DETERMINATION OF BINDING ENERGIES BETWEEN CYCLODEXTRINS AND AROMATIC GUEST MOLECULES BY MICROCALORIMETRY

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ABSTRACT

The inclusion of substituted benzoic acids in β-CD or selectively methylated β-CDs was investigated by titration microcalorimetry. All thermodynamic functions of the inclusion process ΔG°, ΔH° and ΔS° could be obtained very accurately within one experiment. A very strong influence of the substitution pattern at both the host and the guest on the stability of the inclusion compounds was found.

1. INTRODUCTION

The search for a highly selective and strong inclusion of guests by CD hosts became a very important issue, as many applications, e.g. separation processes, stabilization, solubilization and release of active substances make use of molecular recognition. While slim linear alkane derivatives are included by α-CD, benzene and cyclohexane derivatives fit in β-CD. γ-CD is already large enough to accomodate two anthracene moieties [1]. Binding constants can be determined by spectroscopic methods, e.g. UV, fluorescence, NMR, if a suitable chromophore is present in the host or the guest. Also indirect methods, e.g. solubility, competitive binding, chromatography were used in some cases for this purpose. A very versatile and accurate method for the determination of all thermodynamic binding data is titration microcalorimetry [2,3]. We want to present here the microcalorimetric investigation of the inclusion of substituted benzoic acids by β-CD and its methyl derivatives.

2. MATERIALS AND METHODS

Substituted benzoic acids were obtained from *Aldrich*, β-CD **1** from *Wacker AG*, München, Germany, heptakis-(2-*O*-methyl)-β-CD **2** and heptakis-(2,3-di-*O*-methyl)-β-CD **3** from K. Petzold and D. Klemm, Universität Jena, Germany, heptakis-(2,6-di-*O*-methyl)-β-CD **4** from *Cyclolab*, Budapest, Hungary and heptakis-(6-*O*-methyl)-β-CD **5** and heptakis-(2,3,6-tri-*O*-methyl)-β-CD **6** from G. Weseloh and W. A. König, Universität Hamburg.

A titration microcalorimeter OMEGA from *Microcal. Inc.*, Northampton, MA, USA was used. The reference cell was filled completely with buffer. The sample cell

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(volume 1.3 mL) was completely filled with a 2-5 mM solution of one of the hosts **1-6** in aqueous 0.1 M phosphate buffer, pH 7.2. The 20-50 mM solution of one of the guests in the same buffer was injected as 20 portions of 5 μL into the sample cell at 25°C. The heat flow q caused (fig. 1a) was integrated for every peak to give the heat Q which was corrected by the corresponding heat of dilution of the guest Q_0. $\Delta H° = Q - Q_0$ was calculated per mol of added guest and plotted versus the ratio of total concentrations of guest and CD, [G]/[CD]. The data points were fitted by non linear regression, assuming a 1:1 stoichiometry as reported elsewhere [2] (Fig. 1b).

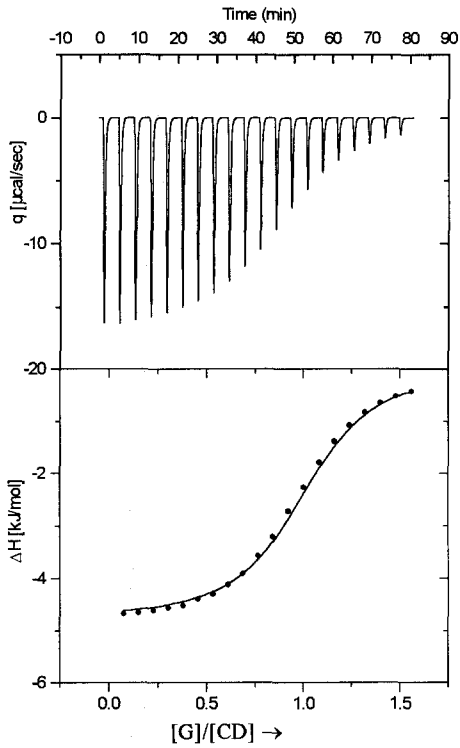

Fig. 1a

Heat flow q detected by an OMEGA microcalorimeter upon the addition of 5 µL portions of a 20 mM solution of 4-t-butylbenzoate to 1.3 mL of a 1 mM solution of heptakis-(2,6-*O*-dimethyl)-β-CD at 25°C.

Fig. 1b

Inclusion enthalpies, calculated by the integration of the heat flows q for the consecutive peaks,

$$\Delta H = Q - Q_0 = \int q dt - \int q_0 dt$$

curve calculated for $\Delta H° = -4.8$ kcal/mol and $K_S = 28'000\ M^{-1}$.

3. RESULTS AND DISCUSSION

An excellent fit of the experimental data by the binding curve, calculated for an 1:1 complex, was found. Therefore we concluded that in any case one guest was included in one CD ring. The stabilities of the inclusion compounds of benzoic acids in β-CD strongly depend on the position and size of substituents at the benzene ring. Free energies of inclusion range from $\Delta G° = -7$ kJ/mol ($K_S = 20$ L/mol) for benzoic acid to $\Delta G° = -24$ kJ/mol ($K_S = 18'400$ L/mol) for 4-t-butyl-benzoic acid (fig. 2). Consequently, a benzene ring alone is too small to fill the β-CD cavity completely.

