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Enantioselective Complexation of Amino Acids by 6A-Deoxy-6A-hydroxyethylamino- β -Cyclodextrin and its Metallo-Derivatives in Aqueous Solution

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Abstract. Enantioselectivity towards several amino acids by metallo-6A-deoxy-6A-hydroxyethylamino-β-cyclodextrins (metallo- β CDea's) was investigated by potentiometric titration of the various amino acid/metallo- β CDea systems with NaOH solution. It was shown that the cyclodextrin derivative is capable of distinguishing between enantiomers of amino acid species in the presence of certain metal ions (Co, Ni, Cu and Zn). Ni- β CDea complexes show the most enantioselectivity,whereas for Cu and Co2C-_CDea complexes less selectivity is observed. As expected, Zn- β CDea complexes exhibit no enantioselectivity. Stability and selectivity, however, do not go hand in hand, since the most stable complexes are formed with Cu2C. Several factors play a role in determining stability and selectivity in binary and ternary complexes and further study is required to gain a more comprehensive understanding of these.

Key words: metallo-cyclodextrin, enantioselectivity, amino acid, binary complex, ternary complex.

1. Introduction

The presence of a hydrophobic cavity in cyclodextrin molecules renders them efficient hosts for a variety of guests. The most stable complexes are usually formed with hydrocarbon type (hydrophobic/lipophilic) species. Since cyclodextrins are composed of D-(+)-glucopyranose units and are chiral, it is expected that they can be used as agents for enantiomeric separation. However, native CDs are poor chiral discriminating agents [1]. To improve their chiral recognition, it is desirable to have a metal ion centre present as well as the chiral cavity in the molecule [2–7]. Native cyclodextrins are also inefficient coordinating ligands because of intramolecular hydrogen bonding. Both of these functions can be enhanced by functionalising the β CD at C(6) with a chelating moiety, in this case ethanolamine, to form 6A-deoxy-6A-hydroxyethylamino- β -cyclodextrin (_CDea).

Determination of stability constants for the formation of binary metallo-_CDeaand metallo-substrate complexes and ternary metallo-substrate-_CDea complexesaffords a mechanism to investigate the role of the metal ion centre and the cyclodextrin cavity interactions on the stability and enantioselectivity of these complexes. Previous work by Lincoln *et al.* [2–3] on enantioselectivity by M-_CDpn (_CDpn = 6A-(3-aminopropylamino)-6A-deoxy-_CD) (M = Co2C, Ni2C, Cu2C, Zn2C) towards phenylalanine (Phe), tryptophan (Trp) and histidine (His) showed that enantioselectivity was largest for Ni2C complexes and that Zn2C complexes possessed no ability for chiral recognition. They also concluded that stabilising interactions of the guest amino acid with

the metal ion centre and with the hydrophobic CD cavity do not reinforce each other. The work presented here looks at the enantioselectivity of corresponding systems using 6A-deoxy-6A-hydroxyethylamino-_-cyclodextrin (_CDea) as the functionalised CD. This deserves investigation because of the important role of aminoalcohols in hormone activity [8–9] and in the biochemistry of aminosugars [10–11]. In many respects, the results mainly support those of the previous authors, however, some differences were also noted and are described later. The various roles of the cyclodextrins, divalent metal ions and amino acids in affecting complexation are discussed.

2. Experimental

2.1. INSTRUMENTATION

Potentiometric titrations were carried out using a Mettler DL25 automatic titrator equipped with a Mettler DG-111-SC-pH electrode that was filled with 3 mol dm-3 KCl (AgCl saturated). All titration solutions were degassed with nitrogen for at least 10 minutes before each titration. During all titrations, a similar stream of nitrogen gas was passed through the solution to prevent CO2 adsorption from the atmosphere, which would cause an extensive drift in EMF. The titration solution was mechanically stirred in a 100 mL titration vessel closed to the atmosphere except for the nitrogen outlet and thermostatted in a waterbath at 25 _C (except where otherwise stated).

