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Thermodynamics of the interaction of cyclodextrins with aromatic and α,ω-amino acids in aqueous solutions: a calorimetric study at 25°C

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Abstract

The interaction in water of α- and β-cyclodextrins with L-phenylalanine, L-tyrosine, L-tryptophan, and L-histidine has been studied calorimetrically at 25°C in pure water and in a phosphate buffer (pH 11.3). The interaction in water of α-cyclodextrin with some α,ω-amino acids was also studied. When a complex forms, calorimetry allows the calculation of both the enthalpy and the association constant, from which the free energy and the entropy of the process can be obtained. Aromatic amino acids form 1:1 inclusion complexes, characterized by low values of the association constants. The association occurs through the insertion of the guest's aromatic ring into the host's cavity, and is stronger at pH 11.3 than in pure water. For α,ω-amino acids the association constants increase with increasing lengths of the alkyl chains between the functional groups. For this class of substances the association is supposed to occur through an interaction mechanism different from inclusion, which involves mainly the exterior of cyclodextrin. The analysis of the signs and values of the thermodynamic parameters obtained permits hypotheses to be made about the forces involved in the association process.

Keywords: Cyclodextrins; Calorimetry; Inclusion; Amino acids

1. Introduction

The most important property of cyclomalto-oligosaccharides (cyclodextrins) is their capability of forming complexes with a great variety of organic substances either in

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solution or in the solid state [1–3]. Notwithstanding the great number of papers on these complexes, much remains to be clarified about the forces involved in these interaction processes [4,5].

The smallest of these cyclodextrins, cyclomaltohexaose (αCD), is the most interesting. In the solid state it has two water molecules entrapped in the cavity, hydrogen-bonded to each other and to two glucopyranose rings [6]. These water molecules relax to the bulk when an inclusion complex forms. At the same time αCD undergoes a conformational transition from a "tense" to a "relaxed" conformation [7]. Cyclomaltoheptaose (βCD) differs from αCD mainly in the size of the cavity, being constituted by seven glucopyranose rings. No conformational transition has been observed for βCD, according to crystallographic studies, which show a more symmetrical conformation with 6.5 water molecules in the cavity [3]. There are few hypotheses concerning the forces involved in these inclusion processes, and many problems concerning the mechanism and the changes experienced by water in the hydration shells of the "guest" and "host" molecules are still unresolved.

In preceding papers we have reported on the binary aqueous solutions of αCD [8] and on its interaction with hydroxylated substances [8,9], alkylureas [10], amino acids [11,12], and other small molecules [13,14]. Our present contribution continues the program aimed at understanding the forces involved in the interaction of cyclodextrins with some amino acids in aqueous solutions. A calorimetric study, at 25°C, is here reported on the interaction of αCD and βCD with the aromatic amino acids L-phenylalanine, L-histidine, L-tyrosine, and L-tryptophan, in water and in a phosphate buffer, pH 11.3. The interaction of αCD with the following amino acids in water was also investigated: 11-amino-undecanoic, 8-amino-octanoic, DL-2-amino-octanoic, 6-aminohexanoic, 5-aminopentanoic, and L-2,6-diaminohexanoic. The aim of this study is to analyse the role of the functional groups in the inclusion process, depending on their position and on their ionization degree. Beyond the detection of the thermal effect, calorimetry permits one to know whether an association process occurs, and, in this case, to evaluate its equilibrium constant. From that, the free energy and entropy of the process can be derived.

2. Experimental

Materials.—The α- and β-cyclodextrins and the amino acids employed were purchased from Sigma and from Merck. The optical rotations of αCD and βCD were in agreement with those reported in the literature. Solutions were prepared by weight using doubly distilled water.

For solutions at pH 11.3, a NaH_2PO_4/NaOH buffer was employed. The choice of this buffer is determined by the need to have anions not interfering with the inclusion process. It is reported in the literature that phosphate and sulphate anions satisfy this requirement in the pH range 2–11 [15].

Calorimetry.—The values of the experimental heats of mixing, ΔH^{mix}, of two binary solutions containing any one of the solutes were determined at 25°C by means of an LKB 10700-1 flow microcalorimeter and by a Thermal Activity Monitor (TAM) from

Thermometric. Experimental details are extensively reported in preceding papers [8–11,16–20].

