

The Copper(II) Complexity of O-Phosphorylethanolamine

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The complex formation between copper(II) and O-phosphorylethanolamine (H_2A) ions has been studied in 0.15 M KCl medium at 25° by pH titration.

By an adaption of Hedström's method³⁹ the concentration of free O-phosphorylethanolamine was calculated. The experimental data could be explained by formation of the complexes: $CuHA^+$, CuA , $Cu(HA)A^-$ and CuA_2^{2-} . The corresponding equilibrium constants were computed (Table 2).

Probable coordination sites involved in the complexes are suggested, and are discussed in relation to copper(II) binding of phosphoproteins and phosphatides.

As pointed out by several authors¹⁻⁵ phosphoproteins and phosphopeptides may under physiological conditions act as strong metal binders. The great metal affinity of these compounds has been partly ascribed to the presence of phosphorus^{2,4,6-9}, which in view of recent investigations^{2,10-18} occurs mainly as monophosphate esters linked through the hydroxyl groups of serine and threonine residues*. Informations of the metal binding power of phosphoproteins and their break-down products may, thus, be obtained from studies on O-phosphorylated model compounds. Apparent stability constants of some metal complexes of O-phosphorylserine have previously been reported^{20,21}. The present paper describes the copper(II) complexity of another model compound, O-phosphorylethanolamine; this ligand itself being an important metabolite existing in living tissues both in free²²⁻²⁹ and combined state²⁸⁻³⁵.

The complex formation was measured by pH titration³⁶⁻³⁸ at 25° in 0.15 M KCl. In this case several titrations for different ligand concentrations were carried out to allow calculation of the free ligand concentration more directly from the experimental data by graphical integration (*cf.* Ref.³⁹), assuming the possible presence of any set of mononuclear equilibria including hydrogen and hydroxo copper(II) complexes of the ligand. An equation required for this calculation was derived by applying the equations formulated by Hedström³⁹.

* Apart from the phosphomonoester bond, the occurrence of other linkages of phosphorus has been suggested for some phosphoproteins¹⁹.

The existing complexes and corresponding overall equilibrium constants were evaluated graphically. The pK values of the ligand, determined under the same experimental conditions⁴⁰, were used in the computations.

SYMBOLS

- A O-phosphorylethanolamine with all dissociable hydrogen removed,
- $$\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{O} \cdot \text{P} \begin{array}{l} \text{O} \\ \parallel \\ \text{O}^- \\ \text{O}^- \end{array}$$
- a molar concentration of free A
- b_q apparent stability constant of the complex $\text{CuA}_q^{(2-2q)+}$ defined by
 $b_q = [\text{CuA}_q^{(2-2q)+}] [\text{CuA}_{q-1}^{(4-2q)+}]^{-1} [\text{Cu}^{2+}]^{-1}$
- b_{CuHA} apparent stability constant of the complex CuHA^+ , defined by
 $b_{\text{CuHA}} = [\text{CuHA}^+] [\text{HA}^-]^{-1} [\text{Cu}^{2+}]^{-1}$
- $b_{\text{Cu(HA)A}}$ apparent stability constant of the complex Cu(HA)A^- , defined by
 $b_{\text{Cu(HA)A}} = [\text{Cu(HA)A}^-] [\text{CuHA}^+]^{-1} [\text{A}^{2-}]^{-1}$
- b_{CuCl} apparent stability constant of the complex CuCl^+ , defined analogical to b_q
- CA total molar concentration of O-phosphorylethanolamine, defined by eqn. 5
- C_{Cu} total molar concentration of copper(II) ions, defined by eqn. 4
- C_{H} total molar concentration of hydrogen ions, defined by eqn. 6
- C_{Cl} total molar concentration of chloride ions
- h molar concentration of free hydrogen ions
- I defined by eqn. 21
- j number of protons bound in the proton complex H_jA
- K_{CuHA} apparent acid ionization constant of the complex CuHA^+ , defined by
 $K_{\text{CuHA}} = h [\text{CuA}] [\text{CuHA}^+]^{-1}$
- $K_{\text{Cu(HA)A}}$ apparent acid ionization constant of the complex Cu(HA)A^- , defined by
 $K_{\text{Cu(HA)A}} = h [\text{CuA}_2^{2-}] [\text{Cu(HA)A}^-]^{-1}$
- L defined by eqn. 22
- M g moles/litre
- p, q, r number of HA^- , A^{2-} , OH^- groups bound in the complex $\text{Cu(HA)}_p\text{A}_q(\text{OH})_r^{(2-p-2q-r)+}$, respectively
- R defined by eqn. 9
- S defined by eqn. 10
- Z ligand number, defined by eqn. 8
- β_2 apparent complexity constant, = $b_1 b_2$
- $\beta_{\text{Cu(HA)A}}$ apparent complexity constant, = $b_{\text{CuHA}} b_{\text{Cu(HA)A}}$
- $\kappa_{1,1p,0q,r}$ apparent overall equilibrium constant defined by eqn. 2
- κ_{j1} apparent complexity constant defined by eqn. 3
- φ defined on page 478
- [] molar concentration of the species indicated

MATERIALS

O-Phosphorylethanolamine, prepared according to Baer and Stancer⁴¹, was a generous gift from Ing. G. Fölsch of this institute. The data of analysis for the present preparation are to be found in Ref.⁴⁰ Aqueous stock solutions of this compound were standardized by potentiometric titrations. When not used, these solutions had to be kept frozen in order to obtain satisfactory reproducibility.