Hydrophobic substituents at the para position, especially t-butyl, contribute to fill the cavity. A methyl substituent in the meta position or more hydrophilic substituents like i-propoxy, are less suited to increase the binding constant.

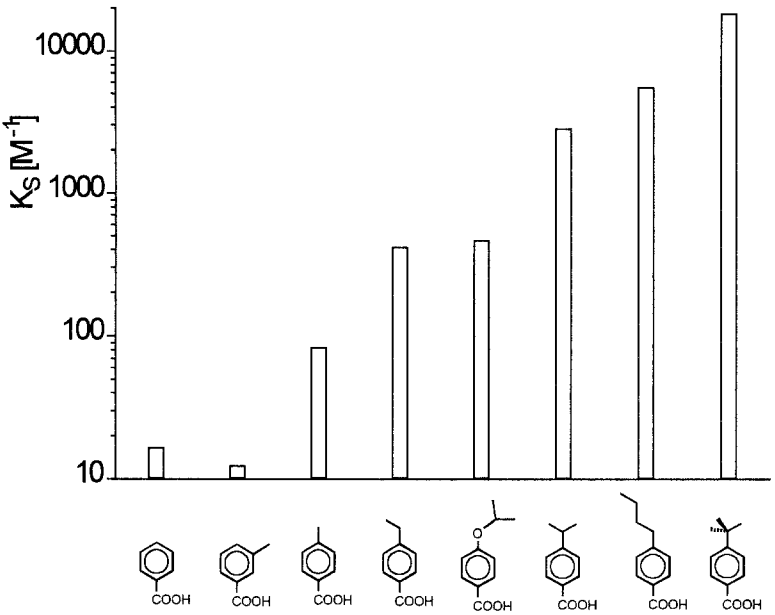

Fig. 2 Stability constants of the inclusion compounds of substituted benzoic acids and β-CD at pH 7.2

We also investigated the influence of methyl substituents at β-CD on its binding capability. 4-t-butylbenzoic acid was chosen as the guest. We found that the binding constant K_s highly depends on the position of the methyl groups. Those CD derivatives methylated at the secondary face of β-CD showed reduced binding constants (fig. 3). Especially methylation at O-3 causes a severe reduction of K_s. This reduction might be caused by a loss of rigidity of the host due to a loss of intramolecular hydrogen bonds. On the other hand, methylation at O-6 increases the binding constant to a remarkable value of K_s = 42'000 L/mol. This may be due to a prolongation of the hydrophobic cavity which allows an additional hydrophobic interaction between the t-butyl group of the guest and the methyl groups of the host. This topography would be in accordance with the crystal structure found for the inclusion compound of β-CD and 4-t-butyl-benzoic acid [4].

4. CONCLUSION

Very high stability constants can be reached for CD inclusion compounds by appropriate attachment of hydrophobic substituents at both host and guest.

5. ACKNOWLEDGEMENTS

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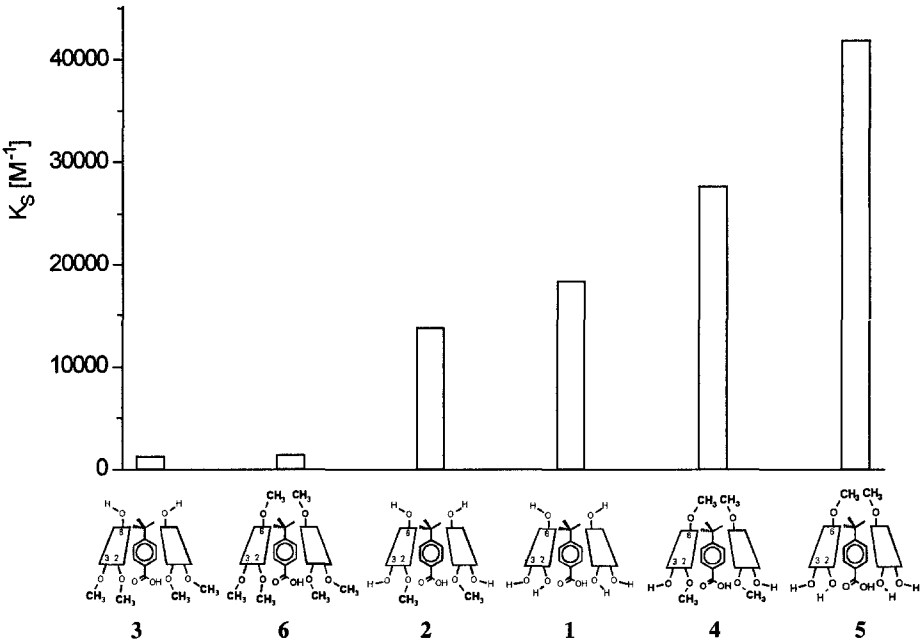

Fig. 3 Infuence of the pattern of methylation of β-CD on the binding constant K_S of t-butyl benzoate at pH 7.2

6. REFERENCES

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