Formation of Metallo-6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrins and Their Complexation of Tryptophan in Aqueous Solution

Carolyn A. Haskard,[1a] Christopher J. Easton,[1b] Bruce L. May,[1a] and Stephen F. Lincoln*,[1a]

Department of Chemistry, University of Adelaide, Adelaide, South Australia 5005, Australia, and Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia

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A pH titration study shows that 6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrin (βCDtren) forms binary metallocyclodextrins, $[M(\beta CDtren)]^{2+}$, for which $\log(K/dm^3\ mol^{-1}) = 11.65 \pm 0.06$, 17.29 ± 0.05, and 12.25 ± 0.03, respectively, when $M^{2+} = Ni^{2+}$, Cu^{2+}, and Zn^{2+}, where K is the stability constant in aqueous solution at 298.2 K and $I = 0.10$ mol dm^{-3} ($NaClO_4$). The ternary metallocyclodextrins $[M(\beta CDtren)Trp]^+$, where Trp^- is the tryptophan anion, are characterized by $\log(K/dm^3\ mol^{-1}) = 8.2 \pm 0.2$ and 8.1 ± 0.2, 9.5 ± 0.3 and 9.4 ± 0.2, and 8.1 ± 0.1 and 8.3 ± 0.1, respectively, where the first and second values represent the stepwise stability constants for the complexation of (*R*)- and (*S*)-Trp^-, respectively, when $M^{2+} = Ni^{2+}$, Cu^{2+}, and Zn^{2+}. From comparisons of stabilities and UV−visible spectra, the binary and ternary metallocyclodextrins appear to be six-coordinate when $M^{2+} = Ni^{2+}$ and Zn^{2+} and five-coordinate when $M^{2+} = Cu^{2+}$. The factors affecting the stoichiometries and stabilities of the metallocyclodextrins, are discussed and comparisons are made with related systems.

Introduction

The formation of a binary metallocyclodextrin through the coordination of a metal ion by a functionalized cyclodextrin, and the formation of a ternary metallocyclodextrin through the binding of a substrate, offers an opportunity to study the effects of metal center and cyclodextrin interactions on metallocyclodextrin stability and substrate binding.[2−17] The ternary metallocyclodextrin annulus can partly encapsulate a substrate which also interacts with the adjacent metal center, and in this respect it resembles the Michaelis complex of some metalloenzymes.[18−21] The catalytic activities of metalloenzymes are very metal center specific, and this may be partly due to the influence of the metal center on the thermodynamic stability of the metalloenzyme and its efficacy in binding substrates. The simpler and more readily manipulated metallocyclodextrins provide an opportunity to study the influence of the metal center on metallocyclodextrin formation and on substrate binding in some detail, and such studies may be relevant to the understanding of some aspects of metalloenzymes. Although a range of metallocyclodextrin studies have appeared,[2−17] only two of these studies incorporate quantitative data on the effect of changing the metal center on binary and ternary metallocyclodextrin formation.[16,17]

We now report a study of the binary metallo-6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrin, $[M(\beta CDtren)]^{2+}$, where $M^{2+} = Ni^{2+}$, Cu^{2+}, and Zn^{2+}, and the ternary metallocyclodextrins $[M(\beta CDtren)Trp]^+$, where Trp^- is the tryptophan anion. Their protonated analogues have also been studied. (Bound water molecules are generally not shown in the metallocyclodextrin formulas in the text, and tryptophan and its protonated form are indicated by TrpH and $TrpH_2^+$, respectively.) The three M^{2+} were selected because Zn^{2+} frequently acts as a metal center in metalloenzymes,[18−21] and while Ni^{2+} and Cu^{2+} fill this role less often, they are closely related in electronic structure and size to Zn^{2+}. The tetradentate 6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino) substituent of βCDtren ensures the formation of stable $[M(\beta CDtren)]^{2+}$. The substrates (*R*)- and (*S*)-Trp^- were chosen for study because their aromatic moieties are of appropriate size to fit into the $[M(\beta CDtren)]^{2+}$ annulus, they bind to metal centers and provide a test for enantioselectivity in $[M(\beta CDtren)Trp]^+$.[16]

It is found that the M^{2+}/βCDtren/Trp^- systems exist as a series of labile equilibria, some of which are shown for the Ni^{2+} system in Figure 1. The truncated cone represents the cyclodextrin moiety where the wide end of the annulus is delineated by fourteen secondary hydroxy groups and the narrow end is delineated by six primary hydroxy groups and the secondary

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(1) (a) University of Adelaide. (b) Australian National University.
(2) Matsui, Y.; Yokoi, T.; Mochida, K. *Chem. Lett*. **1976**, 1037.
(3) Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc*. **1977**, *99*, 7100.
(4) Tabushi, I.; Kuroda, Y.; Mochizuki, A. *J. Am. Chem. Soc.* **1980**, *102*, 1152.
(5) Tabushi, I.; Kuroda, Y.; Mizutani, T. *Tetrahedron* **1984**, *40*, 545.
(6) Akkaya, E. U.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988**, *110*, 8553.
(7) Akkaya, E. U.; Czarnik, A. W. *J. Phys. Org. Chem*. **1992**, *5*, 540.
(8) Schneider, H.-J.; Xiao, F. *J. Chem. Soc., Perkin Trans. 2* **1992**, 387.
(9) Cucinotta, V.; Purello, R.; Rizzarelli, E. *Comments Inorg. Chem.* **1990**, *11*, 85.
(10) Bonomo, R. P.; Cucinotta, V.; D'Alessandro, F.; Impellizzeri, G.; Maccarrone, G.; Vecchio, G.; Rizzarelli, E. *Inorg. Chem.* **1991**, *30*, 2708.
(11) Impellizzeri, G.; Maccarrone, G.; Rizzarelli, E.; Vecchio, G.; Corradini, R.; Marchelli, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1348.
(12) Cucinotta, V.; D'Alessandro, F.: Impellizzeri, G.; Vecchio, G. *J. Chem. Soc., Chem. Commun*. **1992**, 1743.
(13) Bonomo, R. P.; Cucinotta, V.; D'Alessandro, F.; Impellizzeri, G.; Maccarrone, G.; Rizzarelli E.; Vecchio, G. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1993**, *15*, 167.
(14) Corradini, R.; Dossena, A.; Impellizzeri, G.; Maccarrone, G.; Marchelli, R.; Rizzarelli, E.; Sartor, G.; Vecchio, G. *J. Am. Chem. Soc.* **1994**, *116*, 10267.
(15) Brown, S. E.; Coates, J. H.; Easton, C. J.; van Eyk, S. J.; Lincoln, S. F.; May, B. L.; Stile, M. A.; Whalland; C. B.; Williams; M. L. *J. Chem. Soc., Chem. Commun.* **1994**, 47.
(16) Brown, S. E.; Coates, J. H.; Easton, C. J.; Lincoln, S. F. *J. Chem. Soc., Faraday Trans*. **1994**, *90*, 739.
(17) Brown, S. E.; Haskard, C. A.; Easton, C. J.; Lincoln, S. F. *J. Chem. Soc., Faraday Trans*. **1995**, *91*, 1013.
(18) Frausto da Silva, J. J. R.; Williams, R. J. P. *The Biological Chemistry of The Elements*; Oxford University Press: Oxford, U.K., 1991.
(19) Silverman, D. N.; Lindskog, S. *Acc. Chem. Res*. **1988**, *21*, 30.
(20) Christiansen, D. W.; Lipscomb, W. N. *Acc. Chem. Res*. **1989**, *22*, 62.
(21) Kimura, E. *Prog. Inorg. Chem*. **1994**, *41*, 443.