Aqueous stock solutions of copper(II) chloride were prepared from the British Drug Houses Ltd. (A.R.) product and was standardized by an ion exchange method⁴².

Carbonate-free 0.2557 M KOH, containing 0.15 M KCl was prepared and standardized as before¹⁷.

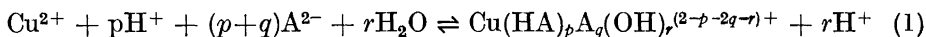
The other reagents used in this work were of analytical grade and were not purified further. Tripple distilled water and weighed-in pipettes were used throughout.

METHOD

The quantities, h and C_H , were varied at each pH titration, while C_{Cu} and C_A were held practically constant. This was carried out by successive delivering KOH to the titration solution, recording pH after each addition. As recording instrument a Radiometer PHM 3j valve potentiometer, equipped with a Radiometer glass electrode G 102 B, and a calomel electrode K 100 was used. The activity coefficients were assumed to be constant in the presence of 0.15 M KCl. All measurements were carried out at $25.00 \pm 0.03^\circ$.

The pH meter was standardized with 0.05 M potassium biphthalate and 0.05 M borax as recommended by Hitchcock *et al.*⁴³. The pH meter readings were transformed to values of $-\log h$ by a calculated activity coefficient⁴⁰ being 0.79 ± 0.02 . It was thought to apply in the pH range, 4–7, involved in the computations below. For routine calibration of the entire measuring system, acetic acid titrations were used, *cf.* Ref.⁴⁰, the deviations never exceeding 0.01 pH units.

Assuming O-phosphorylethanolamine only to interact with copper(II) ions, when both protons are removed from the phosphoryl group, *i.e.* as HA^- and A^{2-} , the complex equilibria can be written



$$(p = 0,1,2,\dots; q = 0,1,2,\dots; r = 0,1,2,\dots; p+q+r = 1,2,3,\dots)$$

Since H_2O is approximately constant, the system is defined by the overall equilibrium constants * (all charges omitted in the following for the sake of clarity):

$$\kappa_{1,1p,0q,r} = \frac{[Cu(HA)_p A_q(OH)_r]}{[Cu] h^{p+r} a^{p+q}} \quad (2)$$

The quantities p, q , and r may attain any integer values given above, restricted by $(p+q+r)_{\max}$, which is equal to the maximum coordination number of copper(II). Occurrence of polynuclear complexes was assumed to be negligible with the use of low values for C_A and C_{Cu} , with C_A in excess of C_{Cu} .

Analogously to eqn. 2, constants for the formation of proton complexes are defined

$$\kappa_{j1} = \frac{[H_j A]}{h^j a} \quad (j = 1,2,3) \quad (3)$$

The values of these constants, used in this work, have been re-calculated from the pK values reported earlier⁴⁰.

Due to presence of bulk electrolyte (0.15 M KCl), the species $CuCl^+$ has also to be considered. The total content of copper(II), ligand and dissociable hydrogen will thus be given by (*cf.* Ref.³⁸)

* The notation of these constants is simplified, when only one or two complex forming groups are bound to the central metal ion. Thus will, *e.g.*, the constants corresponding to $CuOH^+$ and CuA be written $\kappa_{1,1}$ and $\kappa_{1,01}$, respectively.

$$C_{\text{Cu}} = [\text{Cu}](1 + \sum \kappa_{1,1p,0q,r} h^{p-r} a^{p+q} + b_{\text{CuCl}}[\text{Cl}]) \quad (4)$$

$$C_{\text{A}} = a + \sum (p+q)\kappa_{1,1p,0q,r}[\text{Cu}]h^{p-r}a^{p+q} + \sum \kappa_{j1}h^ja \quad (5)$$

$$C_{\text{H}} = h - [\text{OH}] + \sum (p-r)\kappa_{1,1p,0q,r}[\text{Cu}]h^{p-r}a^{p+q} + \sum j\kappa_{j1}h^ja \quad (6)$$

The summations have been carried out in respect of all stated values of p, q and r . The value of C_{H} for each point of titration may be calculated from

$$C_{\text{H}} = 2C_{\text{A}} - [\text{KOH}] \quad (7)$$

The figure $b_{\text{CuCl}}[\text{Cl}]$ in eqn. 4 will be constant as $[\text{Cl}] \approx C_{\text{Cl}} = 0.15$, and $[\text{OH}]$ in eqn. 6 negligible in the $-\log h$ range involved.

From eqns. 4 and 5 we obtain the ligand number defined thus (*cf.* Ref.³⁸)

$$Z = \frac{C_{\text{A}} - a - \sum \kappa_{j1}h^ja}{C_{\text{Cu}}} = \frac{\sum (p+q)\kappa_{1,1p,0q,r}h^{p-r}a^{p+q}}{1 + \sum \kappa_{1,1p,0q,r}h^{p-r}a^{p+q} + b_{\text{CuCl}}[\text{Cl}]} \quad (8)$$

It follows from eqn. 8 that the constants $\kappa_{1,1p,0q,r}$ can be determined, if Z is known as a function of h and a . As Z is given by eqn. 8, if h and a are known, we have, thus, first to compute a . This computation as well as the evaluation of p, q, r and $\kappa_{1,1p,0q,r}$ will be described after the following section.

MEASUREMENTS

Solutions for titration of metal complexes were prepared by pipetting stock solutions of O-phosphorylethanolamine, copper(II) chloride, potassium chloride and aq. dest. in order to obtain the initial concentrations C_{A} , C_{Cu} and 0.15 M KCl. The titrations were continued to pH 10.5. All titration equipment was the same as that described¹⁷. The content (initially 10 ml) of the titration vessel was stirred and freed from carbon dioxide by bubbling nitrogen, pretreated as previously¹⁷.

