The Statistics of Ternary Complex Formation with Special Reference to Biological Fluids

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A simple statistical treatment is applied to the distribution equilibrium of an aqueous solution containing a metal ion (M) which can form kinetically labile complexes with bidentate ligands (A) and (B). Thus for the equilibrium:

$MA_2 + MB_2 \rightleftharpoons 2MAB$

the treatment verifies that the distribution constant $K_d = 4$, assuming the absence of any intraligand interactions or other factors favouring the formation of any of the species. It is shown that for a molecules of A and b molecules of B with a statistical weighting factor w that favours or disfavours A relative to B, that:

$[MA_2]:[MB_2]:[MAB] \equiv (wa)^2:b^2:2wab$

*and**, *that*

$w = (\beta_2^A/\beta_2^B)^{1/2}$

This treatment is applied to Cu(II) and Zn(II) binary and ternary species that are postulated to be of importance in the low molecular weight fraction of blood. One important conclusion is that in the absence of intraligand or other interactions the ternary complex will only predominate over a narrow range of conditions, i.e. $2 > wa/b > 0.5$. *Thus for most situations one of the binary complexes should statistically dominate.*

Introduction

Considerable attention is being paid to mixed ligand chelate complexes, especially the ternary complexes of composition MAB (M = divalent metal ion, A and B are different bidentate or tridentate ligands). First, the study of such species enhances our knowledge of the nature of the intraligand electronic and steric interactions that can occur [1–3]. Second, such species are of importance in biological fluids in which metal ions can coordinate with a large number of possible ligands. Of particular importance in this category are the kinetically labile complexes formed by Cu(II), Mn(II) and Zn(II) with amino acids and other potential, low molecular weight, ligands [4–6].

In aqueous media of the kinetically labile complexes the ratio of ternary to binary complexes at equilibrium reflects the extent of the significance of any intraligand interactions. Two expressions are used to assess the relative importance of the ternary complexes. Sigel [2] in his elegant work on intraligand hydrophobic interactions makes use of the equilibrium (1):

$$MA + MB \rightleftharpoons MAB + M_{aq} \quad (1)$$

However, in biological fluids the ligand concentrations generally far exceed the metal concentrations so the amount of partially chelated or free metal ions is extremely small. In this context it is more appropriate to use the expression (2):

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

For which the equilibrium constant* is given by:

$$K_D = [MAB]^2/[MA_2][MB_2] = (\beta^{AB})^2/\beta_2^A \cdot \beta_2^B \quad (3)$$

Hence,

$$\log \beta^{AB} = 1/2\{\log \beta_2^A + \log \beta_2^B\} + 1/2 \log K_D \quad (4)$$

From consideration of stability constants and assuming *equality of* β_2^A and β_2^B and *equimolar* amounts of A and B it can be shown [7] that K_D has a value of 4. This value was also obtained by Gillard *et al.* [8] for the case when A and B are optical enantiomers of the same ligand. The value of 4 is also based on the assumption that there are no special factors favouring the formation of any one of the possible species; it is hence often referred to as the statistical factor *favouring* ternary complex formation (since the value is positive). Deviations from the value of 4 for experimentally determined K_D are taken as indications of intraligand interactions [1, 2].

*Abbreviations used throughout: β_2^L, overall stability constant of the species ML_2; $K_n^L = [ML_n]/[ML_{n-1}][L]$; $K_L^{LL'} = [MLL']/[ML][L']$; $[X_t]$, total concentration of Ligand X; aa, amino acidate; Cys, cysteinate; His, histidinate; Thr, threoninate.

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However, the conditions used in the above treatment, *i.e.* equality of stability constants and concentrations, are not applicable to most solutions, particularly biological fluids. There is thus a need for a more general, statistical, derivation of the value of K_D. General statistical treatments are already available in the literature [9–11] but are not simply applied to the equilibrium (2). We now wish to present a simple statistical treatment for the derivation of K_D which also highlights some important features concerning the equilibrium process (2).

Statistical Treatment

We assume that the formation of the species MA_2, MB_2 and MAB is completely random. In the first instance we consider the occupation of the coordination sites on M to be equally likely for both A and B ligands. Given that there are a molecules of A and b molecules of B, and that the number of molecules of M is less than a or b, then the probability of forming MA_2 is simply given by:

$$\frac{a}{(a+b)} \times \frac{(a-1)}{(a+b-1)} \tag{5}$$

Likewise, the probability of MB_2 formation is:

$$\frac{b}{(a+b)} \times \frac{(b-1)}{(a+b-1)} \tag{6}$$

and, for the ternary species, MAB is:

$$2 \times \frac{a}{(a+b)} \times \frac{b}{(a+b-1)} \tag{7}$$

The factor of 2 in expression (7) arises from the fact that the ternary species can form in two statistically equal ways, *i.e.* as MAB and MBA.