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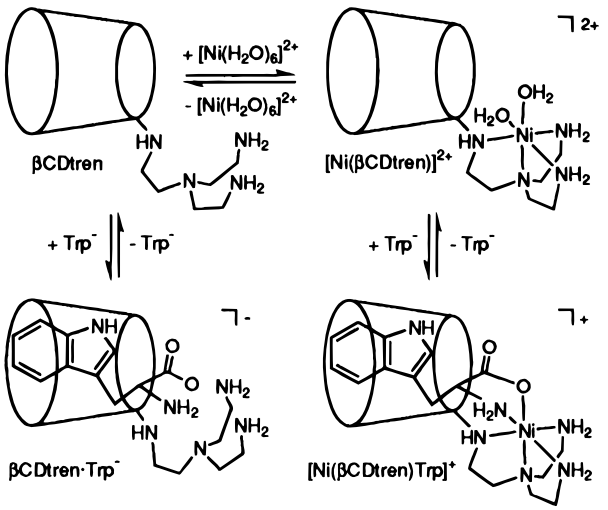


Figure 1. Both $[Ni(\beta CDtren)(H_2O)_2]^{2+}$ and $[Ni(\beta CDtren)Trp]^{+}$ are six-coordinate, as is probably the case for their Zn^{2+} analogues. It is possible that coordination of a cyclodextrin primary hydroxy group may replace one of the two coordinated water molecules in the Ni^{2+} and Zn^{2+} binary metallocylodextrins. The Cu^{2+} metallocyclodextrins are probably five-coordinate as discussed in the text.

amine group of the 6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino) substituent in place of the seventh primary hydroxy group of β-cyclodextrin, βCD. The structures shown for the complex βCDtren·Trp^- and the metallocyclodextrins $[Ni(\beta CDtren)]^{2+}$ and $[Ni(\beta CDtren)Trp]^{+}$ are deduced from this study.

Experimental Section

Preparation of Materials. The tetrakis(hydrochloric acid) salt of 6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrin (βCDtren$(HCl)_4$) was prepared by stirring 6^A-deoxy-6^A-*O*-((4-methylphenyl)sulfonyl)-β-cyclodextrin[22] (8.0 g, 6.2 mmol) and (2-(bis(2-aminoethyl)amino)ethyl)amine (tren, 0.9 cm^3, 6.02 mmol) in pyridine (60 cm^3) at 333 K for 48 h. The solution was evaporated to dryness under reduced pressure, and the residue was triturated with acetone (3×80 cm^3) and dissolved in water (20 cm^3). This solution was added dropwise with stirring to acetone (250 cm^3), and the resulting precipitate was collected by filtration and washed with acetone and ether. The resultant off-white solid was dissolved in water (60 cm^3), and the solution was heated and treated with charcoal (2 g). Filtration of the mixture and evaporation to dryness of the filtrate under reduced pressure gave a white solid that was dissolved in water (200 cm^3) and stirred with Bio-Rex 70 ion-exchange resin in the acid form (50 g) for 16 h at room temperature. The resin was isolated by filtration and was washed with water (1 dm^3) and then aqueous ammonia (10%, v/v, 1 dm^3). The ammonia washings were evaporated to dryness under reduced pressure, the residue was dissolved in water (20 cm^3), and dilute hydrochloric acid (1 cm^3) was added dropwise with stirring. The solution was evaporated to dryness under reduced pressure, and the βCDtren residue was dried to constant weight over P_2O_5 to give βCDtren$(HCl)_4$ as a colorless solid (2.6 g, 31%). Anal. Calcd for $C_{48}H_{90}Cl_4N_4O_{34}$: C, 40.91; H, 6.43; N, 3.97. Found: C, 40.84; H, 6.52; N, 4.06. The tris(methanesulfonic acid) salt of 6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrin, βCDtren$(MeSO_3H)_3$, was prepared by dissolving βCDtren$(HCl)_4$ (2.6 g, 0.15 mmol) in water (15 cm^3), adding methanesulfonic acid (1 cm^3), and adding the mixture to acetone (250 cm^3) with stirring. The resulting off-white precipitate was filtered off, washed with acetone and ether, and dissolved in water (30 cm^3), and the resultant solution was heated with charcoal (1 g). Filtration of the mixture and evaporation to dryness gave $\beta CDtrenH_3(MeSO_3)_3(Me_2CO)_5(H_2O)_8$ as a white solid (2.1 g), which was dried to constant weight and stored over P_2O_5 under vacuum in darkness. Anal. Calcd for $C_{66}H_{144}N_4O_{56}S_3$: C, 39.91; H, 7.26; N, 2.82; S, 4.84. Found: C, 39.85; H, 7.26; N, 2.92; S, 4.99. ^1H NMR (300 MHz, D_2O): δ 2.75 (s, 9H), 2.84 (t, $J = 6$ Hz, 4H), 2.92 (t, $J = 6$ Hz, 2H), 3.10 (t, $J = 6$ Hz, 4H), 3.20 (t, $J = 6$ Hz, 2H), 3.4−4.0 (m, 42H), 5.03 (m, 7H). ^{13}C NMR (75.8 MHz, D_2O): δ 37.8, 39.8, 46.2, 49.5, 50.0, 51.2, 61.6, 62.1, 68.9, 73.1, 73.2, 73.3, 73.6, 74.1, 74.4, 81.7, 82.4, 82.8, 84.4, 102.4, 103.1.

(*R*)- and (*S*)-tryptophan (Sigma) were dried to constant weight and stored in the dark over P_2O_5 in a vacuum desiccator before use. Their enantiomeric purities were determined to be ≥ 99% after HPLC analysis (Pirkle covalent (*S*)-phenylglycine column) of the esters formed with thionyl chloride pretreated methanol. Metal perchlorates (Fluka) were twice recrystallized from water and were dried and stored over P_2O_5 under vacuum. (***Caution***! Anhydrous perchlorate salts are potentially powerful oxidants and should be handled with care.) Stock 0.100 mol dm^{-3} $Ni(ClO_4)_2$, $Cu(ClO_4)_2$, and $Zn(ClO_4)_2$ solutions were standardized by edta titration in the presence of Murexide indicator in the first two cases and Eriochrome Black T in the last case.[23] Deionized water, purified with a MilliQ reagent system to produce water with a specific resistance of >15 MΩ cm, was boiled to remove CO_2 and used in the preparation of all solutions.

Equilibrium Studies. Potentiometric titrations were carried out using a Metrohm Dosimat E665 titrimater, an Orion SA 720 potentiometer, and an Orion 8172 Ross Sureflow combination pH electrode that was filled with 0.10 mol dm^{-3} $NaClO_4$. All titration solutions were saturated with nitrogen by passing a fine stream of nitrogen bubbles (previously passed through aqueous 0.10 mol dm^{-3} $NaClO_4$) through them for at least 15 min before commencement of the titration. During the titrations, a similar stream of nitrogen bubbles was passed through the titration solution that was magnetically stirred and thermostated at 298.2 ± 0.1 K in a water-jacketed 20 cm^3 titration vessel closed to the atmosphere except for a small exit for nitrogen.