Five titrations with different C_{A} were carried out in this manner covering the concentration ranges $C_{\text{A}} = (0.8-3) \times 10^{-3}$, $C_{\text{H}} = (0.8-6) \times 10^{-3}$ and $C_{\text{Cu}} = 7.8 \times 10^{-4}$. Each titration could be reproduced within 0.01 in pH. The data used for the calculations below constituted a mean of at least two titrations.

At $-\log \approx 6.3$ the measuring solutions grew slightly opalescent, developing into a greenish-blue precipitate at $-\log h \approx 7$. Semi-quantitative analysis of the washed and dried precipitate, isolated from a solution of pH 7.8, showed the presence of about 4 % N and 16 % P. This apparently indicates the precipitate to be a copper(II)-O-phosphorylethanolamine compound. Due to this sparing solubility of the present system above $-\log h \approx 6.3$, the computations were not extended further than up to $-\log h = 6.10$.

The results of the measurements are shown in Fig. 1, where the titrations are represented as $-\log h(C_{\text{H}})C_{\text{A}}$ curves.

DETERMINATION OF THE FREE O-PHOSPHORYLETHANOLAMINE CONCENTRATION

No true indications about the kind of complexes formed could be obtained from usual titration curves (as for example in the Cu(II)-O-phosphorylserine

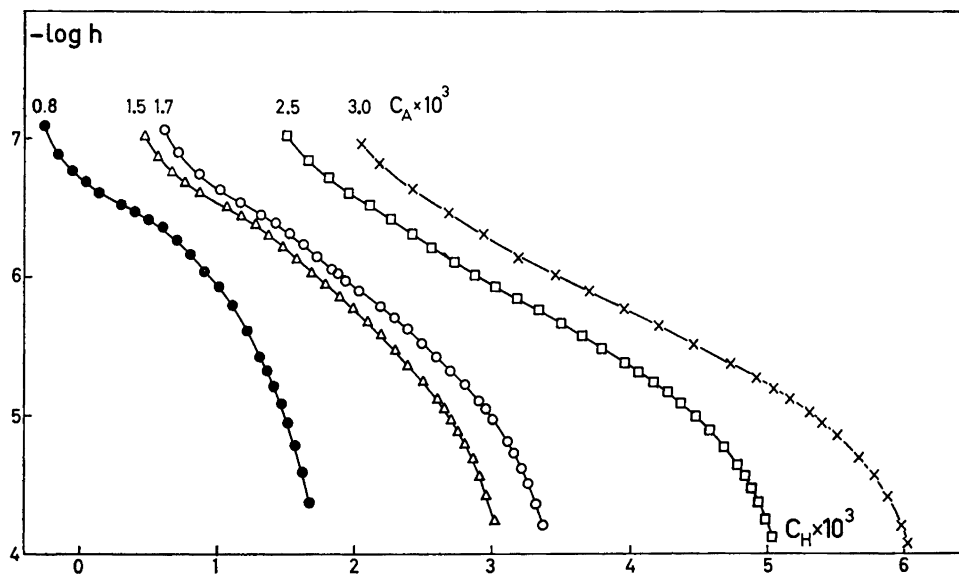


Fig. 1. The potentiometric measurements. $-\log h$ as a function of the total concentration of hydrogen ions, C_H (obtained from eqn. 7), for different total O-phosphorylethanolamine concentration, C_A .

system²¹). The free ligand concentration was therefore computed without any special assumptions about the complexes formed. For this purpose Hedström's method³⁹ was made applicable to measurements and equilibria of the present type.

We define the functions R and S

$$R = \sum \kappa_{j_1} h^j a = \sum [H_j A] \quad (9)$$

$$S = \sum \kappa_{1,1p,0q,r} h^{p-r} a^{p+q} \quad (10)$$

Partial differentiation of R with respect to $\ln h$, followed by partial differentiation with respect to $\ln a$, results in

$$\left(\frac{\partial R}{\partial \ln h} \right)_a = \sum j \kappa_{j_1} h^j a \quad (11)$$

$$\left(\frac{\partial^2 R}{\partial \ln h \partial \ln a} \right) = \sum j \kappa_{j_1} h^j a = \left(\frac{\partial R}{\partial \ln h} \right)_a \quad (12)$$

Partial differentiation of S with respect to $\ln h$ and $\ln a$, followed by partial differentiation with respect to $\ln a$ and $\ln h$, gives

$$\left(\frac{\partial S}{\partial \ln h} \right)_a = \sum (p-r) \kappa_{1,1p,0q,r} h^{p-r} a^{p+q} \quad (13)$$

$$\left(\frac{\partial S}{\partial \ln a}\right)_h = \sum (p+q)\kappa_{1,1p,0q,r}h^{p-r}a^{p+q} \quad (14)$$

$$\left(\frac{\partial^2 S}{\partial \ln h \partial \ln a}\right) = \left(\frac{\partial^2 S}{\partial \ln a \partial \ln h}\right) = \sum (p-r)(p+q)\kappa_{1,1p,0q,r}h^{p-r}a^{p+q} \quad (15)$$

