Complex formation of fenchone with α-cyclodextrin: NMR titrations

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Abstract 13C NMR titration studies of inclusion complexes of bicyclic terpenoid, fenchone enantiomers with α-cyclodextrin revealed their 1:2 guest–host stoichiometry. Sequential binding constants were determined indicating a strong binding cooperativity of two α-cyclodextrin to fenchone. The overall association constants were used to calculate the Gibbs free energies of diastereomeric complex formation, which might be used as a measure of chiral recognition of fenchone by α-cyclodextrin. These results were compared with corresponding data derived for camphor, which is an isomeric bicyclic terpenoid.

Keywords Alpha-cyclodextrin · Fenchone · Inclusion complexes · 13C NMR titration · Sequential association constants · Diastereomeric complexes · Chiral recognition

Introduction

Cyclodextrins (CDs) are macrocyclic oligosaccharides composed of a number of glucopyranoside units bound together by α-1,4 bonds. The naturally occurring α-, β- and γ-cyclodextrins (αCD, βCD, and γCD) consist of six, seven, and eight glucopyranose units, respectively [1]. They are obtained by enzymatic starch degradation [1, 2]. CDs, whose shape remains a truncated cone, contain a lipophilic central cavity and a hydrophilic outer surface. The size of αCD cavity: bottom diameter 0.53 nm, top diameter 0.47 nm, and cone height 0.79 nm [1, 2] allows for accommodating many low molecular weight compounds. In aqueous solutions, CDs can form host–guest inclusion complexes with many partially or fully lipophilic molecules often increasing the guest solubility. Hence their wide application in chemistry, pharmacy, or food industry [1–3]. A number of non-covalent forces is responsible for the stabilization of inclusion complexes [4]. The stoichiometry and stability of such complexes strongly depend on the physicochemical properties of guest molecules [5].

Among many compounds complexed with CDs and their derivatives, the bicyclic monoterpentoid, camphor, has been extensively studied by different experimental methods [6–17]. In contrast CD complexes of camphor isomer - fenchone (1,3,3-trimethylbicyclo[2.2.1]heptan-2-one) have been the subject of few studies mainly devoted to physiological or pharmaceutical applications [18–23]. Hence rigorous physicochemical studies of fenchone—CDs complexes would provide useful information on the variation of complex stabilities with variation in the geometry of isomeric guest compounds. Fenchone is characterized by low solubility in water and a size that is comparable to that of the inner cavity of αCD. Fenchone enantiomers are shown in Fig. 1.

NMR spectroscopy is very well suited to study weak and moderate strength molecular complexes and their properties. It is accepted that taking into account typical NMR sample concentrations, the best accuracy can be obtained for association constants within the range 10−10⁸ M⁻¹ [24, 25]. Therefore, NMR has been widely used for...
studying inclusion complexes formed by CDs. The success of NMR spectroscopy in this field is due to its ability to study complex chemical systems, to determine complex stoichiometry, association constants, and conformations and to obtain information on their symmetry and dynamics [5, 26, 27]. Compared to other techniques, NMR spectroscopy provides a superior method to study complexation phenomena as guest and host molecules can be simultaneously observed at the atomic level. Since the rates of complex formation and decomposition are usually faster than the chemical shift time scale (often misleadingly named NMR time scale), the observed chemical shifts are the mole fraction weighted averages of the chemical shifts existing in the free and complexed molecules [5, 24]. If the assumption of rapid equilibrium is not valid, an analysis of the total lineshape is required [16, 28]. CDs are chiral and, therefore, can form diastereomeric complexes, usually of different stability, with enantiomeric species [29].