In all titrations, standardized 0.100 mol dm^{-3} NaOH was titrated against the species of interest in solutions 0.007 mol dm^{-3} in $HClO_4$ and 0.090 mol dm^{-3} in $NaClO_4$. Thus, the protonation constants for βCDtren were determined from titrations of 10.00 cm^3 aliquots of 0.002 mol dm^{-3} $\beta CDtrenH_3(MeSO_3)_3$ solutions. The stability constants for the formation of $[M(\beta CDtren)]^{2+}$ and related complexes were determined by titration of 10.00 cm^3 aliquots of 0.001 mol dm^{-3} $\beta CDtrenH_4^{4+}$ to which 0.075 cm^3 of $M(ClO_4)_2$ solution had been added. The stability constants for the formation of βCDtren·(*R*)-Trp^-, βCDtren·(*S*)-Trp^-, and related complexes were determined by titration of 5.00 cm^3 each of 0.002 mol dm^{-3} solutions of either (*R*)-$TrpH_2^+$ or (*S*)-$TrpH_2^+$ and $\beta CDtrenH_4^{4+}$. The stability constants for the formation of $[M(\beta CDtren)$-(*R*)-$Trp]^+$, $[M(\beta CDtren)$-(*S*)-$Trp]^+$, and related complexes were determined by titration of 5.00 cm^3 each of 0.002 mol dm^{-3} solutions of either (*R*)-$TrpH_2^+$ or (*S*)-$TrpH_2^+$ and $\beta CDtrenH_4^{4+}$ with 0.075 cm^3 of $M(ClO_4)_2$ solution added. E_0 and pK_w values were determined by titration of 0.010 mol dm^{-3} $HClO_4$ (0.090 mol dm^{-3} in $NaClO_4$) against 0.100 mol dm^{-3} NaOH. Derivations of the stability constants were carried out using the program SUPERQUAD.[24] 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 χ^2 for each run was <12.6 at the 95% confidence level.[24]

Spectrophotometric Studies. All spectra were run in duplicate on a Cary 2200 spectrophotometer in 0.025 mol dm^{-3} NaPIPES buffer at pH 7.00 and $I = 0.10$ mol dm^{-3} ($NaClO_4$) in quartz cells thermostated at 298.2 K against reference solutions containing all components of the solution of interest except the metal salt. The spectra of the Co^{2+} systems were run under nitrogen on solutions prepared under nitrogen in a glovebox.

Results

Several complexes exist in aqueous solutions of βCDtren, M^{2+}, and tryptophan in the pH range 2.0−11.5 (Figure 1 and Tables 1 and 2). Their stabilities were calculated from the differences between the pH profiles arising from titration of

(22) Brown, S. E.; Coates, J. H.; Coghlan, D. R.; Easton, C. J.; van Eyk, S.; Lepore, A.; Lincoln, S. F.; Luo, Y.; May, B. L.; Schiesser, D. S.; Wang P.; Williams, M. L. *Aust. J. Chem.* **1993**, *46*, 953.

(23) Vogel, A. I. *Quantitative Inorganic Analysis*, 3rd ed.; Longmans: London, 1961.

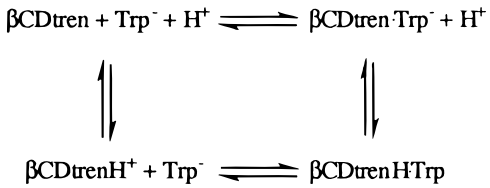
(24) Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195.

Table 1. Protonation and Stability Constants for 6^A-((2-(Bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrin (βCDtren) and Its Complexes and Related Species[a] in Aqueous Solution at 298.2 K and $I = 0.10$ mol dm^{-3} ($NaClO_4$)

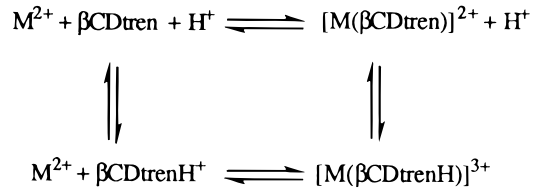
equilibrium	$\log(K/\text{dm}^3\ \text{mol}^{-1})$[b]
$\beta\text{CDtren} + H^+ \rightleftharpoons \beta\text{CDtrenH}^+$	9.85 ± 0.02 (10.14)[c]
$\beta\text{CDtrenH}^+ + H^+ \rightleftharpoons \beta\text{CDtrenH}_2^{2+}$	8.99 ± 0.09 (9.43)[c]
$\beta\text{CDtrenH}_2^{2+} + H^+ \rightleftharpoons \beta\text{CDtrenH}_3^{3+}$	6.89 ± 0.05 (8.41)[c]
$\beta\text{CDtrenH}_3^{3+} + H^+ \rightleftharpoons \beta\text{CDtrenH}_4^{4+}$	2.6 ± 0.3
$\beta\text{CDtren} + (R)\text{-Trp}^- \rightleftharpoons \beta\text{CDtren}\cdot(R)\text{-Trp}^-$	6.36 ± 0.01
$\beta\text{CDpn} + (R)\text{-Trp}^- \rightleftharpoons \beta\text{CDpn}\cdot(R)\text{-Trp}^-$ [d]	3.41
$\beta\text{CD} + (R)\text{-Trp}^- \rightleftharpoons \beta\text{CD}\cdot(R)\text{-Trp}^-$ [d]	2.33
$\beta\text{CDtren} + (S)\text{-Trp}^- \rightleftharpoons \beta\text{CDtren}\cdot(S)\text{-Trp}^-$	6.5 ± 0.1
$\beta\text{CDpn} + (S)\text{-Trp}^- \rightleftharpoons \beta\text{CDpn}\cdot(S)\text{-Trp}^-$ [d]	3.40
$\beta\text{CD} + (S)\text{-Trp}^- \rightleftharpoons \beta\text{CD}\cdot(S)\text{-Trp}^-$ [d]	2.33
$\beta\text{CDtrenH}^+ + (R)\text{-Trp}^- \rightleftharpoons \beta\text{CDtrenH}\cdot(R)\text{-Trp}$	5.85 ± 0.03
$\beta\text{CDtrenH}^+ + (S)\text{-Trp}^- \rightleftharpoons \beta\text{CDtrenH}\cdot(S)\text{-Trp}$	5.9 ± 0.1
$\beta\text{CDtren}\cdot(R)\text{-Trp}^- + H^+ \rightleftharpoons \beta\text{CDtrenH}\cdot(R)\text{-Trp}$	9.34 ± 0.04
$\beta\text{CDtren}\cdot(S)\text{-Trp}^- + H^+ \rightleftharpoons \beta\text{CDtrenH}\cdot(S)\text{-Trp}$	9.3 ± 0.2
$\beta\text{CDtrenH}^+ + (R)\text{-TrpH} \rightleftharpoons \beta\text{CDtrenH}\cdot(R)\text{-TrpH}^+$	5.59 ± 0.05
$\beta\text{CDtrenH}^+ + (S)\text{-TrpH} \rightleftharpoons \beta\text{CDtrenH}\cdot(S)\text{-TrpH}^+$	5.61 ± 0.08
$\beta\text{CDtrenH}\cdot(R)\text{-Trp} + H^+ \rightleftharpoons \beta\text{CDtrenH}\cdot(R)\text{-TrpH}^+$	8.99 ± 0.07
$\beta\text{CDtrenH}\cdot(S)\text{-Trp} + H^+ \rightleftharpoons \beta\text{CDtrenH}\cdot(S)\text{-TrpH}^+$	8.9 ± 0.2
$\text{Trp}^- + H^+ \rightleftharpoons \text{TrpH}$[d]	9.28
$\text{TrpH} + H^+ \rightleftharpoons \text{TrpH}_2^+$ [d]	2.40

