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On the impossibility of determination of stepwise binding constants for the 1 : 2 complex of (+)-camphor with α-cyclodextrin

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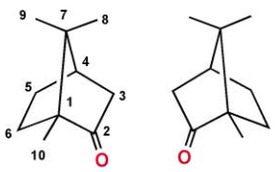
Knowledge of stepwise binding constants for complexes with higher than 1 : 1 stoichiometry would allow one to study the cooperativity of their formation. However, a detailed analysis of partitioning of the overall binding constant β_{12} determined by NMR titrations for the 1 : 2 complex of (+)-camphor with α-cyclodextrin into the stepwise ones K_1 and K_2 carried out analogously to published procedures revealed that the partitioning cannot be carried out unequivocally for $K_1 << K_2$. The programs for partitioning cannot be used as a black box and a satisfactory reproduction of the experimental dependence of relative shifts as a function of relative CD concentration should not be the only criterion of the reliability of the stepwise binding constants obtained using such programs.

Introduction

Knowing the temperature dependence of stepwise binding constants of complexes of higher than 1 : 1 stoichiometry would provide invaluable information on the complexation process, allowing one to determine ΔH and ΔS for the stepwise processes.[1] NMR titrations have been proposed as one of the few methods that can supply these data.[2] Several programs have been applied to analyse specific cases of 1 : 1 and 1 : 2 stoichiometry[3] and a few general programs to solve the problem have been described.[4,5] However, no reliable assessment of the accuracy of the results obtained is given there; the reproduction of experimental dependence of chemical shifts on the concentration of one component (while the second one is in excess at constant concentration) is the only criterion of the reliability of the results obtained by such a fitting and the limitations of the applied methods are not discussed. Moreover, the programs are sometimes used as black boxes, as in our previous work,[6] with little or no attention given to their accuracy and limitations. Therefore, an analysis of the reliability of the results obtained using the NMR titration method seemed of value.

We have recently determined the stepwise binding constants K_1 and K_2 at room temperature for the complexes of camphor enantiomers **1** with α-cyclodextrin, α-CD, **2**[6] using the program developed by the Hunter group.[5] However, a careful inspection of the latter paper revealed that formulae (6), (9) and (10) there (not the program itself) contain errors (the dimensionless quantities are summed up with those having dimensions of concentration). Moreover, as with other papers describing such programs a good reproduction of experimental data is the only criterion of the accuracy of the fitting procedure. (Errors in the formulae (eqns. (1) and (4)) are also contained in the paper by Pistolis and Malliaris describing the analogous procedure.[7])

By applying a method similar to those used in the literature[2] to elucidate K_1 and K_2 values at various temperatures, we encountered fundamental problems, described below, showing that partitioning of the overall binding constant β_{12} into the stepwise ones K_1 and K_2 is equivocal.

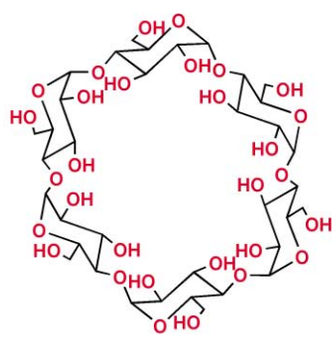


(+)1R,4R

1a

(-)1S,4S

1b



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Method

The following equilibria describe the stepwise complex formation between the substrate (camphor) S and the receptor (α-CD) R:

$$R + S \rightleftharpoons RS \quad (1)$$

$$RS + R \rightleftharpoons R_2S \quad (2)$$

The stepwise binding constants are equal to

$$K_1 = [RS]/([R][S]) \quad (3)$$

$$K_2 = [R_2S]/([RS][R]) \quad (4)$$

where [R], [S], [RS], [R_2S] describe the equilibrium concentrations of the receptor, substrate, 1 : 1 and 1 : 2 complexes, respectively.

The overall binding constant β_{12} is defined as

$$\beta_{12} = K_1K_2 \quad (5)$$

The following relations hold for the total concentrations of the receptor R_0 and substrate S_0