Treatment of the data.—Under the assumption that a 1:1 complex forms, the association process can be represented as follows:

$$CD + L = CD \cdot L \tag{1}$$

where CD indicates either of the cyclodextrins and L any of the guest substances employed. The enthalpy of formation of the complex, ΔH^*, is related to the heat of mixing of two binary solutions, ΔH^{mix}, and to the heats of dilution experienced by the two solutes, ΔH^{dil}, as follows [21]:

$$\Delta H^* = \Delta H^{mix}\left\{\left[(m_{ix})(m_{iy})\right] \rightarrow (m_x, m_y)\right\} - \Delta H^{dil}(m_{ix} \rightarrow m_x) - \Delta H^{dil}(m_{iy} \rightarrow m_y) \tag{2}$$

where m_{ix}, m_{iy}, m_x, and m_y are the initial and final molalities of the x and y solutes. The determination of the ΔH^* value then requires the knowledge of the heats of dilution of the binary solutions. These are known from the following relation:

$$\Delta H^{dil}(m_i \rightarrow m) = h_{xx} m(m - m_i) + h_{xxx} m\left(m^2 - m_i^2\right) + \ldots \tag{3}$$

where ΔH^{dil} (J kg^{-1}) is the heat of dilution of a solute from the initial (m_i) to the final molality (m). The h coefficients appearing in eq (3) are determined by the experimental heats of dilution of binary solutions. To fit the data, a least-squares method was employed choosing the polynomial of highest degree, whose coefficients still exceed their own 95% confidence limits. The standard molar enthalpy of association of the guest molecule, ΔH_a^o, is obtained from:

$$\Delta H_a^o = \Delta H^*/m_{CD \cdot L} \tag{4}$$

where $m_{CD \cdot L}$ is the molality of the adduct formed in the final solution. In the presence of a large excess of the guest molecule $m_{CD \cdot L} \rightarrow m_{CD}$, and at saturation:

$$\Delta H_a^o = \left(\Delta H^*/m_{CD}\right)_{sat} \tag{5}$$

ΔH^*, normalized to the total molality of the dextrin, m_{CD}, can be expressed as a function of the actual molality of the guest molecule, m_L^f, to ΔH_a^o, and to the apparent association constant, K_a', as follows [22]:

$$\Delta H^*/m_{CD} = m_L^f \Delta H_a^o K_a'/\left(1 + K_a' m_L^f\right) \tag{6}$$

or in a linear form, useful for fitting the data:

$$m_{CD}/\Delta H^* = 1/\Delta H_a^o + 1/\Delta H_a^o K_a' m_L^f \tag{7}$$

For each value of ΔH^* the actual concentration of the guest molecule is given by:

$$m_L^f = m_L - \Delta H^*/\Delta H^*(sat) m_{CD} \tag{8}$$

where m_L is the total stoichiometric molality of the guest. The standard enthalpy and the association constant are obtained from eqs (7) and (8) by an iterative least-squares method. The iterations are continued until two successive values of ΔH_a^o differ by less

than 2%. The values of the free energy and entropy were then obtained through the usual thermodynamic relations:

$$\Delta G_a^{o\prime} = -RT \ln K_a'; \quad T\Delta S_a^{o\prime} = \Delta H_a^o - \Delta G_a^{o\prime} \tag{9}$$

It is to be noted that the lack of information about activity coefficients leads to the evaluation of apparent association parameters.

3. Results

In Table 1 the h_{xx} coefficients, as evaluated from eq (3), are given for binary aqueous solutions of L-tryptophan, L-histidine, L-phenylalanine, L-lysine, and for αCD at pH 11.3. For βCD the dilution of the binary solutions gave a null thermal effect. In the same table, the literature value of h_{xx} for αCD in pure water is also reported. These coefficients are necessary to evaluate the ΔH^* values. For the other amino acids studied, the heats of dilution were subtracted instrumentally.

In Table 2 the thermodynamic parameters (association constant, enthalpy, free energy, and entropy) are shown for the inclusion process involving either of the two cyclodextrins and L-histidine, L-tyrosine, L-tryptophan, and L-phenylalanine in pure water and at pH 11.3. For the phenylalanine–αCD system, the data obtained at pH 13.6 are also reported. Note that the data in water for this last system, evaluated under the present experimental conditions, are in very good agreement with those reported in the literature obtained from thermodynamic and nonthermodynamic experiments [12,25].