We may eliminate [Cu] from eqns. 5 and 6 by extension of the expression in those equations, containing [Cu] with C_{Cu} . By substituting eqns. 9–11 and 13–14 into the resulting equations, followed by partial differentiation with respect to $\ln h$ and $\ln a$, respectively, we obtain

$$\left(\frac{\partial C_A}{\partial \ln h}\right)_a = C_{Cu} \left[\frac{(1+S+b_{CuCl}[Cl])\left(\frac{\partial^2 S}{\partial \ln a \partial \ln h}\right) - \left(\frac{\partial S}{\partial \ln a}\right)_h \left(\frac{\partial S}{\partial \ln h}\right)_a}{(1+S+b_{CuCl}[Cl])^2} \right] + \left(\frac{\partial R}{\partial \ln h}\right)_a \quad (16)$$

$$\left(\frac{\partial C_H}{\partial \ln a}\right)_h = C_{Cu} \left[\frac{(1+S+b_{CuCl}[Cl])\left(\frac{\partial^2 S}{\partial \ln h \partial \ln a}\right) - \left(\frac{\partial S}{\partial \ln h}\right)_a \left(\frac{\partial S}{\partial \ln a}\right)_h}{(1+S+b_{CuCl}[Cl])^2} \right] + \left(\frac{\partial^2 R}{\partial \ln h \partial \ln a}\right) \quad (17)$$

From eqns. 12 and 15 follows that the right hand sides of eqns. 16 and 17 are identical. Consequently

$$\left(\frac{\partial C_A}{\partial \ln h}\right)_a = \left(\frac{\partial C_H}{\partial \ln a}\right)_h \quad (18)$$

This equation may be transformed³⁹ to

$$\left(\frac{\partial \ln a}{\partial \ln h}\right)_{C_A} = -\left(\frac{\partial C_H}{\partial C_A}\right)_h \quad (19)$$

Solving for $-\log a$ within the range $-\log h_0$ to $-\log h$ results in

$$-\log a = -\left[\int_{-\log h_0}^{-\log h} \left(\frac{\partial C_H}{\partial C_A}\right)_h d(-\log h) \right]_{C_A} + (-\log a_0) \quad (20)$$

From eqn. 20 values of $-\log a_0/a$ were determined by graphical integration³⁹. The $-\log h(C_H)_{C_A}$ curves of Fig. 1 were thus cut for constant $-\log h$, and C_H drawn against C_A (Fig. 2a). The quantity $\left(\frac{\partial C_H}{\partial C_A}\right)_h$ was determined, and plotted against $-\log h$ (Fig. 2b). The area under the resulting curve from $-\log h = -\log h_0$ to $-\log h$ gave then $-\log a_0/a$. After the calculation of a_0 from

$$a_0 = C_A/(1 + \kappa_{11}h + \kappa_{21}h^2) \quad (5a)$$

at a $-\log h$ value ($= -\log h_0$), where no complex formation could be detected, the corresponding a values were obtained. The a values, determined in this manner, are listed in Table 1. For each a value determined the corresponding Z value was calculated; the result has been compiled in the same table.

In spite the graphical integration method³⁹ by Hedström usually requires many values for the known variables, the rather few C_A values of this study, seem sufficient, due to the constancy of $\left(\frac{\partial C_H}{\partial C_A}\right)_h$ in the C_A range involved (Fig. 2a).

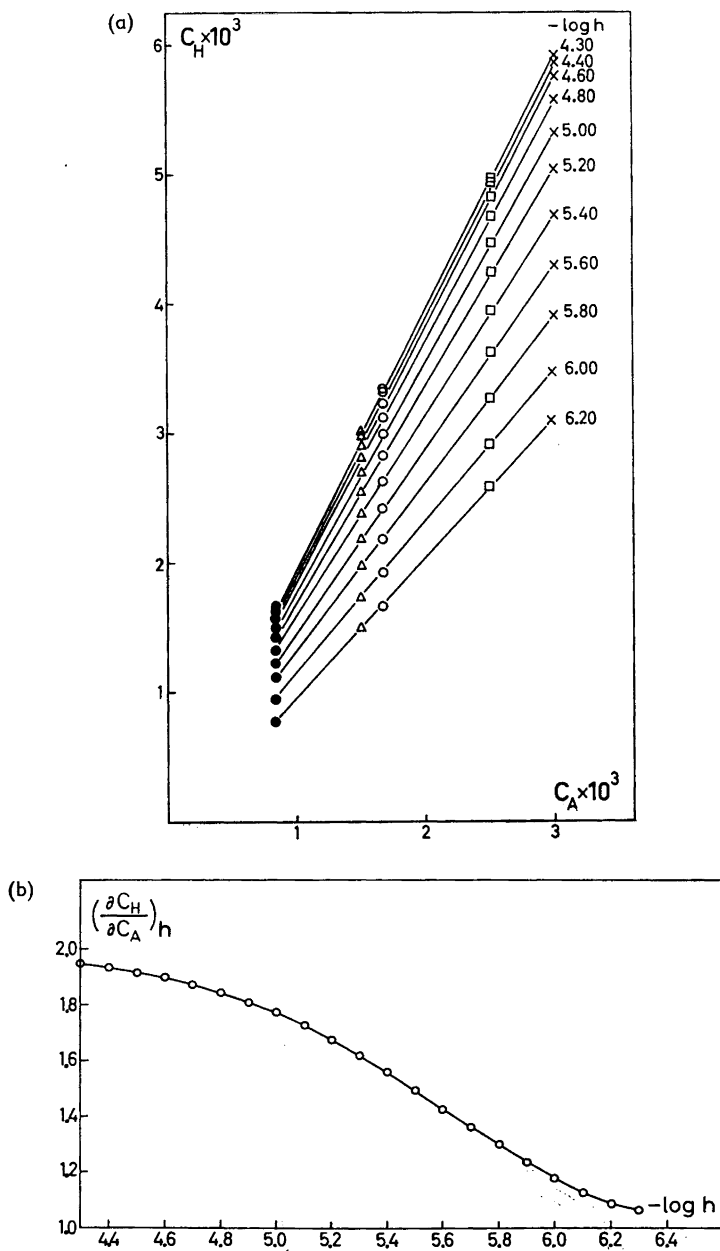


Fig. 2. Evaluation of the free O-phosphorylethanolamine concentration. (a). C_H vs. C_A for different $-\log h$. (b). $\left(\frac{\partial C_H}{\partial C_A}\right)h$ vs. $-\log h$ for $C_A = (0.8-3) \times 10^{-3}$.