$$[R]_0 = [R] + [RS] + 2[R_2S] \quad (6)$$

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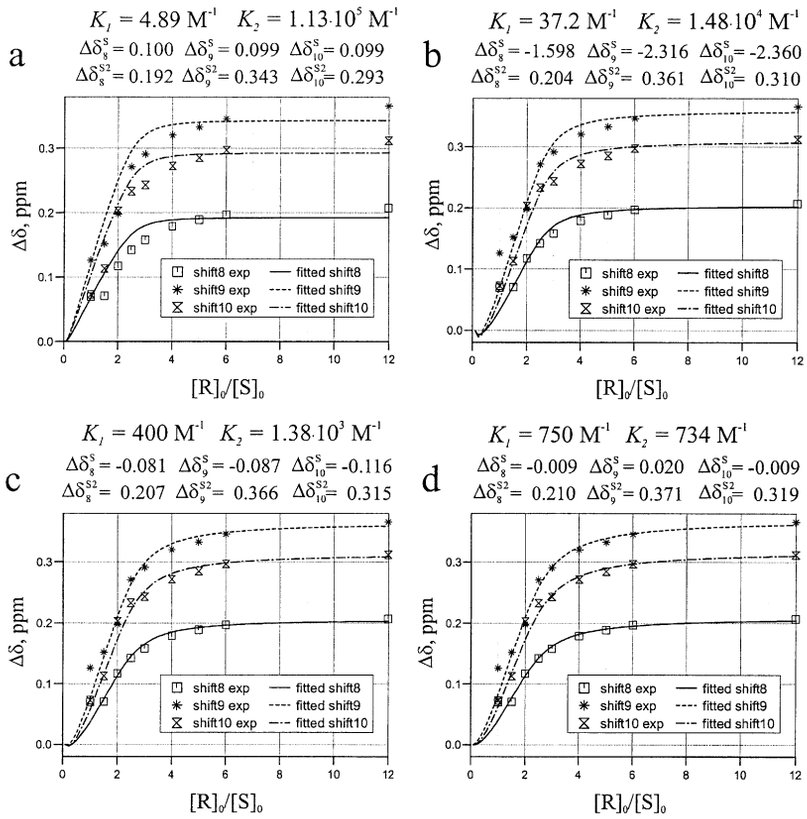

Fig. 1 The dependence of experimental and fitted chemical shifts of proton signals of C8, C9 and C10 methyl groups on the relative concentration $[R]_0/[S]_0$ for selected values of K_1.

$$[S]_0 = [S] + [RS] + [R_2S] \quad (7)$$

In the case of a rapid exchange among the free host and guest, the 1 : 1 and 1 : 2 complexes, the averaged chemical shifts are given by the formulae:

$$\delta^{R}_{av} = (\delta^{R0}[R] + \delta^{R}[RS] + \delta^{R2}[R_2S])/[R]_0 \quad (8a)$$

$$\delta^{S}_{av} = (\delta^{S0}[R] + \delta^{S}[RS] + \delta^{S2}[R_2S])/[R]_0 \quad (8b)$$

where δ^{R0} and δ^{S0} denote signals of the free host and guest, δ^{R} and δ^{S} refer to the corresponding signals of the 1 : 1 complex, while δ^{R2} and δ^{S2} denote the corresponding signals of the 1 : 2 complex in which the guest camphor is embedded inside a capsule formed by two α-CD host molecules.

Usually, chemical shifts $\Delta\delta$ relative to the shifts of the free substrate are analysed. Thus for the guest

$$\Delta\delta = \delta^{S0} - \delta^{S}_{av} \quad (9)$$

For any given $[S]_0$, $[R]_0$, K_1 and K_2, the system of eqns. (3), (4), (6), (7) can be solved with respect to [R], [S], [RS] and $[R_2S]$. Although this system can be analytically solved, the formulae obtained are numerically very costly and unstable. Therefore, it is preferable to reduce this system analytically to a single equation and find its root numerically. In effect, relative shifts $\Delta\delta$ can be computed using eqn. (9) for any given $[R]_0$ and $[S]_0$ depending on the parameters K_1, K_2, δ^{S} and δ^{S2}. Thus, for each experiment with fixed values of $[R]_0$ and $[S]_0$, a new equation for $\Delta\delta$ is obtained and for a sufficient number of experimental values, the desired K_1, K_2, δ^{S} and δ^{S2} can, in principle, be obtained. Taking into account experimental errors, one obtains a standard nonlinear least squares fit problem to be solved *e.g.* by the conjugate gradient method.

In our study, a slightly more complicated experimental setting has been adopted, measuring three sets of chemical shifts for three different protons of the camphor methyl groups $C8H_3$, $C9H_3$, $C10H_3$. This increases the set of parameters from (K_1, K_2, δ^{S}, δ^{S2}) to (K_1, K_2, δ^{S}_8, δ^{S2}_8, δ^{S}_9, δ^{S2}_9, δ^{S}_{10}, δ^{S2}_{10}) but each experiment now yields three equations for $\Delta\delta_8$, $\Delta\delta_9$ and $\Delta\delta_{10}$.

