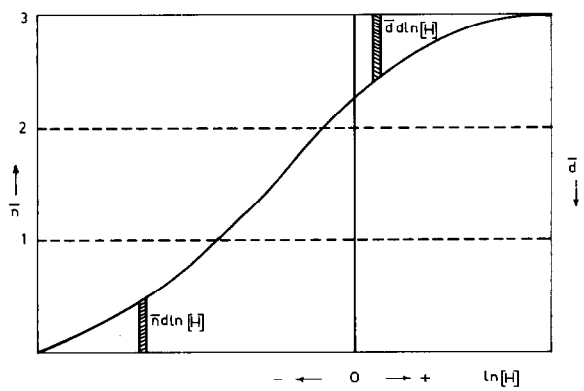

Fig. 1. Formation function $\bar{n}$ and dissociation function $\bar{d}$. The areas of the hatched elements are d ln Σ_A and d ln Σ_A^D respectively.

per total base (Fig. 1). Then by analogy with eqns. (3), (4), (5) and (6) we can write

$$\Delta G^{\ominus}/RT = \Delta G_F^{\ominus}/RT - \Delta G_D^{\ominus}/RT$$
$$= \int_{[H]=0}^{[H]=1} (\partial \ln \Sigma_A/\partial \ln[H]) d \ln[H]$$
$$- \int_{[H]=\infty}^{[H]=1} (\partial \ln \Sigma_A^D/\partial \ln[H]) d \ln[H]$$
$$= \int_{[H]=0}^{[H]=1} \bar{n} \, d \ln[H] - \int_{[H]=\infty}^{[H]=1} \bar{d} \, d \ln[H] \quad (9)$$

The stepwise equilibrium constants are bound to β_t by the relation (factorization)

$$\beta_t = K_1 \cdot K_2 \ldots K_i \ldots K_t \quad (10)$$

and hence the Gibbs free energy can be obtained as the sum of the stepwise chemical potentials

$$\Delta G^{\ominus} = \Delta\mu_1^{\ominus} + \Delta\mu_2^{\ominus} + \ldots + \Delta\mu_i^{\ominus} + \ldots + \Delta\mu_t^{\ominus} \quad (11)$$

each of which corresponds to the partial molar free energy change for the addition of one mole of protons to the previously formed species.

AVERAGE CHEMICAL POTENTIALS

The subdivision of the free energy into single step chemical potentials corresponds in graphical terms to the subdivision of the area under the curve of the Bjerrum plot into several sigmoidal areas (Fig. 2). In order to evaluate the cooperativity effect we can calculate the difference between the average of i stepwise areas and the first area (average cooperativity). We also have to take into account the statistical factor k_{st} which is an entropy factor

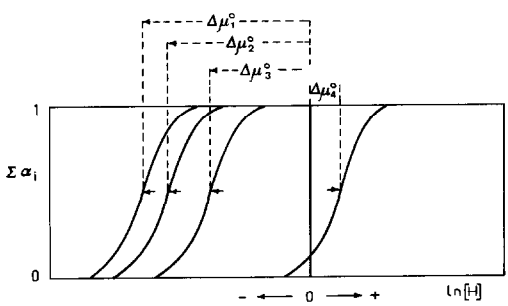

Fig. 2. Stepwise chemical potential $\Delta\mu_i^{\ominus}$. The cooperativity chemical potential $\Delta\mu_{\gamma(i)}^{\ominus}$ is calculated from eqn. (12).

determining the variation in the cumulative constants due to the site occupation probability.

For the average cooperativity we calculate

$$\begin{aligned}\Delta\mu^{\ominus}_{\gamma(i)} &= \left(\Delta\mu_1^{\ominus} + \Delta\mu_2^{\ominus} + \ldots + \Delta\mu_i^{\ominus}\right)/i - \Delta\mu_1^{\ominus} + \Delta\mu_{st}^{\ominus} \\ &= \left(-RT\ \ln\ \beta_i^{1/i} + RT\ \ln\ \beta_1\right) - RT\ \ln\ k_{st} \\ &= -RT\ \ln\ \gamma(i)\end{aligned} \tag{12}$$

The cooperativity chemical potential corresponds to a comparison of the geometric mean $\beta_i^{1/i}$—for the contemporary binding of i protons—with the constant β_1 for the binding of one proton alone.