Table 1. Corresponding values of $-\log h$, C_A , C_H (obtained from $-\log h(C_H)C_A$ curves), a (obtained from eqns. 20 and 5a), and Z (from eqn. 8).

$$C_{Cu} = 7.84 \times 10^{-4}$$

$$\kappa_{11} = 1.259 \times 10^{10} \text{ M}^{-1}, \quad \kappa_{21} = 4.678 \times 10^{15} \text{ M}^{-2}$$

$-\log h$	\times $C_A = 3.010 \times 10^{-3}$ $-\log h_0 = 4.30$			\square $C_A = 2.519 \times 10^{-3}$ $-\log h_0 = 4.30$			\circ $C_A = 1.682 \times 10^{-3}$ $-\log h_0 = 4.30$			\triangle $C_A = 1.507 \times 10^{-3}$ $-\log h_0 = 4.30$			\bullet $C_A = 0.834 \times 10^{-3}$ $-\log h_0 = 4.40$		
	C_H $\times 10^3$	a $\times 10^9$	Z	C_H $\times 10^3$	a $\times 10^9$	Z	C_H $\times 10^3$	a $\times 10^9$	Z	C_H $\times 10^3$	a $\times 10^9$	Z	C_H $\times 10^3$	a $\times 10^9$	Z
5.300	4.870	16.17	0.0973	4.080	13.535	0.0834	2.720	9.039	0.0579	2.457	8.097	0.0523	1.372	4.483	0.0297
5.400	4.687	23.31	0.1261	3.927	19.51	0.1076	2.620	13.035	0.0742	2.366	11.67	0.0671	1.324	6.464	0.0381
5.500	4.498	33.13	0.1596	3.766	27.72	0.1354	2.518	18.52	0.0932	2.270	16.59	0.0841	1.273	9.185	0.0478
5.600	4.300	46.36	0.1985	3.605	38.81	0.1688	2.410	25.92	0.1159	2.177	23.215	0.1044	1.224	12.855	0.0591
5.700	4.105	63.92	0.2455	3.435	53.49	0.2081	2.299	35.73	0.1427	2.071	32.01	0.1285	1.169	17.725	0.0728
5.800	3.904	86.83	0.2974	3.263	72.68	0.2528	2.175	48.55	0.1728	1.965	43.48	0.1555	1.109	24.075	0.0878
5.900	3.703	116.3	0.3564	3.079	97.31	0.3019	2.041	65.00	0.2056	1.850	58.22	0.1851	1.038	32.235	0.1045
6.000	3.479	153.6	0.4227	2.903	128.55	0.3583	1.907	85.86	0.2431	1.725	76.91	0.2194	0.948	42.59	0.1233
6.100	3.280	200.3	0.4947	2.729	167.65	0.4187	1.779	112.0	0.2837	1.611	100.3	0.2552	0.862	55.54	0.1428

* Initial concentration.

EVALUATION OF THE EQUILIBRIUM CONSTANTS

As can be seen from Table 1 all values of Z are below 1. To include all possibly existing complexes, $(p+q)_{\max}$ was therefore in the first place assumed to equal 2. For $p = q = 0$, r was assigned to 1, but otherwise r was set equal to zero. Eqn. 8 will thus be reduced to

$$\frac{Z}{(1-Z)a} \varphi = \kappa_{1,11}h + \kappa_{1,01} + \frac{(2-Z)a}{(1-Z)} (\kappa_{1,12}h^2 + \kappa_{1,11,01}h + \kappa_{1,02}) \quad (8a)$$

where $\varphi = 1 + \kappa_{1,1}h^{-1} + b_{CuCl}[Cl]$

Since the term $b_{CuCl}[Cl]$ is approximately constant in this study (*cf.* p. 474), the expression φ in eqn. 8a will be constant for constant h . Varying values of $\kappa_{1,1}$ in this expression are reported in the literature⁴⁴. In the present study use will therefore be made of a range of values from $10^{-7.5}$ to $10^{-8.25}$ M. From these values follow that the amount of $CuOH^+$ will be rather negligible in the present $-\log h$ range.

To identify complexes formed, $Z(1-Z)^{-1}a^{-1}$ was first plotted against h (Fig. 3). In this plot of eqn. 8a the presence of $CuHA^+$ and CuA only should give a straight line independent of C_A ; existing $CuHA^+$ species making the ordinata values to decrease with h . At the occurrence of higher complexes, *i.e.* $(p+q) > 1$, the plotted data should vary with C_A . As the latter case applies to Fig. 3, we may identify the higher complexes apparently present.

