Functionalized β-cyclodextrins: thermodynamic studies and NMR titration of 6-diethylenetriamine derivative

Vincenzo Cucinotta,[a] Franca D'Alessandro,[b] Giuseppe Impellizzeri,[b] Giuseppe Maccarrone,[b] Enrico Rizzarelli[b,c] and Graziella Vecchio[c]

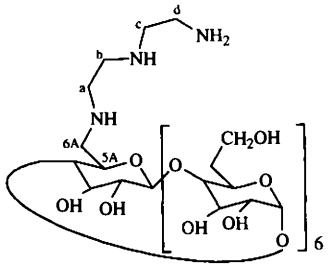
[a] *Dipartimento di Scienza degli Alimentazione, Università di Napoli 'Federico II', Via Università, 100, 80055 Portici, Napoli, Italy*
[b] *Dipartimento di Scienze Chimiche, Università di Catania, viale A. Doria, 6, Catania, Italy*
[c] *Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico, CNR -, viale A. Doria, 6, Catania, Italy*

A thermodynamic and spectroscopic investigation has been carried out on the protonation and copper(II) complexation of the 6-diethylenetriamine derivative of β-cyclodextrins. By ^{13}C NMR titration, the order of protonation of the three nitrogen atoms has been ascertained. The unusual entropy changes accompanying the different steps of protonation, as well as the copper(II) complexation, are discussed.

Introduction

Cyclodextrins (CD), cyclic oligomers of glucose, owing to their interior cavity which provides a relatively hydrophobic environment, may behave as hosts to a series of inorganic and organic molecules.[1-4] In order to increase the selectivity and the effectiveness in their receptor ability, functionalization by different groups may be accomplished by tailor-made synthetic routes.[5]

A further step forward may be taken by using metal complexes of the functionalized cyclodextrin, thus exploiting the residual coordination ability around the metal ion.[6-13] A straightforward way to obtain such complexes is to functionalize the cyclodextrin by a potential coordinating moiety, typically an amine. Some peculiar properties of the CD-derivative–metal complex, due to the cavity, have been described in depth.[6,8,11-21]



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The 6-deoxy-6-(5-amino-3-azapentylamino)cyclomaltoheptaose (CDdien) **1** has been synthesized and its zinc(II) complex has been shown to form a host–guest complex with the 2-oxoadamantan-1-carboxylate 330 times stronger than the parent.[22] Furthermore the CDdien ligand[23] and its zinc(II) complex[21] have been investigated as a nuclease model.

A series of amino-bonded mono- and di-derivatives of β-CDs have been synthesized in our laboratory to investigate their complexing properties.[14-19] The relevance of a full characterization of the metal complex species when these systems are proposed as enzyme models has been underlined.[24]

In order to gain more insight into the different protonated species and the copper(II) complex of the 6-deoxy-6-(5-amino-3-azapentylamino)cyclomaltoheptaose (CDdien) a detailed potentiometric and calorimetric study was carried out. Furthermore, in order to study the conformational characteristics and to identify the successive protonation sites of CDdien, an NMR titration study was carried out. The copper(II) complex EPR parameters were also reported and compared with those of the analogous species of dien (diethylenetriamine), to study the influence of the CD cavity.

Experimental

Materials

Copper(II) nitrate was prepared from copper(II) basic carbonate by adding a slight excess of HNO_3. The concentrations of stock solutions were determined by EDTA titrations with murexide as the indicator. The excess HNO_3 was determined by Gran's method and by ACBA computer program (see Calculations). Stock solutions of HNO_3 and KOH were determined by titration with primary standard tris(hydroxymethyl)aminomethane (THAM) and potassium hydrogen phthalate, respectively. Potassium nitrate (Suprapur Merck) was used without further purification. All solutions were prepared with doubly distilled water.

Commercially available reagents were used directly unless otherwise noted for the CDdien synthesis.