It has been shown [1] that eqn. (12) is related to a model where the ligand–receptor bonds are rearranged. When $\log K_{\gamma(i)}$ is plotted against $i-1$ smooth lines are obtained if the binding affinity depends on a unique continuous cooperativity function as eqn. (1); however, clear discontinuities arise if there are changes in the geometry of the acid or in the intrinsic site affinity or if there are distinct variations in the cooperativity effect. By plotting $(1/i)\log\ \beta_i$ (or $\Delta\mu^{\ominus}_{\gamma(i)}$) against i an idea of the behaviour of the system can be obtained. The features of the plot are similar to the information given by the Scatchard plot, $\bar{n}/[\mathrm{H}] = \mathrm{f}(\bar{n})$, where the appearance of a new class of sites is shown by a rapid change in slope. When such discontinuities appear, it is better to treat the single groups of constants separately. In terms of partition function we can make some approximations. For example for a t site receptor (with t' sites in the first class of high affinity and t'' sites in the second class of low affinity) we can assume that for low concentration of [H]

$$\Sigma_A \approx \Sigma_{A'} = 1 + \beta_1[\mathrm{H}] + \beta_2[\mathrm{H}]^2 + \ldots + \beta_i[\mathrm{H}]^i + \ldots + \beta_{t'}[\mathrm{H}]^{t'} \tag{13}$$

and for high values of [H], the partition function can be factorized

$$\Sigma_A = \Sigma_{A''}\beta_{t'} = \beta_{t'}\left(1 + \beta_1''[\mathrm{H}] + \beta_2''[\mathrm{H}]^2 + \ldots + \beta_i''[\mathrm{H}]^{i''} + \ldots + \beta_{t''}''[\mathrm{H}]^{t''}\right) \tag{14}$$

with $\beta_i'' = \beta_i/\beta_{t'}$, whose terms give the probability of successive saturation of the receptor $\mathrm{H}_{t'}\mathrm{A}$.

Within each class the treatment follows the same lines as for homosite receptors [2]. In each class a model partition function is set, for example for four sites

$$\Sigma_{A.MOD} = 1 + 4k[\mathrm{H}] + 6\gamma_2^2k^2[\mathrm{H}]^2 + 4\gamma_3^3k^3[\mathrm{H}]^3 + \gamma_4^4k^4[\mathrm{H}]^4 \tag{15}$$

By comparison of eqns. (14) and (15) we obtain $\beta_2 = 6\gamma_2^2k^2$, $\beta_3 = 4\gamma_3^3k^3$ and $\beta_4 = \gamma_4^4k^4$. If the cooperativity coefficients γ_2, γ_3 and γ_4 are related to one another by eqn. (1), then a cooperativity function holds and the coefficients can be calculated. The experimental β_i values can be corrected for γ_2, γ_3 and γ_4 and a partition function for independent equal sites is obtained. If

the correction through the cooperativity function is appropriate, the resulting Scatchard plot calculated with the corrected β_i values should be linear.

A computer program for processing the data is available from the authors on request.

APPLICATIONS

Ethylenediaminetetraacetic acid (EDTA)

The treatment of the data for EDTA is summarized in Table 1. The values of $(1/i)\log \beta_i$ (or $\Delta\mu^{\ominus}_{\gamma(i)} = -(1/i)RT \ln \beta_i$, average chemical potentials) are plotted in Fig. 3. The points clearly belong to two subsets: one for the protonation of the two amine nitrogens and the other for the protonation of the four carboxylato groups. The Scatchard plot (Fig. 3) shows a clear change in slope at $\bar{n} = 2$, after which a completely flat distribution of points appears.

The two subsets can be treated separately. For the first subset with equilibrium constants $\beta'_1 \equiv \beta_1$ and $\beta'_2 \equiv \beta_2$ a value of $\log \gamma_2 = -0.719$ is obtained. By correcting the original β_i values, values for the protonation of the amine groups are obtained without the cooperativity effect (Table 1). The correction produces the rectification of the Scatchard plot. The site constant that results is $\log k = 8.69$.