In Fig. 4 the quantity $Z(1-Z)^{-1}a^{-1}$ has therefore been plotted against $(2-Z)a(1-Z)^{-1}$ for constant h . As shown in the figure data are best represented by a serie of straight lines, indicating the true value of $(p+q)_{\max}$ to be 2. Conse-

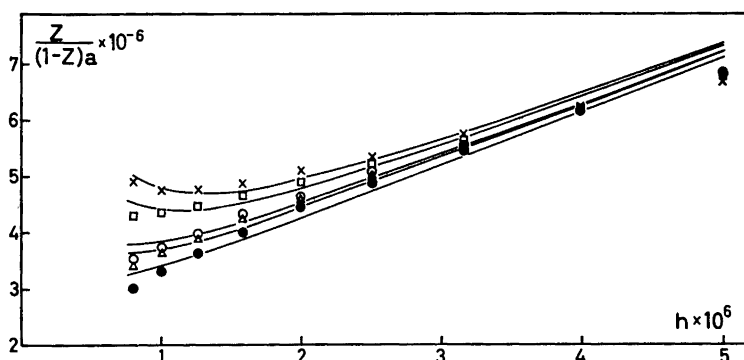


Fig. 3. $Z(1-Z)^{-1}a^{-1}$ vs. h for different C_A (eqn. 8 a). The curves are calculated for $\kappa_{1,11} = 1.1 \times 10^{12} \text{ M}^{-2}$, $\kappa_{1,01} = 2.45 \times 10^6 \text{ M}^{-1}$, $\kappa_{1,11,01} = 2.3 \times 10^{18} \text{ M}^{-3}$, $\kappa_{1,02} = 2.45 \times 10^{12} \text{ M}^{-2}$; $\kappa_{1,1} = 10^{-7.73} \text{ M}$, $b_{\text{CuCl}} = 1.0 \text{ M}^{-1}$. The symbols of the plotted points are those indicated in Table 1.

quently we may consider the probable existence of $\text{Cu}(\text{HA})_2$, $\text{Cu}(\text{HA})\text{A}^-$ and CuA_2^{2-} apart from CuHA^+ and CuA . In Fig. 4 there is, however, a slight upward trend for increasing C_A at high $-\log h$. For this reason the plotted points corresponding to $C_A > 2.52 \times 10^{-3}$ and $-\log h > 6.0$ were excluded at the calculation of slopes and intercepts of the straight lines.

From these intercepts (I) and slopes (L) the following functions were formed:

$$I\varphi = \kappa_{1,11}h + \kappa_{1,01} \tag{21}$$

$$L\varphi = \kappa_{1,12}h^2 + \kappa_{1,11,01}h + \kappa_{1,02} \tag{22}$$

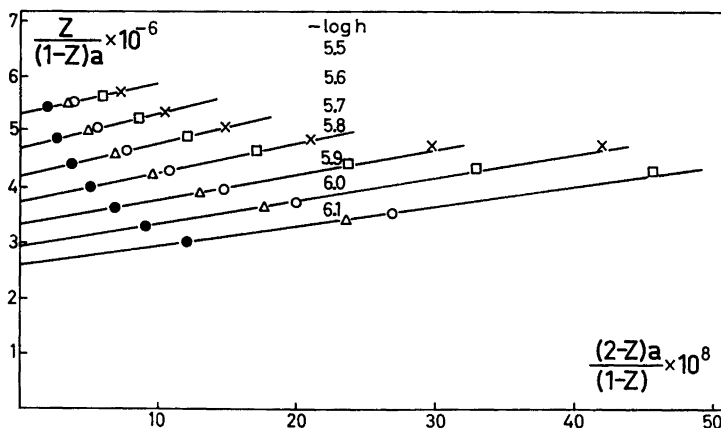


Fig. 4. $Z(1-Z)^{-1}a^{-1}$ as a function of $(2-Z)a(1-Z)^{-1}$ with h as parameter (eqn. 8a). The symbols are the same as indicated in Table 1.

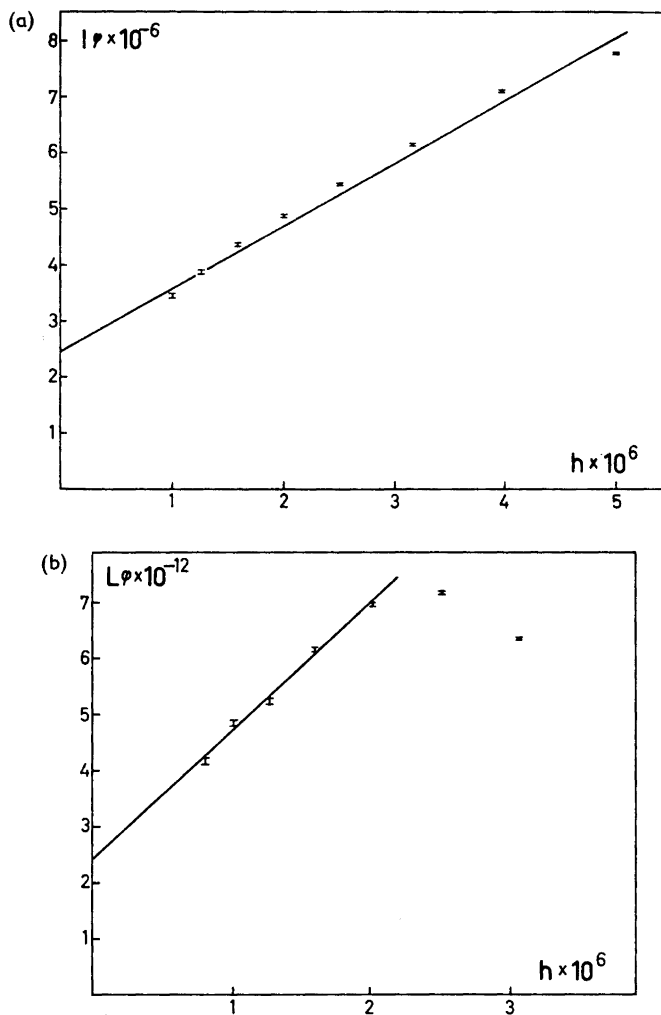


Fig. 5. Graphical evaluation of the overall equilibrium constants, assuming $\kappa_{1,1} = 10^{-7.73}$ M (indicated range $10^{-7.5} - 10^{-8.25}$ M) and $b_{\text{CuCl}} = 1.0 \text{ M}^{-1}$.

