Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **22:** 15–32, 1995.

Solubility and Molecular Modeling of Triflumizole–β-Cyclodextrin Inclusion Complexes

CH. TH. KLEIN, G. KÖHLER, B. MAYER, K. MRAZ, S. REITER, H. VIERNSTEIN and P. WOLSCHANN

Institut für Pharmazeutische Technologie and Institut für Theoretische Chemie und Strahlenchemie der Universität Wien, Althanstraße 14, A-1090 Wien, Austria.

(Received: 10 November 1994; in final form: 1 March 1995)

Abstract. Solubility enhancement of the fungicide triflumicole by β-cyclodextrin is explained using a thermodynamic approach. The influence of organic cosolvents on the overall equilibrium constants of triflumizole complexation with β-cyclodextrin in aqueous solutions has been investigated. Their variance in mixed solvents is only partly explained by a competitive inclusion of substrate and cosolvent molecules in β-cyclodextrin. The geometries of host–guest complexes have been estimated by molecular mechanics calculations. Their broad structural variety caused by the flexibility of host and guest molecules and different association possibilities of triflumizole have been analysed by a dynamic Monte Carlo docking method. The hydrophobic effect has been simulated by cominimization of the hydrophobic contributions to the solvation energy, calculated from the solvent accessible surface area of the complex and the conformational (potential) energy.

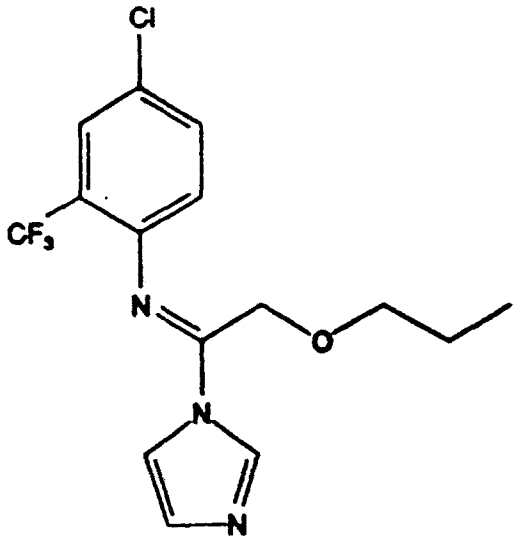
Key words. β-Cyclodextrin, triflumizole, host-guest complex, inclusion complex, solvent accessible surface, solubility enhancement, hydrophobic effect, dynamic Monte Carlo molecular docking.

1. Introduction

The solubilizing effect of cyclodextrins (CDs) is of particular interest in the pharmaceutical industry and chemical technology. Enhancement of the solubility of drugs or pesticides, conferring subsequent bioavailability and improved activity, is caused by complexation of the hydrophobic compounds with appropriate host molecules. Because of the variability in size of the interior cavity, association complexes of CDs have been widely studied. The stability of the inclusion complexes is determined by the fit of the molecular shape of the guest molecule to the complementary molecular surface of the cavity, attractive van der Waals and electrostatic forces, as well as by interactions with the environment. Such complexes are mainly investigated in aqueous solution, but organic cosolvents influence the association process drastically. As a quantitative measure of the complex stability, association constants have been estimated for a series of compounds [1–6].

In continuation of previous studies on the solubility enhancement of the systemic fungicide triflumizole by β-CD [7], thermodynamic considerations are pre-

sented, with the aim of describing the solubilizing effect of the complexing agent. Triflumizole (TF) (Formula 1) has curative and protective actions against *Gymnosporangium* and *Venturia* spp. in pome fruit, against powdery *Erysiphaceae* in fruits and vegetables, and against *Fusarium*, *Fulvia* and *Monilinia* spp. as well as *Helminthosporium*, *Tilletia* and *Ustilago* spp. in cereals. The saturation concentration of this compound in pure water is very low and thus standard formulations are usually applied as suspension concentrates. To improve the solubility as well as the activity of TF, the substance was encapsulated in CDs as a clathrate on the molecular level.



1

The equilibrium constants for complex formation were found to depend on the addition of small amounts of organic solvents and this effect is interpreted in terms of a competitive reaction system. Furthermore, the structure of the association complex was investigated by molecular modeling methods. As the flexibility of CD rings is rather large and many different structures have to be assumed for the association complexes, the conformational space was analysed by a dynamic Monte Carlo method. Solvent interactions were modeled by minimizing the hydrophobic contribution to the solvation energy, in addition to the potential (conformational) energy.