**Experimental**

NMR measurements

αCD (Sigma, 99 % purity) and both enantiomers of fenchone (the gift from prof. H. Dodziuk) were used without further purification. The 2H2O (Armar Chemicals, 99.8 at.% D) solutions of fenchone enantiomers contained small amount of acetone (Chempur, pure p.a.) whose NMR signal was used as the indicator of external magnetic field inhomogeneity and internal secondary reference: δH = 2.22 and δC = 30.89 [30]. All measurements were performed at magnetic field of 9.4 T, using a Varian Unity Inova 400 MHz, spectrometer. NMR measurements were performed at a temperature carefully adjusted to 300.6 K with an accuracy of 0.1 K and was checked by an ethylene glycol reference sample (composition: 80 % ethylene glycol, Aldrich/20 % dimethyl sulfoxide-D6, Armar Chemicals).

The changes in 1H and 13C chemical shifts of three methyl signals as a function of αCD concentration were analyzed assuming either simple 1:1 or complex 1:1 and 1:2 guest–host stoichiometry. In the latter case stepwise (sequential) binding [26] was assumed. Sequential macroscopic association constants were defined by the following eqns.:

\[ K_{1,c} = \frac{[\text{GH}]}{[\text{G}][\text{H}]} \quad \text{and} \quad K_{2,c} = \frac{[\text{GH}_2]}{[\text{GH}][\text{H}]} \]

with square brackets [ ] denoting molar concentrations of appropriate species, [G]—guest (fenchone), [H]—host (αCD), [GH] and [GH2]—complexes with stoichiometry...
1:1 and 1:2, respectively. Averaged chemical shifts, $\delta_a$, were calculated using the formula [24]

$$\delta_a = \delta_f + \sum_{i=1}^{N} x_i (\delta_i - \delta_f) = \delta_f + \sum_{i=1}^{N} x_i \Delta \delta_i$$

where $\delta_f$ is chemical shift in uncomplexed fenchone, whereas $x_i$ and $\delta_i$ are mole fractions and chemical shifts of $i$-th complex species. Association constants $K_{i,c}$ and complexation-induced shifts $\Delta \delta_i$ were determined by fitting the experimental dependence of $\delta_{exp}$ in fenchone molecules versus $M$ various concentrations of $\alpha$CD. The least-squares procedure used a Fortran routine written in-house optimizing the model parameters that consisted of minimization through a grid search of the target function $\chi^2$ given by:

$$\chi^2 = \sum_{i=1}^{M} (\delta_{exp} - \delta_a)^2$$

Confidence limits of fitted parameters were estimated by use of constant $\chi^2$ boundaries [31]. Fisher–Snedecor statistics ($F$ test) was used for the stoichiometry selection at the probability 0.01.

**Results and discussion**

The fenchone signal assignments had to be done *de novo* on the basis of COSY, NOESY and $^1$H/$^13$C HSQC spectra since the literature values [32, 33] corresponded to a different solvent. $^1$H and $^{13}$C chemical shifts of free fenchone are collected in Table 1. 1D $^1$H-NMR and 2D $^1$H/$^{13}$C HSQC spectra for (−)-fenchone in D$_2$O and CDCl$_3$ are shown in Figs SF3 and SF4, respectively (Supplementary Materials). Three methyl signals exist, with the farthest and easiest to detect $^1$H and $^{13}$C chemical shifts on complexation. Their $^{13}$C resonances with complexation shifts, exceeding those of $^1$H signals, are especially convenient for quantitative analysis of NMR titration data. In order to provide satisfactory signal dispersion and signal-to-noise ratios of fenchone methyls at the concentration of 1 mM and the natural abundance of $^{13}$C isotope, the 2D $^1$H/$^{13}$C correlation spectra with $^1$H detection are the method of choice. Superposition of a series of HSQC spectra showing C10 correlations in (−)-fenchone–$\alpha$CD complex is shown in Fig. 2. So derived $^{13}$C methyl chemical shift changes upon variable ratios of $\alpha$CD to fenchone enantiomers were used in a numerical procedure yielding best estimates of the association constants.

