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Thermodynamic parameters of the formation of a complex between cyclodextrins and steroid hormones

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Abstract

The association constants of four steroids with β-and γ-cyclodextrins were measured in methanol–water (45:55, v/v) and acetonitrile–water (30:70, v/v) by the Hummel–Dreyer method as a function of temperature. Enthalpies and entropies of the complex formation were calculated, as well as enthalpies of partitioning of the solutes from the solution to the stationary phase. The reasons for less negative enthalpies of complex formation and for lower association constants in the acetonitrile–water medium as compared to methanol–water are discussed.

Keywords: Thermodynamic parameters; Complex formation; Cyclodextrins; Steroid hormones; Hormones

1. Introduction

The number of studies on the determination of thermodynamic parameters from chromatographic retention data is increasing continuously. One of these parameters is the association constant of complexes formed in the liquid phase. It has been evaluated chiefly by two methods: the first is based on the change of retention time [1–6], and the second is that of Hummel and Dreyer [7–10]. Association constants of steroid hormones with cyclodextrins (CD) were determined both by the first method [5,6] and by the second [9,10].

In the Hummel–Dreyer method a column is equilibrated with an eluent carrying a solute (substrate) molecule (here a steroid hormone). A sample of ligand (or complexing agent, here CD) is dissolved in the eluent and applied to the column. If sufficient complexation occurs and if the complex and complexing agent move faster than the substrate, the chromatogram will contain a positive peak of the complex and behind it, at the elution position of the free substrate, a negative peak that corrresponds to the amount of substrate consumed to produce the complex. The area of the positive peak is a measure of the concentration of the complex formed. The measurement, by UV absorption, is easier if the complexing agent itself does not absorb light. The amount of the bound substrate is determined by an internal or external calibration technique [11]. The method works well if the rate of complex formation is much higher than the speed of the chromatographic process itself. The method is relatively simple and fast and the phenomenon investigated, namely complexation, can be seen immediately. Another advantage of the method is the very low consumption of complexing agents so this method is frequently used for studying binding of various molecules (mainly drugs) to bio-macromolecules.

Investigation of various complexes in different solvents was accompanied by a great deal of speculation about the mechanisms and driving forces for complexation. Knowledge of the enthalpy and entropy changes of this reaction leads to a better

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understanding of the interactions leading to complex formation [12]. Chromatography was also used for the determination of these parameters for some hydroxyl aromatics with β-cyclodextrin (β-CD) on the grounds of the dependence of the capacity factor k' on absolute temperature T in the methanol–water mobile phase with and without β-CD [13]. Mohseni and Hurtubise derived an equation that related ΔH_a (enthalpy change accompanying association), ΔS_a (entropy change accompanying association), K_a (the association constant), ΔH and ΔS (enthalpy and entropy changes as a solute, in this case a hydroxyl aromatic, partitions from the mobile to the stationary phase). Calculation of ΔH_a and ΔS_a was possible when it was assumed that K_a changed very little with T that is, the dependence of K_a on $1/T$ could be neglected.

The simplest method for the determination of the enthalpy and entropy changes is the straightforward measurement of the dependence of the association constant K_a on T. The thermodynamic parameters ΔG_a, ΔH_a, and ΔS_a are related to K_a by the equation $\Delta G_a = -RT \ln K_a = \Delta H_a - T\Delta S_a$. A plot of $\ln K_a$ vs. $1/T$ yields a slope of $\Delta H_a/R$ and a y-intercept of $\Delta S_a/R$.

For this purpose K_a was measured by different methods: fluorimetry [14–17], pH potentiometry [18], UV absorption spectroscopy [19–21], flow microcalorimetry [22], gas–liquid chromatography [23], liquid chromatography [8], and NMR [24]. In this paper the thermodynamic parameters of the association reaction of four steroid hormones with β- and γ-cyclodextrins were determined by measurement of the association constants by the Hummel–Dreyer method as a function of T.

2. Experimental

2.1. Materials

β-CD was purchased from Sigma, (St. Louis, MO, USA) and γ-CD from Merck (Darmstadt, Germany). The water content in CDs was determined by the Karl–Fisher method. Stock solutions of steroids containing about 0.6 mM of the compounds for estradiol, ethinyloestradiol and estriol, and about 0.3 mM for estrone in methanol–water (45:55, v/v) or acetonitrile–water (30:70, v/v) were prepared. The concentration of the estrone stock solution was lower because of its lower solubility. The stock solutions were diluted tenfold or fivefold (estrone) to produce eluents. Solutions for calibration were obtained in a similar manner but the concentrations of steroids in them were twice as large as in the eluents. Solutions for injection containing CDs were obtained by dissolving a weighed amount of CD (about 40 mg) in the mobile phase, with gentle warming in the case of methanol–water mobile phases.

