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Thermodynamics of inclusion complexes of natural and modified cyclodextrins with propranolol in aqueous solution at 298 K

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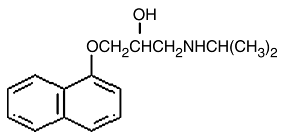
Abstract—The association constant, standard Gibbs energy, enthalpy and entropy for formation of inclusion complexes of propranolol, a β-blocker, with various natural and modified cyclodextrins have been determined by calorimetry at 298 K. Both natural and methyl-modified α-cyclodextrins do not form complexes, while β- and γ-cyclodextrins do. Complexing ability of 2-hydroxypropyl-β-cyclodextrin depends on the average substitution degree. For γ-cyclodextrin, hydrophobic interactions play the major role in binding the guest. The association of natural and modified β-cyclodextrins is ruled by van der Waals interactions and hydrogen bonding because of the tighter fit of the guest into the cavity. Decreasing pH determines increasingly negative values of the association enthalpies.

1. Introduction

Natural cyclodextrins (CDs) are cyclic oligomers of α-D-glucose characterized by a fairly polar exterior and by a relatively nonpolar cavity. The most important property of CDs is their ability to form complexes with a great variety of organic substances in solution and in the solid state.[1–5] The physicochemical properties of the included substances are altered upon complexation and cyclodextrins are widely used for enhancement of aqueous solubility, stability and bioavailability of apolar drugs.[6–9] Cyclodextrin derivatives have been prepared to modify the inclusion ability and to improve the properties of the natural macrocycles as drug carriers.[10] From a pharmaceutical point of view, CD/drug inclusion complexes reduce the concentration of free drug, with the consequent reduction of side effects. Since how strongly the drug is held by cyclodextrin determines the bioavailability and therapeutic effects, it is important to know the association constants for the inclusion process.[10]

Extensive studies on the complexes of cyclodextrins exist: however, it is still a matter of debate as to which contribution is responsible for the overall free energy of association. Complexation is determined by hydrophobic interactions, hydrogen bonds, van der Waals interactions, conformational energy, dipole–dipole and ion–dipole interactions[1,11–13] and the rearrangement of water molecules originally surrounding both cyclodextrin and guest molecule.[14–16] The present contribution is aimed at understanding the factors determining the formation of the complexes between cyclodextrins and substances of pharmacological interest in aqueous solution. To that end, the interaction of α-cyclodextrin (αCD), β-cyclodextrin (βCD) and γ-cyclodextrin (γCD), 2-hydroxypropyl-β-cyclodextrin (HPβCD) and methyl-α-cyclodextrin (MαCD) with propranolol, a β-blocker, is investigated by a calorimetric method at 298 K, in water and in acidic solution, pH 1.3 and 3.0.



Propranolol

The role of the cavity size in the formation of the complexes with propranolol will be analyzed. The mode of inclusion will be examined through the interaction of iso-

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Keywords: Cyclodextrins; Propranolol; Inclusion complexes; Isothermal calorimetry; Thermodynamic parameters.

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propylamine and 1-naphthylacetic acid with α-, β- and γ-cyclodextrins. The D-isomer of propranolol is nearly 40 times more potent as a β-blocker than the L-isomer and appears to mediate the antiarrhythmic and antihypertensive activity of the racemic mixture, while only L-propranolol appears to be beneficial in treating angina pectoris. Differential complexation of the stereoisomers has been investigated by liquid chromatography employing β-cyclodextrin-bonded media.[17] Resolution values were greater than 1, thus indicating a moderate resolution.[18] Other authors investigated the complexes between cyclodextrins and propranolol stereoisomers by fluorescence measurements.[19,20] Here, we examine whether differences in the thermodynamic parameters for association between cyclodextrins and the two stereoisomers of propranolol can be detected by calorimetry.

