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The binary pyrene/heptakis-(6-amino-6-deoxy)-β-cyclodextrin complex: a suitable chiral discriminator. Spectrofluorimetric study of the effect of some α-amino acids and esters on the stability of the binary complex

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Abstract—The effect of some α-amino acids and their esters on the stability of the binary pyrene/heptakis-(6-amino-6-deoxy)-β-cyclodextrin (py/am-β-CD) complex has been studied by means of fluorescence spectroscopy at two pH values (8.0 and 9.0). The binary complex was generally stabilized by adding the ternary agent at pH 8.0. A more varied substrate effect is observed at pH 9.0 where am-β-CD is present in the uncharged form. The conditional constant (β_2) values determined by L/D α-amino acids show that the binary complex is a suitable receptor for chiral recognition. The enantiomer selectivity values obtained, ranging from 1.2 up to 7.4, are generally higher than those reported for α-amino acids and their derivatives by modified cyclodextrins.

1. Introduction

Enantioselective receptor–substrate interactions are extremely important in biochemical systems and form the basis of chiral recognition, which is a major goal in current supramolecular chemistry research.[1] As has been reported recently, supramolecular systems such as cyclodextrins (CDs) are potential chiral discriminators.[2] CDs constitute a homologous series of non-reducing, cyclic D-(+)-glucose oligomers ranging in size from six- to twelve-ring structures.[3] They are produced by the action of *Bacillus macerans* amylase on starch.[4] Of this series, the torus-shaped β-CD has been studied the most. β-CD has a moderately polar cavity that enables it to bind guests of low water solubility.[5]

The binding ability of β-CD, combined with its chirality, renders it suitable for enantiomeric discrimination, due to the formation of diastereomeric host–guest complexes from a racemic mixture.[6] Indeed, β-CD has been employed as a mobile–phase modifier in the liquid chromatographic separation of enantiomers.[7] The potential of CD-bonded phases in high-performance liquid chromatography (HPLC) has also been demonstrated in the separation of optical, geometrical and structural isomers.[8]

The binding ability of the native β-CD can be varied by replacing one or more hydroxyl groups with, for example, amino groups.[9] Indeed, this should cause variations in hydrophobicity and the cavity shape of the β-CD. Amino-β-CDs are very interesting hosts, since their molecular binding ability depends on the acidity of the solution.[10] Therefore, the host–guest interactions can be modulated by changing the charge on the amino group. Using native or modified CD can alter both the chiral recognition as well as the binding ability. Liu et al. have collected data, by means of spectrophotometric titration, relating to enantiomer selectivity (the ratio between stability constants for the complexes of the (*R*)- and (*S*)-enantiomers) of α-amino acids using 6-anilino-6-deoxy-β-cyclodextrin and 6-1-pyridinio-6-deoxy-β-cyclodextrin.[11] Kano et al. have determined the enantiomer selectivity of 6-amino-6-deoxy-β-cyclodextrin and heptakis-(6-amino-6-deoxy)-β-cyclodextrin (am-β-CD) with α-amino acid derivatives (*N*-acetylated and methyl esters) by means of ^{1}H NMR spectroscopy.[12] Using microcalorimetric titration techniques Inoue et al. have studied the chiral recognition of several anionic, cationic and neutral guests by β-CD and 6-amino-6-deoxy-β-cyclodextrin,[13] which showed that aminated CDs have higher enantiomer selectivity than β-CD.

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On moving from binary substrate–CD complexes to ternary complexes (two substrate molecules included in the CD cavity) the host–guest interactions change. We have found it intriguing to study by means of fluorescence spectroscopy, the chiral recognition and the complexation of some α-amino acids by binary pyrene/heptakis-(6-amino-6-deoxy)-β-cyclodextrin (py/am-β-CD) complex. A few years ago, Bohne used the fluorescence spectroscopy to study the effect of amino acids on the stability of the binary py/β-CD complex.[14]

In the present study the L/D α-amino acids were chosen as the ternary agents in order to examine the effects of the size, shape and chirality of the guest upon its inclusion. The measurements were carried out in aqueous borate buffered solutions (pH 8.0 and 9.0) where all of the amino acids studied were in the anionic form (as can be easily calculated using their pK values). The chosen amino acids can be grouped according to the polarity and charge of the side chain. Those with a non-polar side chain were leucine (Leu), isoleucine (Ile), proline (Pro), valine (Val) and phenylalanine (Phe). Methionine (Met) was used as an amino acid with a polar uncharged side chain. Finally, an amino acid with a polar charged side chain was histidine (His). In order to evaluate how the host–guest interaction can be affected by the charge we have also collected data relative to the methyl esters of α-amino acids.