Since a and b $\gg 1$ then from (5)–(7) we obtain:

$$[MA_2]:[MB_2]:[MAB] \equiv a^2:b^2:2ab \tag{8}$$

It thus follows from (3) that:

$$K_D = (2ab)^2/a^2b^2 = 4 \tag{9}$$

The value of 4 for K_D is seen to be independent of the starting concentrations of A and B, as of course any true equilibrium constant should be; we shall be paying more attention to this point concerning concentrations later. It is very simple to extend this treatment to the more general case when occupation of the coordination sites by A and B is not equally likely. Statistically this can be treated as a weighting factor favouring either A or B occupying the coordination sites; let there be a weighting factor w favouring or disfavouring A relative to B. The factor a in the above expressions is now replaced by wa, leading to:

$$[MA_2]:[MA_2]:[MAB] \equiv (wa)^2:b^2:2(wa)b \tag{10}$$

and

$$K_D = \{2(wa)b\}^2/(wa)^2 \cdot b^2 = 4 \tag{11}$$

Thus, the statistical factor of 4 is seen to be independent of any weighting factor favouring any of the binary complexes.

The statistical factor w must of course be a reflection of the differences of the thermodynamic stabilities of the complexes, the relationship between w and the chemical equilibrium constants can be established in the following way. The factors a and b in eqns. (5)–(7) are now multiplied by the respective K_n^L value. Thus in (5) a and (a – 1) now become $K_1^A a$ and $K_2^A(a-1)$ respectively, and since a $\gg 1$, their product becomes $K_1^A K_2^A a^2$ or $\beta_2^A a^2$. Expression (8) thus becomes:

$$[MA_2]:[MB_2]:[MAB] \equiv \beta_2^A a^2:\beta_2^B b^2:\beta^{AB} ab \tag{12}$$

From this $K_D = (\beta^{AB})^2/\beta_2^A\beta_2^B$, which is eqn. (3).

Note, the value of 2 in the quotient '$\beta^{AB}ab$' in (12) is absent, this is in fact inherent in β^{AB} ($=2[\beta_2^A \cdot \beta_2^B]^{1/2}$), and is also evident from the thermodynamic relationship:

$$K_1^A K_A^{AB} = K_1^B K_B^{AB} = \beta^{AB} \tag{13}$$

reflecting the two pathways for the formation of MAB.

From eqns. (11) and (12), it is evident that:

$$w = (\beta_2^A/\beta_2^B)^{1/2} \tag{14}$$

Applications

Having shown that $K_D = 4$ is generally applicable, then from equation (4) it can be seen that the contribution of this factor (0.5 log $K_D = 0.301$) to the formation of the ternary complexes is negligible compared to the contribution from the stability constants of the binary species. In fact, assuming that no intraligand interactions nor other kinetic or thermodynamics factors arise, the stability constant of the ternary species is virtually the geometric mean of the product of the stability constants of the contributing binary species. This factor is of particular importance in terms of the relative concentrations of the species in any given solution. This relative distribution can also readily be obtained from expression (10) replacing numbers of molecules with total concentrations of the ligands, $[X]_t$. We shall now illustrate how useful and important is the evaluation of the relative distribution of the species. The examples chosen are all species that have been postulated as being present in the low molecular weight fraction of human blood. This fraction has received considerable attention because of its importance in trace metal metabolism [4–6].

Example 1: Cu(His)(Thr)

Early chromatographic studies [12] suggested that the ternary complex Cu(His)(Thr) was a significant species of Cu(II) in the low molecular weight fraction. This finding was not supported by computer modelling of the equilibria in this fraction [4, 5] but did receive some support from a potentiometric study of the aqueous Cu–His–Thr system [13, 14]. If we now consider the equilibrium:

$$Cu(His)_2 + Cu(Thr)_2 \rightleftharpoons 2Cu(His)(Thr) \qquad (15)$$

Using the data from May *et al.* [4], *i.e.* at 37 °C and in 0.15 mol dm^{-3} NaCl solution log $\beta_2^{His} = 17.5$, log $\beta_2^{Thr} = 14.0$, for blood plasma $[His]_t = 8.5 \times 10^{-5}$ mol dm^{-3}, $[Thr]_t = 15 \times 10^{-5}$ mol dm^{-3}, then from eqns. (10) and (14):

$$[Cu(His)_2]:[Cu(Thr)_2]:[Cu(His)(Thr)] = 1013:1:64$$

This supports the results from the computer calculations that $Cu(His)_2$ rather than Cu(His)(Thr) is a major species. Freeman *et al.* [13], however, found the concentration order to be $[Cu(His)(Thr)] > [Cu(His)_2] > [Cu(Thr)_2]$ from potentiometric analysis at 25 °C (0.10 mol dm^{-3} KNO_3). Their log stability constant for the ternary species, corrected to 37 °C, is 16.9, this compares to the statistically expected value of 16.05 from eqn. (4). So it would appear that a small favourable intraligand interaction is enhancing the thermodynamic stability of the ternary species; the nature of this interaction has still to be established [14].