The analysis of the second subset (i.e. carboxylato groups) can be performed in the same manner. The plot of the average chemical potentials against i'' (Fig. 4(a)) reveals a discontinuity at the fourth value, that may be interpreted as being due to a difference in the intrinsic binding constants of the carboxylato groups. The calculation of $\log \gamma(i'')$ (Fig. 4(b)) confirms a change in behaviour. The equilibrium constants β''_2 and β''_3 show zero

TABLE 1

Analysis of cooperativity in the protonation of $EDTA^{4-}$ at 25 °C ($I = 3.0$ M $NaClO_4$ [a])

i	$\log \beta_i$	$(1/i)\log \beta_i$	i'	$(1/i')\log \beta_{i'}$	$\log k_{st}$	$\log(\beta_i^{1/i'}/k_{st})$	$\log \gamma(i')$
1	9.04	9.04	1	9.04	0	9.04	0
2	16.04	8.02	2	8.02	−0.301	8.321	−0.719
			i''	$(1/i'')\log \beta_{i''}$	$\log k_{st}$	$\log(\beta_{i''}^{1/i''}/k_{st})$	$\log \gamma(i'')$
3	18.55	6.18	1	2.51	0	2.51	0
4	20.68	5.17	2	2.32	−0.213	2.533	0.023
5	22.38	4.48	3	2.11	−0.401	2.511	0.001
6	22.81	3.80	4	1.69	−0.602	2.292	−0.218

EDTA = ethylenediaminetetraacetic acid. [a] Reference 7. $\log \gamma(i'')^{calc} = a + b(i'' - 1)$; $a = 0.026 \pm 0.008$; $b = -0.008 \pm 0.017$.

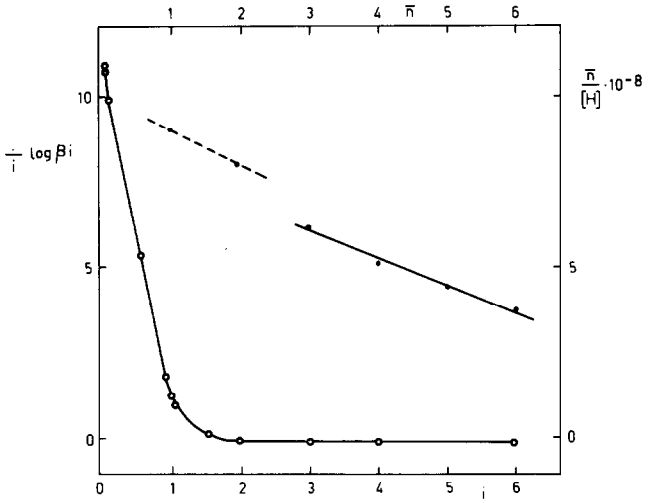

Fig. 3. EDTA: average protonation constants $(1/i)\log \beta_i = f(i)$ and corresponding Scatchard plot.

cooperativity effect, whereas β_4'' presents a very high negative cooperativity. This behaviour can be explained by the fact that the fourth value refers to the addition of a proton to a positively charged species H_5A^+. Therefore the apparent difference in the average chemical potentials is due to induced rather than to intrinsic heterogeneity of sites. The assignment of $\log \gamma_4 = -0.218$ leads to rectification of the Scatchard plot (Fig. 5) with a site binding constant $\log k = 1.91$.

Ethylenediaminetetraacetohydroxamic acid (EDTH)

The molecule of EDTH is similar to that of EDTA, but with different acidic groups: acetohydroxamato instead of carboxylato. The calculations

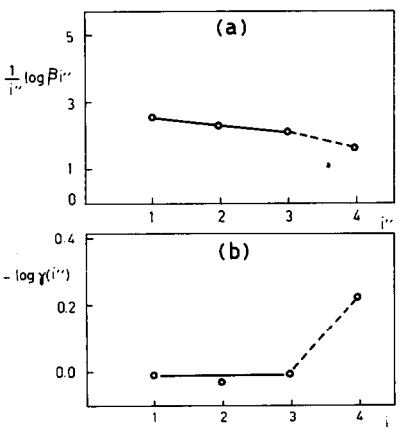

Fig. 4. EDTA, carboxylato subset: (a) average protonation constants $(1/i'')\log \beta_{i''}$; (b) cooperativity function $\log \gamma(i'')$.