Magnetic moment determinations were performed on a Sherwood Scientific magnetic susceptibility balance, standardised with mercury(II)tetrathiocyanatocobaltate(II). Infrared spectra of solids (KBr-disc) were recorded using a Perkin Elmer Paragon 1000 FT-IR spectrometer. NMR spectra were measured by a Varian Gemini 2000 instrument (200 MHz). Atomic Absorption (AA) experiments were conducted on a Shimadzu AA-670 spectrophotometer.

2.2. PREPARATION OF MATERIALS

The 6A-deoxy-6A-hydroxyethylamino-_-cyclodextrin was prepared as follows, by a modification of a previous method [12]. 6A-deoxy-6A-*O*-*p*-toluenesulphonyl--cyclodextrin [13] (_CDOTs) (2.7 g, 2 mmol) and potassium iodide (0.8 g, 4mmol) in aqueous solution (500 mL) were stirred for one hour on a steambath. Ethanolamine (1.44 mL, 20 mmol) was added and the reaction continued for 5 hours under atmospheric pressure until the solution was evaporated to 40 mL. This solution was cooled to room temperature and acetone (200 mL) was gradually added. The resulting precipitate was collected by filtration and washed with acetone. The resultant off-white solid was recrystallised from water and dried in vacuo for 4 hours at 50 _C. Anal. Calcd for _CDea: C. 39.23, H. 7.43, N. 1.04; found: C. 38.97, H. 6.92, N. 1.35. 1H-NMR (200 MHz, DMSO): _ 4.80, 4.00–2.85, 2.05, 1.02. 13C-NMR (200 MHz, DMSO): _ 102.05, 81.61, 73.15–72.10, 60.00, 56.11, 39.44, 30.73, 18.53 [14–16]. FTIR-spectra were recorded for _CD, _CDOTs and _CDea. Two new bands appear in the _CDOTs spectrum due to the asymmetric and symmetric SO2-stretches at 1375 cm□1 and 1182 cm□1 respectively [17]. As expected, these bands are absent in the spectrum of _CDea. (R)- and (S)-amino

acids (Sigma) were dried to constant weight prior to use. Metal perchlorates (Aldrich) were purchased and used without further treatment. Stock 0.100 mol dm□3 Ni(ClO4)2, Cu(ClO4)2, Co(ClO4)2 and Zn(ClO4)2 solutions were standardised by EDTA titration. Deionised water was used in the preparation of all solutions.

2.3. POTENTIOMETRIC TITRATIONS

In all titrations, standardised 0.100mol dm□3 NaOH was titrated against the species of interest in solutions which were 0.010 mol dm□3 in HClO4 and 0.090 mol dm□3 in NaClO4. Thus, the protonation constants for _CDea and the amino acids were determined from titrations of 25 mL aliquots of 0.001 mol dm□3 _CDeaHC2 or 0.001 mol dm□3 amino acid solutions respectively. The stability constants for the binary metallo-cyclodextrin or metal-amino acid complexes were determined by titration of 25 mL aliquots of 0.001 mol dm□3 _CDeaHC2 or 0.001 mol dm□3 amino acid solution to which 0.240 mL and 0.120 mL of 0.100 mol dm□3 M(ClO4)2 solution had been added, respectively. The stability constants for the formation of complexes between _CDea and the (R)- or (S)-amino acid were determined by titration of 10 mL of 0.001 mol dm□3 solutions of either (R)- or (S)-amino acid and 10 mL of 0.001 mol dm□3 of _CDeaHC2 solution. The stability constants for the ternary complexes between the metals, _CDea and (R)- or (S)-amino acid were determined by titration of 10 mL of 0.001 mol dm□3 solutions of either (R)-or (S)-amino acid and 10 mL of 0.001 mol dm□3 _CDeaHC2 solution with 0.100 mL of 0.100 mol dm□3 M(ClO4)2 solution added. pKw values were determined by titration of 0.010 mol dm□3 HClO4 (0.090 mol dm□3 in NaClO4) against 0.100 mol dm□3 NaOH. Derivations of the stability constants were carried out using the program SUPERQUAD [18]. At least three runs were performed for each system, and at least two of these runs were averaged; the criterion for selection for this averaging being that _2 for each run was <12.6 at the 95% confidence level. Several complexes exist in aqueous solutions of _CDea, M2C and amino acids in the pH-range 2.0–11.5. The stabilities of these complexes were calculated from the differences between the pH-profiles arising from titration against NaOH of solutions containing different combinations of the complexing species. Three such pH-profiles are shown in Figure
The sequence of these titrations was as follows: the determination of (i) the pKa values of the amino acids and _CDeaHC2 (ii) the stability constants of the complexes in solution of (a) _CDeaHC2 and either (R)- or (S)-amino acid, (b) M2C and the amino acid, (c) M2C and _CDeaHC2 and (d) the ternary system M2C,_CDeaHC2 and either (R)- or (S)-amino acid. The pKa values determined in (i) were used as constants in the determination of stability constants in (ii). The stability constants determined in (ii-a)–(ii-c) were also employed as known values in the determination of stability constants in (ii-d). The titration data were fitted to equilibria containing the minimum number of species required for a good fit. A plot of the major Cu2C species present in the system Cu2C-_CDea-(R)-phenylalanine is shown in Figure 2. This is typical of the other systems studied.