In Table 3 the same thermodynamic parameters are reported for the interaction in pure water of αCD with the following acids: 11-amino-undecanoic, 8-amino-octanoic, 6-aminohexanoic, 5-aminopentanoic, DL-2-amino-octanoic, and L-2,6-diaminohexanoic (L-lysine). Only the last substance was used to carry out the calorimetric titration with βCD.

Table 1
Values of the pairwise enthalpic interaction coefficients, h_{xx}, for the binary aqueous solutions of some of the amino acids employed and for αCD at 25°C

Substance	h_{xx} [a,b]
αCD (pH 7) [c]	−3920(65)
αCD (pH 11.3)	−3348(219)
βCD	$\Delta H^{dil} = 0$
L-Phenylalanine	1140(30)
L-Lysine [d]	1533(37)
L-Histidine [e]	−622(14)
L-Tryptophan [e]	2885(172)

[a] Units: J mol^{-2} kg.
[b] Figures in parentheses are the 95% confidence limits. For all other amino acids not reported in this table, heats of dilution were instrumentally subtracted.
[c] Ref. [8].
[d] Ref. [23].
[e] Ref. [24].

Table 2
Thermodynamic parameters for the association between αCD and βCD and the aromatic amino acids reported, at different pH, at 25°C

System	K'_a [a,b]	$-\Delta H^{o}_a$ [b,c]	$-\Delta G^{o\prime}_a$ [c,d]	$T\Delta S^{o\prime}_a$ [c,e]
L-Phenylalanine–αCD				
Water	15(1)	7.0(0.4)	6.7(0.2)	−0.3(0.6)
pH 11.3	25(1)	13.0(0.4)	8.0(0.1)	−5.0(0.5)
pH 13.6	8(3)	2.1(0.6)	5(1)	3.0(1.6)
L-Phenylalanine–βCD				
Water	18(3)	9(1)	7.2(0.4)	−1.8(1.4)
pH 11.3	106(12)	5.2(0.3)	11.6(0.3)	6.4(0.6)
L-Tyrosine–αCD				
Water		N.A. [f]		
pH 11.3		N.A. [f]		
L-Tyrosine–βCD				
Water		— [g]		
pH 11.3	147(6)	6.7(0.2)	12.4(0.1)	5.7(0.3)
L-Histidine–αCD				
Water	9(4)	14(6)	5.4(1)	−8.6(7)
pH 11.3	12(1)	3.2(0.3)	6.2(0.2)	3.0(0.5)
L-Histidine–βCD				
Water		N.A. [f]		
pH 11.3		N.A. [f]		
L-Tryptophan–αCD				
Water	19(4)	9(1)	7.3(0.6)	−1.7(1.6)
pH 11.3	28(1)	8.0(0.2)	8.3(0.1)	0.3(0.3)
L-Tryptophan–βCD				
Water		— [g]		
pH 11.3		— [g]		

[a] kg mol^{-1}.
[b] Figures in parentheses are the standard deviations as obtained by fitting the data to eqs (7) and (8).
[c] kJ mol^{-1}.
[d] Errors are half the range obtained from the extreme values of the association constants.
[e] Errors are the sum of the errors on free energy and enthalpy.
[f] N.A. means that measurements have been performed, but no association was detected.
[g] The sign means that measurements have not been performed for the very low solubility of both substances.

The ΔH^{o}_a and K'_a values are evaluated through an iterative least-squares method. The stoichiometry was assumed to be 1:1; imposing different stoichiometries did not lead to conclusive results. Association enthalpies are negative for both series of substances employed. For α,ω-amino acids they increase with increasing length of the alkyl chain between the amino and carboxy groups. For the aromatic amino acids the values of the association constants are low in pure water, but they increase on passing to pH 11.3.