2.2. Apparatus

The LC system used was a Shimadzu pump (LC-10AS), oven (CTO-10AC), UV detector (SPD-10A), and integrator R6A. The columns used were Partisil ODS and Vydac C_4 (both 25 cm×4.6 mm I.D., d_p = 10 μm). The volume of the injected samples was 50 μl. Detection was effected at 280 nm, i.e. the absorption maximum of the eluent.

3. Results and discussion

The association constants for four steroid hormones with β- and γ-CD were obtained in the temperature range 15–70°C, using methanol–water (45:55, v/v) (MeOH–H_2O) and acetonitrile–water (30:70, v/v) (MeCN–H_2O) mobile phases. As usual, K_a decreased with increasing T. By way of example the results for estriol are shown in Fig. 1 and Fig. 2, respectively, presented in the form of van't Hoff plots ($\ln K_a$ vs. $1/T$). From the slopes and intercepts of such plots the corresponding values ΔH_a and ΔS_a were calculated for all four compounds. They are listed in Table 1 and Table 2. For all linear regression plots between $\ln K_a$ and $1/T$ the correlation coefficients were higher than 0.99 in methanol–water and in acetonitrile–water in the case of β-CD complexes. So we have a good reason to infer that corresponding ΔH_a values are constant in the studied temperature range. For the complexes with γ-CD in acetonitrile–water these correlation coefficients were also high (average value for four plots being 0.9907) however linear regression between K_a and $1/T$ was better – the average value of the correlation coefficient being 0.9981. This might suggest that ΔH_a is

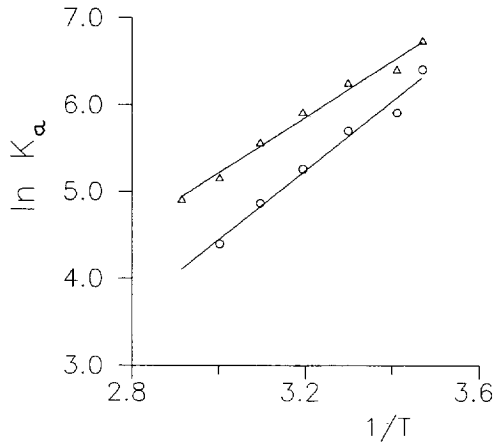

Fig. 1. Van't Hoff plots for complexes of estriol with β-cyclodextrin (○) and γ-cyclodextrin (△) in methanol–water (45:55 v/v).

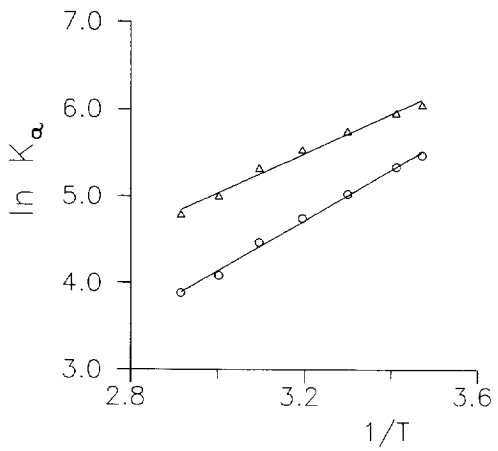

Fig. 2. Van't Hoff plots for complexes of estriol with β-cyclodextrin (○) and γ-cyclodextrin (△) in acetonitrile–water (30:70 v/v).