2. Results

In Table 1, the thermodynamic parameters (association constant, standard Gibbs energy, enthalpy and entropy) for the 1:1 inclusion complexation are reported. α-Cyclodextrin does not associate with the guest molecule, nor does methyl-α-cyclodextrin. At the lowest pH, 1.3, where enthalpy is largest, experiments were carried out with both L- and D-propranolol. β- and γ-cyclodextrins form complexes with the two stereoisomers characterized by very similar association constants but with different contributions from the enthalpic and entropic terms. For β-cyclodextrin, both enthalpy and entropy are negative and large, while for γ-cyclodextrin, enthalpy is still negative, but small, and entropy is positive. Enantioselectivity was not detected: within the experimental error, the association constants are the same for the two enantiomers of propranolol and low, not allowing to distinguish between the two forms. A racemic mixture was, then, employed for measurements at higher pH and in pure water, with the intent of using the enantiomers in the case high association constants would be obtained. At pH 3.0, the complex formed by β-cyclodextrin is characterized by less negative enthalpy and entropy, while the constant is higher than that at pH 1.3. In water (pH 5.8), the complexes formed are characterized by even smaller negative enthalpies and positive entropies. In these experimental conditions, the constants for βCD and γCD are well differentiated.

In the literature,[19] the apparent association constants for the association of β-cyclodextrin with propranolol stereoisomers were evaluated through fluorometric methods.[19] In the absence of achiral modifiers, at pH 2.5, the values of the constants are 166 ± 13 and 138 ± 26, in good agreement with the value (111 ± 8) presently obtained for the mixture of (±)-propranolol interacting with βCD at pH 3.0.

The part of propranolol which includes into the cavity of a cyclodextrin was identified through the study of the interactions with isopropylamine and 1-naphthylacetic acid (Table 1). The former substance does not associate with α-, β- and γ-cyclodextrins, while 1-naphthylacetic acid forms complexes with β- and γ-cylodextrins. The association was studied at elevated pH (11.3) because of the very low solubility of the acid at lower pHs.

Table 1. Thermodynamic parameters for the association of various cyclodextrins with propranolol in aqueous solutions at 298.15 K

System	K'_{a} [a,b]	$-\Delta_{\mathrm{a}}H^{\circ\prime}$ [b,c]	$-\Delta_{\mathrm{a}}G^{\circ\prime}$ [c,d]	$T\Delta_{\mathrm{a}}S^{\circ\prime}$ [c,e]
H_2O				
(±)-Propr + αCD		N.A.[f]		
(±)-Propr + βCD	239 ± 41	9 ± 1	13.6 ± 0.4	5 ± 1
(±)-Propr + γCD	88 ± 30	1.6 ± 0.4	11.1 ± 0.9	9 ± 1
(±)-Propr + MαCD		N.A.[f]		
(±)-Propr + HPβCD (DS[g] = 6.3)	120 ± 30	7 ± 1	11.9 ± 0.6	5 ± 2
(±)-Propr + HPβCD (DS[g] = 3.0)	264 ± 23	6.4 ± 0.3	13.8 ± 0.2	7.4 ± 0.5
H_3PO_4 0.05 mol kg^{-1}, pH 3.0				
(±)-Propr + βCD	111 ± 8	14.6 ± 0.8	11.7 ± 0.2	−3 ± 1
H_3PO_4 0.5 mol kg^{-1}, pH 1.3				
D-Propr + αCD		N.A[f]		
L-Propr + αCD		N.A.[f]		
D-Propr + βCD	64 ± 8	24 ± 2	10.3 ± 0.3	−14 ± 2
L-Propr + βCD	64 ± 10	28 ± 4	10.3 ± 0.4	−18 ± 4
D-Propr + γCD	78 ± 8	4.1 ± 0.2	10.8 ± 0.3	6.7 ± 0.5
L-Propr + γCD	79 ± 11	3.2 ± 0.2	10.8 ± 0.3	7.6 ± 0.5
Phosphate buffer 0.5 mol kg^{-1}, pH 11.3				
1-Naphthylacetic acid + βCD	702 ± 73	8.9 ± 0.3	16.2 ± 0.3	7.3 ± 0.6

[a] kg/mol.

[b] Errors reported are the standard deviations as obtained by fitting the data to Eq. 2.

[c] kJ/mol.

[d] Errors are half the range of $\Delta_{\mathrm{a}}G^{\circ\prime}$ calculated from the upper and lower error in K'_{a}.

[e] Errors are the sum of the errors on free energy and enthalpy.

[f] N.A. means that experiments have been performed, but no association was detected.

[g] Degree of substitution.

2-Hydroxypropyl-β-cyclodextrin was studied at two different substitution degrees, 6.3 and 3.0. In both cases, an inclusion complex with the racemic mixture of propranolol is formed. Enthalpy is the same, while the association constant is higher for the smaller substitution degree.