The sigmoidal shape of all titration curves (Figs. 3, 4) strongly suggests a composite stoichiometry of the studied complexes and a possibility of cooperative binding [34, 35]. In fact, the best reproduction of experimental chemical shifts has been obtained assuming a sequential binding model, whereas a simple 1:1 stoichiometry was precluded on the basis of Fisher–Snedecor statistics. The best fit estimates of the association constants are collected in Table 2. Their $K_{1,c}$ values are smaller than those averaged for a variety of many 1:1 inclusion complexes built up of $\alpha$CD host molecules [36]. Nevertheless, an association of second $\alpha$CD molecule to 1:1 fenchone–$\alpha$CD complexes significantly increases their stability.

Association constants expressed on the molar concentration scale, $K_{i,c}$, are not suitable for determining thermodynamic quantities. Therefore, recalibration of association constants $K_{i,c}$ on molar fraction scale, $K_{i,a}$, has to be done [16, 26]. The $K_{i,a}$ values and estimates of the corresponding Gibbs free energies $\Delta G_0$ for complex formation of both fenchone enantiomers with $\alpha$CD are given in Table 3. A comparison of these data with earlier results obtained for camphor complexes with $\alpha$CD [8, 16] reveals that the overall association constants, $\beta_{12,a} = K_{1,a} K_{2,a}$, for camphor complexes are three orders of magnitude higher than the corresponding values for fenchone complexes. On the other hand, chiral recognition, (i.e., differentiation of enantiomeric species, forming diastereomeric complexes which are, quantitatively expressed as $\Delta \Delta G_0 = \Delta G_{0(-)} - \Delta G_{0(+)}$) for camphor complexes is lower than that observed for fenchone complexes.

The systems with at least two binding sites can exhibit a complex behavior that depends not only on the affinities for each site but also on the interaction between the sites. For instance, the facing rims of two cyclodextrin molecules may interact forming dimers via hydrogen bonds linking their hydroxyls at C2 and C3 glucopyranose units and promoting additional 1:2 complex stabilization. If the binding to one site enhances the affinity for a second site,

| Position | $^1$H$^a$ | $^1$H$^b$ | $^1$H$^c$ | $^{13}$C$^a$ | $^{13}$C$^b$ |
|----------|-----------|-----------|-----------|-------------|-------------|
| 4        | 2.19      | 2.14      | 2.14      | 45.84       | 45.3        |
| 5x       | 1.76      | 1.72      | 1.80      | 24.74       | 25.0        |
| 5n       | 1.76      | 1.82      | 1.70      |             |             |
| 6x       | 1.68      | 1.56      | 1.54      | 32.41       | 31.8        |
| 6n       | 1.34      | 1.39      | 1.37      |             |             |
| 7a       | 1.61      | 1.53      | 1.54      | 41.81       | 41.6        |
| 7s       | 1.89      | 1.79      | 1.80      |             |             |
| 8        | 1.04      | 1.03      | 1.04      | 22.89       | 23.3        |
| 9        | 1.03      | 1.03      | 1.04      | 21.37       | 21.7        |
| 10       | 1.11      | 1.14      | 1.15      | 14.16       | 14.6        |

$^a$ D$_2$O solution, this work
$^b$ CDCl$_3$ solution, Ref. [32]
$^c$ CDCl$_3$ solution. Ref [33]
the so called positive cooperativity takes place. Since cooperativity factors are specific for microscopic description of multisite association processes, it is not always possible to extract them from macroscopic association constants which are usually obtained experimentally [26, 35, 37]. A qualitative analysis, however, can be performed easily once the macroscopic association constants have been determined. For a system with two binding sites, the cooperativity factor $a$ can be estimated from [37]:

$$a = \frac{K_2,c}{K_1,c}$$
the two lower order microscopic association constants, has to bear in mind that this equation is strictly valid only if one considers the differential intramolecular rotations of the methyl groups [10].

