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The Statistics of Ternary Complex Formation. Inclusion of Ligand Protonation

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We recently published [1] a simple statistical treatment for determining the distribution of binary and ternary species in aqueous media which obey the equilibrium:

$$MA_2 + MB_2 \rightleftharpoons 2MAB \quad (1)$$

where M is a divalent metal ion, A and B are different bidentate or tridentate ligands. It was shown that if A and B have total concentrations (or amounts) a and b respectively, then the ratio of concentrations at equilibrium is:

$$\{MA_2\}:\{MB_2\}:\{MAB\} = (wa)^2 : b^2 : 2wab \quad (2)$$

where, w is a statistical weighting factor given by*:

$$w = (\beta^{A}_{102}/\beta^{B}_{102})^{1/2} \quad (3)$$

The assumptions in this treatment are first, that there are no significant intraligand interactions and hence the distribution is purely statistical, and second, that the probability of forming a species is proportional to the *total* concentration of ligand present. This second assumption means that the ligands must either be aprotic, or their pK_a values must be at least an order of magnitude less than the solution pH, or the two ligands must have similar pK_a values.

An interesting example of the latter condition is the aqueous copper(II)–L-alanine–L-valine system examined by I and Nancollas [2]. Using the same concentrations that these authors used in their potentiometric study and the stability constants obtained by them, for neutral pH we obtain from (2) and (3):

$$\{Cu(Ala)_2\}:\{Cu(Val)_2\}:\{Cu(Ala)(Val)\} = 1.28:1:2.67.$$

This is in remarkable agreement with the experimental value [2] at pH 6.5 of 1.12:1:2.73. Their $\log\beta$ value of 15.20 for the ternary complex is very close to the statistically expected value of 15.09 (from expression (3) in [1]) confirming the lack of any intraligand interactions in the ternary complex. A further interesting feature of this system is that the quotient wa/b = 1.13 and so is within the range predicted [1] for the ternary species to dominate.

The protonation constants of alanine and valine are very similar, however, for ligands with significantly different protonation properties it becomes clear that expression (2) is no longer valid. This will be demonstrated with ternary metal–cysteine–histidine systems (*vide infra*). We shall first show that it is relatively easy to take into account the amount of ligand protonation in our statistical treatment.

In our original treatment [1] the probability of forming a species was related to the *total* amount of ligands present. For the case of weakly acid or weakly basic ligands this probability will be proportional to the *de-protonated* form of the ligand, *i.e.* the form in which it complexes to the metal ion. For those systems, like biological fluids, in which the ligand concentrations are far in excess over the metal ion concentrations, the amount of de-protonated ligand $\{X\}$ can be related to the total amount $\{X\}_t$ present, by:

$$\{X\} = \{X\}_t/\{1 + \sum_n \beta_{0n1}\{H\}^n\} \quad (4)$$

Inserting (4) into (2) gives:

$$\{MA_2\}:\{MB_2\}:\{MAB\} \equiv (w\{A\}_t/\{1 + \sum_n \beta^{A}_{0n1}\{H\}^n\})^2 : (\{B\}_t/\{1 + \sum_m \beta^{B}_{0m1}\{H\}^m\})^2 : 2w\{A\}_t\{B\}_t/\{1 + \sum_n \beta^{A}_{0n1}\{H\}^n\}\{1 + \sum_n \beta^{B}_{0m1}\{H\}^m\} \quad (5)$$

Note, that from (5) the equilibrium constant K_D for reaction (1) again has the value of 4, the statistically expected value. We shall now apply eqn. (5) to the ternary systems considered in the earlier paper [1] and to the nickel(II)–cysteine–histidine system. Since we are considering their formation in human blood serum the conditions are: pH 7.4, 37 °C and 0.15 mol dm^{-3} ionic strength background. The total concentrations of amino acids are as given earlier [1]. The species considered and their stability constants are listed in Table I.

The calculated statistical ratios from both eqn. (2) and eqn. (5) are given in Table II. The inclusion of ligand protonation is seen to dramatically affect systems I and IV. The predominance of $Ni(His)_2$ over $Ni(Cys)_2^{2-}$ in system I is in accord with the experimental observations of Luccassen and Sarkar [3] on Ni(II) complexation in human serum and parallels the results from a computer simulation exercise [4] on blood serum equilibria (using program ECCLES [5]) which gave the concentration order $\{Ni(His)_2\} > \{Ni(Cys)(His)^-\} > \{Ni(Cys)_2^{2-}\}$. The difference in

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*Abbreviations used: $\beta^{X}_{pqr} = \{M_pH_qX_r\}/\{M\}^p\{H\}^q\{X\}^r$; aa, amino acidate; Ala, alaninate; Cys, cysteinate; His, histidinate; Thr, threoninate; Val, valinate.

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TABLE I. Species Considered and Their Stability Constants at 37 °C and 0.15 mol dm^{-3} Ionic Strength.

Species[a]	log β	Reference
$HCys^-$	10.11	6
H_2Cys	18.08	6
$Ni(Cys)_2^{2-}$	19.02[b]	7
$Zn(Cys)_2^{2-}$	17.98	8
HHis	8.92	8
$Ni(His)_2$	14.97[b]	9
$Cu(His)_2$	17.50	8
$Zn(His)_2$	11.68	8
HThr	8.71	8
$Cu(Thr)_2$	14.01	8
Haa	9.33[c]	8
$Cu(aa)_2$	14.7[c]	8

[a] Only protonation constants >7 need be considered. [b] Corrected to 37 °C and 0.15 mol dm^{-3} ionic strength. [c] Averaged values.

TABLE II. Calculated Statistical Ratios $\{MA_2\}:\{MB_2\}:\{MAB\}$.

System				Concentration Ratios Using	
No.	M	A	B	Equation (2)	Equation (5)
I	Ni	Cys	His	821:1:28.7	1:6.2:2.5
II	Cu	His	Thr	1013:1:6.4	391:1:19.8
III	Cu	His	aa	3.1:1:1.6	4:1:2
IV	Zn	His	Cys	1:1.47 × 10^5:7.65 × 10^3	1:29:5.4

conclusions resulting from the inclusion of ligand protonation can be attributed to the significant difference in basicities between cysteine and histidine. For systems II and III, where the differences in ligand basicities are not so large the concentration ratios are of the same order for both calculations, further confirming our previous conclusions [1].

It is again worth noting that despite the equilibrium constant, K_D, for equilibrium (1) being positive in none of the above systems does the ternary complex predominate. For systems I and III where the ternary species are significant the factor wa/b is 0.4 and 2.0 respectively, close to the range 2 > wa/b > 0.5 in which the ternary complex will be the dominant species.

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