Using the Job method[8] we have recently found[6] that the 1 : 2 stoichiometry is heavily prevailing for the complex of (+)-camphor **1** with α-CD **2**. Thus, for a large excess of the host the relative concentration of the free guest and that of the 1 : 1 complex (in relation to the 1 : 2 one) are negligible. Consequently, eqns. (8b) and (9) can be reduced to

$$\Delta\delta \approx \delta^{S0} - \delta^{S2} \quad (10)$$

for a large excess of α-CD, giving an immediate estimation of an initial δ^{S2} value. To further reduce the number of unknown parameters, the overall binding constant $\beta_{12} = K_1K_2$ was estimated on the basis of the Benesi–Hildebrand procedure.[9] An average value of 5.51×10^5 M^{-2} of β_{12} was calculated from the values obtained for the signals of the $C8H_3$, $C9H_3$, $C10H_3$ methyl groups. Then, only K_1, δ^{S}_8, δ^{S}_9, δ^{S}_{10} remain as the unknown parameters to be determined by the fitting procedure. However, it should be stressed that δ^{S2}_8, δ^{S2}_9 and δ^{S2}_{10} values have also been fitted, resulting in very close values of these parameters as shown below.

Since the nonlinear fitting problem has multiple minima, it has been essential to allow the user of the fitting program an easy, intuitive choice of initial approximation. A standard conjugate gradient nonlinear minimization algorithm has been wrapped into an interactive graphical application written in the AVS Express data visualization system.[10]

Results

The calculated dependences of the shifts of the $C8H_3$, $C9H_3$, $C10H_3$ methyl groups on the relative concentration of the host c_{rel} are presented in Fig. 1a–d while the amount of the free host and the complexes in the mixture as a function of relative CD concentration c_{rel} is presented in Fig. 2a–d. The K_1, K_2

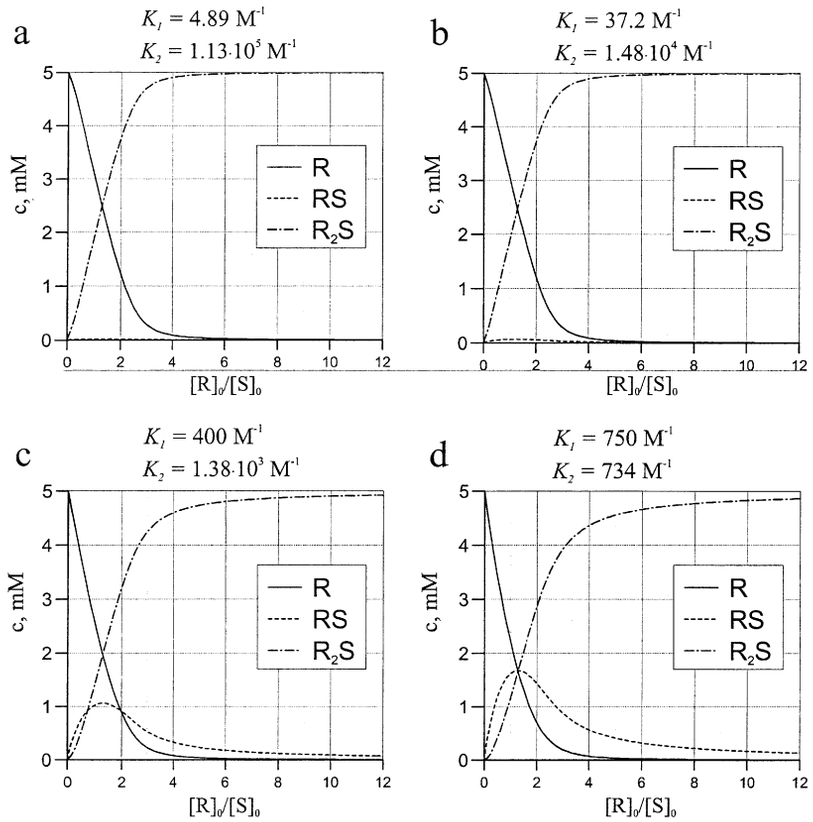

Fig. 2 The dependence of concentrations of free host (R) and those of 1 : 1 and 1 : 2 complexes (RS and R_2S, respectively) on the relative host to guest concentration for the same K_1 values as those shown in Fig. 1.

values given in the figures are the fitted values with one to three significant digits.