TLC was carried out on silica gel plates 60F-254 (Merck). CD derivatives were detected by the anisaldehyde reagent.

Synthesis of 6-deoxy-6-(5-amino-3-azapentylamino)cyclomaltoheptaose 1

6-Deoxy-6-iodocyclomaltoheptaose (CDI) (1 g, 0.8 mmol)[15] was dissolved in dien (5 ml, 31 mmol) under a N_2 stream, and the reaction was carried out at 60 °C for 7 h, the end-point of the reaction (disappearance of CDI) was checked by TLC. The dien solution was added to acetone whilst stirring the solid obtained was collected by filtration, washed with acetone and applied on a CM-Sephadex C-25 (NH_4^+ form) column (20 × 700 mm). Water (500 ml) and then a linear gradient 0–0.25 M of NH_4HCO_3 (1.6 l, total volume) were used as eluent. The appropriate fractions were combined to give CDdien (0.250 g, yield 25%). (R_f 0.2, PrOH–H_2O–NH_3, 5:3:1); δ_H(200 MHz, D_2O), 5.0 (1-H), 4–3.8 (5-, 3-, 6-H), 3.7–3.5 (2-, 4-H), 3.38 (4A-H), 3.04 (6'A-H) and 2.9–2.7 (6A-H, and CH_2 of dien chain); δ_C(50 MHz, D_2O) 104.5 (1-C), 104.3 (1A-C), 86.3 (4A-C), 83.8 (4-C), 75.7 (2-C), 74.7 (3-C), 74.5 (5-C), 73.1 (5A-C), 63.0 (6-C), 52.0 (6A-C), 51.8 (c-C), 50.5 (*a*-C), 50.1 (b-C) and 42 (d-C); FABMS *m/z* 1221 (M + 1).

Potentiometric measurements

Computer-controlled potentiometric titrations were performed with a Metrohm digital pH meter (Model 654). The titration cell (2.5 ml) was thermostatted at 25.0 ± 0.2 °C and all solutions were kept under an atmosphere of nitrogen, which was bubbled through a solution of the same ionic strength and temperature as the solution under study. Alkali was added from a Hamilton burette equipped with 0.25 or 0.50 cm^3 syringes. The ionic strength of all solutions were 0.10 M (KNO_3). The concentrations of the β-cyclodextrin derivative (L) and copper(II) ion (M) ranged from 0.003 to 0.006 M. Duplicate or triplicate titrations were carried out at an L : M ratio of 1 : 1 and 1 : 2. The titrant was KOH. Other details were as previously described.[25,26]

The titration of HNO_3 with KOH was performed before and after each set of experiments to convert reading values to pH and to calculate log K values. The ionic strength was kept at 0.1 M (KNO_3).

Calorimetric measurements

Calorimetric measurements were performed at 25.000 + 0.001 °C using a Tronac 450 isoperibolic calorimeter equipped with a titration Dewar (25 ml). The calorimetric system was calibrated by titrating THAM with HCl in accordance with Grenthe. The calorimetric titrations were carried out by titrating the solution and the ligands with HNO_3 (0.2–0.4 M). Reaction heats, corrected for the heat of dilution, determined by separate experiments, were calculated by considering the calorie unit as equivalent to 4.184 J. Other experimental details were as previously reported.[25,26]

Spectroscopic measurements

^1H NMR spectra (200.13 MHz) and ^{13}C NMR spectra (50.32 MHz) were recorded with a Bruker AC-200 spectrometer on D_2O solutions without a reference compound; the ^1H NMR spectra were referenced to water since most of the usual reference compounds interact with the CD cavity. The solutions of protonated CDdien were prepared by adding a stoichiometric amount of DCl in D_2O solution. A suitable excess of DCl was used in the case of the three-protonated species.

