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Spectrophotometric study on the thermodynamics of binding of α- and β-cyclodextrin towards some *p*-nitrobenzene derivatives †

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Received 14th January 2003, Accepted 17th March 2003
First published as an Advance Article on the web 3rd April 2003

Binding properties of native α- and β-cyclodextrin towards some nitrobenzene derivatives have been studied by means of UV-vis spectrophotometry. The former host is able to form complexes having 1 : 1 and 1 : 2 stoichiometric ratios with these guests, while only 1 : 1 complexes are detected with the latter host. A careful analysis of the thermodynamic parameters for complexation equilibria, under the perspective of the enthalpy–entropy compensation effect, reveals that binding abilities of the two different hosts are subject to different features.

Introduction

Binding properties of both native and modified cyclodextrins towards organic compounds have been the object of a large number of studies,[1] with respect to their potential or actual applications in several research and industrial fields—pharmaceuticals,[2] foods and cosmetics,[3] separation,[4] chiral discrimination,[5] enzyme mimics,[6] stereo-selective syntheses,[7] and so on. Work on these topics is constantly increasing and is periodically reviewed. Despite an enormous amount of experimental as well as theoretical[8] work, analysis of the ultimate factors governing the binding phenomenon and particular aspects at the molecular level, such as chiral recognition,[9] are still the object of intense debate, and up until now cannot be considered fully understood. Different approaches, from QSAR to molecular modelling,[8] have been used to this end.

In particular, several efforts have been devoted in recent years to a systematic analysis of thermochemical data pertinent to the inclusion process.[10] Since the seminal paper by Tabushi and co-workers,[11] the thermodynamics of binding has been generally discussed in terms of a combination of ideal steps, which can be summarised as: i) desolvation of the guest (ideal transfer from bulk solution into the gas phase); ii) internal desolvation of the host (ideal transfer of some or all its internal "high energy" water molecules in the gas phase and then into the bulk solvent); iii) host–guest binding (ideal transfer of the guest from the gas phase into the host cavity) and iv) reorganisation of the solvent around and inside the cavity. Binding properties towards several classes of organic guests[9b,10,12] (aliphatic and alicyclic alcohols, acids, amines, aminoacids and their derivatives, mono- and polycyclic aromatics, natural and semi-natural products) have been examined and some general rules have been assessed. Until it is a somewhat diffused opinion that van der Waals and hydrophobic interactions may be in most cases the best candidates for the driving force of the binding processes,[13] the importance of conformational strain release, of electrostatic, polar and hydrogen bonding interactions and of solvation effects cannot be ignored, and it has been often shown that no obvious hierarchy among all the possible factors can be unambiguously identified.[5b,9a,12b,14]

In this context we have already been interested[5b,14] in elucidating the various aspects of the binding properties of native and some (alkyl)amino- modified β-cyclodextrins towards nitro- and amino-benzene derivatives. As a proceeding of this work, we compared the thermodynamics of binding of native α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) towards some *p*-nitrobenzene derivatives **1**–**13** (Fig. 1), measuring by means of UV-vis spectrophotometry the binding constants at various temperatures ranging from 288.15 to 318.15 K.

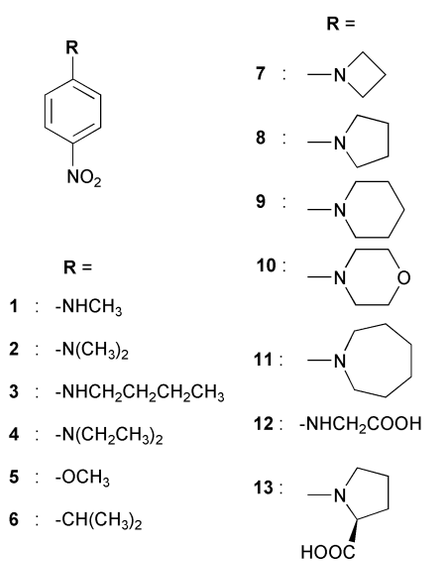

Fig. 1 *p*-Nitrobenzene guests **1**–**13**.

As will be discussed below, consideration of the binding constants alone does not allow a full understanding of the binding phenomenon characteristics, and it must be completed by a careful examination of all related thermodynamic parameters. Substrates **1**–**13** differ by the ancillary chain *para* to the nitro-group. The ancillary chains range from aliphatic primary (**1**, **3**), secondary (**2**, **4**) and cyclic amines (**7**–**11**) to amino acids (**12**–**13**); furthermore *p*-nitroanisole (**5**) and *p*-nitroisopropylbenzene (**6**) were added as useful comparisons. The guests were chosen in such a way to have significant variations, depending on the ancillary chain, in properties such as molecular volume, hydrophobicity, polarity, ability to act as a hydrogen bond donor and electric charge as a function of the solvent medium. In particular, among the examined guests **1** and **5** are isosteres, as well as **2** and **6**; substrates **3**, **4** and **8** are comparable with respect to their molecular volume, but have different conform-

† Electronic supplementary information (ESI) available: Values of inclusion constants at different temperatures. See http://www.rsc.org/suppdata/ob/b3/b300330b/