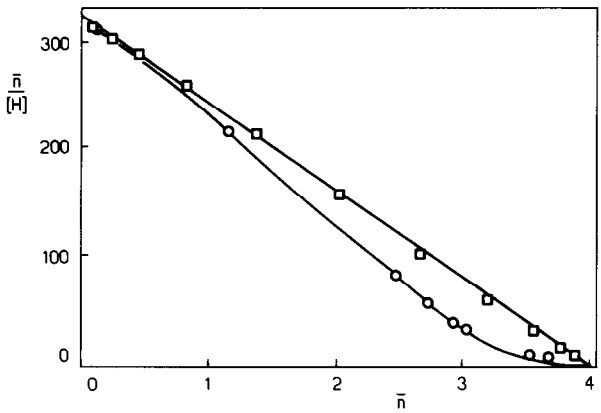

Fig. 5. EDTA, carboxylato subset: ○, uncorrected and □, corrected Scatchard plot.

for EDTH are summarized in Table 2. The plot of $(1/i)\log \beta_i$ against i shows the same discontinuity at $i = 2$ as for EDTA. The subdivision of the equilibrium constants into two subsets seems to be appropriate. In the first subset the cooperativity effect between the amine groups is practically zero ($\log \gamma_2 = 0.051$), a result which is at variance with EDTA. The intrinsic basic constant ($\log k = 10.81$) of EDTH is also different from that of the amine groups ($\log k = 8.69$) of EDTA.

For the second subset the plot of $(1/i'')\log \beta''_{i''}$ against i'' (Fig. 6(a)) shows a continuous linear behaviour. The monotonic behaviour is confirmed by the plot of $\log \gamma(i'')$ against $i''-1$ (Fig. 6(b)). The cooperativity among the hydroxamato groups in EDTH is higher ($\log \gamma(i'') = 0.020 - 0.093(i'' - 1)$) than that between carboxylato groups in EDTA. The Scatchard plot is shown in Fig. 7.

TABLE 2

Analysis of cooperativity in the protonation of $EDTH^{4-}$ at 20 °C ($I = 0.1$ M $NaClO_4$ [a])

i	$\log \beta_i$	$(1/i)\log \beta_i$	i'	$(1/i')\log \beta_{i'}$	$\log k_{st}$	$\log(\beta_i^{1/i'}/k_{st})$	$\log \gamma(i')$
1	11.10	11.10	1	11.10	0	11.10	0
2	21.70	10.85	2	10.85	−0.301	11.151	0.051
			i''	$(1/i'')\log \beta_{i''}$	$\log k_{st}$	$\log(\beta_i^{1/i''}/k_{st})$	$\log \gamma(i'')$
3	28.93	9.69	1	7.23	0	7.23	0
4	35.60	8.90	2	6.95	−0.213	7.163	−0.067
5	41.65	8.33	3	6.65	−0.401	7.051	−0.179
6	47.20	7.87	4	6.375	−0.602	6.977	−0.253

EDTH = ethylenediaminetetraacetohydroxamic acid. [a] Reference 8. $\log \gamma(i'')^{calc} = a + b(i'' - 1)$; $a = 0.020 \pm 0.011$; $b = -0.093 \pm 0.024$.

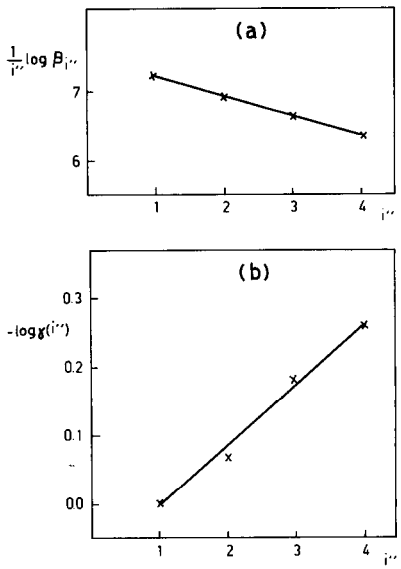

Fig. 6. EDTH, acetohydroxamato subset: (a) average protonation constants $(1/i'')\log\beta_{i''}$; (b) cooperativity function $\log\gamma(i'')$.