- (a) Eqn. 21 plotted as $I\varphi$ vs. h .
 (b) Eqn. 22 plotted as $L\varphi$ vs. h .

In Fig. 5a, eqn. 21 is plotted as the curve $I\varphi(h)$. For each h -value the above-mentioned range of $\kappa_{1,1}$ is indicated (mean value $10^{-7.73}$ M). The constant b_{CuCl} was set equal to 1.0 M^{-1} (Ref.⁴⁴). As shown in the figure, data can be fitted to a straight line with finite slope and intercept. The species CuHA^+ and CuA are thus obviously existing. The intercept ($\kappa_{1,01}$) and the slope ($\kappa_{1,11}$) of the best straight line furnished the equilibrium constants:

$$\begin{aligned}\kappa_{1,01} &= (2.45 \pm 0.2) \times 10^6 \text{ M}^{-1} \\ \kappa_{1,11} &= (1.1 \pm 0.2) \times 10^{12} \text{ M}^{-2}\end{aligned}$$

In a similar manner $L\varphi$ has been plotted against h in Fig. 5b. Even in this case, data can be fitted to a straight line, which may provide evidence of existence of the species $\text{Cu}(\text{HA})\text{A}^-$ and CuA_2^{2-} . Formation of $\text{Cu}(\text{HA})_2$, in measurable quantities can reasonable be ruled out. In the present plot data should then have been represented as a parabola with its vertex downwards. The values of equilibrium constants related to $\text{Cu}(\text{HA})\text{A}^-$ and CuA_2^{2-} were computed from the best straight line to be:

$$\begin{aligned}\kappa_{1,02} &= (2.45 \pm 0.2) \times 10^{12} \text{ M}^{-2} \\ \kappa_{1,11,01} &= (2.3 \pm 0.2) \times 10^{18} \text{ M}^{-3}\end{aligned}$$

The logarithms of the computed overall equilibrium constants, $\kappa_{1,1p,0q,r}$, were converted into logarithms of apparent stability constants and apparent complexity constants of the complexes, as well as into $\text{p}K$ values of the hydrogen copper(II) complexes. This is evident from Table 2, where all these constants are given in logarithmic form.

DISCUSSION

As can be seen in Fig. 3, the curves calculated from the computed constants reasonably agree with the plotted data. As expected the deviations are most apparent at high h , as the pH decrements due to complex formation are least pronounced there.

The complexes evaluated as formed in this study, *i. e.* CuHA^+ , CuA , $\text{Cu}(\text{HA})\text{A}^-$ and CuA_2^{2-} , may not exclude the existence of other complexes for true, and at least some other possibilities may therefore be discussed.

An indication of the existence of complexes with more than two O-phosphorylethanolamine groups per central ion, thus, seems to be the upward trend of the curves of Fig. 4 at high $-\log h$. This trend is, however, too slight to allow any accurate proof of the existence of such species.

In the present computations, only a hydroxo complex CuOH^+ was considered, which apparently is the only inorganic hydroxo species formed in the present concentration ranges⁴⁴. Other monohydroxo complexes, *i. e.* in the first place $\text{Cu}(\text{HA})\text{OH}$ and $\text{CuA}(\text{OH})^-$, would at $-\log h = 6.1$ (the upper limit of $-\log h$ used in this work) apparently constitute less than 5 % of the corresponding species CuHA^+ and CuA , *cf.* Ref.⁴⁵. These hydroxo complexes, consequently, cannot appreciably change the proposed reaction scheme. This is also evident from the curves of Figs. 3 and 5a, as the occurrence of a large amount of $\text{CuA}(\text{OH})^-$ would have been indicated as an upward trend for decreasing h . The right hand side of eqn. 8a should then include the figure $\kappa_{1,01,1}h^{-1}$. The presence of $\text{Cu}(\text{HA})\text{OH}$ on the other hand, cannot be excluded, as these measurements do not distinguish this complex from CuA .