DOI: 10.1039/b300330b

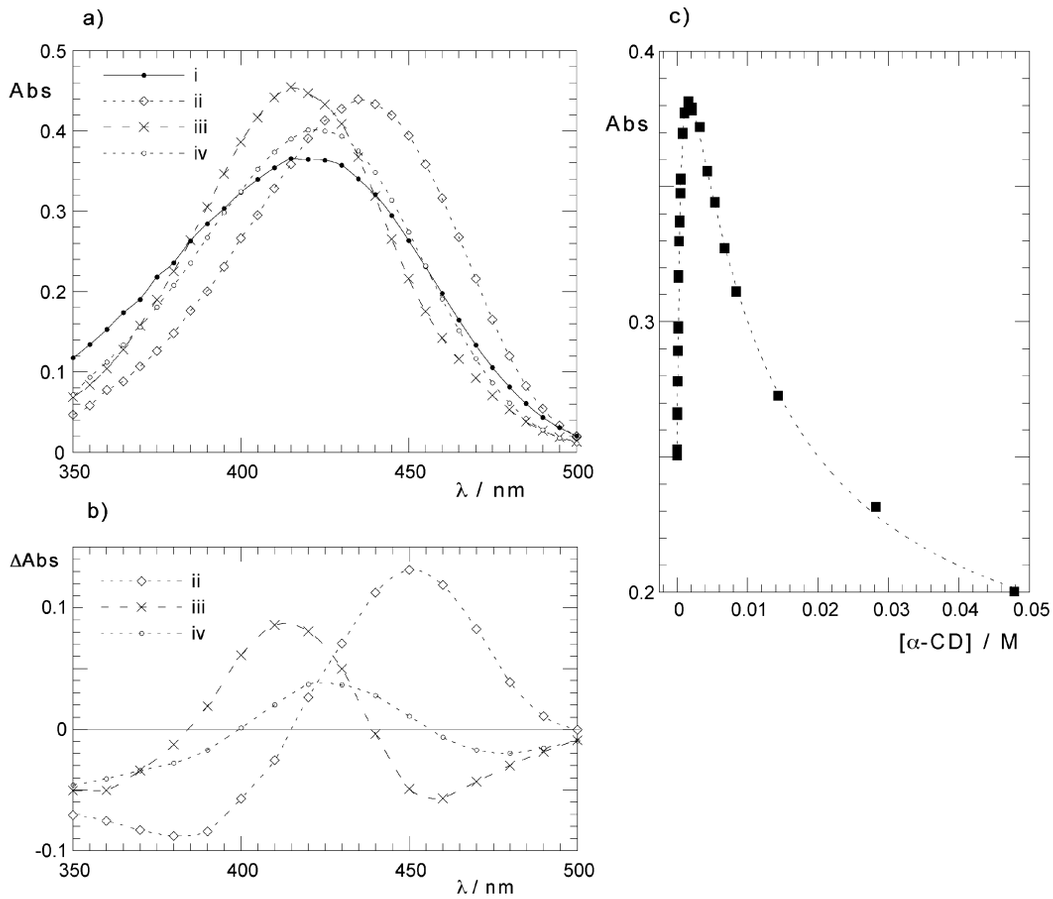

Fig. 2 Recorded spectra (a), difference spectra (b) and absorption trend (c) for a typical measurement experiment. Data refer to guest **7** (experiment performed at 288.15 K). In a) and b) curves i, ii, iii, iv are the spectra of the guest free or in the presence of 0.002 M α-CD, 0.048 M α-CD or 0.004 M β-CD respectively. In c) the absorption intensity trend at 452 nm in the presence of an increasing amount of α-CD is presented; the dotted line is obtained by fitting of eqn. (2).

ational freedom; **1**, **3** and **12** are the only guests having the aniline-like nitrogen atom able to act as a hydrogen bond donor; **6** has a lower dipole momentum than **5**, which in turn is less polar then all other guests. Intense UV-vis absorptions make molecules **1**–**13** ideal substrates for a spectrophotometric study. Measurements were generally performed in phosphate buffer solution at pH = 6.0 because, according to our previous studies,[5b,14] slightly stronger association is observed at this pH than at higher pH values. However at pH = 6.0 guests **12** and **13** should be in deprotonated anionic forms, according to their pK_a values (*vide infra*); so association constants with these substrates were also studied in phosphate buffer at pH = 2.5.

Results and discussion

As a principle, guests **1**–**13** can get into the cyclodextrin cavity with either the nitro group or the ancillary chain directed towards the host primary rim. Simple nitrobenzene guests adopt the former inclusion mode,[15–17] although some nitrobenzene derivatives may present a more articulated behaviour. For example, evidence from NMR,[15] circular dichroism[16] and also kinetics measurements,[17] indicate that *p*-nitrophenol and *p*-nitrophenyl short-chain alkanoates are actually included with the nitro group directed towards the primary rim. Differently, long-chain *p*-nitrophenyl alkanoates preferentially include their alkyl chains. Also on the basis of computational models[14] (both dynamic and "simulated annealing" simulations) we may reasonably assume that guests **1**–**13** should prefer the former inclusion mode, as a consequence of the remarkable interaction between the polarised *p*-nitrophenyl moiety and the intrinsic dipole momentum owned by the cyclodextrin cavity.[18] Thus, owing to the local electric field effect, and in agreement with the observed behaviour of *p*-nitroaniline in solvents of increasing dielectric constant,[19] inclusion of studied substrates in the cyclodextrin cavity may generally be expected to cause a bathocromic shift of the UV-vis absorption for the *p*-nitroaniline-like chromophore (Fig. 2a).

On the basis of the "difference spectra" (Fig. 2b), recorded comparing solutions of each guest in the absence and in the presence of a suitable amount of cyclodextrin (see Experimental section), we observed that inclusion in the α-CD cavity generally induces stronger bathochromic shifts than inclusion in the β-CD cavity, with maximum variations in the absorption intensity ranging up to about 40–80% in the former case but only up to 20–30% in the latter case. This suggests that our guests experience harder environmental changes upon inclusion in the narrowest α-CD cavity than in the β-CD cavity, in agreement with the idea that the effectiveness of non-bonding interactions in modifying the properties of an included guest strictly depend on the distance from the host inner wall.[12b] Simple molecular models easily predict that the average diameter of the α-CD cavity is hardly large enough to contain the aromatic moiety of the guests. Furthermore, it should also be mentioned that the α-CD cavity is able to hold up to two or three water molecules,[11,20] while the wider β-CD cavity can accommodate seven water molecules.[20] Thus displacement of water molecules upon complexation from the α-CD cavity is likely to be complete, but this is not necessarily true for β-CD.