Polyprotic acids

The analysis of the cooperativity effect has been extended to a group of polyprotic acids: orthophosphoric acid (H_3PO_4), pyrophosphoric acid ($H_4P_2O_7$), mellitic (benzenehexacarboxylic) acid, pyromellitic (1,2,4,5-benzenetetracarboxylic) acid, 1,3,5-benzenetricarboxylic acid, hemimellitic (1,2,3-benzenetricarboxylic) acid, trimellitic (1,2,4-benzenetricarboxylic) acid, 1,2,3,4-butanetetracarboxylic acid, phthalic (1,2-benzenedicarboxylic) acid

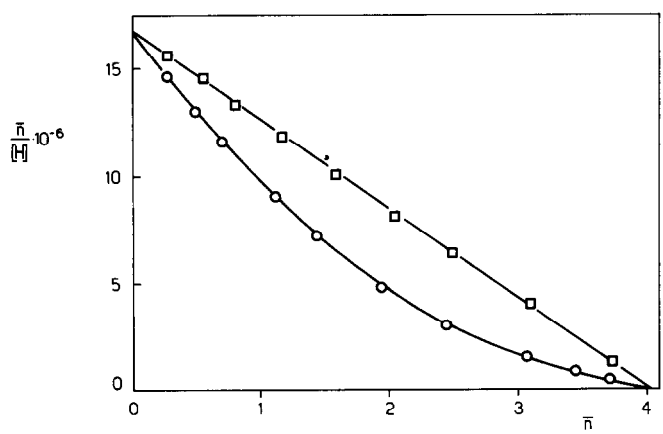

Fig. 7. EDTH, acetohydroxamato subset: ○, uncorrected and □, corrected Scatchard plot.

TABLE 3

Site affinity constants k and cooperativity functions $\gamma(i)$ in polyprotic acids [a]

Acid	log β_i				log k	log $\gamma(i)$ [b]	
	$i=1$	$i=2$	$i=3$	$i=4$		a	b
Benzenehexacarboxylic (i')	7.49	13.81	–	–	7.17	–	−0.284
Benzenehexacarboxylic (i'')	5.09	8.61	10.82	11.52	4.49	−0.051	−0.518
1,2,4,5-Benzenetetracarb.	6.23	11.15	14.27	15.97	5.63	0.144	−0.597
1,2,3,4-Butanetetracarb.	7.16	13.01	17.59	21.02	6.56	−0.019	−0.431
1,2,3-Benzenetricarb.	5.51	9.33	11.95	–	5.03	−0.164	−0.443
1,2,4-Benzenetricarb.	5.01	8.72	11.12	–	4.53	0.002	−0.414
1,3,5-Benzenetricarb.	5.18	9.28	12.40	–	4.70	−0.033	−0.269
1,2-Benzenedicarb.	5.41	8.36	–	–	5.13	–	−0.928
1,3-Benzenedicarb.	4.50	8.00	–	–	4.19	–	−0.199
Orthophosphoric	11.74	17.46	19.46	–	11.26	−0.767	−2.005
Pyrophosphoric (i')	9.00	15.19	–	–	8.70	–	−1.104
Pyrophosphoric (i'')	2.00	2.80	–	–	1.70	–	−0.299
EDTA [c] (i')	9.04	16.04	–	–	8.69	–	−0.719
EDTA [c] (i'')	2.51	4.64	6.34	6.77	1.91	0.026	−0.008
EDTH [d] (i')	11.10	21.70	–	–	10.81	–	0.051
EDTH [d] (i'')	7.23	13.90	19.95	25.50	6.63	0.020	−0.093

[a] Data from ref. 5. [b] $\log \gamma(i)^{calc} = a + b(i-1)$. [c] Reference 7. [d] Reference 8.

and isophthalic (1,3-benzenedicarboxylic) acid (Table 3). An example of a Scatchard plot is shown in Fig. 8 for 1,3,5-benzenetricarboxylic acid. For mellitic and pyrophosphoric acids (Fig. 9) the linearization can only be achieved if two classes of sites are considered. This kind of behaviour can also be revealed by a careful examination of the plot $(1/i)\log \beta_i$; this sometimes reveals the behaviour in compounds where the subdivision of classes is not immediately apparent. Cadmium(II) forms complexes with