4. Discussion

Inclusion complexes with aromatic amino acids.—Considerable attention has been directed to the inclusion complexes of cyclodextrins with aromatic amino acids because

Table 3
Thermodynamic parameters in water for the association between αCD and the reported amino acids at 25°C

Amino acid	K'_a [a,b]	$-\Delta H^\circ_a$ [b,c]	$-\Delta G^{\circ\prime}_a$ [c,d]	$T\Delta S^{\circ\prime}_a$ [c,e]
11-Amino-undecanoic (pH 6.7)	2200(200)	26.6(0.5)	19.0(0.2)	−7.6(0.7)
8-Amino-octanoic (pH 7.1)	76(3)	14.8(0.4)	10.7(0.1)	−4.1(0.5)
2-Amino-octanoic (pH 7.8)	630(30)	17.7(0.3)	16.0(0.2)	−1.7(0.5)
6-Aminohexanoic (pH 7.0)	22(6)	1.5(0.3)	7.6(0.7)	−6(1)
2-Aminohexanoic [g] (L-norleucine)	46(2)	8.9(0.3)	9.5(0.1)	0.6(0.4)
5-Aminopentanoic		N.A. [f]		
2-Aminopentanoic [g] (L-norvaline)	12(7)	2(1)	6(2)	4(3)
2,6-Diaminohexanoic (lysine)–αCD				
water (pH 10)	5(2)	4(1)	4(1)	0(2)
pH 11.3	10.7(0.3)	18.1(0.5)	5.8(0.1)	−12.4(0.6)
2,6-Diaminohexanoic (lysine)–βCD				
water (pH 10)		N.A. [f]		
pH 11.3	15(2)	14(1)	6.7(0.4)	−7.3(1.4)

[a] kg mol^{-1}.
[b] Figures in parentheses are the standard deviations as obtained by fitting the data to eqs (7) and (8).
[c] kJ mol^{-1}.
[d] Errors are half the range of the extreme values of the association constants.
[e] Errors are the sum of the errors on free energy and enthalpy.
[f] No association detected.
[g] Ref. [11].

of their features similar to enzyme–substrate complexes [12,25,26]. The prevailingly hydrophobic cavity of the macrocycles leads to association with the guest molecule: for this reason cyclodextrins are good models for studying the molecular and thermodynamic basis in protein–ligand interactions.

The shape-matching between the host cavity and the guest inserting group should be one of the factors leading to the association between the two molecules. In fact, the data reported in Table 2 show that the dimensions of the cyclodextrin ring greatly influence the formation of the complexes. Literature data suggest that the benzene ring penetrates shallowly into the αCD cavity, because the diameter of the dextrin is too small to give a deep and tight inclusion. The internal diameters of αCD and βCD are ca. 5 and 7 Å [5], respectively, while the diameter of a benzene ring, including van der Waals radii, is 6.8 Å. Then, the αCD cavity is too small to accommodate the benzene ring, while βCD has the best dimensions for a stronger association. The values of the association constants are very low, and similar to those derived for substances bearing very short alkyl chains. Histidine forms a complex with αCD, characterized by the smallest K'_a value, while it does not with βCD, probably because the small imidazole ring would be statistically disordered in the interior of the larger cavity, i.e., the dimensions of the imidazole ring

are determining in the formation of the complexes. By contrast, tyrosine does not associate with αCD, neither in water nor in a buffer, thus indicating that the presence of the hydroxyl group on the benzene ring heavily disturbs the association. This is in agreement with previous findings on positional isomers of alkanols, according to which the hydroxyl group is not included into the cavity, but rather halts the further penetration of the alkyl chain [9]. Instead, the association of tyrosine with βCD at pH 11.3 is characterized by the largest constant found for these complexes. At this pH, the hydroxyl group is ionized and, probably, it is the phenolate ion inside the cavity that increases the association with respect to phenylalanine. These results agree with NMR data reported in the literature indicating that there is no detectable interaction of αCD with tyrosine, which instead forms a complex with βCD [26].

In a previous paper concerning the inclusion of α-amino acids bearing unsubstituted alkyl chains in αCD, it was found that the association constants are smaller than those characterizing the interaction with 1-alkanols having comparable alkyl chains: the presence of the zwitterion lowers the value of the constant by ~ 50% [11]. Then, the strength of the association is highly dependent on the nature of the hydrated functional group. With varying pH there will be a change in the nature and solvation of the functional groups. The data in Table 2 show that at pH 11.3, where the amino group is the prevailing species, the association constants increase. Phenylalanine associates with both αCD and βCD: at pH 11.3, the association constant of the latter interaction increases by an order of magnitude. At pH 13.6, the secondary hydroxyl groups of αCD (pK 12) are dissociated [12,27], and they interact repulsively with the carboxylate anion of the amino acid. This explains the lower values of the association constant and enthalpy found for the system αCD–phenylalanine.