The overall association constant $\beta_{12,a} = K_{1,a} K_{2,a}$

If $a > 1$, the binding sites exhibit positive cooperativity reflecting the favorable energy loss due to a simultaneous host binding to both sites of the guest molecule [26, 37]. One has to bear in mind that this equation is strictly valid only if the two lower order microscopic association constants, $\kappa_{1i}$, are identical. It is a consequence of the relation between microscopic and macroscopic association constants: $K_{1e} = \kappa_{1A} + \kappa_{1B}$ [26]. Fortunately, the cooperativity factor reaches a minimum at $\kappa_{1A} = \kappa_{1B} = K_{1,c}/2$, where the conclusion about positive cooperativity based on the inequality $a > 1$ remains valid. Therefore, formation of the two chiral (+)- and (−)-fenchone–αCD complexes is characterized by strong cooperativity since their lower limit cooperativity factors are equal to 42.6 and 9.9 for (+)-fenchone and (−)-fenchone complexes, respectively (cf. Table 2). Moreover, it might seem intuitively obvious that a stronger complex is characterized by a larger cooperativity.

The stepwise association constants $K_{i,c}$ in complexes of fenchone with α-cyclodextrin differ by one order of magnitude. This result is in contrast with the data obtained for corresponding complexes of camphor studied by similar approach [8]. It has been estimated that their stepwise association constants differ by four orders of magnitude, thus, precluding their separation but supporting conclusion about strong cooperative binding in camphor–αCD complexes.

Table 3: Values of the association constants, ($K_{i,a}$; $i = 1, 2$), in mole fraction scale, Gibbs free energies, $\Delta G_{0}$, for complex formation of both fenchone enantiomers with αCD and chiral recognition, $\Delta \Delta G_{0}$, compared with corresponding data for camphor complexes taken from Ref. [16]

| Enantiomer       | $K_{1,a}$         | $K_{2,a}$         | $\beta_{12,a}$       | $\Delta G_{0}$ (kJ/mol) | $\Delta \Delta G_{0}$ (kJ/mol) |
|------------------|-------------------|-------------------|----------------------|-------------------------|---------------------------------|
| (+)-fenchone     | 550.8 ± 26.5      | 5870 ± 295        | (3.23 ± 0.22) $10^6$ | -37.5 ± 0.2             | 4.0 ± 0.2                       |
| (−)-fenchone     | 523.5 ± 26.0      | 1290 ± 67         | (0.68 ± 0.05) $10^6$ | -33.5 ± 0.2             |                                 |
| (+)-camphor      | (2.07 ± 0.01) $10^9$ |                  |                      |                         |                                 |
| (−)-camphor      | (0.86 ± 0.01) $10^9$ |                  |                      |                         |                                 |

The overall association constant $\beta_{12,a} = K_{1,a} K_{2,a}$

All three methyl carbons in either (+)- or (−)-fenchone–αCD complexes exhibit complexation 13C chemical shift displacement for the 1:2 complex (Δ$\delta_2$) that is larger than that of the 1:1 complex (Δ$\delta_1$). One can argue that two αCD molecules surrounding a fenchone molecule may exert stronger perturbation to the environment of a guest molecule than a single αCD molecule, thus resulting in a relatively larger complexation 13C chemical shift displacements. In the absence of detailed information on the geometries of fenchone–αCD complexes, however, a detailed interpretation of Δ$\delta_i$ values seems problematic. Nevertheless, all but one Δ$\delta_i$ values are larger for the more stable (+)-fenchone–αCD complex than for the (−)-fenchone–αCD complex, thus the tighter the complex, the larger is the perturbation and hence the chemical shift displacement.

Conclusions

Stoichiometry and sequential association constants have been determined for diastereomeric complexes of fenchone enantiomers with α-cyclodextrin by means of NMR titrations. Estimation of stepwise association constants makes it possible to evaluate and confirm the presence of positive cooperativity for 1:2 complex formation, if any.

For both terpenoids, fenchone and camphor, the (+)-enantiomers form more stable complexes with αCD than the corresponding (−)-isomers. Both fenchone complexes, however, are comparatively much less stable than those of camphor. In contrast, chiral recognition by αCD for fenchone is larger in comparison with camphor. It can be expected that the two geminal methyl groups attached to the C3 carbon atom in fenchone impose more steric hindrance to complex formation with αCD than their counterparts in camphor located at the C7 carbon.

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