3. Results

The acid dissociations of TrpHC2 (pKa1 = 2.37 _ 0.04 and pKa2 = 9.39 _ 0.02), PheHC2 (pKa1 = 2.70 _ 0.02 and pKa2 = 9.19 _ 0.01) and HisH2C 3 (pKa1 = 1.99 _ 0.03, pKa2 = 6.11 _ 0.01 and pKa3 = 9.21 _ 0.01) were derived from data obtained in the pH-range

2.0–11.5. These pKa values compare favourably with literature values [19]. For _CDeaHC2, pKa1 = 8.08 _ 0.07 and pKa2 = 10.69 _ 0.05 were derived from data obtained in the pH-range 5.0–8.5. The constants determined for each system studied are defined here for the phenylalanine system. Similar definitions apply to the constants for the tryptophan and histidine systems.

_CDeaH + (R)-Phe□ *K*1R T_CDea.(R)-Phe]2□ + HC (1)

_CDeaH + (S)-Phe□ *K*1S T_CDea.(S)-Phe]2□ + HC (2)

_CD + (R)-Phe□ *K*2R T_CD.(R)-Phe]□ (3)

_CD + (S)-Phe□ *K*2S T_CD.(S)-Phe]□ (4)

2M2C + 2_CDea□ + 2OH□ *K*3 [M2(_CDea)2(OH)2] (5)

M2C + (S)/(R)-Phe□ *K*4 TM.(S)/(R)Phe]C (6)

[M.(S)/(R)Phe]C + (S)/(R)-Phe□ *K*5 TM-((S)/(R)Phe)2] (7)

M2C C _CDea□ C (R)-Phe□ *K*6R TM:_CDea.(R)-PheU (8)

M2C C _CDea□ C (S)-Phe□ *K*6S TM._CDea.(S)-PheU (9)

Log *K* (*K* = stability constants) for ternary and binary metallo-complexes as defined above are listed in Table I. Table II contains logK values for non-metallo systems and suggests that the level of enantioselectivity achieved in these systems ranges from very poor in the case of _CD to fair for _CDea. Table III shows the enantioselectivity for the ternary systems as derived from Table I.

Table I. Stability constants log(*K*/dm3 mol□1)a for metallo-6Adeoxy- 6A-hydroxyethylamino-_-cyclodextrins and related complexes in aqueous solution at 25 _C and I = 0.10 (NaClO4).