Table 1
The ΔH_a and ΔS_a values of the complexes of four steroids with β- and γ-cyclodextrins in methanol–water (45:55,v/v). The enthalpy and entropy units are kcal mol^{-1} and cal mol^{-1} deg^{-1}, respectively

Solute	β-CD		γ-CD	
	ΔH_a	ΔS_a	ΔH_a	ΔS_a
Estriol	−7.9	−14.9	−6.4	−8.7
Estradiol	−8.9	−17.3	−6.3	−7.4
Ethinyloestradiol	−8.5	−16.2	−7.4	−10.6
Estrone	−8.0	−15.3	−6.5	−9.8

Table 2
The ΔH_a and ΔS_a values of the complexes of four steroids with β- and γ-cyclodextrins in acetonitrile–water (30:70, v/v)

Solute	β-CD		γ-CD	
	ΔH_a	ΔS_a	ΔH_a	ΔS_a
Estriol	−5.7	−8.9	−4.5	−3.5
Estradiol	−6.0	−9.5	−4.2	−2.0
Ethinyloestradiol	−5.2	−7.5	−4.2	−1.9
Estrone	−5.2	−7.7	−4.2	−3.4

The enthalpy and entropy units are kcal mol^{-1} and cal mol^{-1} deg^{-1}, respectively.

not rigorously constant in this case. Such an effect was expected for the enthalpy change of hydrophobic bond formation because of the dependence of water structure on temperature [12]. It does not mean that the hydrophobic effect is significant for this class of complexes; there are other processes present which can also give a temperature dependent contribution to ΔH_a.

It was noted that values of ΔH_a in MeOH–H_2O were in all cases more negative than in MeCN–H_2O medium. Free energy of complex formation is due to several processes: (1) medium–medium interactions (solvophobic effect and cavity closure), (2) removing the solvent molecules from the CD cavity, (3) adding the solute to CD molecule, and (4) solvation of the complex. Of these processes, only the third is medium independent. So the overall free energy change (at least in the first approximation) can be written as the equation [25]:

$$\Delta G_a = \Delta G_{MM} + \Delta G_{MS} + \Delta G_{SS}$$

where ΔG_{MM} is the contribution arising from medium–medium interactions, ΔG_{MS} includes all solute–medium interactions, that is, all solvatation phenomena; and ΔG_{SS} includes all solute–solute contributions. Similar additive expressions can be written for ΔH_a and ΔS_a.

Solvophobic interaction M–M results in (at least partial) closure of a cavity in the medium occupied by a free solute molecule. Expression for the free energy of cavity formation, ΔG_{cav}, is given in [26] as a function of the heat of vaporization of the solvent, solute and solvent molar volume, and surface tension of the solvent. Heat of vaporization and molar volume of the solvent were calculated assuming a

linear relationship for the dependence of the two values on the mole fraction of the two components in the mixture. Surface tension data were taken from [27]. It appeared that the difference in ΔG_{cav} between MeOH–H_2O (45:55) and MeCN–H_2O (30:70) is only 0.21 kcal/mol, the value for cavity closure in MeCN being more negative. Such difference in ΔG_a would produce at 30°C the ratio of association constants in MeOH–H_2O and MeCN–H_2O equal to 0.7. In this work association constants in MeOH–H_2O are greater than in MeCN–H_2O so the contribution of the process of cavity closure can not be responsible for the differences between both media. This finding is in contradiction with the point of view, promoted by Sinanoğlu and Fernández [28] that the "cavity term" provides the driving force for the association. Another opinion expressed in [15] is that the surface tension of solutions has a substantial effect on the stability of complexes. In the present case the surface tension of both media is the same (37.1 dyne cm^{-1}) nevertheless association constants of complexes and their formation enthalpies differ considerably.

Another contribution to the overall process of complex formation is the solvophobic effect. The effect increases with increasing solvent polarity. Unfortunately there is no unequivocal measure of solvent polarity. If one takes into consideration the retention volumes of solutes in both media on the same column, their polarity is very similar, being slightly greater in the case of MeOH–H_2O. On the other hand the dielectric constant is greater for the MeCN–H_2O mixture. If one takes this property as a measure of polarity, the solvophobic effect contribution should be more stabilizing in MeCN–H_2O (30:70) than MeOH–H_2O (45:55) medium, contrary to the observed results.