3. Discussion

As the data reported in Table 1 show, at pH 1.3 stereoisomers of propranolol do not form complexes with α-cyclodextrin, but they associate with β- and γ-cyclodextrins with almost the same association constant. Complexation of a hydroxyalkylated guest molecule with a cyclodextrin occurs by inclusion of the alkyl chain into the hydrophobic cavity.[21–24] Propranolol has two possible points to interact with the cavity of a cyclodextrin, the isopropylamine group and the naphthyl group. Calorimetric titrations of the three natural cyclodextrins with isopropylamine and 1-naphthylacetic acid proved that formation of a complex is achieved only with the latter substance interacting with β- and γ-cyclodextrins, through the insertion of the naphthyl group into the cavity. Probably, the naphthyl group is too large to be included into the cavity of the smallest α-cyclodextrin, whose internal diameter is about 4.9 Å. That is not unexpected, literature data report that the benzene ring penetrates shallowly into the narrower αCD cavity, whose diameter is too small to give a deep and tight inclusion with the guest molecule, while fitting deeper into the wider cavity of βCD.[25,26] β-Cyclodextrin, whose internal diameter is about 7 Å, seems to have the best dimensions towards the association with propranolol.

Association parameters greatly vary depending on the medium employed. In the presence of 0.5 mol L^{-1} phosphoric acid, pH 1.3, enthalpies and entropies are both negative and very large, meaning that the contributions from van der Waals interactions and hydrogen bonds are prevailing.[2,11,23] Entropy is large and negative. Hence, the loss of degrees of freedom occurring upon inclusion overwhelms the contribution due to the relaxation of ordered water molecules from the cavity and from the hydration shell of the guest to the bulk. Consequently, the association constants for the L and D stereoisomers are small, similar to those derived for substances bearing short alkyl chains.

Complexation by γ-cyclodextrin is characterized by negative and small enthalpies and by positive entropies, in water and at pH 1.3. With respect to βCD, the internal diameter of γ-cyclodextrin is rather large, so that the naphthyl group will adapt loosely to the cavity with a smaller negative enthalpic contribution from van der Waals forces. However, the association constant is not very different from that of the smaller cyclodextrin because of the positive entropic term originating from the relaxation to the bulk of water molecules from the cavity and the hydration shell of the including guest.

Large changes occur in the values of association enthalpies and entropies at decreasing pH (see Table 1). The former quantity becomes markedly more negative and the latter less positive or even changes sign passing from positive to negative. Those differences cannot be ascribed to changes in the ionization state of the secondary amino group, that is almost fully protonated at the pH used.[27] At the moment, a valid explanation for those differences cannot be proposed. However, the extent of alteration induced in the structure of water and in the hydration shells of the interacting substances by the different nature of the solvent medium could be hypothesized to play a major role in determining the values of the thermodynamic parameters.

Thermodynamic association parameters are influenced by the average degree of substitution (Table 1). In fact, the inclusion process involving the hydroxypropylated cyclodextrin having a larger substitution degree is characterized by a smaller association constant, probably because of the steric hindrance exerted by a higher number of hydroxypropyl groups. Other similar cases are reported in the literature. For instance, correlations have been found between the stability of inclusion complexes of HPβCD with phenolphthalein and the degree of substitution; in particular, stability constants decrease with increasing degree of substitution probably because of the disturbance of the three-site interaction characteristic for the molecular recognition of phenolphthalein by cyclodextrins.[28,29]

For many drugs, only racemic mixtures are available for clinical use, while the use of pure isomers could ameliorate the therapeutic effect. Cyclodextrins are chiral media, so different association parameters are expected towards the enantiomers of a chiral guest. To separate racemic mixtures, highly specific chromatographic techniques, that used β-cyclodextrin-bonded media, were developed for stereoselective drug separation of a wide variety of clinically relevant cyclic and heterocyclic drugs.[17] Resolution values greater than 1.0 were obtained for propranolol.[18] That pulled us to further investigate the forces determining the formation of the complexes. Propranolol satisfies the requirements necessary for chiral recognition by cyclodextrins: it forms inclusion complexes, and a relatively tight fit exists between the complexed moiety and the cyclodextrin. Beyond that, the chiral centre, namely the carbon bearing the hydroxyl group, is near and interacts with the rim of the cyclodextrin cavity. The data reported in Table 1, relative to βCD and γCD, show that at pH 1.3 the constants for the association of the two isomers are the same, and $\Delta G^{\circ}{}_{\mathrm{D}} = \Delta G^{\circ}{}_{\mathrm{L}}$. The equality of the Gibbs energy for the two enantiomers means that there is no enantioselective binding. On the other hand, computer-generated projections from X-ray crystallographic data of the lowest free energy inclusion complexes of D- and L-propranolol with β-cyclodextrin show that the two guests are placed identically within the cyclodextrin cavity.[18] The structures are overlaid exactly to the point of the chiral carbon atom: however, in the L-propranolol complex the secondary amino group is less favourably positioned for hydrogen bonding to both 2- and 3-hydroxyl groups of the cyclodextrin. That would determine the different retention time of the