log *K*	Co	Ni	Cu	Zn
*K*3	2.69 _ 0.05	3.72 _ 0.06	5.84 _ 0.04	3.41 _ 0.09
tryptophan				
*K*4	4.37 _ 0.01	5.78 _ 0.02	7.65 _ 0.03	5.26 _ 0.02
*K*5	3.57 _ 0.04	4.97 _ 0.07	7.67 _ 0.06	4.77 _ 0.06
*K*6R	6.14 _ 0.02	7.02 _ 0.02	8.43 _ 0.04	7.20 _ 0.07
*K*6S	6.39 _ 0.06	7.75 _ 0.07	8.68 _ 0.06	7.20 _ 0.03
phenylalanine				
*K*4	4.42 _ 0.03	5.19 _ 0.01 =	7.80 _ 0.02	4.39 _ 0.02
*K*5	3.44 _ 0.05	4.39 _ 0.06	6.92 _ 0.05	4.02 _ 0.04
*K*6*R*	5.85 _ 0.03	5.95 _ 0.02	7.87 _ 0.06	6.10 _ 0.06
*K*6*S*	5.53 _ 0.05	6.58 _ 0.03	7.75 _ 0.07	6.10 _ 0.05
histidine				
*K*4	6.96 _ 0.06	8.54 _ 0.07	10.45 _ 0.05	
*K*5	5.42 _ 0.11	6.90 _ 0.12	8.71 _ 0.10	
*K*6*R*	6.55 _ 0.04	7.49 _ 0.04b	8.91 _ 0.05	
*K*6*S*	6.72 _ 0.05	8.01 _ 0.04b	8.98 _ 0.03	

a Errors quoted for K (means of N runs) represent the standard deviation. b At 45 _C.

binary

4. Discussion

4.1. ENANTIOSELECTIVITY IN BINARY NON-METALLO SYSTEMS

For the complexation of the amino acids by _CDea (Table IIb) (Equations 1 and 2), log(*K*1*R*/dm3mol□1) = 4.82 _ 0.04, 3.91 _ 0.04 and 3.72 _ 0.04 and log(*K*1*S*/dm3mol□1) = 4.81 _ 0.05, 4.17 _ 0.03 and 3.54 _ 0.03 were derived in the pH-range 8.5–11.5 for Trp□, Phe□ and His□ respectively. The order of complexation with _CDea, i.e., Trp□ > Phe□ > His□ is in accordance with expectations based on the hydrophobic interactions alone. The increased stability of these complexes over those of corresponding native _CD complexes [3] (Table IIa) is surprising. The repulsion between the negative charges could possibly be minimised by having the amino acid approach the CD from the secondary side. Derivatisation of CD extends the hydrophobicity of the cavity and this may contribute to the higher stability. Some enantioselectivity is indicated for the _CDea systems, with the exception of Trp□ (Table IIb). This may suggest that in the latter case the hydrophobic effect is the most important factor in stabilising the complex. Native _CD shows no enantioselectivity.

Table II. Enantioselectivity in binary non-metallo systems.

	CD			CDea		
	Trp	Phe	His	Trp	Phe	His
log *K*R	2.33	2.91	–	4.82	3.91	3.72
log *K*S	2.33	2.83	–	4.81	4.17	3.54
select_	0.00	0.08(R)	–	0.01 (R)	0.26 (S)	0.18 (R)

The isomer for which the _CD or _CDea is enantioselective is given in parentheses.

4.2. FORMATION OF BINARY METALLO-COMPLEXES

There is ample evidence in the literature for the structures of metal ion complexes with ethanolamine [20–26]. In light of this and also on the basis of AA studies (5.56% Cu2C, 1:1 ratio _CDea:Cu2C) and full elemental analysis (Anal. Calcd: Cu2C.4.72, C.39.21, H.6.31, O.48.72, N.1.04; found: Cu2C.5.02, C.38.84, H.6.14 N.1.28) carried out on the amorphous blue solid isolated from the binary system Cu2C:_CDea at pH = 9.5, several structures were proposed for the Cu2C-_CDea complex. SUPERQUAD results allowed the authors to favour the structure shown in Figure 3, i.e., a binuclear hydroxy-bridged structure with _CDea□ moieties acting as bidentate ligands to the metal ion centres. The magnetic moment (_ = 1.08 BM/metal ion) determined at room temperature on the blue powder suggests antiferromagnetism and lends support to the presence of a binuclear hydroxy-bridged structure which facilitates magnetic coupling via a superexchange mechanism [27]. Complexes with a similar bridging system have been shown to change colour from blue to green when exposed to air [28]. This colour change is also observed for the Cu(II)-_CDea complex providing further support for the proposed structure. Similar models also satisfied the requirements of the SUPERQUAD program for the remainder of the metal ions to the exclusion of other possible models. The formation of the metallo-_CDea complexes (Equation 5) has been characterized by log(*K*3/dm3