The fitting procedure was carried out for several sets of K_1, δ_8^S, δ_9^S, δ_{10}^S and the set of δ_8^{S2}, δ_9^{S2} and δ_{10}^{S2} values chosen on the basis of the spectra obtained for a large excess of CD showing that:

1. The fitting procedure did not converge for the starting value of $K_1 < 5$ M^{-1}. Starting from the latter K_1 value one obtains 4.89 M^{-1} as the result. However, as shown in Fig. 1a, the reproduction of experimental curves is very poor.

2. A slightly better fit was obtained for K_1 = 37.16 M^{-1} (Fig. 1b). However, the procedure yielded very high, negative values of the shifts of the 1 : 1 complex as compared to those of the 1 : 2 one. These big absolute values are unreasonable. However, the fact that the values are negative cannot be checked against experiment.

3. A good reproduction (shown in Fig. 1c) of experimental shifts was obtained for the assumed K_1 value of *ca.* 400 M^{-1} (K_2 values equal to 1377 M^{-1}). This is a reasonable relation although our Job experiments carried out earlier[6] point to a much lower K_1 value. The fitting procedure in this case yielded reasonable, although perhaps still too large, values of the shifts of the signals of the 1 : 1 complex as compared with those of the 1 : 2 one. Again, the fact that all the former values are negative cannot be checked against experiment.

4. A still better reproduction of the experimental shifts (Fig. 1d) was obtained for K_1 values ranging from 750 to 1534 M^{-1} (which results in the K_2 values equal to 734 to 359 M^{-1}) leading to a predominance of the 1 : 1 complex for low concentrations of α-CD. These data are completely unreliable since they contradict the results of Job experiments carried out earlier for the complex with (1*S*,4*S*)-camphor[6] showing a very strong predominance of the 1 : 2 complex. For all these values, the shifts of the signals of the methyl groups of the 1 : 1 complex were, in agreement with expectations, significantly lower than the corresponding shifts of the 1 : 2 complex. However, only positive values of the former shifts were obtained for the latter complex while for the K_1 value of 750 M^{-1} small negative values were calculated for $C8H_3$ and $C10H_3$ methyl groups. As stated before, the experimental results do not allow one to make any choice between the negative and positive values.

5. In attempts to reproduce $K_1 = 9.0 \pm 1.8$ M^{-1} and $K_2 = 7.1 \pm 0.9$ M^{-1} obtained in our previous work[6] with the Hunter group program,[5] the starting K_1 values of 5 to 20 M^{-1} were tried while K_2 was calculated from the estimated β_{12} value. All these attempts yielded poorer fits than those briefly discussed above. Moreover, some of them yielded unreasonably high values of δ_8^R, δ_9^R, δ_{10}^R shifts of the 1 : 1 complex. It should be stressed that the latter values were not given in the output of the program described in Ref. 5.

To rationalize these data let us look at the dependence of the amounts of the free guest, 1 : 1 and 1 : 2 complexes on the relative CD concentration $c_{rel} = [R]_0/[S]_0$ for different values of K_1 presented in Fig. 2a–d. In all four curves, for c_{rel} higher than 10 the solution under study consists practically only of the 1 : 2 complex and free α-CD. On the other hand, for small concentrations the accuracy of the shift determinations for small signals is low (due to a low intensity and the signals superposition it is especially poor for the c_{rel} equal to 1). In addition, for $K_1 << K_2$ the concentration of the 1 : 1 complex is practically negligible for all values of α-CD concentrations. Thus, the influence of the δ^R term describing the 1 : 1 complex in the formula (8a) is insignificant and consequently no reliable determination of the stepwise binding constants K_1 and K_2 could be carried out in this case.

Conclusions

Knowledge of stepwise binding constants for complexes of stoichiometry equal to or higher than 1 : 2 would be invaluable for their characterization. However, it cannot be carried out for $K_1 << K_2$. In general, we strongly discourage using the fitting programs as a black box as well as using a satisfactory

reproduction of the experimental dependence of relative shifts as a function of relative CD concentration as the only criterion of the reliability of the stepwise binding constants obtained using such programs.

Experimental

Series of 1 mM solutions of (1*R*,4*R*)-(+)-camphor (Fluka, purity > 97.0%) in D_2O with α-CD (Wacker) were prepared in such a way that the concentration of α-CD was 1 to 12 times higher than that of the corresponding camphor enantiomer.

All spectra were measured on a Varian Unity Plus 500 spectrometer, using the ID_PFG probehead with actively shielded z-gradient coil. The samples were inserted in the magnet and left for at least 15 minutes for the equilibration. The temperature was controlled by a standard VT unit. In all cases a 7.2 μs high power π/2 1H pulse was used and 32 scans were acquired with a relaxation delay of 2 s and an FID acquisition time of 1.4 s.

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