Example 2: Cu(His)(aa)

Those amino acids with non-coordinating side-groups form complexes with Cu(II) of similar stability (average log $\beta_2^{aa} = 14.7$ at 37 °C and 0.15 mol dm^{-3} NaCl solution [5]). Although this stability constant is far less than that of $Cu(His)_2$ the relatively large concentration of these amino acids (*ca.* 2.69×10^{-3} mol dm^{-3}) could make them potential ligands, particularly in ternary complex formation. Using the information given we obtain:

$$[Cu(His)_2]:[Cu(aa)_2]:[Cu(His)(aa)] = 3.1:1:1.6$$

This result parallels our own observations on aqueous Cu^{II}–His–aa solutions [15] and those from computer modelling [4, 5] that these amino acids are not of significance in the blood milieu in the form $Cu(aa)_2$ but can make some contribution as the Cu(His)(aa) complexes.

From this example it is also interesting to speculate as to why the complex Cu(His)(cystine) should be a major Cu species in the blood fraction [4, 5]. Cystine is present at a lower concentration (4.0×10^{-5} mol dm^{-3}) than is histidine. Unfortunately, reliable stability constants for the Cu(II)–cystine equilibria are not available because of problems with low solubility, however, by analogy with the similar ligand penicillamine disulphide [16] we can expect simple bidentate glycine-like coordination with respect to each metal ion. From the computer model calculations it would appear that protonation of cystine at the non-coordinating end has a negligible influence on the amount of ternary complex formation which further confirms the simple glycine-like behaviour. The stability constant used in these calculations for the ternary Cu(His)(Cystine) species was obtained experimentally by Hallman *et al.* [4]. The log value obtained at 37 °C and 0.15 mol dm^{-3} KNO_3 was 18.51. Inserting this value into equation (4) using the data already given leads to log $\beta = 18.92$ for $Cu(cystine)_2^{2-}$. This is remarkably high for such a species, it can be compared to the value of 14.31 obtained for the analogous bispenicillamine disulphide complex [16] at 25 °C (*ca.* 13.8 at 37 °C) which is more in line with the expected glycine-like behaviour [17].

Example 3: Zn(His)(Cys)

Hallman *et al.* [4] calculated this species to be the major zinc(II) species in the low molecular weight fraction of human blood followed by $Zn(His)^+$ and $Zn(Cys)_2^{2-}$. May *et al.* [5] using a slightly different data base for the total ligand concentrations and including citrate obtained the order $Zn(cys)(citrate)^{3-} > Zn(cys)_2^{2-} > Zn(His)(Cys)^-$. Both computations used the same stability constants. In a later study Williams and his co-workers [18] re-determined the Zn(II)–citrate equilibria and from this concluded that the major species is $Zn(Cys)_2^{2-}$ followed by Zn(His)(Cys).

Taking, from these above studies, the values log $\beta_2^{His} = 11.68$, log $\beta_2^{Cys} = 17.98$, $[His]_t = 8.5 \times 10^{-5}$ mol dm^{-3} and $[Cys]_t = 2.3 \times 10^{-5}$ mol dm^{-3}, then from equation (10) we obtain:

$$[Zn(His)_2]:[Zn(Cys)_2]:[Zn(His)(Cys)] \equiv 1:1.47 \times \times 10^5:7.65 \times 10^3$$

and, from (4), we calculate log $\beta\{Zn(His)(Cys)\}$ to be 15.13, which is very close to the experimentally measured value [4] of 15.23. The predominance of the $Zn(Cys)_2$ complex from these calculations is thus in accord with the conclusions of Williams and his group [5, 18]. Likewise, since log $\beta\{Zn(citrate)_2\}$ was found [18] to be only 7.36 and since the log $\beta\{Zn(Cys)(citrate)^{3-}\}$ of 12.35 is less than the statistical value (from (4)) of 12.97, the insignificance of any citrate-containing Zn species is only to be expected.

Conclusions

The foregoing examples illustrate the usefulness of the simple statistical approach outlined here. Not

only does it verify the statistical factor of 4 for the equilibrium (2) in general but it gives the statistically expected concentration distribution of the binary and ternary species. This distribution together with the calculated log β^{AB} value from eqn. (4) allows a valuable comparison with experimental values for the prediction of intraligand effects.

An important conclusion from the above examples and a fact which is evident from examination of expression (10) is that, in the absence of highly favourable intraligand effects, in the vast majority of cases one of the binary complexes will be the predominant species. From equation (10) it can be seen that the ternary species will only predominate under the condition $2 > wa/b > 0.5$, which is an extremely narrow range. Thus the statement "...under purely statistical conditions one would expect the ternary complex to be formed to the extent of 50% while the binary complexes should occur to the extent of 25% each." [2], applies *only* to the situation when wa = b, *i.e.*, only under the fortuitous circumstance of $(\beta_2^A/\beta_2^B)^{1/2} = [B]_t/[A]_t$. A condition which would be rarely met in biological or any other fluid.

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