The apparent negligible amount of these mononuclear hydroxo species may exclude the existence of *ol* complexes^{7,46-50}. Polynuclear complexes, where one copper(II) ion is coordinated through either the phosphoryl and amino group of the ligand, can reasonably also be excluded. In the $-\log h$ range involved the $\text{p}K$ value of the ligand amino group (=10.10) does not allow for such complex formation due to the relatively low copper(II)-ammine affinity (see, *e. g.*, $\log b_1$ for the $\text{Cu}(\text{II})$ -ammine system⁴⁴), *cf.* Ref.⁵¹

Table 2. Logarithms of $\kappa_{1,1p,0q,r}$ constants, apparent stability constants, and apparent complexity constants of the complexes, and apparent pK values of the acid complexes in 0.15 M KCl medium at 25°.

$$C_A = (0.8-3) \times 10^{-3}, \quad C_{Cu} = 7.84 \times 10^{-4}, \quad -\log h = 5.30-6.10.$$

$$\log \kappa_{11} = 10.10, \quad \log \kappa_{21} = 15.67, \quad \kappa_{1,1} = 10^{-7.73} M, \quad b_{CuCl} = 1.0 M^{-1}$$

Reaction		Log. equilibrium constant		
$Cu^{2+} + H^+ + A^{2-}$	$\rightleftharpoons CuHA^+$	$\log \kappa_{1,11}$	=	12.04
$Cu^{2+} + HA^-$	$\rightleftharpoons CuHA^+$	$\log \frac{\kappa_{1,11}}{\kappa_{11}}$	= $\log b_{CuHA}$	= 1.94
$Cu^{2+} + A^{2-}$	$\rightleftharpoons CuA$	$\log \kappa_{1,01}$	= $\log b_1$	= 6.39
$Cu^{2+} + H^+ + 2A^{2-}$	$\rightleftharpoons Cu(HA)A^-$	$\log \kappa_{1,11,01}$	=	18.36
$Cu^{2+} + HA^- + A^{2-}$	$\rightleftharpoons Cu(HA)A^-$	$\log \frac{\kappa_{1,11,01}}{\kappa_{11}}$	= $\log \beta_{Cu(HA)A}$	= 8.26
$CuHA^- + A^{2-}$	$\rightleftharpoons Cu(HA)A^-$	$\log \frac{\kappa_{1,11,01}}{\kappa_{1,11}}$	= $\log b_{Cu(HA)A}$	= 6.32
$Cu^{2+} + 2A^{2-}$	$\rightleftharpoons CuA_2^{2-}$	$\log \kappa_{1,02}$	= $\log \beta_2$	= 12.39
$CuA + A^{2-}$	$\rightleftharpoons CuA_2^{2-}$	$\log \frac{\kappa_{1,02}}{\kappa_{1,01}}$	= $\log b_2$	= 6.00
$CuHA^+$	$\rightleftharpoons CuA + H^+$	$\log \frac{\kappa_{1,11}}{\kappa_{1,01}}$	= pK_{CuHA}	= 5.65
$Cu(HA)A^-$	$\rightleftharpoons CuA_2^{2-} + H^+$	$\log \frac{\kappa_{1,11,01}}{\kappa_{1,02}}$	= $pK_{Cu(HA)A}$	= 5.97

The existence of $CuHA^+$ and $Cu(HA)A^-$ provides evidence for the ligand phosphoryl group to act complexing. This is in contrast to the $Ag(I) - O$ -phosphorylethanolamine system⁵², where the species AgA^- and AgA_2^{3-} only could be detected in a similar concentration range as the present. The amino group of the ligand was suggested as single coordination site for the latter species⁵², which may be attributed to $Ag(I)$ preferably forming linear complexes with this type of ligand, *cf.* Ref.⁵³. The great difference between b_{CuHA} and b_1 in this work (*cf.* Table 2) indicates the ligand amino group to be introduced as dentate site, when $CuHA^+$ is converted to CuA . The species CuA and CuA_2^{2-} may, thus, be coordinated through the ligand phosphoryl and amino group or the amino group only. The former alternative is more probable, at least for CuA , as $\log b_1$ of the $Cu(II)$ -amine system⁴⁴ is reported to be about 2 log. units lower than that of the present. The effect of the increased net negative charge, caused by the presence of the phosphoryl group, cannot reasonable alone explain this great difference in b_1 . — Due to the same fact

copper(II) reasonably interacts with the phosphoryl group also in the corresponding O-phosphorylserine 1:1 complex²¹. The central copper(II) ion is, however, in this complex apparently mainly coordinated through a five-membered chelate ring formed by the amino and carboxyl donor groups. The value of $\log b_1$ ($= 9.64$) in this system is about 1.5 — 2 log. units greater than $\log b_1$, in copper(II) systems of similar unphosphorylated amino acids^{38,54,55}.

Klotz *et al.*^{6,56} studied the copper(II) binding properties of α - and β -casein. The α -casein was found to have the greater copper(II) binding capacity, being greater than that of any other protein investigated⁶. The difference in copper(II) affinity of α - and β -casein paralleled the phosphorus content of those proteins, suggested to be due to difference in net negative charge⁶. The apparent complexing action of the phosphoryl group in the present complexes, and in the O-phosphorylserine complexes²¹ may indicate that the greater copper(II) binding power of these phosphoproteins is partly due to complex formation through phosphoryl groups.

In the phospholipids, phosphatidylethanolamine and phosphatidylserine, where the phosphoryl group is diesterified, the same groups are free to react as those proposed as main dentate sites in O-phosphorylethanolamine and O-phosphorylserine. Since the pK values of the amino group in these phosphatides are of the same size⁵⁷ as those of the corresponding low molecular weight phosphates^{21,40}, both types of compounds may at a physiological pH form copper(II) complexes of nearly the same strength. The complexes formed by those ligands will then be those, where the dissociable proton is removed from the amino nitrogen group due to the pK value of the hydrogen copper(II) complexes (*cf.* Table 2 and Ref.²¹). This gives evidence for a considerable copper(II) complexity of these phosphatides, which may have biological significance. At inhibition of tromboplastinase action by copper(II) ions⁵⁸, for instance, complexes will obviously be formed between Cu(II) ions and the thromboplastical active lipid substrate, mainly constituting phosphatidylethanolamine and phosphatidylserine⁵⁹.

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