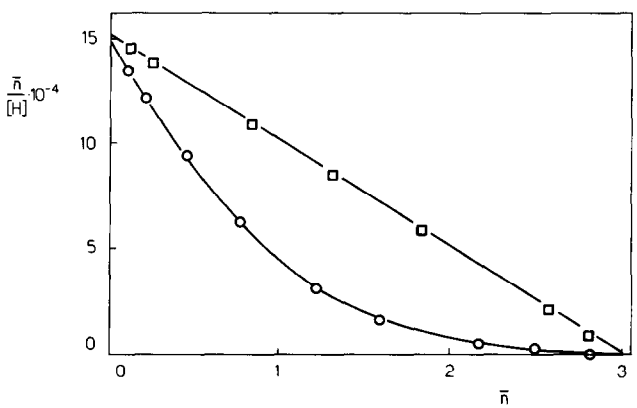

Fig. 8. 1,3,5-Benzenetricarboxylic acid: ○, uncorrected and □, corrected Scatchard plot.

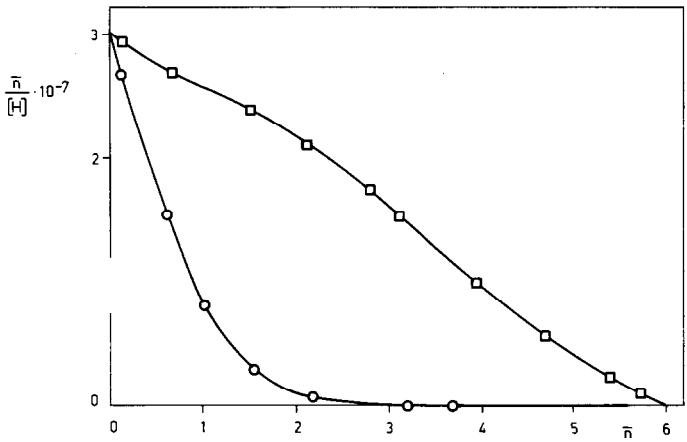

Fig. 9. Mellitic (benzenehexacarboxylic) acid. Six carboxylic groups treated as a unique set: ○, uncorrected and □, inappropriately corrected Scatchard plot.

ammonia up to the ratio 1 : 6. The analysis clearly shows that there are two subsets, one of four sites and the other of two sites [2].

The values of the site constants k and the cooperativity functions $\log \gamma(i)$ for the acids examined are reported in Table 3. EDTA and EDTH are also included.

The cooperativity effect corresponds to a change in chemical potential $\Delta\mu^{\ominus}_{\gamma(i)}$ and can be broken into enthalpic and entropic contributions. The

TABLE 4

Cooperativity enthalpy $\Delta h^{\ominus}_{\gamma(i)}$ (kJ mol^{-1}) for protonation in some polyprotic acids [a]

Acid		$i=1$	$i=2$	$i=3$	$i=4$
Benzenehexacarboxylic [b]	$\Delta H^{\ominus}/i$	4.73	8.12	10.42	15.15
Benzenehexacarboxylic [b]	$\Delta h^{\ominus}_{\gamma(i)}$	0	3.39	5.69	10.41
1,2,4,5-Benzenetetracarb.	$\Delta H^{\ominus}/i$	6.69	5.00	5.52	7.40
1,2,4,5-Benzenetetracarb.	$\Delta h^{\ominus}_{\gamma(i)}$	0	−1.69	−1.17	0.71
1,2,3,4-Butanetetracarb.	$\Delta H^{\ominus}/i$	−5.02	−3.56	−2.79	−2.30
1,2,3,4-Butanetetracarb.	$\Delta h^{\ominus}_{\gamma(i)}$	0	1.46	2.23	2.72
1,2,3-Benzenetricarb.	$\Delta H^{\ominus}/i$	−1.55	−1.21	0.67	–
1,2,3-Benzenetricarb.	$\Delta h^{\ominus}_{\gamma(i)}$	0	0.34	2.22	–
1,2,4-Benzenetricarb.	$\Delta H^{\ominus}/i$	3.97	2.18	3.18	–
1,2,4-Benzenetricarb.	$\Delta h^{\ominus}_{\gamma(i)}$	0	−1.79	−0.79	–
1,3,5-Benzenetricarb.	$\Delta H^{\ominus}/i$	4.90	3.47	1.10	–
1,3,5-Benzenetricarb.	$\Delta h^{\ominus}_{\gamma(i)}$	0	−1.43	−3.80	–
Orthophosphoric	$\Delta H^{\ominus}/i$	−14.64	−9.00	−3.35	–
Orthophosphoric	$\Delta h^{\ominus}_{\gamma(i)}$	0	5.64	11.29	–