EPR frozen solution spectra were performed on a Bruker instrument type ER 200D, driven by the 3220 data system. All spectra were recorded at a temperature of 150 K on Cu–CDdien complexes in solution (3×10^{-3} M), at pH 7. Up to 5% methanol was added to these solutions to increase resolution. Parallel spin Hamiltonian parameters were calculated directly from the experimental spectra. The DPPH radical ($g_{||} = 2.0036$) was used to standardize the klystron frequency, the magnetic field being also monitored by a Bruker gaussmeter type ER 0.35 M.

Calculations

The calculations concerning the electrode system as well as the eluent slope were performed by the computer program ACBA,[27] which refines the parameters of acid–base titrations by using a non-linear least-squares method minimizing the function $U = \Sigma(V_i - V_{icalc})^2$ where V_i is the volume of the titrant added. All other potentiometric data were handled by the program SUPERQUAD.[28] The above program minimizes the error-square sum based on the measured electrode potentials. The enthalpies of formation were computed by means of the least-squares program DOEC.[29]

Results and discussion

CDdien was synthesized using a slightly different procedure to that reported in the literature.[22] The ^1H NMR and the ^{13}C NMR spectra are shown in Figs. 1 and 2, respectively.

A ^{13}C NMR titration study was carried out in order to ascertain the protonation sites and the possibility of the formation of more mono- and di-protonated species, due to the contemporary protonation at different sites of the molecule.[30–32] The results are graphically summarized in Fig. 3. As concerns the assignment, the 5A-carbon (the 5-carbon of the glucose functionalized ring, called A) is easily distinguishable, being the only one bonded to a methine group and thus being at a lower field in comparison to all the other observed carbon atoms. The d-carbon (see Fig. 3), as well, is shifted upfield in the titration as it is the only one attached to an NH_2 group. The other four carbon atoms (6A, a, b and c) on the contrary have very similar chemical shifts and the assignment was made step-by-step. It is known that the protonation shift of ^{13}C in amino compounds exhibits certain trends depending on the number of bonds separating the carbon and the amino group. A larger upfield protonation shift is generally observed for the carbon β to the nitrogen which undergoes the protonation.[30]

From the very first additions of DCl the d-carbon undergoes an upfield shift thus suggesting that the first protonation occurs on one of the nitrogen atoms nearer to the d-carbon atom. However, the largest shift is shown by another carbon atom, which therefore should be in the β-position to the protonating nitrogen, while the d-carbon should be in the α-position. Thus, we must draw the conclusion that the first protonation occurs on the NH_2 group and that the most affected carbon atom is the c-carbon, as indicated in the figures. Interestingly, after five