2. Results and discussion

2.1. Fluorescence spectral titrations

Upon inclusion of the fluorophore (pyrene) into the CD cavity, the luminescence is enhanced because the guest molecule is shielded from quenching and non-radiative decay processes that occur in the bulk solution.[15] The fluorescence intensity of the pyrene gradually increases with increasing concentration of am-β-CD. Typical fluorescence spectral changes upon addition of am-β-CD to a pyrene solution are shown in Fig. 1.

It has been claimed that in the presence of β-CD, the pyrene gives a binary complex having stoichiometry 1:2.[16] Also in our case variations in the fluorescence intensity (ΔI) of the pyrene with increasing am-β-CD concentration have been interpreted on the grounds of the formation of a binary complex with 1:2 stoichiometric ratio. This hypothesis has been confirmed through a Job's plot method.[17] The complexation of the pyrene with β-CD (am-β-CD in our case) has been described[14] by the sequential complexation of β-CD molecules.

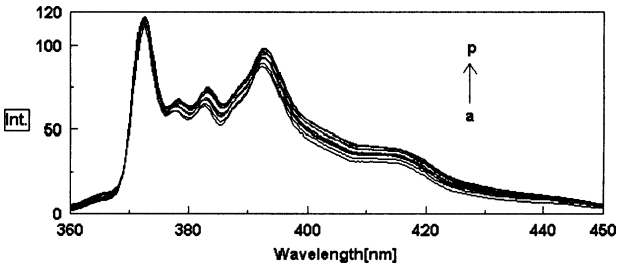

Figure 1. Fluorescence spectra of pyrene in the absence (a) and in the presence of increasing concentrations (p) of am-β-CD in borate buffer solution at pH 8.0.

$$\mathrm{Py+CD} \overset{K_1}{\rightleftharpoons} \mathrm{PyCD}$$

$$\mathrm{PyCD+CD} \overset{K_2}{\rightleftharpoons} \mathrm{PyCD_2}$$

In the presence of a ternary agent, K_1 and K_2 are conditional equilibrium constants, since they include a term related to the ternary agent concentration. The overall association constant, β_2, is defined as:

$$\beta_2 = K_1 K_2 = \frac{[\mathrm{PyCD_2}]}{[\mathrm{Py}][\mathrm{CD}]^2} \quad (1)$$

If $[\mathrm{CD}] > [\mathrm{PyCD_2}]$, the change in the fluorescence intensity of the pyrene with increasing am-β-CD concentration can be related to β_2 as follows:

$$\Delta I = \frac{\beta_2 \Delta\alpha [\mathrm{Py}]_0 [\mathrm{CD}]_0^2}{1+\beta_2 [\mathrm{CD}]_0^2} \quad (2)$$

where ΔI is the difference in molar absorption coefficients for the free and complexed pyrene, and $[\mathrm{Py}]_0$ and $[\mathrm{CD}]_0$ are the total concentrations of the pyrene and the am-β-CD, respectively. Eq. (2) is the non-linearized version for Benesi–Hildebrand treatment.[18] A typical plot of ΔI as a function of $[\mathrm{CD}]_0^2$ is shown in Fig. 2.

The geometry of the py/CD complex has been represented by many models. It has been reported that in the presence of β-CD, the pyrene penetrates into the moderately polar cavity, but models show that half of the pyrene molecule protrudes. A second cyclodextrin molecule can then enclose the protruding part, giving a complex having a 1:2 stoichiometric ratio (Fig. 3).

Neither of the cavities is completely filled by the pyrene molecule and therefore some water molecules are not displaced. This process is not thermodynamically favored and the stability of the complex can be altered by adding a ternary agent such as an alcohol,[19] an alkyl sulfate[20] or an α-amino acid.[14] Formation of a ternary complex is deduced by changes in the fluorescence spectrum on adding the ternary agent. The β_2 values for the ternary complex can be either higher or lower than the binary complex. Enhancement of the complexation efficiency has been attributed to the formation of a ternary complex where a ternary agent replaces some water molecules inside the CD cavity. A decrease in the complexation constant is attributed to the opposite situation, where the ternary agent partially displaces the pyrene within the CD cavity.