Nevertheless the behaviour of several substrates towards α-CD appears complex, because on increasing the host concentration the observed bathocromic shift effect seems to regress (Fig. 2a). Indeed only at low α-CD concentrations recorded spectra show good isosbestic points, as well as a regular increase of the bathochromic shift. Depending on the chosen

Table 1 Thermodynamic parameters for the first binding process between α-CD and guests **1**–**13**

Guest	pH	$K_{\alpha,1}$/M^{-1} at 298.15 K	$\Delta H^{\circ}_{\alpha,1}$/kJ mol^{-1}	$T\Delta S^{\circ}_{\alpha,1}$/kJ mol^{-1}	$\Delta G^{\circ}_{\alpha,1}$/kJ mol^{-1}
1	6.0	990 ± 25	−37.8 ± 0.6	−20.7 ± 0.6	−17.1 ± 0.1
2	6.0	1180 ± 40	−38.8 ± 1.0	−21.3 ± 1.0	−17.5 ± 0.1
3	6.0	1455 ± 35	−32.6 ± 0.6	−14.6 ± 0.6	−18.0 ± 0.1
4	6.0	1145 ± 60	−34.8 ± 1.4	−17.4 ± 1.4	−17.4 ± 0.1
5	6.0	315 ± 50	−35.9 ± 0.4	−21.7 ± 0.6	−14.2 ± 0.3
6	6.0	505 ± 70	−28.5 ± 2.3	−12.9 ± 2.3	−15.4 ± 0.4
7	6.0	1200 ± 30	−38.0 ± 0.3	−20.5 ± 0.3	−17.6 ± 0.1
8	6.0	2120 ± 120	−36.4 ± 0.5	−17.5 ± 0.5	−19.0 ± 0.1
9	6.0	1345 ± 25	−38.9 ± 1.0	−20.9 ± 1.0	−17.8 ± 0.1
10	6.0	930 ± 10	−34.0 ± 1.3	−17.1 ± 1.3	−16.9 ± 0.1
11	6.0	3610 ± 90	−42.4 ± 0.1	−22.1 ± 0.1	−20.3 ± 0.1
12	6.0	1010 ± 40	−30.6 ± 0.9	−13.5 ± 0.9	−17.4 ± 0.1
13	6.0	1185 ± 15	−35.7 ± 1.1	−18.1 ± 1.1	−17.6 ± 0.1
12	2.5	1010 ± 60	−20.4 ± 0.8	−3.3 ± 0.8	−17.2 ± 0.1
13	2.5	1040 ± 25	−30.8 ± 0.8	−13.6 ± 0.8	−17.2 ± 0.1

Table 2 Thermodynamic parameters for the second binding process between α-CD and guests **1**–**13**

Guest	pH	$K_{\alpha,2}$/M^{-1} at 298.15 K	$\Delta H^{\circ}_{\alpha,2}$/kJ mol^{-1}	$T\Delta S^{\circ}_{\alpha,2}$/kJ mol^{-1}	$\Delta G^{\circ}_{\alpha,2}$/kJ mol^{-1}
1	6.0	15.1 ± 1.2	−23.2 ± 1.4	−16.5 ± 1.4	−6.7 ± 0.2
3	6.0	33.1 ± 1.4	−15.4 ± 0.1	−6.7 ± 0.2	−8.7 ± 0.1
7	6.0	76.9 ± 1.9	−23.8 ± 1.0	−13.1 ± 1.0	−10.7 ± 0.1
8	6.0	235 ± 15	−38.9 ± 1.7	−25.4 ± 1.7	−13.5 ± 0.2
12	6.0	25.5 ± 1.2	−42.7 ± 0.5	−34.7 ± 0.5	−8.0 ± 0.1
12	2.5	86 ± 5	−54.9 ± 0.7	−43.9 ± 0.7	−11.0 ± 0.2
2,**4**,**9**,**10**	6.0	<10	—	—	—

operative wavelength, we can observe that absorbances firstly increase (or decrease), then pass through a maximum (or a minimum) and finally decrease (or increase) on increasing the host concentration (Fig. 2c). Differently, spectra of all guests recorded in the presence of an increasing concentration of β-CD always show a monotonical shift of the absorption maximum and good isosbestic points. The latter behaviour accounts for the formation of only one host–guest complex having a 1 : 1 stoichiometric ratio, according to the reaction shown in Scheme 1.

$$\text{Guest} + \beta\text{-CD} \overset{K_{\beta}}{\rightleftharpoons} \text{Guest}\cdot\beta\text{-CD}$$

Scheme 1

Thus absorbances at a given wavelength then show a regular hyperbolic trend, which can be processed to give the value of the association constant K_{β} according to eqn 1:

$$Abs = Abs_o + \frac{\left(\varepsilon_{G,\beta CD} - \varepsilon_G\right)[\text{Guest}]_o \; K_{\beta}[\text{CD}]}{1 + K_{\beta}[\text{CD}]} \qquad (1)$$

where Abs is the recorded absorbance as a function of the host concentration, Abs_o is the absorbance expected in absence of cyclodextrin, $\varepsilon_{G\cdot\beta CD}$ and ε_G are the molar absorption coefficients of the included and free guest respectively, $[\text{Guest}]_o$ is the overall analytical concentration of the guest, and finally [CD] is the concentration of the free host (noticeably, this equation is the non-linearised version of the well-known Benesi–Hildebrand[21] treatment). On the other hand, the absorption trend observed in the presence of α-CD can be explained admitting that two different complexes, having respectively 1 : 1 and 1 : 2 stoichiometric ratios, are formed, according to the reactions shown in Scheme 2.