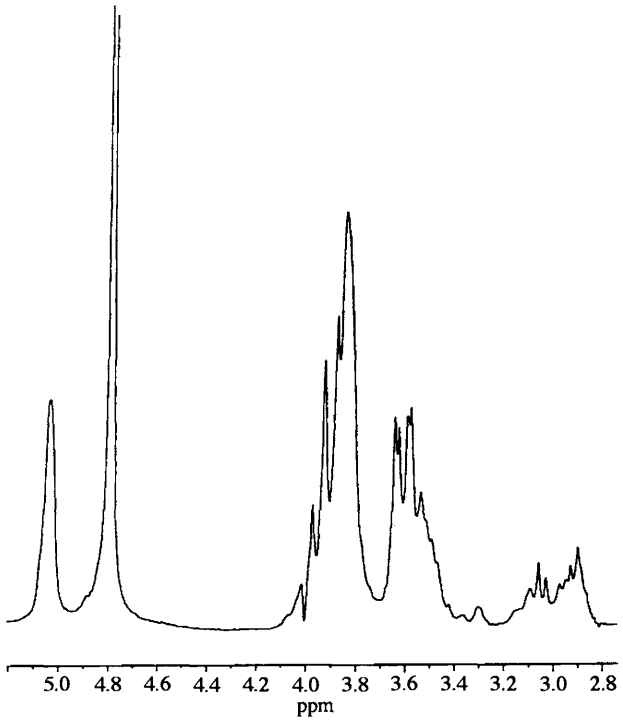

Fig. 1 ^1H NMR spectra (D_2O, 200.13 MHz) of CDdien

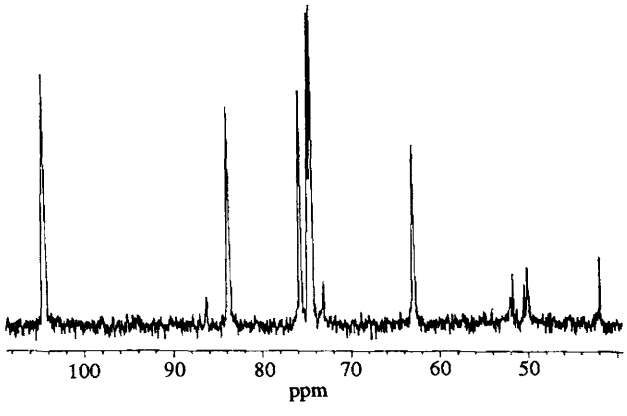

Fig. 2 ^{13}C NMR spectra (D_2O, 50.33 MHz) of CDdien

Table 1 ^{1}H NMR data of selected protons of CDdien

δ						
Species	6 A	6′ A	5 A	4 A	CH_2 (b), CH_2 (c)	CH_2 (a), CH_2 (d)
CDdien	2.85	3.05	3.90	3.40	2.70	2.80
$[(CDdien)H]^+$	3.10	3.30	4.05	3.45	2.90	3.10
$[(CDdien)H_2]^{2+}$	3.40	3.60	4.15	3.60	3.30	3.409
$[(CDdien)H_3]^{3+}$	> 3.30	> 3.30	4.15		> 3.3	> 3.3

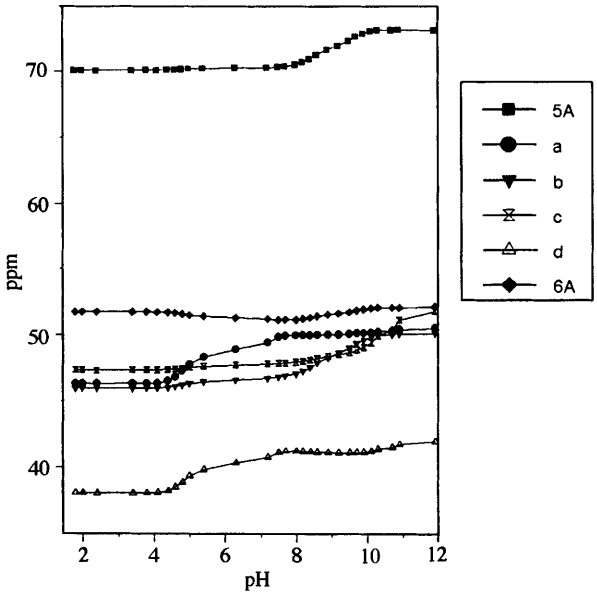

Fig. 3 ^{13}C NMR chemical shift (δ) as a function of pH (a-, b-, c-, d-, 5A- and 6A-carbon)

additions from the start, the 5A-carbon starts to shift upfield too, and this shift goes on until the sixteenth addition. Since a 1 : 1 molar addition of acid corresponds to about the eighth addition, clearly the shift starts during the first protonation and lasts until the end of the second protonation, thus suggesting that the second protonation occurs on the nitrogen bonded to the cyclodextrin and is partly overlapped to the first. Coherently, not only the 5A-carbon, but all the other carbon atoms start to shift after the fifth addition, since all of them are in the α- or β-position to one of the two nitrogen atoms involved in the protonation. Besides the 5A-carbon, another carbon atom shows a larger shift, suggesting to be in the β-position to the cyclodextrin-bonded nitrogen, and this should be the b-carbon, as shown in the figures.