mol□1) = 2.69 _ 0.05, 3.72 _ 0.06, 5.84 _ 0.04, and 3.41 0.09 for Co2C, Ni2C, Cu2C and Zn2C respectively (Table I). Stability variation for the binary metallo-cyclodextrins with the nature of M2C is as anticipated from the Irving–Williams sequence (Co2C < Ni2C < Cu2C > Zn2C) [29] which arises through a combination of the variation of M2C size and ligand field effects. In thecase of Ni2C, the stability constants were determined at 45 _C as interference from metal hydroxide precipitation occurred at 25 _C.

It is interesting to note the higher stability of the binary metallo-_CDea complexes over the corresponding M(ea) complexes [20]. The amino function in _CDea (pKa = 8.08) (Section 3) is certainly less basic towards protonation than that in ea (pKa = 9.50) [30]. This is accounted for in terms of the close proximity of the hydrophobic cavity inhibiting solvation of the protonated species. The increased stability of the M-_CDea complex may be a function of the proposed binuclear bridged system rather than of the donor ability of the amino function. The stability constants determined for the metallo-amino acid complexes (Equations 6 and 7) (Table I) are in reasonable agreement with those in the literature [19], and also exhibit variations anticipated from the Irving–Williams series [29]. The stability constants *K*4 and *K*5 were derived from data obtained in the pH-range 6.5–8.5, 5.5–8.0, 4.0–7.0 and 5.5–7.5 when M2C = Co2C, Ni2C, Cu2C and Zn2C respectively. In all these systems, K4 > K5 as anticipated for sequential binding of ligands. No enantioselectivity was observed with these binary metallo systems.

4.3. ENANTIOSELECTIVITY IN TERNARY SYSTEMS

The stability constants (Equations 8 and 9) in Table I for the ternary complexes show that the complexes formed with Cu2C are the most stable, whereas complexes formed with Ni2C are the most enantioselective (Table III). Due to the precipitation of metal hydroxide at 25 _C in the case of Ni2C, the experiments were carried out at 45 _C. The stability constants for the ternary complexes are derived from dataobtained in the pH-ranges 7.5–8.5, 7.5–9.5, 6.0–10.0 and 6.7–7.5 when M2C = Co2C, Ni2C, Cu2C and Zn2C respectively. The stabilities of the ternary complexes are greater than those of the analogous metal-amino acid and _CDea-amino acid complexes. This is consistent with theproposal that the binding of the carboxylic acid moiety of the amino acid by M2C and the hydrophobic interaction between the aromatic moiety and the interior of the cyclodextrin annulus reinforce each other to stabilise the ternary complex. Previous work by Lincoln *et al*. [2–3], however, did not show reinforcement of these two interactions except in the case of Zn2C.

Table III. Enantioselectivity in ternary metallo-systems.

	Co2C	Ni2C	Cu2C	Zn2C
Tryptophan	0.25 (S)	0.73 (S)	0.25 (S)	0.00
Phenylalanine	0.32 (R)	0.63 (S)	0.12 (R)	0.00
Histidine	0.28 (S)	0.52 (S)	0.07 (S)	

Enantioselectivity_ = |log *K*R □ log *K*S|. The isomer for which enantioselectivity is observed is shown in parentheses.