In Table 1 the conditional overall equilibrium constants (β_2) are listed for py/am-β-CD complexes collected in aqueous borate buffer at pH 8.0 and 9.0, in the absence and presence of ternary agent. Furthermore, the previously published β_2 values for py/β-CD/amino acid complexes[14] are also included. These values, although determined under different experimental conditions (see Table 1), are interesting and useful for comparison. All

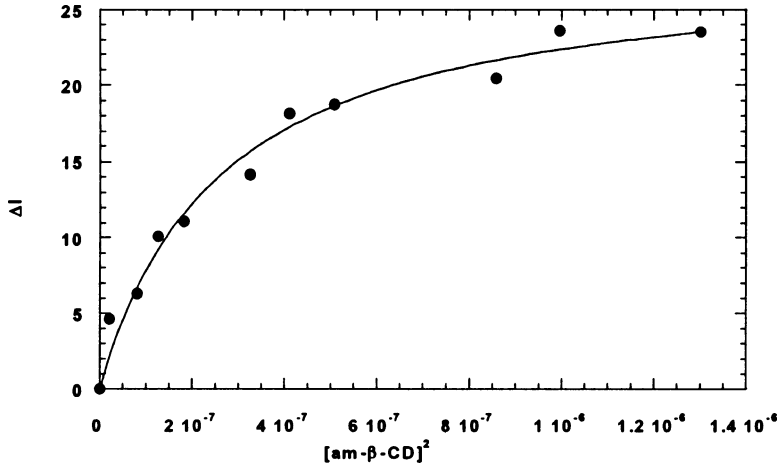

Figure 2. Curve fitting analysis of fluorescence spectral titration of pyrene with am-β-CD in borate buffer solution at pH 8.0.

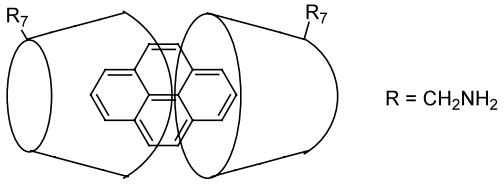

Figure 3. Model of binary complex pyrene/am-β-cyclodextrin.

β_2 values were obtained by analyzing (using a non-linear least squares method) the spectrofluorimetric titration curves.

The β_2 values show that the py/am-β-CD ($\beta_2 = 1.7\times10^6$ and 4.8×10^6) complex, at the two pH values examined, is more stable than the py/β-CD ($\beta_2 = 4.7\times10^4$)[14] complex.

This is probably due to the difference in the hydrophobicity and shape of the cavity between the two CDs. am-β-CD, which is less soluble in water than β-CD, should be more hydrophobic. Furthermore, the py/am-β-CD complex is more stable at pH 9.0 than at pH 8.0, due to the fact that am-β-CD is partially protonated at pH 8.0 but is not protonated at pH 9.0, as established from pK_a values.[21] Previously, Kano has reported[12] that, due to the expansion of the NH_3^+ group side, am-β-CD cannot include a neutral guest such as pyrene, whereas its partially cationic or neutral forms are able to incorporate such a guest.

2.2. L-Amino acids

Regarding the ternary py/CD/amino acid complexes, the am-β-CD forms, both at pH 8.0 and 9.0, more stable aggregates than β-CD. Actually, the latter gives stable ternary complexes only in the presence of L-Leu, L-Phe, L-Met and L-Ile. For L-His it has been reported that the lack of interaction between the amino acid and the py/β-CD complex is due to the more difficult desolvation process of this ternary agent (which has a negatively charged side chain).[14] Interactions between amino acids having a non-polar side chain and the py/β-CD complex have been explained on the grounds of molecular volume, hydrophobicity and side chain structure.

It is interesting to note that the β_2 values for ternary py/am-β-CD/amino acid complexes depend on the pH of the solution as well as on both the chirality and structure of the ternary agent. Indeed the L-amino acids studied form more stable complexes at pH 8.0 than at pH 9.0, which seems to indicate that the electrostatic interactions present at pH 8.0 between the ammonium groups of am-β-CD and the carboxylate group of amino acids, are more efficient than the hydrogen bonds that stabilize the system at pH 9.0. Regarding the effects of amino acid structure on β_2, we can observe that amino acids having a polar side chain (L-His and L-Met) stabilize the py/am-β-CD binary complex. The stabilization increases (L-His<L-Met) as the polarity of the side chain decreases. Furthermore, unlike β-CD, am-β-CD forms stable ternary complexes in the presence of L-His. Presumably this is due to both the strong electrostatic interactions between the host and the side chain of the ternary agent at pH 8.0, and the hydrogen bond network at pH 9.0.