In order to distinguish between the two carbon (6A or a) atoms, both in the α-position to the cyclodextrin-bonded NH, we have to look to the third and last protonation step that, for exclusion, occurs on the middle NH. The more affected carbon atom, just on the basis of this data, has been attributed to the a-carbon and the remaining atom must be the 6A-carbon atom. The fact that the already assigned d-carbon shows a large upfield shift during this third protonation, confirms the hypothesized overall assignment. Lastly, it is interesting to observe the significant downfield shift of the 6A-carbon, due to the middle NH protonation. Since no through-chain-effect may explain such a surprising shift, we should invoke a through-space-effect, presumably due to the cyclodextrin cavity.

The ^{1}H NMR spectra of CDdien in the different protonation states were assigned on the basis of the COSY spectra, and the corresponding data are listed in Table 1. Just as in the case of other derivatives such as CDen,[18] CDhm,[15] the substitution of an OH group by an NH group causes a large downfield shift of the two 6-, 5- and 4-H of the A glucose ring. However, in contrast to previous systems, CDdien does not show any evidence of an analogue intrachain hydrogen bond.[15,18] The spectra suggest that the first protonation step involves the NH_2 group, while the second step involves the NH bound to cyclodextrin. In fact, a downfield shift of the 5A proton has been observed in other derivatives[15,18] when the NH bound to the cavity was protonated. As described above for the ^{13}C NMR spectra, a partial overlapping of the first two protonation steps is suggested, since a slight downfield shift of the 5A proton after the first protonation is also observed.

Since the ^{1}H and ^{13}C NMR spectra were independently assigned by a heterocorrelation spectrum carried out for the unprotonated species, we verified the agreement of the above described assignments, at least for the unprotonated species. The thermodynamic data concerning the protonation and the copper(II) complexation of CDdien are summarized in Table 2, where the data of dien (diethylenetriamine) are also reported for comparison. Furthermore, in order to have a more strict comparison, the *N*-methyl derivative was added as well, but unfortunately only the free energy data are available. As observed for other amino-derivatives of cyclodextrin[15,18] the cavity disturbs the proton complex formation, since the protonation constants all have lower values. Interestingly, with respect to dien, CDdien always shows a more favourable entropic contribution and a less favourable enthalpic contribution. It is just this exceptionally high entropic contribution that permits CDdien to show a low decrease in basicity with respect to dien, in spite of the very high decrease in the enthalpic change. In order to explain such behaviour, let us first of all look at the influence of the protonation state of such molecules on the solvent organization. If we look at the usual entropy changes associated with the protonation of triamines, we see that while the value concerning the first protonation is positive, the second is small (close to zero) and generally negative and the third is negative.[33] This data suggests that solvent organization is higher in the unprotonated amine, the lowest in the mono- and di-protonated species, and increases again when forming the three-protonated species. Since it is known that an electric charge in water is surrounded by a certain number of water molecules, one could expect that the entropy changes concerning all the protonations should be negative. To explain the experimental behaviour we must invoke a stronger solvent organization in the uncharged amine, and such organization is what is commonly called the 'hydrophobic effect'. Thus, the positive charge on the amino group has not only the effect of organizing water molecules around it, but, what is more relevant, is that it destroys the hydrophobic organization of the uncharged species by making such a molecule more hydrophilic and less hydrophobic. The successive protonations make a species that is neither sharply hydrophilic nor hydrophobic, and consequently, organizes fewer water molecules. In such a hypothesis, it is the large size of CDdien, in comparison with the other commonly studied triamines, that gives rise to the exceptionally high entropic contribution. A different source of entropic gain may be ascribed to the solute (CDdien) conformation. Though the NMR spectra gives no evidence of dien moiety inclusion inside the cavity, interactions (hydrogen bonds) of unprotonated nitrogen atoms of the chain with the 6-OH of the upper rim of the cavity may be hypothesized and consequently the chain

Table 2 Thermodynamic parameters[a] for proton and copper(II) complexes of CDdien and dien at 25 °C and I = 0.1 M