The values of the association constants and enthalpies for the complexes between tryptophan and αCD are similar, in pure water and at pH 11.3. As often emphasized, these complexes form through the shallow inclusion of the aromatic ring into the cavity, and their association parameters are altered by the ionization state of the amino acid functional group. For tryptophan, the presence of a system constituted by two condensed aromatic rings removes the functional groups from the ring of the cyclodextrin. Consequently, the interactions with the hydroxyl groups of the dextrin rim are weaker (for instance, with respect to phenylalanine), independent of the ionization state of the functional group, and without significant influence on enthalpy.

Complexes with α,ω-amino acids.—From preceding studies carried out in this laboratory on mono- and poly-hydroxylated substances reacting with αCD, it was inferred that it is the alkyl chain that penetrates the cyclodextrin cavity, while the functional group forms hydrogen bonds with the external hydroxyl groups of the macrocycle [8,9]. When the hydroxyl group occupies more-central positions, it prevents the remainder of the alkyl chain from penetrating the cavity. When the hydroxyl groups are confined to the extremities of the alkyl chain, as in the case of α,ω-diols, a more complex process occurs. It is unlikely that the complex forms by the introduction into the cavity of one of the functional groups. Instead, we suggest that the α,ω-diol caps the cavity, with the two hydroxyl groups forming hydrogen bonds with the external hydroxyl groups of cyclodextrin. The alkyl chain between the functional groups would point toward the interior of the cavity. This capping mechanism has already been

proposed by other authors for the interaction of these macrocycles with some bifunctional substances [28].

We think that the data shown in Table 3 are such that the same mechanism can be invoked for the α,ω-amino acids here studied, owing to the presence of the functional groups at the extremities of the alkyl chain. As emphasized before, the inclusion of these groups into the cavity of the dextrin can be excluded. At increasing length of the alkyl chain the association constant increases, indicating the optimization of the interactions with the cavity. When the alkyl chain is not long enough, as for 5-aminopentanoic acid, interactions are very weak and association does not occur. Preceding studies on the complexation of αCD with α-amino acids [11] have shown that the unsubstituted alkyl chain penetrates into the macrocycle cavity, leading to complexes stronger than those formed by the α,ω-isomers. 2-Aminopentanoic acid (norvaline) associates detectably with αCD, while the complex formed by 2-aminohexanoic acid (norleucine) is characterized by a higher K'_a value with respect to the α,ω-isomer. Even greater is the difference in the values of the constants for 8-amino-octanoic acid and 2-amino-octanoic acid (Table 3), which differ by an order of magnitude. These findings suggest that the alkyl chain does not penetrate the cavity with one of the terminal groups, but that association occurs prevailingly through interactions with the exterior of the cyclodextrin. For 11-amino-undecanoic acid an alternative explanation is possible, namely the formation of a 2:1 αCD–amino acid complex. The alkyl chain could be long enough to be included into one αCD molecule, with the polar ends "outside" and available for the interaction with the exterior of another molecule of αCD. However, there is insufficient evidence to favour the latter hypothesis.

Lysine (2,6-diaminohexanoic acid) is at the same time an α- and ω-amino acid. At pH $\sim$ 10 it is 50% in the zwitterionic form in aqueous solutions, with the terminal amino group ca. 66% ionized: at this pH it forms a weak complex with αCD only. On the other hand, lysine forms complexes with both αCD and βCD at pH 11.3, where the percentage of zwitterion and protonated amino group is reduced. The association constant increases (see Table 3), and entropy and enthalpy are large and negative. Under the hypothesis that association occurs as for α,ω-amino acids, the values of these parameters could indicate ameliorated hydrogen bonds of the terminal groups with the hydroxyl groups of the exterior of cyclodextrins.

It is commonly believed that hydrophobic interactions are the main driving forces for complex formation. However, they often do not play the major role, as evidenced by the positive or negative values of the entropies. There is an enthalpy–entropy compensation effect, a phenomenon frequently observed in water and generally ascribed to the modification of the solvent in the hydration spheres of the interacting substances [29–31]. This compensation leads to the same free energy for different compounds, even though enthalpies and entropies are different. This means that the affinities are determined primarily by the contributions of the parts that are included into the cavity, whereas the other parts of the molecules influence only the enthalpies and entropies. As a conclusion, in some cases there is a significant contribution from the classical hydrophobic effect (i.e., a positive ΔS contribution), while in some other cases this contribution is masked by a predominant binding force characterized by negative ΔH and ΔS.

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