[a] β-Cyclodextrin and 6^A-((3-aminopropyl)amino)-6^A-deoxy-β-cyclodextrin are represented by βCD and βCDpn, respectively. $\beta\text{CDtrenH}_n^{n+}$ indicates the degree of protonation of the title cyclodextrin, and βCDpnH_n^{n+} has an analogous meaning. Trp^-, TrpH, and TrpH_2^+ represent the anionic, neutral, and protonated forms of tryptophan. The complex formed between βCDtren and $(R)\text{-Trp}^-$ is represented by $\beta\text{CDtren}\cdot(R)\text{-Trp}^-$, and other complexes are represented in a similar manner. [b] This work unless otherwise indicated. Errors quoted for K (the mean of N runs) represent the standard deviation, $\sigma = \sqrt{((\sum(K_i - K)^2)/(N-1))}$, where K_i is a value from a single run for the best fit of the variation of pH with added volume of NaOH titrant obtained through SUPERQUAD and $i = 1, 2, ..., N$. [c] Data for the analogous equilibria $\text{tren(H)}_n^{n+} + H^+ \rightleftharpoons \text{tren(H)}_{n+1}^{n+1}$ where $n = 0$, 1, and 2, respectively, from ref 31. [d] References 15 and 16.

Scheme 1



Scheme 2



acidified solutions, containing different combinations of the complexing species, against NaOH using the program SUPERQUAD.[24] The titrimetric technique depends either on the protonation constant of an equilibrium constituent changing on complexation or on the complexation constants for the constituent and its protonated form differing, or both, to produce a pH change. This is exemplified by the βCDtren/Trp^-/H^+ system (Scheme 1) where the protonation constants of βCDtren and its complex $\beta\text{CDtren}\cdot\text{Trp}^-$ differ as do the stability constants of $\beta\text{CDtren}\cdot\text{Trp}^-$ and $\beta\text{CDtrenH}\cdot\text{Trp}$ (Table 1). Similarly, for the M^{2+}/βCDtren/H^+ system (Scheme 2) both the protonation constants of βCDtren and $[M(\beta\text{CDtren})]^{2+}$ and the stability constants of $[M(\beta\text{CDtren})]^{2+}$ and $[M(\beta\text{CDtrenH})]^{3+}$ differ (Tables 1 and 2).

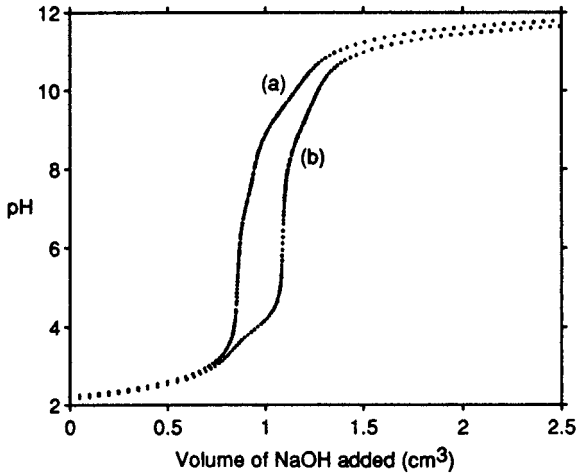


Figure 2. Titration profiles for (a) $\beta\text{CDtrenH}_4^{4+}$ (8.25×10^{-4} mol dm^{-3}) and $(R)\text{-TrpH}_2^+$ (1.03×10^{-3} mol dm^{-3}) and (b) $\beta\text{CDtrenH}_4^{4+}$ (8.25×10^{-4} mol dm^{-3}), $(R)\text{-TrpH}_2^+$ (1.03×10^{-3} mol dm^{-3}), and $Cu(ClO_4)_2$ (7.64×10^{-4} mol dm^{-3}), each in aqueous 0.007 mol dm^{-3} $HClO_4$ and 0.090 mol dm^{-3} $NaClO_4$ against 0.101 mol dm^{-3} NaOH at 298.2 K.

The sequence of titrations was (i) protonation constant determinations for βCDtren, followed by determination of the stability constants of the complexes formed from (ii) βCDtren and either $(R)\text{-Trp}^-$ or $(S)\text{-Trp}^-$ and their protonated analogues, (iii) M^{2+} and either βCDtren or $\beta\text{CDtrenH}^+$, and (iv) M^{2+} and either βCDtren or $\beta\text{CDtrenH}^+$ and either $(R)\text{-Trp}^-$ or $(S)\text{-Trp}^-$ and their protonated analogues. The protonation constants determined in (i), and those previously determined[16] under the same conditions for Trp^-, together with the stability constants determined in (ii) and (iii) and those for the complexation of tryptophan by M^{2+} previously determined under the same conditions,[16] were used where appropriate in the determination of stability constants from (ii)−(iv). The pH titration data were fitted to equilibria containing the minimum number of species required for a good fit, and any newly determined species found to be <5% of the total cyclodextrin or amino acid concentrations were considered to be insignificant. Two such pH titration profiles are shown in Figure 2. The protonation and stability constants derived in this study appear in Tables 1 and 2, and the speciation plots of the major species present in the Cu^{2+} system (Figures 3 and 4) exemplify those generated from these data.

Discussion

Formation of 6^A-((2-(Bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrin tryptophan complexes. The stability constants (Table 1) for $\beta\text{CDtren}\cdot(R)\text{-Trp}^-$ and $\beta\text{CDtren}\cdot(S)\text{-Trp}^-$ are $\sim 10^3$ times greater than those for $\beta\text{CDpn}\cdot(R)\text{-Trp}^-$ and $\beta\text{CDpn}\cdot(S)\text{-Trp}^-$ [16] (where βCDpn is 6^A-((3-aminopropyl)-amino)-6^A-deoxy-β-cyclodextrin), which are ~ 10 times greater than those for $\beta\text{CD}\cdot(R)\text{-Trp}^-$ and $\beta\text{CD}\cdot(S)\text{-Trp}^-$.[15] The phenyl moiety of Trp^- probably resides largely within the hydrophobic region of the cyclodextrin annuli of these complexes (Scheme 1), as has been shown to be the case for a range of cyclodextrin complexes formed with other aromatic guests.[25−28] Polar guests tend to align their dipole moments antiparallel to that of the

(25) Saenger, W. *Inclusion Compd.* **1984**, *2*, 231.
(26) Szejtli, J. *Cyclodextrin Technology*; Kluwer: Dordrecht, The Netherlands, 1988.
(27) Clarke, R. J.; Coates, J. H.; Lincoln, S. F. *Adv. Carbohyd. Chem. Biochem.* **1989**, *46*, 205.
(28) Harata, K. *Inclusion Compd.* **1991**, *5*, 311.