Species	log K	$\Delta G°$/kcal mol^{-1}	$\Delta H°$/kcal mol^{-1}	$\Delta S°$/cal mol^{-1} deg^{-1}	Ref.
CDdien + $H^+ \rightleftharpoons$ [(CDdien)H]$^+$	9.41(1)	−12.83	−10.1(5)	9(2)	*b*
CDdien + $2H^+ \rightleftharpoons$ [(CDdien)H_2]$^{2+}$	16.82(1)	−22.93	−17.1(6)	19(2)	*b*
CDdien + $3H^+ \rightleftharpoons$ [(CDdien)H_3]$^{3+}$	20.79(7)	−28.34	−22.9(7)	18(2)	*b*
CDdien + $Cu^{2+} \rightleftharpoons$ [Cu(CDdien)]$^{2+}$	13.79(1)	−18.80	−9.64(8)	30.7(2)	*b*
dien + $H^+ \rightleftharpoons$ [(dien)H]$^+$	9.84	−13.29	−11.2	7	33
dien + $2H^+ \rightleftharpoons$ [(dien)H_2]$^{2+}$	18.86	−25.58	−23.2	8	33
dien + $3H^+ \rightleftharpoons$ [(dien)H_3]$^{3+}$	23.09	−35.29	−34.4	3	33
dien + $Cu^{2+} \rightleftharpoons$ [Cu(dien)]$^{2+}$	15.9	−21.58	−18.0	12	33

[a] 3σ in parentheses. [b] This work.

freedom degrees are dramatically reduced. If, as it is likely, the nitrogen protonation breaks down such interactions, the chain should gain conformational freedom by the possibility of assuming different conformations. Such behaviour appears to be confirmed by the NMR results that show a progressive loss of fine structure in the signals due to the methylene protons of the chain following the successive protonation steps of CDdien.

Probably both the considered hypotheses are correct, if we think of the two separate contributions, one from the solute conformation and the other from the solvent organization, though it is not possible to quantify each contribution separately. As regards the copper(II) system, in Table 2 we report the thermodynamic data for the [Cu(CDdien)]$^+$ complex formation, the only species necessary to describe the titration curve for this system.

A decrease in the enthalpic gain and an exceptionally high entropic contribution is observed. Just as in the case of protonation, complexation with nitrogen atoms could break down the hypothesized interactions of the side-chain with the CD upper rim, in keeping with the $-\Delta H°$ value for the complexation reaction being smaller than that of the dien ligand. Furthermore, the chain should gain conformational freedom and consequently the positive entropic contribution for the complexation reaction [large in comparison with the dien copper(II) complexation] can be explained.

In order to characterize the copper(II) CDdien complex, the system was also investigated by EPR spectroscopy and compared with the dien–Cu(II) system. The magnetic parameters found at pH 6.5 [$g_{\|}$ = 2.236 (2); $A_{\|}$ = 0.0187 (2) cm^{-1}] suggest a square planar coordination of the [Cu(CDdien)]$^{2+}$ complex, with the three nitrogen atoms and a water oxygen in the equatorial plane. The slight increase in $g_{\|}$ and decrease in $A_{\|}$ in comparison with the [Cu(dien)]$^{2+}$ complex [$g_{\|}$ = 2.229 (2), $A_{\|}$ = 0.0189 (2) cm^{-1}] can be ascribed to the bulkiness of the CD cavity which produces a slight distortion of the square planar arrangement as typically observed for copper(II) complexes of CD derivatives.[15,18]

Conclusions

The determination of the enthalpic and entropic changes accompanying both the protonation and the copper(II) complexation of the CDdien ligand allowed us to ascertain the influence of the CD cavity on these processes. Furthermore, the ^{13}C NMR titration showed an interesting case of partial overlapping between the protonation of the two different sites in the molecule. This last result appears connected with the cavity influence, through the modification of the basicity of the nitrogen atoms.

Acknowledgements

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