In this case data have to be processed by means of equation (2) (where $\varepsilon_{G\cdot\alpha CD}$ and $\varepsilon_{G\cdot(\alpha CD)_2}$ are the molar absorption coefficients of the 1 : 1 or the 1 : 2 complexes respectively) from which the values of the partial association constants $K_{\alpha,1}$ and $K_{\alpha,2}$ can easily be obtained.

$$\text{Guest} + \alpha\text{-CD} \overset{K_{\alpha,1}}{\rightleftharpoons} \text{Guest}\cdot\alpha\text{-CD}$$

$$\text{Guest}\cdot\alpha\text{-CD} + \alpha\text{-CD} \overset{K_{\alpha,2}}{\rightleftharpoons} \text{Guest}\cdot(\alpha\text{-CD})_2$$

Scheme 2

In Tables 1–3 we report the values of the association constants $K_{\alpha,1}$, $K_{\alpha,2}$ and K_{β} determined at 298.15 K, as well as the related thermodynamic parameters ΔH°, $T\Delta S^{\circ}$ and ΔG°.

Considering the formation of 1 : 1 complexes only, α-CD appears in general a more effective host than β-CD towards examined guests, with few exceptions (guests **1**, **6**, **9** and **11**). Nonetheless most of the $K_{\alpha,1}$ values appear quite close to each other, while K_{β} values seem to vary in a slightly wider range. From a thermodynamic viewpoint we notice that most of the $\Delta G^{\circ}_{\alpha,1}$ values (excepting values for **5**, **6** and **11**) are restricted in a range of about 2 kJ mol^{-1}. Correspondingly, ΔG°_{β} values (excepting the same guests) span approximately 4 kJ mol^{-1}. However the apparent small variations of ΔG° values are misleading. In fact $\Delta H^{\circ}_{\alpha,1}$values as well as ΔH°_{β} values span approximately 20 kJ mol^{-1}; thus correct comparisons cannot be performed on the basis of the equilibrium constants alone, but should rather involve the complete sets of thermodynamic parameters.

The existence of linear enthalpy–entropy compensation effects (isokinetic relationships) in host–guest inclusion phenomena has been largely assessed[9c,12,22] and its origin and meaning is still controversial.[9b,13b,23] In general the intercept ($T\Delta S^{\circ}_o$) and slope (T/T_{isok}) of the linear $T\Delta S^{\circ}$ *vs.* ΔH° correlations have been interpreted as a measure respectively of the extent of desolvation and of the loss of conformational freedom for the host.[22b,23a] Nonetheless Liu and Guo[13b] have recently supported with the thermodynamic arguments the idea that solvent reorganization upon complexation should be the actual

$$Abs = \frac{Abs_o + \varepsilon_{G.\alpha CD}\, K_{\alpha,1}[\text{Guest}]_o[\text{CD}] + \varepsilon_{G.(\alpha CD)_2}\, K_{\alpha,1}K_{\alpha,2}\,[\text{Guest}]_o[\text{CD}]^2}{1 + K_{\alpha,1}\,[\text{CD}] + K_{\alpha,1}\,K_{\alpha,2}[\text{CD}]^2} \qquad (2)$$

Table 3 Thermodynamic parameters for the binding process between β-CD and guests **1**–**13**

Guest	pH	K_β/M^{-1} at 298.15 K	ΔH°_β/kJ mol^{-1}	$T\Delta S^\circ_\beta$/kJ mol^{-1}	ΔG°_β/kJ mol^{-1}
1	6.0	1080 ± 50	−12.9 ± 0.7	4.4 ± 0.7	−17.3 ± 0.1
2	6.0	710 ± 25	−10.9 ± 0.6	5.4 ± 0.6	−16.3 ± 0.1
3	6.0	915 ± 50	−17.9 ± 0.8	−1.1 ± 0.8	−16.9 ± 0.1
4	6.0	590 ± 30	−14.1 ± 0.9	1.7 ± 0.9	−15.8 ± 0.1
5	6.0	175 ± 25	−5.8 ± 0.2	7.0 ± 0.4	−12.8 ± 0.3
6	6.0	1450 ± 65	−6.3 ± 0.6	11.7 ± 0.6	−18.0 ± 0.1
7	6.0	765 ± 30	−11.9 ± 0.4	4.5 ± 0.4	−16.5 ± 0.1
8	6.0	1300 ± 40	−12.7 ± 0.6	5.0 ± 0.6	−17.8 ± 0.1
9	6.0	2640 ± 35	−13.1 ± 1.2	6.4 ± 1.2	−19.5 ± 0.1
10	6.0	650 ± 25	−14.7 ± 0.6	1.3 ± 0.6	−16.0 ± 0.1
11	6.0	17300 ± 310	−24.2 ± 0.4	0.1 ± 0.4	−24.1 ± 0.1
12	6.0	350 ± 45	−22.4 ± 1.2	−7.9 ± 1.3	−14.5 ± 0.3
13	6.0	665 ± 50	−13.1 ± 0.4	3.0 ± 0.4	−16.1 ± 0.2

Table 4 Enthalpy–entropy compensation correlations (eqn. 3)

Host	Data set	$T\Delta S^\circ_o$/kJ mol^{-1}	Slope	T_{isok}/K	r	n	Remarks
α-CD	$K_{\alpha,1}$	13.9 ± 2.2	0.90 ± 0.06	332 ± 23	0.968	15	
α-CD	$K_{\alpha,1}$	7.7 ± 2.6	0.94 ± 0.07	319 ± 23	0.989	6	
β-CD	K_β	16.6 ± 1.7	1.01 ± 0.12	295 ± 35	0.935	12	**11** excluded
Both	$K_{\alpha,1}$, K_β	15.8 ± 0.7	0.96 ± 0.02	313 ± 8	0.992	27	β-CD·**11** excluded

ultimate source of the observed compensation. A simultaneous plot of $T\Delta S^\circ_{\alpha,1}$, $T\Delta S^\circ_{\alpha,2}$ and $T\Delta S^\circ_\beta$ *vs.* $\Delta H^\circ_{\alpha,1}$, $\Delta H^\circ_{\alpha,2}$ and ΔH°_β respectively is shown in Fig. 3. Good linear correlations can be clearly found (it should be noticed that our data refer to a set of homogeneous guests, in agreement with the criterion suggested by Linert and co-workers[24]); fitting analyses according to eqn. 3 give the results shown in Table 4.