Table 2. Protonation and Stability Constants for Metallocyclodextrins of 6^A-((2-(Bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrin (βCDtren) and Related Species[a] in Aqueous Solution at 298.2 K and $I = 0.10$ mol dm^{-3} (NaClO4)

	log(K/dm^3 mol^{-1})[b]		
equilibrium	$M^{2+} = Ni^{2+}$	$M^{2+} = Cu^{2+}$	$M^{2+} = Zn^{2+}$
$M^{2+} + \text{tren} \rightleftharpoons [M(\text{tren})]^{2+}$ [c]	14.6	18.5	14.5
$M^{2+} + \text{pn} \rightleftharpoons [M(\text{pn})]^{2+}$ [c]	6.31	9.75	
$M^{2+} + \beta\text{CDtren} \rightleftharpoons [M(\beta\text{CDtren})]^{2+}$	11.65 ± 0.06	17.29 ± 0.05	12.25 ± 0.03
$M^{2+} + \beta\text{CDpn} \rightleftharpoons [M(\beta\text{CDpn})]^{2+}$ [d]	5.2	7.35	4.96
$M^{2+} + \beta\text{CDtrenH}^+ \rightleftharpoons [M(\beta\text{CDtrenH})]^{3+}$	8.46 ± 0.06	11.56 ± 0.02	7.92 ± 0.02
$M^{2+} + \beta\text{CDpnH}^+ \rightleftharpoons [M(\beta\text{CDpnH})]^{3+}$ [d]	3.1	3.09	3.0
$[M(\beta\text{CDtren})]^{2+} + H^+ \rightleftharpoons [M(\beta\text{CDtrenH})]^{3+}$	6.65 ± 0.09	4.11 ± 0.05	5.51 ± 0.04
$[M(\beta\text{CDtren})\text{OH}]^+ + H^+ \rightleftharpoons [M(\beta\text{CDtren})]^{2+}$	9.68 ± 0.09	8.48 ± 0.04	8.9 ± 0.6
$M^{2+} + \text{Trp}^- \rightleftharpoons [M(\text{Trp})]^+$ [d]	5.42	8.11	4.90
$[M(\text{Trp})]^+ + \text{Trp}^- \rightleftharpoons [M(\text{Trp})_2]$[d]	4.67	7.20	
$[M(\beta\text{CDtren})]^{2+} + (R)\text{-Trp}^- \rightleftharpoons [M(\beta\text{CDtren})\text{-}(R)\text{-Trp}]^+$	8.2 ± 0.2	9.5 ± 0.3	8.1 ± 0.1
$[M(\beta\text{CDpn})]^{2+} + (R)\text{-Trp}^- \rightleftharpoons [M(\beta\text{CDpn})\text{-}(R)\text{-Trp}]^+$ [d]	4.1	7.85	5.3
$[M(\beta\text{CDtren})]^{2+} + (S)\text{-Trp}^- \rightleftharpoons [M(\beta\text{CDtren})\text{-}(S)\text{-Trp}]^+$	8.1 ± 0.2	9.4 ± 0.2	8.3 ± 0.1
$[M(\beta\text{CDpn})]^{2+} + (S)\text{-Trp}^- \rightleftharpoons [M(\beta\text{CDpn})\text{-}(S)\text{-Trp}]^+$ [d]	5.1	8.09	5.3
$[M(\beta\text{CDtren})]^{2+} + (R)\text{-TrpH} \rightleftharpoons [M(\beta\text{CDtren})\text{-}(R)\text{-TrpH}]^{2+}$	4.6 ± 0.2	4.3 ± 0.3	
$[M(\beta\text{CDtren})\text{-}(R)\text{-Trp}]^+ + H^+ \rightleftharpoons [M(\beta\text{CDtren})\text{-}(R)\text{-TrpH}]^{2+}$	5.6 ± 0.3	4.0 ± 0.5	
$[M(\beta\text{CDtren})]^{2+} + (S)\text{-TrpH} \rightleftharpoons [M(\beta\text{CDtren})\text{-}(S)\text{-TrpH}]^{2+}$	4.3 ± 0.2	4.2 ± 0.2	
$[M(\beta\text{CDtren})\text{-}(S)\text{-Trp}]^+ + H^+ \rightleftharpoons [M(\beta\text{CDtren})\text{-}(S)\text{-TrpH}]^{2+}$	5.4 ± 0.3	4.0 ± 0.3	
$[M(\beta\text{CDtrenH})]^{3+} + (R)\text{-TrpH} \rightleftharpoons [M(\beta\text{CDtrenH})\text{-}(R)\text{-TrpH}]^{3+}$	3.56 ± 0.07	4.4 ± 0.2	4.82 ± 0.06
$[M(\beta\text{CDtren})\text{-}(R)\text{-TrpH}]^{2+} + H^+ \rightleftharpoons [M(\beta\text{CDtrenH})\text{-}(R)\text{-TrpH}]^{3+}$	5.6 ± 0.3	4.3 ± 0.4	
$[M(\beta\text{CDtrenH})]^{3+} + (S)\text{-TrpH} \rightleftharpoons [M(\beta\text{CDtrenH})\text{-}(S)\text{-TrpH}]^{3+}$	3.6 ± 0.3	4.4 ± 0.2	4.96 ± 0.05
$[M(\beta\text{CDtren})\text{-}(S)\text{-TrpH}]^{2+} + H^+ \rightleftharpoons [M(\beta\text{CDtrenH})\text{-}(S)\text{-TrpH}]^{3+}$	6.0 ± 0.4	4.3 ± 0.3	
$[M(\beta\text{CDtren})((R)\text{-Trp})\text{OH}] + H^+ \rightleftharpoons [M(\beta\text{CDtren})\text{-}(R)\text{-Trp}]^+$	7.86 ± 0.02	8.58 ± 0.02	8.7 ± 0.3
$[M(\beta\text{CDtren})((S)\text{-Trp})\text{OH}] + H^+ \rightleftharpoons [M(\beta\text{CDtren})\text{-}(S)\text{-Trp}]^+$	7.77 ± 0.03	8.53 ± 0.08	8.76 ± 0.08

[a] In addition to the abbreviations given in the footnote to Table 1, the following abbreviations apply: tren = (2-(bis(2-aminoethyl)amino)ethyl)amine, pn = 1,3-diaminopropane, and their complexes are represented by $[M(\text{tren})]^{2+}$ and $[M(\text{pn})]^{2+}$, respectively. The binary metallocyclodextrin formed by the title cyclodextrin is represented by $[M(\beta\text{CDtren})]^{2+}$, and $[M(\beta\text{CDtren})\text{-}(R)\text{-Trp}]^+$ is the ternary cyclodextrin formed with (R)-Trp$^-$. Analogous representations refer to the metallocyclodextrins of 6^A-((3-aminopropyl)amino)-6^A-deoxy-β-cyclodextrin (βCDpn). Metallocyclodextrin protonation is indicated by the addition of protons to the abbreviations and appropriate changes of charge. [b] This work unless otherwise indicated. Errors quoted for K (the mean of N runs) represent the standard deviation, $\sigma = \sqrt{((\sum(K_i - K)^2)/(N-1))}$, where K_i is a value from a single run for the best fit of the variation of pH with added volume of NaOH titrant obtained through SUPERQUAD and $i = 1, 2, ..., N$. [c] Reference 31. [d] Reference 16.