The process which seems to have the greatest contribution is that of removing the solvent molecules from the CD cavity. β-CD is known to form weak complexes with alcohols [21], the complex becoming stronger as the alcohol polarity decreases. The association constant of MeOH with β-CD is 0.32 M^{-1} [21], whereas the association constant of MeCN with this ligand is 6.0 M^{-1} [29]. Let us take into consideration the terms of ΔH_a describing interactions which involve steroid and CD molecules (ΔH_{MS} and ΔH_{SS}). Matsui and Mochida [21] found that the enthalpy for association of β-CD with alkanols is positive, decreasing in the order 1-butanol>1-pentanol>1-hexanol. So the enthalpy of removing these alcohols from the CD cavity is negative, and the same is probably true for methanol. On the other hand for more bulky (and less polar) alcohols the enthalpy sign is opposite [21], and this is probably true for MeCN, because MeCN is more bulky than MeOH and it can be considered "less polar" at least on the basis of its chromatographic elution strength, as compared to MeOH. So, if a part of ΔH_{MS}, i.e. the enthalpy of removing solvent molecules from the CD cavity, is more negative in MeOH–H_2O than in MeCN–H_2O medium, one can expect that the same holds for the ΔH_a of the overall process of complex formation with β-CD, in accordance with the experimental data in Table 1 and Table 2. These data show the same trend for γ-CD.

This implication is justified by another finding that for all four steroids studied the complexes in MeCN–H_2O were weaker than in MeOH–H_2O. It is consistent with the statement that if a cosolvent is added to water which also binds to CD it can compete with a particular substrate for binding to CD. Thus, stronger binding of solvent molecules to CD results in weaker complexation of the solute molecules. For comparison, the values of K_a at 30°C in both media are given in Table 3. The same association constants were measured previously [10], but in the case of estriol the value of K_a was overestimated and was slightly higher than with γ-CD. Values obtained in this work are in good agreement with those evaluated on the basis of the decrease of retention time [6]. It

Table 3
Comparison of association constants of four steroids with β- and γ-CD in MeOH–H_2O (45:55, v/v) and MeCN–H_2O (30:70) media at 30°C

Solute	K_a MeOH–H_2O		K_a MeCN–H_2O	
	β-CD	γ-CD	β-CD	γ-CD
Estriol	300	523	152	313
Estradiol	465	882	191	384
Ethinyloestradiol	364	910	125	414
Estrone	267	352	92	182

must be added that these association constants were found without taking the concentration of CDs bound by solvent molecules into consideration, as if all CD not bound to steroid molecules was free. Such association constants are sometimes termed "apparent" ones.

Table 1 and Table 2 show us also that the entropy change, ΔS_a, accompanying complex formation in MeOH–H_2O is more negative than in MeCN–H_2O medium. The entropy of β-CD – n-alkanol complex formation is positive, and the reaction is not enthalpy but entropy driven [21]. This might suggest that MeOH in the β-CD cavity is less ordered than in solution. So the entropy of exchanging the MeOH molecules with the steroid molecules has a negative contribution to the process of removal of MeOH molecules and their transference to the solution ($\Delta S_{MS}<0$). The greater value of ΔS_a for formation of the complex steroid–CD in the case of MeCN–H_2O is an indication that the entropy of complexing β-CD by MeCN is less positive (or even negative) than in the case of MeOH–H_2O . With γ-CD the overall changes in entropy ΔS_a are shifted towards less negative values as compared to ΔS_a with β-CD in both media, and ΔS_a is less negative in MeCN–H_2O than in MeOH–H_2O. Nevertheless the partial contribution ΔS_{MS} is not known in the case of interaction not only for MeCN but also for MeOH with γ-CD. So we can not speculate if these differences are due to changes in ΔS_{SS} (steroid–CD) or ΔS_{MS}. We do not even know if the negative sign of ΔS_a in the case of γ-CD has any physical meaning because the change of concentration scale (from molarity used here) to mole fractions shifts ΔS values to more positive values [25], in MeCN–H_2O, 30:70 solution by 7.5 units. There is not unanimous agreement as to which concentration scale is better for the interpretation of entropy changes [25].

From Table 3 it can be seen that the association constants of the complexes with γ-CD are greater than with β-CD. One of the reasons for this fact is probably the difference in the interior cavity dimensions of the two CDs. The diameter of the wider opening of the CD torus is 6.5 Å for β-CD and 8.3 Å for γ-CD [30], their height is 7.8 Å. In the interior of CD there is not enough room for any whole steroid molecule, because their length is ca. 12 Å, and width ca. 7 Å (between centers of Van der Waals spheres, calculated with the help of a Spartan package, using a molecular modelling approach). So a steroid molecule can get deeper into γ-CD and occupy all its length whereas only ring A or D of the solute molecule can enter the β-CD cavity.