$$T\Delta S^\circ = T\Delta S^\circ_o + (T/T_{isok})\,\Delta H^\circ \qquad (3)$$

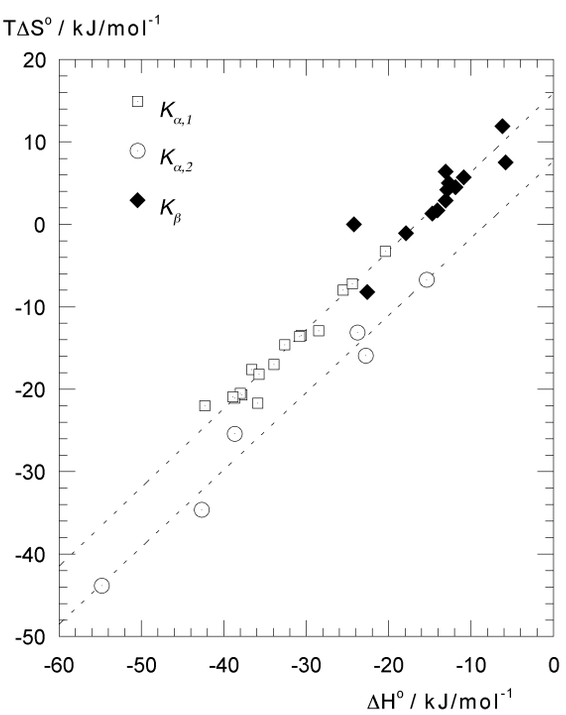

Fig. 3 Enthalpy–entropy compensation plot.

It should be remarked that in the correlation ΔH°_β *vs.* $T\Delta S^\circ_\beta$ the point for guest **11** falls significatively out of the fitting line, which is object of later discussion. Correlation slopes are close to 1, so isokinetic temperatures are actually near to 298.15 K, explaining why equilibrium constants appear similar. Also intercept values for $T\Delta S^\circ_{\alpha,1}$ and $T\Delta S^\circ_\beta$ are similar within the experimental uncertainty, so the two data sets may be joined to give an excellent overall correlation.

Despite the apparently similar enthalpy–entropy correlations results, inspection of data given in Tables 1 and 3 shows that the inclusion processes in the α-CD or β-CD cavity respectively present quite different characteristics. As a matter of fact, no correlation between $K_{\alpha,1}$ and K_β values can be found as well as between $\Delta G^\circ_{\alpha,1}$ and ΔG°_β. Both $\Delta H^\circ_{\alpha,1}$ and $T\Delta S^\circ_{\alpha,1}$ are negative, so inclusion in α-CD appears as an essentially enthalpy-driven process. Inclusion in β-CD shows again negative ΔH°_β values but positive $T\Delta S^\circ_\beta$, so both enthalpy and entropy variations contribute favourably. Comparisons between $\Delta H^\circ_{\alpha,1}$ and ΔH°_β or between $\Delta S^\circ_{\alpha,1}$ and ΔS°_β are interesting, because they show that in general a fair inverse correlation seems to exist, in the sense that values for β-CD decrease as the corresponding values for α-CD increase. Significative exceptions to this main trend are found only for guests **5** and **6** (which are not *p*-nitroaniline derivatives and consequently have different electronic characteristics than the other guests) and for guest **11** (falling out of the enthalpy–entropy correlation). Thus it seems that on passing from α- to β-CD the same factors (solvation effects, dipolar or hydrophobic interactions, and so on) may affect the energetics of binding in somewhat opposite ways. This agrees with the observations by Matsui and Mochida[25] on the stability of complexes with branched and cyclic alcohols, studied by means of a QSAR approach. Using the water–octanol partition coefficients (Log P_e) and the Taft's steric parameters (E_s) as molecular descriptors, these authors found that the regression coefficients of E_s for α-CD or β-CD complexes are opposite in sign, suggesting that a bulky guest experiences van der Waals repulsions upon binding in the former case, attractions in the latter case.

Further insights are provided by a detailed analysis of thermochemical data. Let us consider first the two isosteric couples **1**,**5** (having –$NHCH_3$ and –OCH_3 as ancillary chains respectively) and **2**,**6** (having –$N(CH_3)_2$ and –$CH(CH_3)_2$ as ancillary chains respectively). We can easily compare their behaviour on the basis of properties such as their polarity, hydrophobicity or ability to act as hydrogen bond donors–acceptors. For the inclusion in α-CD the corresponding $\Delta H^\circ_{\alpha,1}$ values follow the order **1**≈ **2** < **5** < **6**. This is the same order expected on the basis of the polarity of the guest aryl moieties, irrespective of the hydrophobicity of the ancillary chain (**6** shows a less favourable $\Delta H^\circ_{\alpha,1}$ value than the other guests) and of the possibility to provide hydrogen bond donation (**1** and **2**

have nearly the same $\Delta H^{\circ}_{\alpha,1}$ within the experimental indetermination). This suggests that dipolar interactions between the aryl group and the cavity local electric field assume a major role in determining the affinity of the guests for the α-CD host. On passing from α-CD to β-CD, however, we observed that differences in binding enthalpy values between these four guests undergo significative variations. For example the differences between **2** and its more hydrophobic isostere **6** decrease from $\Delta\Delta H^{\circ}_{\alpha,1} = -10.3$ kJ mol^{-1} to $\Delta\Delta H^{\circ}_{\beta} = -4.6$ kJ mol^{-1}. This means that, with β-CD as host, stabilising hydrophobic interactions are able to partly compensate the differences in polar effects. On the other hand, enthalpy differences between **1** and **5**, increasing from $\Delta\Delta H^{\circ}_{\alpha,1} = -1.9$ kJ mol^{-1} to $\Delta\Delta H^{\circ}_{\beta} = -7.1$ kJ mol^{-1}, account for an increased importance of hydrogen bond donation. Furthermore, it is interesting to notice that enthalpy differences between **5** and **6** revert their signs and pass from $\Delta\Delta H^{\circ}_{\alpha,1} = -7.4$ kJ mol^{-1} to $\Delta\Delta H^{\circ}_{\beta} = +0.5$ kJ mol^{-1}, confirming the increased importance of hydrophobic effects over polar interactions with the β-CD. Also differences between **1** and **2** revert their signs and pass from $\Delta\Delta H^{\circ}_{\alpha,1} = +1.0$ kJ mol^{-1} to $\Delta\Delta H^{\circ}_{\beta} = -2.0$ kJ mol^{-1}, despite hydrophobic interactions which should work in the opposite way. Therefore we may deduce that guest hydrogen bond donation is as well as or even more effective than van der Waals effects in influencing the affinity of the guest for β-CD.