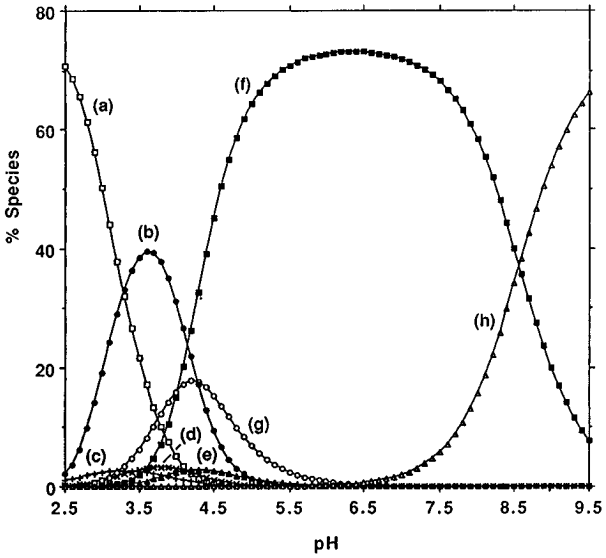


Figure 3. Plot of percentage of Cu^{2+} species in a solution 7.64×10^{-4}, 8.25×10^{-4}, and 1.03×10^{-3} mol dm^{-3} in total Cu^{2+}, βCDtren, and (R)-TrpH, respectively, calculated from the data in Tables 1 and 2 and plotted relative to total $[(R)\text{-TrpH}] = 100\%$: (a) Cu^{2+}; (b) $[Cu(\beta\text{CDtrenH})\text{-}(R)\text{-TrpH}]^{3+}$; (c) $[Cu((R)\text{-Trp})]^+$; (d) $[Cu(\beta\text{CDtrenH})]^{3+}$; (e) $[Cu(\beta\text{CDtren})]^{2+}$; (f) $[Cu(\beta\text{CDtren})\text{-}(R)\text{-Trp}]^+$; (g) $[Cu(\beta\text{CDtrenH})\text{-}(R)\text{-Trp}]^{2+}$; (h) $[Cu(\beta\text{CDtren})((R)\text{-Trp})\text{OH}]$. No other Cu^{2+} species are present at >5%.

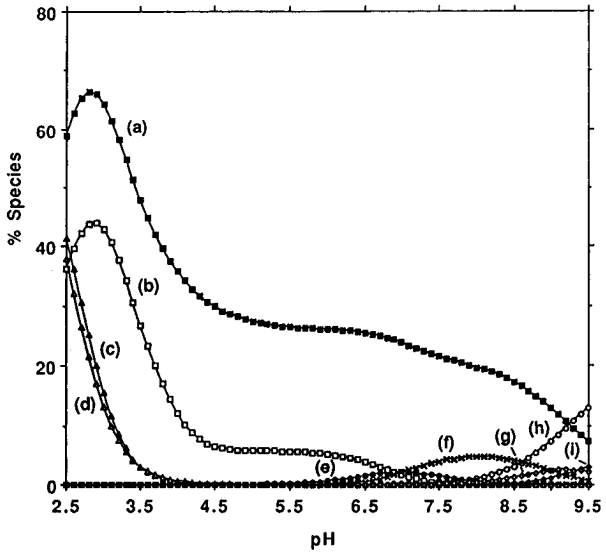


Figure 4. Plot of percentage of non-Cu^{2+} species in a solution 7.64×10^{-4}, 8.25×10^{-4}, and 1.03×10^{-3} mol dm^{-3} in total Cu^{2+}, βCDtren, and (R)-TrpH, respectively, calculated from the data in Tables 1 and 2 and plotted relative to total $[(R)\text{-TrpH}] = 100\%$: (a) (R)-TrpH; (b) $\beta\text{CDtrenH}_3^{3+}$; (c) $\beta\text{CDtrenH}_4^{4+}$; (d) $(R)\text{-TrpH}_2^+$; (e) $\beta\text{CDtrenH}_2^{2+}$; (f) $\beta\text{CDtrenH}\cdot(R)\text{-TrpH}^+$; (g) $\beta\text{CDtrenH}\cdot(R)\text{-Trp}$; (h) $(R)\text{-Trp}^-$, (i) $\beta\text{CDtren}\cdot(R)\text{-Trp}^-$. No other non-$Cu^{2+}$ species are present at >5%.

cyclodextrin, which for α-cyclodextrin has a magnitude of 10−20 D with the positive and negative poles near the centers of the narrow and wide ends of the annulus, respectively.[29,30] Similar dipole orientations are assumed for the cyclodextrins considered here. Thus, the increase in stability of the complexes with change in nature of the cyclodextrin in the sequence βCD < βCDpn < βCDtren is attributable to the interaction of the

(29) Sakurai, M.; Kitigawa, M.; Hoshi, H.; Inoue, Y.; Chûjô, R. *Chem. Lett.* **1988**, 895.

(30) Sakurai, M.; Kitigawa, M.; Hoshi, H.; Inoue, Y.; Chûjô, R. *Carbohydr. Res.* **1990**, *198*, 181.

Trp^- amino carboxylate group with the narrow end of the cyclodextrin annulus.

The higher stability of the βCDtren complexes may arise because either (i) βCDtren has a greater dipole and a consequently stronger interaction with Trp^-, (ii) the greater bulk of the 6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino) substituent hinders egress more than ingress of Trp^-, or (iii) it hydrogen bonds more strongly to Trp^-, or a combination of these factors. As no complexation of Trp^- by (2-(bis(2-aminoethyl)amino)-ethyl)amine (tren) was detected by the pH titrimetric method employed in this study, it appears that the interaction of the phenyl moiety of Trp^- with the interior of the cyclodextrin annulus is the essential contribution to complex stability on which the stabilizing effect of the 6^A-((2-(bis(2-aminoethyl)-amino)ethyl)amino) substituent is superimposed. The similarity of the βCDtren·(*R*)-Trp^- and βCDtren·(*S*)-Trp^- stabilities is also consistent with this interaction dominating the complexation free energy and any free energy differences arising from matching of the opposite chiralities of (*R*)-Trp^- and (*S*)-Trp^- with the homochirality of βCDtren being small by comparison. A similar dominance applies for the analogous βCD and βCDpn complexes.

Protonation decreases the stabilities of βCDtrenH·(*R*)-Trp, βCDtrenH·(*S*)-Trp, βCDtrenH·(*R*)-$TrpH^+$, and βCDtrenH·(*S*)-$TrpH^+$ (Table 1) despite an anticipated increase in the dipolar character of βCDtrenH$^+$. This may reflect either a decreased ability of βCDtrenH$^+$ to hydrogen bond with Trp^- and TrpH or an increased hydration of βCDtrenH$^+$, by comparison with that of βCDtren, diminishing the hydrophobic interaction with the tryptophan phenyl moiety.

Formation of Binary Metallocyclodextrins. The stabilities of the binary metallocyclodextrins, $[M(\beta CDtren)]^{2+}$, are lower than those of the analogous $[M(tren)]^{2+}$ complexes when M^{2+} = Ni^{2+}, Cu^{2+}, and Zn^{2+} [31] (Table 2). This probably reflects a difference in the electron-donating powers of the secondary amine group in βCDtren and a primary amine group in tren and the greater steric hindrance to metal binding caused by βCDtren. However, the stabilities of $[M(\beta CDtren)]^{2+}$ are substantially greater than those of $[M(\beta CDpn)]^{2+}$ because of the tetradentate nature of βCDtren. The stability variations for both binary metallocyclodextrins with the nature of M^{2+} are as anticipated from the Irving−Williams sequence[32] ($Ni^{2+} < Cu^{2+} > Zn^{2+}$) which arises through a combination of the variation of M^{2+} size and ligand field effects. The stabilities of $[M(\beta CDtrenH)]^{3+}$ are decreased by comparison with those of $[M(\beta CDtren)]^{2+}$ because the protonation of an amino group decreases the denticity of βCDtrenH$^+$ to 3 and causes charge repulsion of M^{2+}. The acidity of $[M(\beta CDtrenH)]^{3+}$ (Table 2) is greatly increased by comparison with that of βCDtrenH$^+$ (Table 1) because of the coordination of M^{2+}. The most acidic is $[Cu(\beta CDtrenH)]^{3+}$, coincident with its being the most stable of the protonated binary cyclodextrins formed in the equilibria between M^{2+} and βCDtrenH$^+$ (Table 2). The formation of $[M(\beta CDtren)OH]^+$ arises from the protolysis of a coordinated water molecule that has a pK_a of 9.68, 8.48, and 8.9 when M^{2+} = Ni^{2+}, Cu^{2+}, and Zn^{2+}, respectively.