[a] Data from ref. 5. [b] Last four protonation steps.

values of the cooperativity enthalpy are obtained from the cumulative enthalpy $\Delta H_i^{\ominus}$ as

$$\Delta h_{\gamma(i)}^{\ominus} = (1/i)\Delta H_i^{\ominus} - \Delta H_1^{\ominus} \qquad (16)$$

and are then plotted against $i - 1$.

The results of the analysis of the enthalpy data of some of the polyprotic acids examined are reported in Table 4. The values of $\Delta h_{\gamma(i)}^{\ominus}$ have to be compared with the experimental error before considering their significance. This holds for the entropy contribution also.

DISCUSSION

The main factors which are generally taken into account to explain the variations in equilibrium constants with either "small" or "large" molecules are electrostatic effects [9–12], inductive, polar and steric effects [13–15], solvent or medium effects [16,17] and conformational effects [18,19].

An evaluation of the character of the cooperativity effect can be inferred from the comparison of the values of the intrinsic binding constants and cooperativity functions in the two compounds EDTA and EDTH.

The behaviour of the EDTH molecule is in agreement with an interpretation based on inductive rather than electrostatic effects. In fact the groups $-CH_2(CO)-NHO^-$ enhance the affinity of nitrogen for the proton as shown by the site constant $\log k = 10.81$ in comparison with $\log K = 10.64$ for methylamine [5]. The addition of the two protons to the amine groups is not affected by cooperativity probably because the addition of the first proton does not alter the distribution of charge in the second half of the molecule. The addition of protons to $-CH_2(CO)-NHO^-$ clearly changes the induction of electrons on the binding sites.

The EDTA molecule behaves as if it is strongly influenced both by inductive and electrostatic effects. The amine nitrogens ($\log k = 8.69$) are weaker bases than those of ethylenediamine [5] ($\log k = 9.49$) because of the attraction of electrons by the carboxylato groups through the bonds. The electrostatic interactions cause strong cooperativity between amine groups. The carboxylato groups are characterized by zero cooperativity effect probably because the inductive effects compensate for the affinity loss due to the electrostatic effect. The last proton is more loosely bound because of the electrostatic repulsion in the reaction $AH_5^+ + H^+ \rightleftharpoons AH_6^{2+}$.

The data on the polyprotic acids add some very important information useful for the understanding of the relations between structure of molecules and thermodynamic affinity. These acids are model compounds from which an indication (and in the future possibly a quantitative evaluation) of the electrostatic, inductive and steric factors affecting specific site affinity can be gained. The slopes of the lines are proportional to the probable charge

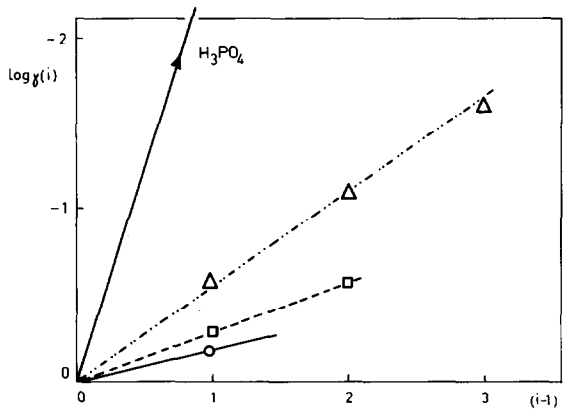

Fig. 10. Cooperativity function $\log \gamma(i)$ for acids of different charge density: ——, orthophosphoric acid; △, pyromellitic (1,2,4,5-benzenetetracarboxylic) acid; □, 1,3,5-benzenetricarboxylic acid; ○, isophthalic (1,3-benzenedicarboxylic) acid.