On the basis of NMR [5] and thermodynamic [6] evidence structures were proposed for ternary complexes of histamine monofunctionalised Cu(II) complexes with amino acids having a cis arrangement of the amino groups of the histamine moiety and the amino acid. This results in the aromatic moiety of the acid achieving inclusion in the preferred enantiomer only. This was supported by the reported crystal structure [7] of histamine monofunctionalised Cu(II)-L-Trp complex showing the aromatic moiety of the acid ligand outside the CD cavity. For the preferred enantiomer the structure proposed is that shown in Figure 4 [2–4]. The aromatic moiety of the amino acid resides in the cyclodextrin annulus, with the amino acid chiral centre in the vicinity of the primary hydroxyl groups of the cyclodextrin and the amine and carboxylate groups coordinated to M2C. The ethanolamine moiety of _CDea is also coordinated to M2C. The variation of stability with the nature of M2C coincides with the variation of the ionic radii of six-coordinate Co2C, Ni2C, Cu2C and Zn2C[29]. However, stability and enantioselectivity do not go hand in hand. As mentioned earlier, complexes with Ni2C are the most enantioselective (Table III), in all cases showing greater selectivity for the S-isomer over the R-isomer. The selectivity varies from three-fold for histidine to more than five-fold for tryptophan. The Co2C and Cu2C analogues show a significant, but lesser degree of selectivity. Enantioselectivity varies with the geometric constraints arising from ligand field effects in Co2C, Cu2C and Ni2C, and the lack of such constraints in d10 Zn2C. It is difficult to explain why Ni(II)-complexes are the most selective. It may be simply a function of the ionic size and steric constraints. The colour (blue-green) suggests an octahedral field with solvent molecules occupying the vacant sites. However, colour is not a very reliable criterion of structure. Indeed an equilibrium between octahedral and square planar arrangement may be present. The smaller enantioselectivity observed in the more stable complexes demonstrates that increasing complex stability does not necessarily induce a corresponding increase in enantioselectivity.

Finally, the presence of a metal ion can either reinforce or reverse the enantioselectivity. In the case of the Cu2C and Co2C complexes, the presence of the metal ion reverses the selectivity order of the corresponding non-metallo complex. With Ni2C, enantioselectivity is always towards the (S)-isomer. This suggests that in the case of the Co2C and Cu2C complexes the nature of the metal ion centre is predominant in determining selectivity, whereas for Ni2C complexes the nature of the amino acid is also important.

5. Conclusion

While the relative stabilities of the binary metallo-_CDea complexes vary with M2C in the sequence Co2C < Ni2C < Cu2C > Zn2C and are dominated by the nature of the metal ion, the subsequent binding of an amino acid is greatly influenced by its interaction with the cyclodextrin annulus. Thus the combined effects of _CDea and metal ion produce a greater binding of the amino acid in the ternary complex (which also varies in the same metal sequence) than that in either the metallo-amino acid or _CDea-amino acid complexes. The most stable complexes are not necessarily the most enantioselective. Enantioselectivity also varies with the nature of the metal and the geometric constraints due to ligand field effects. It is intended to further investigate the various influences of

metal ion coordination, hydrogen bonding, cavity size and amino acid structure on the enantioselectivity demonstrated in these ternary complexes.

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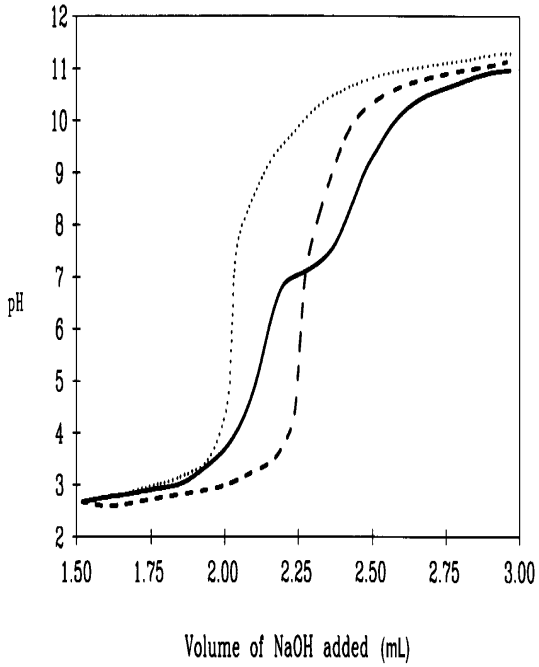