In aqueous solution, $[Ni(tren)(H_2O)_2]^{2+}$ is six-coordinate, but the five-coordinate stoichiometry, $[M(tren)H_2O]^{2+}$, is observed when $M^{2+} = Cu^{2+}$ and Zn^{2+}.[33−35] Over the wavelength range

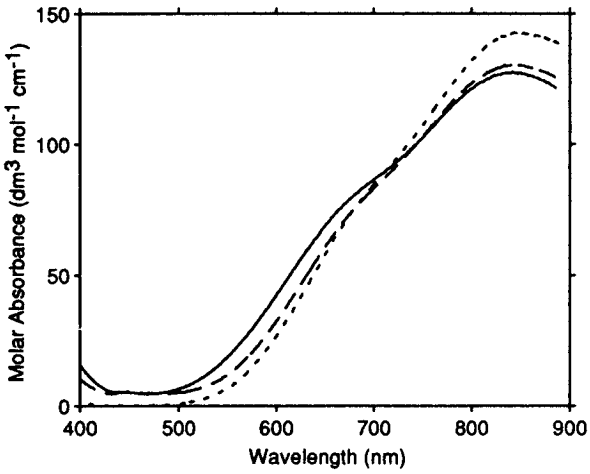


Figure 5. Absorbance spectra for $[Cu(tren)H_2O]^{2+}$ (dotted curve), $[Cu(\beta CDtren)H_2O]^{2+}$ (dashed curve), and $[Cu(\beta CDtren)Trp]^+$ (solid curve) in aqueous 0.025 mol dm^{-3} NaPIPES buffer at pH 7.00 and I = 0.10 mol dm^{-3} ($NaClO_4$) at 298.2 K.

400−900 nm, $[Ni(tren)(H_2O)_2]^{2+}$ exhibited a major absorbance maximum at 560 nm with a molar absorbance of 10 dm^3 mol^{-1} cm^{-1} assigned to the $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ transition in reasonable agreement with the literature.[36] The spectra of $[Ni(\beta CDtren)(H_2O)_2]^{2+}$ and $[Ni(\beta CDtren)Trp]^+$ differ only slightly in molar absorbance in the range 400−900 nm, and both show maxima at 567 nm with molar absorbances of 6 dm^3 mol^{-1} cm^{-1}, consistent with six-coordination. (It appears that a metal center bound to a polyamine substituent at the 6^A site of a modified cyclodextrin may simultaneously coordinate a cyclodextrin primary hydroxy group, but it was not possible to distinguish between such coordination and that of a water molecule from our data.[7])

The spectrum of $[Cu(tren)H_2O]^{2+}$ (Figure 5) shows a shoulder at ∼720 nm and a maximum at 847 nm (molar absorbance = 143 dm^3 mol^{-1} cm^{-1}) assigned to the $^2A_1' \rightarrow {}^2E''$ and $^2A_1' \rightarrow {}^2E'$ transitions, respectively, in reasonable agreement with literature data.[36] The spectra of $[Cu(\beta CDtren)H_2O]^{2+}$ and $[Cu(\beta CDtren)Trp]^+$ exhibit shoulders at ∼698 and ∼690 nm, respectively, and maxima at 841 nm, with molar absorbances of 131 and 128 dm^3 mol^{-1} cm^{-1}, consistent with Cu^{2+} being five-coordinate in these metallocyclodextrins. UV−visible spectroscopy provides little information about the environment of Zn^{2+} because of its d^{10} electronic configuration. While the formation of five-coordinate $[Zn(tren)H_2O]^{2+}$ in solution[37] indicates the possibility of five-coordinate $[Zn(\beta CDtren)H_2O]^{2+}$ and $[Zn(\beta CDtren)Trp]^+$ forming, an analysis of stability data indicates that six-coordination is more probable. Thus, the differences between the $\log(K/dm^3\ mol^{-1})$ values for $[M(\beta CDtren)]^{2+}$ and $[M(tren)]^{2+}$ are 2.95, 1.21, and 2.25 when M^{2+} = Ni^{2+}, Cu^{2+}, and Zn^{2+}, respectively (Table 2). The first difference corresponds to the effect of the βCD substituent on a six-coordinate metal center, whereas the second corresponds to its effect on a five-coordinate metal center. The difference when $M^{2+} = Zn^{2+}$ is intermediate between the other two values, which may result from the βCD substituent causing a change from five- to six-coordination, consistent either with Zn^{2+} in $[Zn(\beta CDtren)H_2O]^{2+}$ being six-coordinate through the coordination of a cyclodextrin primary hydroxy group as discussed above or with the stoichiometry being $[Zn(\beta CDtren)(H_2O)_2]^{2+}$.

The spectra of solutions of $[Co(tren)H_2O]^{2+}$, Co^{2+}/βCDtren, and Co^{2+}/βCDtren/Trp^- and their protonated analogues ob-

(31) *Critical Stability Constants*; Smith, R. M., Martell, A. E., Eds.; Plenum Press: New York, 1975; Vol. 1.
(32) Irving, H.; Williams, R. J. P. *J. Chem. Soc.* **1953**, 3192.
(33) Coates, J. H.; Gentle, G. J.; Lincoln, S. F. *Nature* **1974**, *249*, 773.
(34) Hunt, J. P. *Coord. Chem. Rev.* **1971**, *7*, 1.
(35) Rablen, D. P.; Dodgen, H. W.; Hunt, J. P. *J. Am. Chem. Soc.* **1972**, *94*, 1771.
(36) Jørgensen, C. K. *Acta Chem. Scand.* **1956**, *10*, 887.
(37) Paoletti, P.; Ciampolini, M.; Sacconi, L. *J. Chem. Soc.* **1963**, 3589.

served under the same saturating nitrogen conditions as those applying in the titrations exhibited significant charge transfer bands extending from 400 to 500 nm, which are absent from the spectra of completely oxygen free solutions of $[Co(tren)H_2O]^{2+}$.[37] These bands probably arise from the formation of μ-peroxo complexes that are well established for tetra- and pentaamminecobalt(II) complexes.[38] While the proportion of the complex existing as the μ-peroxo form is probably small, the effect of this on the measured stability constants is uncertain, and accordingly the Co^{2+} system is not further discussed.

Formation of Ternary Metallocyclodextrins. The stepwise stability constants for the formation of the ternary metallocyclodextrins [M(βCDtren)-(*R*)-Trp]$^+$ and [M(βCDtren)-(*S*)-Trp]$^+$ from [M(βCDtren)]$^{2+}$ and (*R*)- and (*S*)-Trp$^-$ are substantially greater than the analogous stability constants for [M(βCDpn)-(*R*)-Trp]$^+$ and [M(βCDpn)-(*S*)-Trp]$^+$. This reflects the differing interactions of Trp$^-$ with the βCDpn and βCDtren that produced the $\sim 10^3$-fold greater stability of βCDtren·(*R*)-Trp$^-$ and βCDtren·(*S*)-Trp$^-$ by comparison with that of βCDpn·(*R*)-Trp$^-$ and βCDpn·(*S*)-Trp$^-$, as discussed above. The probability of substitution of Trp$^-$ on [M(βCDpn)]$^+$ where four water molecules are available for substitution compared to the one or two available in [M(βCDtren)]$^{2+}$, depending on the identity of M^{2+}, should be higher for the former species on a statistical basis. However, this is insufficient to offset the differences in the contributions to ternary metallocyclodextrin stability arising from the interaction of Trp$^-$ with βCDpn and βCDtren.