Amino acids having a non-polar side chain stabilize the binary py/am-β-CD complex at pH 8.0. The observed order of the β_2 values (L-Pro>L-Phe>L-Val>L-Leu>L-Ile) cannot be put down to only one property of the amino acid. The high stabilization calculated for L-Pro is probably connected to the lesser capacity of the secondary amino group (cf. a primary amino group) to form hydrogen bonds both with water molecules and amino groups on the primary rim of CD. Instead, the hydrophobicity could be the factor that determines the stabilizing effect of L-Phe. A combination of the hydrophobicity, size and the structure of the side chain should determine the different stability of the ternary complex in the case of L-Val, L-Leu and L-Ile.

At pH 9.0, the β_2 values show that of the amino acids bearing a non-polar side chain, only L-Phe stabilizes the binary complex. This infers that strong electrostatic interactions are able to compensate for the lack of hydrophobic interactions, but in the absence of Coulombic interactions the higher stabilization is due to greater hydrophobicity.

Table 1. Conditional overall equilibrium constants (β_2) for py/am-β-CD complexes collected in aqueous borate buffer at pH 8.0 and pH 9.0 in the absence and presence of ternary agent

Ternary agent	CD	$\beta_2 \pm s^a/10^6$ (M^{-2}) pH 8.0	$\beta_2 \pm s^a/10^6$ (M^{-2}) pH 9.0
	β-CD	0.047[b]	0.047[b]
	am-β-CD	1.7±0.4	4.8±2.1
L-Leu	β-CD	0.43[b]	0.43[b]
L-Leu	am-β-CD	3.8±1.3	2.2±0.3
D-Leu	am-β-CD	0.8±0.5	5.4±0.9
L-Ile	β-CD	0.032[b]	0.032[b]
L-Ile	am-β-CD	3.0±1.4	2.1±0.3
D-Ile	am-β-CD	1.3±0.3	12.3±2.6
L-Phe	β-CD	0.38[b]	0.38[b]
L-Phe	am-β-CD	8.9±2.3	5.2±0.8
D-Phe	am-β-CD	1.2±0.5	7.6±1.8
L-Met	β-CD	0.058[b]	0.058[b]
L-Met	am-β-CD	13.4±4.5	8.8±2.6
D-Met	am-β-CD	2.2±0.6	1.5±0.5
L-Pro	am-β-CD	11.9±3.9	1.8±0.6
D-Pro	am-β-CD	3.1±0.4	2.6±0.5
L-Val	am-β-CD	4.9±1.3	2.0±0.6
D-Val	am-β-CD	3.4±1.0	12.1±1.4
L-His	am-β-CD	7.9±1.3	6.2±1.8
D-His	am-β-CD	6.5±2.7	3.5±0.9
L-Leu-Me ester	am-β-CD	3.2±0.6	1.5±0.3
L-Ile-Me ester	am-β-CD	8.7±3.5	2.2± 0.7
L-Phe-Me ester	am-β-CD	2.7±0.4	3.9±0.6
L-Met-Me ester	am-β-CD	2.6±0.8	1.6±0.4
L-Pro-Me ester	am-β-CD	5.1±0.6	12.1±0.3
L-Val-Me ester	am-β-CD	4.6±1.3	6.7±1.7
L-His-Me ester	am-β-CD	1.7±0.6	3.2±0.5

[a] Standard deviation.
[b] This value was calculated for a non buffered solution. See Ref. 14.

2.3. L-Amino esters

The influence of electrostatic interactions on stabilization of ternary complexes has been investigated by esterification of amino acids. The methyl esters, with the exception of the L-His derivative, which does not influence the stability of the binary complex, stabilize the binary py/am-β-CD complex, at pH 8.0. The β_2 values for py/am-β-CD/esters, with the exception of L-Ile derivative, are lower compared to the respective ternary complexes of amino acids. The above data indicates that generally the increase in hydrophobic interactions going from amino acid to methyl ester does not counterbalance the decrease in electrostatic interactions by ion-pairing between amino acid anion and protonated amino groups of am-β-CD. The increase in stabilization of binary complex observed for L-Ile ester could be consequence of a deeper inclusion of side chain respect to those of L-Phe or L-Leu esters. Going from pH 8.0 to pH 9.0 causes the failure of ion–dipole interactions so dipole–dipole and hydrophobic interactions determine the observed β_2 values from which it is possible to conclude that only L-Pro and L-Val esters stabilize the binary complex.

2.4. D-Amino acids

Data collected for D-amino acids show that only D-Pro, D-His and D-Val stabilize the binary complex. Furthermore, the β_2 values for L- and D-isomers show that the chirality plays an important role in determining the degree of amino acid–binary complex interactions. Indeed, all of the D-amino acids examined form less stable ternary complexes relative to those formed with the corresponding L-enantiomers at pH 8.0. Amino acids with a polar side chain (D-His and D-Met) give, at pH 9.0, ternary complexes again less stable than those of the corresponding L-isomers. Instead, D-Leu, D-Ile, D-Phe, D-Val and D-Pro present an opposite behavior.