Figure 1. Titration profiles for (. . .) _CDeaHC2 (9.941 _ 10□4 mol dm□3, 25 mL), (- - -) _CDeaHC2 (1.003 _ 10□3 mol dm□3, 10 mL) and (R)-PheHC2 (1.010 _ 10□3 mol dm□3, 10 mL) and (—) _CDeaHC2 (1.003 _ 10□3 mol dm□3, 10mL), (R)-PheHC2 (1.010 _ 10□3 mol dm□3, 10 mL) and Cu(ClO4)2 (9.93 _ 10□2 mol dm□3, 0.100 mL), each in aqueous 0.010 mol dm□3 HClO4 and 0.090 mol dm□3 NaClO4 against 0.100 mol dm□3 NaOH at 25 _C.

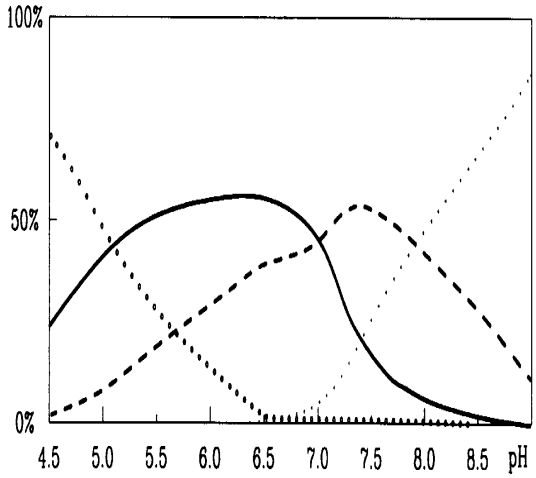

Figure 2. Percentage of Cu2C-species in a solution containing 0.100 mL of 9.93 _ 10□2 mol dm□3 Cu2C, 10 mL of 9.941 _ 10□4 mol dm□3 _CDeaHC2 and 1.010 _ 10□3 mol dm□3 R-PheHC2 , plotted against pH and relative to [Cu2C] = 100%. (R)-PheH (_ _ _), Cu.[(R)-Phe]□ (—), Cu.[(R)-Phe]2 (- - -) and Cu._CDea.(R)-Phe (. . .)

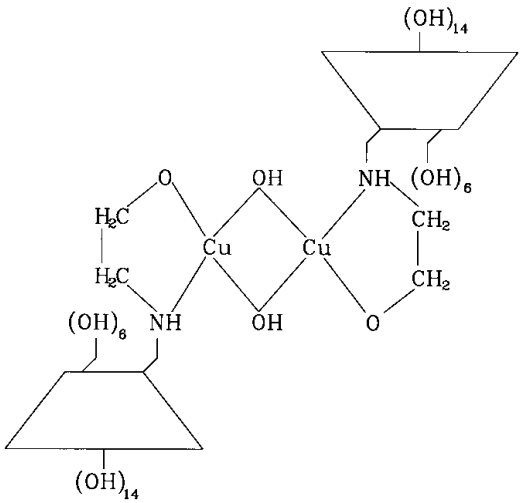

Figure 3. Proposed structure for the complex of _CDea with Cu2C.

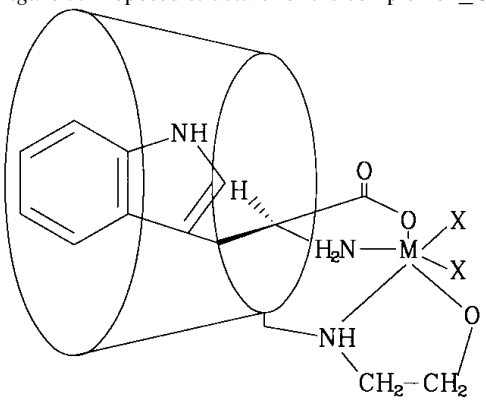

Figure 4. Typical structure proposed for the ternary complex between the metal M, _CDea and amino acid (X1,2 = OH□ or H2O) [2–3].