D-stereoisomer with respect to L-propranol.[18] The data reported here show that chiral recognition does not occur, probably because the effect involving the amino group is lost in the experimental conditions employed.

To conclude, the thermodynamic parameters relative to the formation of these complexes are determined mainly by the changes experienced by solvent water upon the desolvation of the cavity and dehydration of the including substance.[1–4,11,30,31] The negative enthalpies indicate that the reconstitution of a hydration shell of the complex and van der Waals interactions operating between the included alkyl region and the cavity are the prevailing effects. In some cases, as for γ-CD, there is a significant contribution from the classical hydrophobic interactions (a positive ΔS contribution), while in some other cases, as for β-CD in phosphate buffer, this contribution is overwhelmed by other association forces characterized by negative ΔH and ΔS.

4. Experimental

4.1. Materials

Natural α-, β- and γ-cyclodextrins, as well as propranolol hydrochloride, were purchased from Sigma. The optical rotations of CDs were in agreement with those reported in the literature. Methyl-α-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin were purchased from Cyclolab. The mean substitution degree is 10.5–14.7 for the former and 6.3 and 3.0 for the latter, as determined by NMR.

4.2. Calorimetry

Measurements of the heats of mixing, ΔH_{mix}, of solutions of the cyclodextrins (titrant, 0.01 to 0.08 molal) with solutions of propranolol (titrate, 0.001 to 0.006 molal) were made at 298.15 ± 0.03 K with a Thermal Activity Monitor (TAM) from Thermometric, equipped with a 3 mL titration vessel. Solutions of titrant and titrate to be mixed were made in the same solvent (water or H_3PO_4 0.5 mol kg^{-1}, pH 1.3, or H_3PO_4 0.05, as reported in Table 1). For every calorimetric experiment, the pH value was carefully controlled before and after the association process and found to be invariant. Approximately 30 injections (50 μL each) were made in each experiment, and at least two experiments were performed for each reaction. The estimated uncertainties are 1% in the molalities and 2% in the heat effects. Enthalpies of dilution of the titrant were determined, using the same number of injections and concentrations as in the titration experiments, and were subtracted from the enthalpies of mixing the reactants. The dilution of the titrate in the cell was assumed negligible.

4.3. Treatment of the data

The heats of dilution were fit to the following power-law expansion:[32–34]

$$\Delta H_{\mathrm{dil}}(\mathrm{m^i} \rightarrow \mathrm{m^f}) = h_{\mathrm{xx}}\mathrm{m^f}(\mathrm{m^f} - \mathrm{m^i}) + h_{\mathrm{xxx}}\mathrm{m^f}(\mathrm{m^{f2}} - \mathrm{m^{i2}}) + \ldots\ldots, \tag{1}$$

where ΔH_{dil} (J kg^{-1}) is the heat of dilution of a solute from the initial ($\mathrm{m^i}$) to the final molality ($\mathrm{m^f}$). The enthalpic interaction coefficients, h, appearing in Eq. 1 are used to correct the total enthalpy changes for dilution of the solutes.

Assuming a 1:1 complex is formed, the association process can be represented as:

$$\mathrm{CD} + \mathrm{L} = \mathrm{CD} \cdot \mathrm{L},$$

where CD indicates a cyclodextrin and L a guest molecule. The enthalpy of formation of a complex, ΔH^*, is related to the heat of mixing two binary solutions, ΔH_{mix}, as follows:

$$\Delta H^* = \Delta H_{\mathrm{mix}}\{[(\mathrm{m^i_{CD}})(\mathrm{m^i_L})] \rightarrow (\mathrm{m^f_{CD}}, \mathrm{m^f_L})\} - \Delta H_{\mathrm{dil}}(\mathrm{m^i_{CD}} \rightarrow \mathrm{m^f_{CD}}) - \Delta H_{\mathrm{dil}}(\mathrm{m^i_L} \rightarrow \mathrm{m^f_L}), \tag{2}$$