We can now extend our consideration to guests **3**, **4** and **8**. Their ancillary groups (–$NHCH_2CH_2CH_2CH_3$, –$N(CH_2CH_3)_2$ and pyrrolidine respectively) have the same number of carbon atoms. So we may assume the groups as comparable in volume, but they obviously experience a progressively decreasing conformational freedom along the series. Furthermore they show different hydrophobicities, and in particular **3** differs from **4** and **8** because it is a hydrogen bond donor. Both **4** and **8** show more negative $\Delta H^{\circ}_{\alpha,1}$ values but less negative ΔH°_{β} values than **3**. Thus the major conformational freedom of the latter guest allows the occurrence of effective hydrophobic interactions with the β-CD cavity, as well as the β-CD allowing, in turn, more effective hydrogen bond interactions with the guest. Noticeably, enthalpy differences between the two hydrogen bond donors **1** and **3** revert their sign and pass from $\Delta\Delta H^{\circ}_{\alpha,1} = -5.2$ kJ mol^{-1} to $\Delta\Delta H^{\circ}_{\beta} = +5.0$ kJ mol^{-1}, once again confirming the more important role assumed for β-CD by the occurrence of effective hydrophobic interactions as outlined above.

All previous observations allow us to delineate a first model for the interaction between the two different hosts and the guests taken into account. As a matter of fact, data may be rationalised assuming that the narrower α-CD cavity is actually able to bind only the aryl moiety of the guests, in a somewhat rigid way, and leaving the ancillary chain quite free and exposed to the solvent bulk. Differently, the wider β-CD cavity allows the guest to achieve the most effective disposition (energetically speaking) within the cavity or near its secondary rim. However, further considerations, based also on entropy variations, make this simple picture more articulated. In fact, if the interaction with the α-CD host actually gave rise to a strictly rigid situation, negligible differences should be found on comparing guests having the same polar aromatic moiety but differing ancillary chain. This is not in agreement with the rather different $\Delta H^{\circ}_{\alpha,1}$ and $T\Delta S^{\circ}_{\alpha,1}$ values observed. Furthermore, in striking contrast with the well assessed rule that the addition of methylene groups on a linear chain causes a regular increase in inclusion enthalpies,[10,12b] we found the $\Delta H^{\circ}_{\alpha,1}$ value for **3** is less favourable by 5.2 kJ mol^{-1} than the one for **1** (but is largely compensated by a more favourable entropy variation of 6.1 kJ mol^{-1}); differently, ΔH°_{β} and $T\Delta S^{\circ}_{\beta}$ values follow the correct trend. Thus we have to think that either the interaction model with the α-CD is not really so rigid, or there is some other very likely interaction feature to be taken into account.

The aforesaid rule for chain inclusion is also followed when the chain length slightly exceeds the cavity depth. This fact has been explained through the concept of an "expanded hydrophobic sphere".[10] In other words it may be assumed that the properties of the water molecules in proximity of the cyclodextrin rims substantially differ from those of bulk solvent because of the structurising effect of the rims themselves. For our guests it is clear that interaction of their ancillary chains with the expanded hydrophobic sphere should heavily affect both inclusion enthalpy and the entropy, in agreement with the viewpoint that the compensation effect is actually related to solvent reorganisation.[13b,21b] Thus the differences in $T\Delta S^{\circ}_{\alpha,1}$ values between **1** and **3** or between **3** and **4** can easily be accounted to a more unfavourable effect on the structuring of the "hydrophobic sphere" exerted by the conformationally free –$NHCH_2CH_2CH_2CH_3$ group of **3**, as compared to both the –$NHCH_3$ group in **1** and the –$N(CH_2CH_3)_2$ group in **4**. Differently, interaction with the hydrophobic expanded sphere is not strictly necessary in order to explain the characteristics of β-CD inclusion and probably has a negligible effect in most cases. These considerations help us to rationalise the behaviour of guests **7**–**13**.

Guests **7**–**11** illustrate the effect of ring expansion when the ancillary group is a cyclic amine. As simply expected on the basis of the increasing hydrophobicity (due to molecular volume), inclusion in the β-CD cavity is regularly improved along the series **7**, **8**, **9**, **11**. The morpholine derivative **10** is less strongly included in β-CD than the piperidine derivative **9**. This behaviour may be explained considering that the presence of an oxygen atom in the ancillary group makes it less hydrophobic and confers a dipole momentum as opposed to the one owned by the host cavity. However, a less favourable entropy variation is found for **10** than for guests **7**–**9** (enthalpy variation is more favourable, according to the compensatory effect). Therefore we may conclude that a structuring effect of the morpholine ancillary group on its surroundings upon inclusion should also be important. The behaviour of guest **11** towards β-CD is surprising, because of the exceptionally high value of K_{β} (as compared with the series **7**, **8**, **9**) and because it strikingly falls out of the enthalpy–entropy correlation. There is no obvious explanation for this finding, which could be accounted, for example, as a competition with an inclusion mode bearing the highly hydrophobic azepine moiety directed towards the inner part of the host, or alternatively to an interaction of the large ancillary group with the expanded hydrophobic sphere (not occurring in all other cases). It could also be suggested that, owing to the dimension and the conformational freedom of the azepine ancillary group, some unexpected effect of "improved fit" with the β-CD cavity is induced upon complexation, enhancing the usual effect of hydrophobicity. A similar enhanced binding affinity trend for cyclic alcohols has already been observed.[12b] Noticeably the $T\Delta S^{\circ}_{\beta}$ value for **11** is significantly lower than for guests **7**–**9**. Further investigations are needed in order to clarify this topic.