2.5. Chiral recognition

The fact that L- and D-isomers give different β_2 values allows the use of the py/am-β-CD system for chiral recognition. Indeed, from data in Table 1, values of enantiomer selectivity can be calculated ranging from 1.2 for His up to 7.4 for Phe at pH 8.0 and from 1.5 for Phe up to 6.0 for Val at pH 9.0. A comparison among enantiomer selectivity values that can be calculated by our β_2 data and those reported in the literature points out the different ability of the studied system respect to that of modified CDs to recognize central chirality. From the data in Table 1 we can calculate enantioselectivity values of 1.4 (pH 8.0) and 6.0 (pH 9.0) for Val and 4.8 (pH 8.0) and 2.5 (pH 9.0) for Leu. Previously for the same amino acids Liu found enantioselectivity values of 2.1 (Val) and 1.7 (Leu) using the 6-1-pyridino-6-deoxy-β-CD for complexation.[11] The best enantioselectivity value found by Kano[12] for *N*-acetylated amino acids using charged am-β-CD as host was 1.6 for Val

derivative. Lower values were observed for *N*-acetylated Leu and Phe.

In several cases we have observed that structural variation of the amino acid leads to both stronger binding with the binary complex and an enhancement in chiral recognition. This is in disagreement with Inoue's assertion that stronger binding leads to a loss of chiral recognition.[13] Actually, we think that direct substrate–CD interaction is not comparable with substrate–binary complex interaction. Indeed, the former leads to the best host–guest fit, whereas the latter should consist of an acceptable arrangement of substrate into the available residual CD cavity of the binary complex.

3. Conclusions

This work shows that am-β-CD forms a more stable complex with pyrene than β-CD. The effects of α-amino acids on the stability of the binary complex can be rationalized on the basis of either electrostatic interactions (pH 8.0) or hydrophobicity factors (pH 9.0 and amino esters). The β_2 values for L- and D-α-amino acids show that the binary complex is a good system for spectrofluorimetric chiral recognition. The enantiomer selectivity values determined in this work are high compared to literature reports and make the py/am-β-CD complex better for enantioselective binding than both simple am-β-CD and binary py/β-CD complexes. This confirms that modified CDs are very promising materials for applications in several fields.

The data collected demonstrates that the degree of enantiodiscrimination is determined by the location of the penetrating side chain of the guest inside the py/am-β-CD cavity. Finally, the type of substrate–binary complex interaction leads us to conclude that by changing the fluorophore it should be possible to develop other receptors with different chiral recognition abilities.

4. Experimental

4.1. Materials

The heptakis-(6-amino-6-deoxy)-β-cyclodextrin was synthesized and purified according to the procedure described in the literature.[22] The product was dried for 24 h in a dryer under vacuum over phosphorous pentoxide at 60°C and then was stored in the same apparatus at 40°C.

All amino acids and corresponding methyl esters were purchased from Aldrich and used without further purification. Pyrene (≥99% for fluorescence) was purchased from Fluka and used without further purification.

Borate buffer solutions (0.05 M) were prepared according to the standard procedure, using freshly double-distilled decarbonized water. The actual pH of the solutions was recorded using a pH M82 Radiometer equipped with a GK2401C combined electrode.

4.2. Spectrometric measurements

The solution of am-β-CD in borate buffer (1.4×10^{-3} M) was filtered before use by a Millipore 0.45 μm filter. Pyrene aqueous solution (2×10^{-6} M) was prepared injecting a pyrene methanolic solution (2×10^{-3} M) into a buffer solution, containing the ternary agent (1×10^{-2} M). Measurement solutions were prepared by adding increasing volumes of the am-β-CD to 1 mL of the pyrene and ternary agent solution into a volumetric flask. In these solutions, the concentrations of the pyrene and the ternary agent were constant and equal to 2×10^{-7} and 1×10^{-3} M, respectively, while the concentration of the am-β-CD increased from 1.4×10^{-4} to 1.3×10^{-3} M. All measurement solutions were deareated, before use, by Ar for 12 min.

Steady-state fluorescence spectra were acquired with a JASCO FP-777W spectrofluorimeter. Excitation and emission slits were set at 1.5 nm and excitation wavelength was 337 nm. Spectra were recorded from 360 to 450 nm. Every spectrum was averaged on 50 scans. A suitable wavelength was chosen after recording a 'difference spectrum' by comparison to a sample without cyclodextrin and one with the highest CD concentration.

Acknowledgements

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