where $\mathrm{m^i_{CD}}$, $\mathrm{m^i_L}$, $\mathrm{m^f_{CD}}$ and $\mathrm{m^f_L}$ are the initial and final molalities of the cyclodextrin and guest molecule. ΔH^*, normalized to the total molality of the guest, $\mathrm{m_L}$, can be related to the actual molality of the cyclodextrin host molecule, $\mathrm{m^f_{CD}}$, to the standard molar enthalpy of association, $\Delta H^\circ_{\mathrm{a}}$, and to the apparent association constant, K'_{a}, as follows:[35]

$$\Delta H^*/\mathrm{m_L} = (\Delta H^\circ_{\mathrm{a}} K'_{\mathrm{a}} \mathrm{m^f_{CD}})/(1 + K'_{\mathrm{a}} \mathrm{m^f_{CD}}). \tag{3}$$

Eq. 3 can be rewritten in a linear form, more useful for fitting the data, as follows:

$$\mathrm{m_L}/\Delta H^* = 1/\Delta H^\circ_{\mathrm{a}} + 1/(\Delta H^\circ_{\mathrm{a}} K'_{\mathrm{a}} \mathrm{m^f_{CD}}). \tag{4}$$

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

$$\mathrm{m^f_{CD}} = \mathrm{m_{CD}} - [\Delta H^*/\Delta H^*(\mathrm{sat})]\mathrm{m_L}, \tag{5}$$

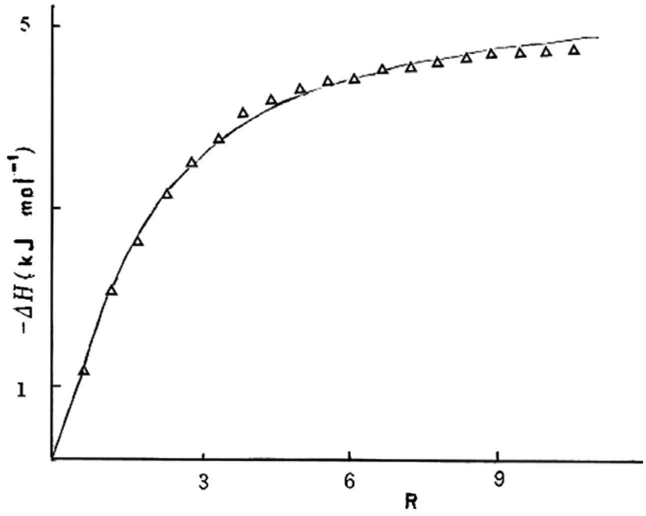

Figure 1. Enthalpies of association, ΔH, as a function of the ratio between the final molalities of hydroxypropyl-β-cyclodextrin (DS = 3) and (±)-propranolol, R = $\mathrm{m_f}$ (HPβCD)/$\mathrm{m_f}$ (propranolol), at 298.15 K. The experimental points are indicated by triangles, while the curve was drawn employing the constant and enthalpy obtained through Eqs. 4 and 5 by the iterative least-squares method described in the text.

where m_L is the total stoichiometric molality of the guest. The standard enthalpy and the constant are obtained from Eqs. 4 and 5 by an iterative least-squares method. The iterations are continued until two successive values of ΔH°_a differ by less than 2%. The values of the free energy and entropy are then obtained through the usual thermodynamic relations. The absence of any information about the activity coefficients leads to the evaluation of association parameters that are thermodynamically not exactly defined. Only an apparent constant, K'_a, can be determined, and consequently the standard free energy and entropy, $\Delta G^{\circ\prime}_a$ and $\Delta S^{\circ\prime}_a$, suffer the same limitations.

If the simple association model discussed above does not describe the studied systems, Eqs. 4 and 5 cannot be used: the least-squares iterative procedure does not converge, and saturation is not easily detected.

In Figure 1, the association enthalpy is reported as a function of the ratio, R, between the final molalities of propranolol and cyclodextrin for (±)-propranolol/HPβCD (DS = 3) system, at 298.15 K in water. The solid line through the points is obtained using the association constant and standard enthalpy evaluated through Eqs. 4 and 5 by the described iterative least-squares method.

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