Behaviour of guests **7**–**11** towards α-CD appears less easy to rationalise. In the series **7**, **8**, **9**, **11**, on increasing the dimensions of the ancillary ring chain both $\Delta H^{\circ}_{\alpha,1}$ and $T\Delta S^{\circ}_{\alpha,1}$ values seem to pass through a maximum. This is probably the result of the combined and contrasting effects of the ancillary group on the expanded hydrophobic sphere and of the progressively reduced affinity of the guest for the solvent bulk. Because these effects are not perfectly parallel, the overall $\Delta G^{\circ}_{\alpha,1}$, and consequently the $K_{\alpha,1}$ values, do not follow a monotonical trend. The behaviour of the morpholine derivative **10** is interesting, because the reduced $\Delta H^{\circ}_{\alpha,1}$ value with respect to **9** seems to account for both the best affinity of the former guest for the solvent bulk and for the effect of the dipole momentum of the morpholine group opposed to the momentum of the α-CD cavity.

Amino acid derivatives **12** and **13** allow us to consider the effect of the introduction of an ionisable carboxylic group on the guest structure. Their measured dissociation pK_a values are

3.52 ± 0.08 and 3.18 ± 0.01 respectively, so they are completely ionised at pH 6.0. Therefore we chose to also investigate binding equilibria at pH 2.5, in order to achieve information about the behaviour of the two guests in their neutral form. The apparent similar values of $K_{\alpha,1}$ observed for the interaction with α-CD treacherously conceal dramatic variations in $\Delta H^{\circ}_{\alpha,1}$ and $T\Delta S^{\circ}_{\alpha,1}$ values on changing the pH of the solution, as well as compared with the other guests. In comparison with **1**, guest **12** (which may be considered as deriving from **1** by substitution of a methyl H atom with the –COOH group) shows much less favourable $\Delta H^{\circ}_{\alpha,1}$ values both at pH 6.0 and 2.5. In the former case this may be very easily explained considering the strongly enhanced affinity of the guest for the water bulk, due to the introduction of the charged group. However at pH 2.5 further decrease in absolute value of $\Delta H^{\circ}_{\alpha,1}$ is observed. This anomalous behaviour should be accounted for, in our opinion, by the interaction of the host with the acidic buffer. The α-CD probably could be partly protonated at this pH value, which makes host desolvation a more difficult (as observed for amino-modified β-cyclodextrins)[14] and enthalpy-demanding process. The introduction of the charged carboxylate group on a cyclic ancillary chain such as in **13** at pH 6.0 – as compared with **8** – strangely does not seem to cause remarkable variations in $\Delta H^{\circ}_{\alpha,1}$ values. This may probably be justified considering that the carboxylate group is forced to be placed near the secondary rim of the host and can quite effectively interact with it, favouring also the structuring of the solvent molecules in the surroundings. Affinity of guests **12** and **13** for β-CD at pH 6.0 is significatively reduced as compared with **1** and **8** respectively. It should be noticed that the difference in ΔH° values between **12** and **13** at pH 6.0 reverts from $\Delta\Delta H^{\circ}_{\alpha,1} = +5.1$ kJ mol^{-1} to $\Delta\Delta H^{\circ}_{\beta} = -9.3$ kJ mol^{-1} on passing from α-CD to β-CD. This may probably be explained considering that the conformational freedom of **12** allows its carboxylate group to have an effective structuring effect on its surroundings within the complex with β-CD (with an effect similar as discussed for **10**), and that this effect compensates for its difficult desolvation.

A very interesting feature of the behaviour of α-CD is the possibility of also forming 1 : 2 complexes with the examined guests. Noticeably, although the formation of 1 : 2 complexes with different cyclodextrins is a well known process, thermodynamic studies on this topic are rare.[26] Analysis of the absorption curves allowed us to get a good estimation of the second complexation constant $K_{\alpha,1}$ (Table 2) only with guests **1**, **3**, **7**, **8** and **12** (the latter at both pH 6.0 and 2.5). With guests **2**, **4**, **9** and **10** we were able to detect qualitatively the formation of the 1 : 2 complex, unless the constant resulted too low to be measured by our method. So in Table 2 we left the generic indication $K_{\alpha,2} < 10$. No evidence for the formation of the 1 : 2 complex with **5**, **6**, **11** and **13** was detected. It is clear that dimensions of the guest ancillary chain have a crucial role in allowing the approach of the second host unit. In particular, the pyrrolidine moiety of **8** seems to meet the conditions for the best fit into the cavity of the second α-CD unit. Indeed reducing **7** or expanding **9** ring dimensions by only one methylene unit decreases dramatically the $K_{\alpha,1}$ value, while for **11** the formation of the 1 : 2 complex is completely suppressed, obviously owing to steric hindrances. Noticeably, **12** shows a better inclusion in the neutral (pH 2.5) than in the ionised form (pH 6.0) as a consequence of its easier desolvation. It is also interesting to notice that the introduction of a carboxylate group in **13** completely suppresses the second complexation process as compared to **8**. We have already observed that also $\Delta H^{\circ}_{\alpha,2}$ and $T\Delta S^{\circ}_{\alpha,2}$ values for the formation of the 1 : 2 complex show a good compensation effect, with a smaller value for the intercept $T\Delta S^{\circ}_{o}$ than for the formation of the 1 : 1 complexes (Table 4). This should indicate that upon the formation of the 1 : 2 complex the second approaching α-CD unit undergoes internal desolvation to a somewhat lesser extent than the first one. However at this point it appears clear that "internal desolvation" should not be intended strictly as the loss of the inner-cavity water molecules, but in some way involves also the "expanded hydrophobic sphere" as defined above. Anyway, the apparent regression of the batochromic shift suggests that the guest chromophore experiences within the 1 : 2 complex a rather different environment than in the 1 : 1 complex. In our opinion the two α-CD units are very likely to assume a head-to-head arrangement in the 1 : 2 complex, causing the annihilation of the local electric field effect discussed above. Analysis of the thermodynamic parameters reveals the most favourable enthalpy variations for **12** than for all other guests, once again indicating the importance of the polarity and the structurising characteristics of the guest. $\Delta H^{\circ}_{\alpha,2}$ values for **7**, **8** and **1** clearly vary in the same way as hydrophobicity, indicating that their inclusion is mostly influenced by van der Waals interactions, while the conformational freedom of the ancillary chain in **3** obviously affects its $\Delta S^{\circ}_{\alpha,2}$ value, with a consequent unfavourable effect on $\Delta H^{\circ}_{\alpha,2}$.