The values of ΔH_a were compared with ΔH, i.e. the enthalpy of solute partitioning from the mobile to the stationary phase, determined with the help of measurement of the capacity factor of the steroids on a C_{18} column in MeOH–H_2O, (45:55), as a function of temperature [13]. The last values were considerably lower and amounted to −4.3, −4.6, −4.8, and −4.0 kcal/mol, respectively for estriol, estradiol, ethinyloestradiol, and estrone in MeOH–H_2O (45:55). In MeCN–H_2O the corresponding values were −1.1, −2.3, −2.8, and −2.8 kcal/mol. On the assumption that the solvent cavity terms are similar for both processes, it can be seen than the stabilizing contributions due to the transfer of a solute to the interior of CD are greater than those due to the partitioning into the quasi-phase of alkyl chains. The changes of ΔH are also much lower in MeCN–H_2O than in MeOH–H_2O medium.

The values of ΔH_a in a methanolic mobile phase were also determined according to Mohseni and Hurtubise [13]. For this purpose the capacity factors of steroids were measured as a function of T in methanol–water (45:55) and methanol–water (45:55)+3 mM β-CD using a C_{18} column. The values obtained (according to Eqs. 3 and 9 of their work) were −2.6, −4.2, −3.7, and −2.9 kcal/mol, respectively for estriol, estradiol, ethinyloestradiol, and estrone. These values are similar to those reported by Mohseni and Hurtubise for hydroxyl aromatics but they are considerably lower than the values determined in this work (Table 1). This is probably due to the fact that in Eq. 9 of [13] describing the temperature dependence of ln k', where k' is the capacity factor in the mobile phase containing β-CD, the term $\{1/K_a+[CD]\}$ is assumed to be temperature independent. In the present case this assumption is groundless. Let us take as an example estradiol at 40 and 60°C. The measured value of K_a for this compound was 321.4 M^{-1} and 107.8 M^{-1} respectively at the two temperatures. The concentration of β-CD was ca. 3 mM. So this term is

equal to ln $\{1/321.4+0.003\}=-5.097$ at 40°C, and ln $\{1/107.8+0.003\}=-4.400$ at 60°C, that is the difference is 0.697 (ln $\{1/K_a+[CD]\}$ really means ln $\{M^{-1}/K_a+[CD]M^{-1}\}$, for the last expression is a pure number). The corresponding difference in ln k' is only 0.152.

Mohseni and Hurtubise in their calculations used the so-called equilibrium concentration of β-CD, $[\beta\text{-CD}]_m$, in the the mobile phase, i.e. the concentration of β-CD which is not complexed with MeOH. Due to the high molar concentration of MeOH, the concentration of β-CD bound to MeOH is 3.5 times greater than that of free β-CD, $[\beta\text{-CD}]_m$. If the association constant of MeOH – β-CD were temperature independent, the use of $[\beta\text{-CD}]$ instead of $[\beta\text{-CD}]_m$ wouldn't have any influence on the thermodynamic functions ΔG_a, ΔH_a, and ΔS_a, because they are determined through $\Delta \ln K_a$. Unfortunately we do not know the temperature dependence of the association constant of MeOH either with β-CD or with γ-CD but the error in the case of β-CD and MeOH can be estimated. On the basis of ΔH_a and ΔS_a values for 1-butanol and 1-pentanol from [21] we found that the association constants of these alcohols with β-CD are about 1.2 times greater at 70°C than at 15°C. If the ratio for the MeOH–β-CD complex is similar, the estimated association constants are 0.33 at 15°C and 0.4 at 70°C. The error introduced by neglecting the amount of CD bound by MeOH would produce the difference in $\Delta H_a \approx 0.3$ kcal/mole, which is of the order of experimental error.

For complexes with γ-CD in MeOH–H_2O and both CDs in MeCN–H_2O we can not evaluate the error due to the neglecting of binding of MeOH or MeCN to CD because we have no data on the stability of these complexes.

4. Conclusions

The values of enthalpy changes due to complex formation with β- and γ-CD are more negative in MeOH–H_2O (45:55) than in MeCN–H_2O (30:70) medium for the four steroids studied. The values of the association constants K_a are greater in the first medium than in the second. These facts are interpreted as being due to the more positive enthalpy change connected with the removal of the cosolvent molecules from the CD cavity, and thus the different competition of MeOH and MeCN with the solute (steroid) for binding with CD. The complexes of the four compounds with γ-CD are stronger than with β-CD at all temperatures in the range 15–70°C.

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