The stabilities of [M(βCDtren)-(*R*)-Trp]$^+$ and [M(βCDtren)-(*S*)-Trp]$^+$ are greater than those of the analogous [M(Trp)]$^+$ and βCDtren·Trp$^-$ complexes (Tables 1 and 2). This is consistent with the binding of the Trp$^-$ amino acid moiety by M^{2+} and the hydrophobic interaction between the Trp$^-$ aromatic moiety and the hydrophobic interior of the cyclodextrin annulus (Figure 1) reinforcing each other to stabilize [M(βCDtren)-(*R*)-Trp]$^+$ and [M(βCDtren)-(*S*)-Trp]$^+$. The variation of the stepwise stability constants for the binding of Trp$^-$ in the ternary metallocyclodextrins with the nature of M^{2+} in the sequence $Ni^{2+} < Cu^{2+} > Zn^{2+}$ is similar to that for the formation of [M(Trp)]$^+$,[31] consistent with the size[39] and electronic configuration[40] of M^{2+} exerting a major influence in this complexation step. The visible spectral data for [Ni(βCDtren)Trp]$^+$ and [Cu(βCDtren)Trp]$^+$ show that the metal centers are six- and five-coordinate, respectively. In the first case the structure is probably six-coordinated as indicated in Figure 1, but for [Cu(βCDtren)Trp]$^+$ the possibility arises that either the amine or the carboxylate group of Trp$^-$ may be bound, or both may be bound and one of the amine groups of the 6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino) substituent may not be bound.

The differences between the log(K/dm^3 mol^{-1}) values for [M(βCDtren)-(*R*)-Trp]$^+$ and [M(βCDtren)]$^+$ are 3.45, 7.79, and 4.15, and the analogous data for the (*S*)-Trp$^-$ analogue are 3.55, 7.89, and 3.95 when $M^{2+} = Ni^{2+}$, Cu^{2+}, and Zn^{2+}, respectively (Table 2). In both cases, the first and third values are quite similar, whereas there is about twice the difference in the case of Cu^{2+}. This is consistent with similar coordination changes occurring for [Ni(βCDtren)]$^+$ and [Zn(βCDtren)]$^+$ on complexation of Trp$^-$, and with both metal centers being six-coordinate.

No enantioselectivity was found in the formation of [M(βCDtren)-(*R*)-Trp]$^+$ and [M(βCDtren)-(*S*)-Trp]$^+$. This contrasts with the formation of [M(βCDpn)-(*R*)-Trp]$^+$ and [M(βCDpn)-(*S*)-Trp]$^+$, where a 10-fold enantioselectivity for (*S*)-Trp$^-$ was found when $M^{2+} = Ni^{2+}$ and a lesser enantioselectivity arose when $M^{2+} = Cu^{2+}$ (Table 2).[15,16] (A similar variation was found in the enantioselective complexation of (*R*)- and (*S*)-phenylalanine anions by [M(βCDpn)]$^{2+}$.[17]) Despite the high stabilities of [M(βCDtren)-(*R*)-Trp]$^+$ and [M(βCDtren)-(*S*)-Trp]$^+$ by comparison with those of [M(βCDpn)-(*R*)-Trp]$^+$ and [M(βCDpn)-(*S*)-Trp]$^+$, the opposed chiralities of (*R*)- and (*S*)-Trp$^-$ generate too small a free energy difference through interaction with the homochiral annulus of the metallocyclodextrin for thermodynamic enantioselectivity to be observed. (Thermodynamic enantioselectivity may reverse with change in the metal binding group as is shown by (6^A-histamino-6^A-deoxy-β-cyclodextrin)copper(II), which forms ternary complexes with (*R*)-Trp$^-$ and the (*R*)-phenylalanine anion that are 2.2 and 1.5 times more stable than those formed with the corresponding (*S*)-enantiomers,[11,14] or it may disappear for the same chiral substrates as is found for (6^A-((2-aminoethyl)amino)-6^A-deoxy-β-cyclodextrin)copper(II).[13])

The pair of protonated species [M(βCDtren)-(*R*)-TrpH]$^{2+}$ and [M(βCDtren)-(*S*)-TrpH]$^{2+}$ are more stable than or similarly stable to the [M(βCDtrenH)-(*R*)-TrpH]$^{3+}$ and [M(βCDtrenH)-(*S*)-TrpH]$^{3+}$ pair when $M^{2+} = Ni^{2+}$ and Cu^{2+}, respectively, or have decreased stabilities by comparison with those of [M(βCDtren)-(*R*)-Trp]$^+$ and [M(βCDtren)-(*S*)-Trp]$^+$. Only [M(βCDtrenH)-(*R*)-TrpH]$^{3+}$ and [M(βCDtren)-(*R*)-Trp]$^+$ and their (*S*) analogues were detected for Zn^{2+}, and the latter metallocyclodextrin is much more stable. These stability variations probably arise because TrpH acts as a monodentate ligand and the major contribution to stability arises from the interaction of the substrate aromatic moiety with the hydrophobic interior of the cyclodextrin annulus.

Conclusions

While the relative stabilities of [M(βCDtren)]$^{2+}$ vary with M^{2+} in the sequence $Ni^{2+} < Cu^{2+} > Zn^{2+}$ and are dominated by the nature of M^{2+}, the subsequent binding of Trp$^-$ is greatly influenced by its interaction with the cyclodextrin annulus. Thus, the combined effects of βCDtren and M^{2+} produce a greater binding of Trp$^-$ in [M(βCDtren)Trp]$^+$ (which also varies with M^{2+} in the sequence $Ni^{2+} < Cu^{2+} > Zn^{2+}$) than that in either [M(Trp)]$^+$ or βCDtren·Trp$^-$, but no enantioselectivity between (*R*)- and (*S*)-Trp$^-$ is observed. The closely related [M(βCDpn)]$^{2+}$ bind (*S*)-Trp$^-$ enantioselectively over (*R*)-Trp$^-$ when $M^{2+} = Ni^{2+}$ and Cu^{2+} but with lower stabilities that also vary with M^{2+} in the sequence $Ni^{2+} < Cu^{2+} > Zn^{2+}$.[16] This enantioselectivity is coincident with the weaker interaction of βCDpn with Trp$^-$ (by comparison with βCDtren) allowing M^{2+} to exert more influence on the binding of Trp$^-$. These observations indicate the subtle relationship between the nature of the cyclodextrin and M^{2+} in substrate binding in ternary metallocyclodextrins. Similarly subtle relationships are probably partly responsible for the high degree of metal ion specificity observed for metalloenzyme activity.

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(38) Mori, M.; Weil, J. A. *J. Am. Chem. Soc.* **1967**, *89*, 3732.
(39) Shannon, R. D. *Acta Crystallogr., Sect. A: Cryst. Phys. Diffr., Theor. Gen. Crystallogr.* **1976**, *A32*, 751.
(40) Schriver, D. F.; Atkins, P. W.; Langford, C. H. *Inorganic Chemistry*, 2nd ed.; Oxford University Press: Oxford, U.K., 1994.