Conclusions

The present study focuses on the different features shown by the inclusion properties of α-CD and β-CD towards guests **1**–**13**. The formation of 1 : 1 complexes with the former host involves a rather rigid inclusion of the aromatic moiety, but the thermodynamics of binding is strongly affected by the interaction between the ancillary chain and the "expanded hydrophobic sphere" of the host itself; the overall process is essentially enthalpy-driven. Furthermore α-CD is also able to form 1 : 2 complexes along with 1 : 1 complexes, depending on the ancillary chain of the guest. Inclusion in the β-CD host, on the other hand, is both an enthalpy- and entropy-driven process; the thermodynamics of binding suggests that the wider host cavity is able to interact effectively with the ancillary chain of the guest.

Experimental

Materials

Commercial α-CD and β-CD (Fluka) were dried in a desiccator *in vacuo* over phosphorus pentoxide at 90 °C for at least 24 hours and stored in the same apparatus at 40 °C; they were then used as such. Commercial **5** (Fluka) was crystallised twice from methanol before use; commercial **6** (Aldrich) was purified by distillation before use. Guests **1**–**4** and **7**–**11** were prepared according to literature reports.[27]

Amino acid derivatives **12**[28] and **13** were prepared according to the following procedure: the amino acid [(10 mmol), glycine for **12** and (L)-proline for **13**] was treated with an equimolar amount of tetrabutylammonium hydroxide in methanol; the solvent was removed *in vacuo*, and the residue was dissolved in 20 ml of DMSO. A slight excess of *p*-fluoronitrobenzene (11 mmol) and potassium carbonate (11 mmol) was added and the mixture was allowed to react under gentle warming (45 °C) with stirring until completeness (TLC). The mixture was then poured into cold water, acidified, and extracted with ethyl acetate; the organic extract was evaporated *in vacuo* and the residue finally purified by chromatography on silica gel with light petrol–ethyl acetate mixtures as eluents (yield 60–80%).

N-(*p*-nitrophenyl)-(L)-proline (13)

Orange–brown crystals; mp >250 °C (decomp.) (Found: C, 56.0; H, 5.1; N, 11.9; O, 27.0. $C_{11}H_{12}N_2O_4$ requires C, 55.9; H, 5.1; N, 11.8; O, 27.2); ν_{max}(nujol mull)/cm^{-1} 1720 (C=O). ^{1}H NMR (250 MHz, DMSO): δ_H(250 MHz; DMSO; Me_4Si) 1.96–2.40 (4 H, m, –CH_2–CH_2CH_2–CH<), 3.43–3.64 (2 H, m, >N–CH_2–), 4.47 (1 H, dd, *J* 8.6 and 2.4, >C*H*–COOH), 6.63 (2 H, d, *J* 9.3, Ar), 8.12 (2 H, d, *J* 9.3, Ar).

All other commercial reagents (Fluka, Aldrich) and materials needed were used as such without further purification. Stock

phosphate buffer solutions were prepared according to literature reports and used within a few days, after checking the actual pH value with a PHM82 Radiometer equipped with a GK2401C combinated electrode. Freshly double-distilled water was used for the preparation of the buffers, which were in turn used as solvents for the preparation of the measurement solutions. All fitting analyses were performed by means of the KALEIDAGRAPH™ 3.0.1 software delivered by Abelbeck Software.

Measurement of pK_a of 12 and 13

A weighed amount (about 40 μmol) of **12** or **13** was introduced in a water-jacketed vessel thermostated at 298.1 ± 0.3 K and was dissolved with a 0.0025 M standardised NaOH solution (20 ml) under magnetic stirring. A stream of fine Argon bubbles was passed for 15 min through the solution, which was then titrated with a 0.1 M standardised HCl solution introduced into the vessel by a microsyringe. The titration was performed following the pH value with the apparatus described above. Data were finally processed fitting the pH *vs.* added base curve by means of the proper equation obtained analytically.

Measurement of binding constants

Solutions for measurements were prepared at a fixed concentration of guest (usually about 30 mM) and at a concentration of host ranging up to 0.05 M for α-CD, or up to 0.008 M for β-CD (according to the maximum solubility of the two cyclodextrins). Uv-vis spectra were recorded at different temperatures ranging from 288.15 to 318.15 K on a Beckmann DU-7 spectrophotometer equipped with a peltier temperature controller, able to keep the temperature within a ±0.1 K error. Suitable work wavelengths for each guest were chosen after recording some "difference spectra" by comparison of the samples without cyclodextrin and in presence of given amounts of cyclodextrin. The absorbances of the different solutions at the work wavelength were processed by direct non-linear regression analysis[29] according to eqns (1) and (2).

Acknowledgements

Financial support from the University of Palermo (funds for selected research topics) and Italian MIUR within the National Research Project "Non-aromatic Heterocycles in stereocontrolled processes" is gratefully acknowledged.

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