density on the sites of the acids. The plots for some representative acids are drawn in Fig. 10. The charge density should be very high in orthophosphoric acid ($b = -2.005$) which has a charge of -3 spread over the surface of a small anion in comparison with pyromellitic acid ($b = -0.597$) which has a charge of -4 spread over a large molecule. 1, 3, 5-Benzenetricarboxylic acid

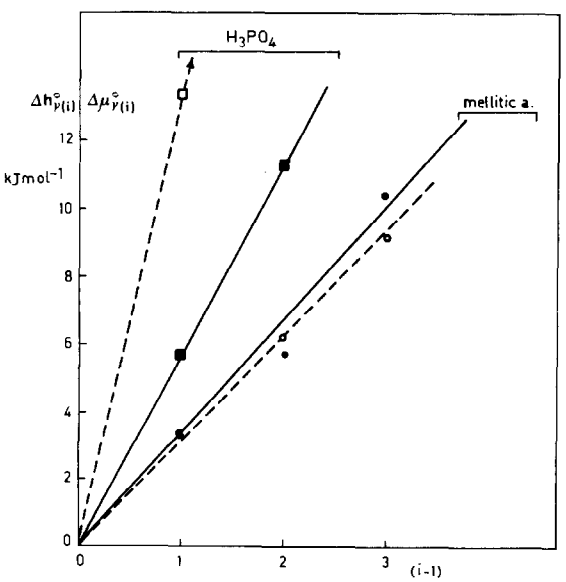

Fig. 11. Cooperativity chemical potential $\Delta\mu^{\ominus}_{\gamma(i)}$ (broken lines) and cooperativity enthalpy, $\Delta h^{\ominus}_{\gamma(i)}$ (full lines) for orthophosphoric and mellitic (second subset) acids.

presents a lower slope ($b = -0.269$) because of its smaller charge and 1,3-benzenedicarboxylic acid presents an even smaller slope ($b = -0.199$). The change in b on going from 1,2,3-benzenetricarboxylic acid ($b = -0.443$) to 1,2,4-benzenetricarboxylic acid ($b = -0.414$) to 1,3,5-benzenetricarboxylic acid ($b = -0.269$) can also be explained by the charge density. The electrostatic effect therefore seems to be the main factor dominating both binding and cooperativity.

The analysis of the cooperativity enthalpies of the acids examined does not produce a homogeneous response (Table 4). Some compounds, such as mellitic acid and orthophosphoric acid, show a high positive enthalpy effect (Fig. 11). Other compounds (1,2,4-benzenetricarboxylic and 1,2,4,5-benzenetetracarboxylic acids) show a small negative contribution in the first protonation step and then change their trend. 1,3,5-Benzenetricarboxylic acid shows a negative enthalpy contribution which regularly varies in every step. The subdivision of the free-energy change into enthalpic and entropic components has raised a long debate. Krug et al. [20] have claimed that the so-called isokinetic principle (the correlation between ΔH° and $T\Delta S^{\circ}$) is exclusively due to transmission of experimental errors. Christensen et al. [21] have shown a good correlation between structure and free energy, but the enthalpic and entropic contributions follow unpredictable paths and compensate each other. Lumry [22] has strongly supported the idea that the compensation is real and has its origin in the solvent surrounding the macromolecules. From the results on the acids examined here no definite conclusion can be drawn. More precise work is necessary in the field of calorimetric determination of the cooperativity enthalpy.

CONCLUSIONS

The interpretation (in terms of the partition function) of the cumulative formation constants for the equilibria between metal and ligand or between proton and base can also be extended to ligands and bases with different classes of sites. The analysis of the data leads to the determination of specific site binding constants and specific cooperativity functions for the different classes of sites.

The values of the site affinity constants and of the coefficients of the cooperativity functions in polyprotic acids are strongly dependent on the electrostatic charge density.

This method of treating the data seems to be highly promising and may promote new measurements, particularly by calorimetry. New calculation procedures need to be developed to obtain a detailed physicochemical interpretation of the factors determining the change in affinity with molecular structure in polysite receptors.

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