2. Experimental

Triflumizole (TF) (E) - 4 - chloro - α,α,α - trifluoro - *N* - (1 - imidazol - 1 - yl - 2 - propoxyethylidene) - *o* - toluidine (IUPAC), (E) - 1 - [1 - [[4 - chloro - 2 - (trifluoromethyl)phenyl] - imino] - 2 - propoxyethyl] - 1*H* - imidazole (C.A.), CAS No. *99 387-89-0*, was obtained from Nippon Soda Co. Ltd. (Japan) with a purity of

>99%. β-CD was provided by Roquette Frères (Lestrem, France) as Kleptose® with a humidity of 14 wt.-%. Ethanol (EtOH), dimethyl sulfoxide (DMSO) and dioxane (DIO) were of analytical reagent grade; the water used in this study was bidistilled.

The electronic absorption spectra were recorded on a Hitachi U3501 spectrophotometer at 25 ± 1 °C. Constant triflumizole concentrations were obtained by 1 : 1 dilution of a saturated solution. β-CD was added as solid. Due to the instability of the solutions, the spectra were recorded immediately after dilution. Spectra in DIO, EtOH and DMSO solutions and their mixtures with water, were obtained by dilution of a 10^{-3} M stock solution in the respective organic solvent.

Solubility measurements and the determination of saturation concentrations were carried out adding excess amounts of triflumizole in the concentration range 4×10^{-4} to 6×10^{-3} M to water, water/cosolvent mixtures and β-CD-solutions. After stirring the samples at 25 °C until equilibrium was reached (36 h), the concentration of dissolved triflumizole was determined by electronic absorption spectroscopy using a Perkin Elmer UV/vis Lambda 16 Spectrometer (Perkin Elmer, Norwalk, CT, USA) at a wavelength of 295 nm.

The overall complexation constants, K', were estimated by a modified Hildebrand–Benesi procedure [7, 8], varying the β-CD concentration. K' was also calculated from experimental data according to the solubility method, assuming a one-step equilibrium. It is based on monitoring solubility changes of triflumizole by adding a complexing agent. The obtained saturation concentration of the guest, c^{s}_{TF} (see Table I) is plotted as a function of the CD concentration, and the apparent K' is calculated from a straight line approximation of the resulting diagram according to the equation of Higuchi and Connors [9].

3. Thermodynamic and Spectroscopic Experiments

The saturation concentration of triflumizole in water was estimated as 4.2×10^{-5} M at 25 °C (see Table I). Addition of β-CD increases the solubility of the compound and an equilibrium constant $K' = 470 \mp 20\ M^{-1}$ was evaluated [7]. The spectrophotometrically determined concentrations are in agreement with the following expression,

$$K_C = \frac{c_{TFCD}}{c^{s}_{TF} \cdot c_{CD}}$$

when K_C is set equal to K', c_{CD} and c_{TFCD} are the concentrations of CD and the association complex at equilibrium, c^{s}_{TF} is the saturation concentration of triflumizole in equilibrium with the solid precipitate. Addition of small amounts of organic solvents also increases the solubility, which is documented in Figure 1.

There is, however, a significant difference between the magnitude of the solubility enhancement caused by apolar and aprotic DIO as cosolvent, when compared to that of polar and protic EtOH or polar, but aprotic DMSO. On the other hand, the

TABLE I. Saturation concentrations (c^s_{TF}) of triflumizole, the one-step association constants (K') and the two-step equilibrium constant, K'_2, for triflumizole complexation with β-CD in different mixtures of water with organic cosolvents.

Solvent	%	c^s_{TF} [M]	K' [M^{-1}]	K'_2 [M^{-1}]
Ethanol	0	0.42×10^{-4}	469	
EtOH	5	0.61×10^{-4}	226	334
	10	0.83×10^{-4}	141	256
	15	1.22×10^{-4}	101	207
Dimethylsulfoxide	1	0.46×10^{-4}	339	402
DMSO	2	0.49×10^{-4}	264	346
	4	0.56×10^{-4}	189	270
	10	0.81×10^{-4}	86	162
Dioxane	1	0.50×10^{-4}	120	293
DIO	2	0.54×10^{-4}	77	209
	5	0.78×10^{-4}	49	113
	10	1.45×10^{-4}	12	63

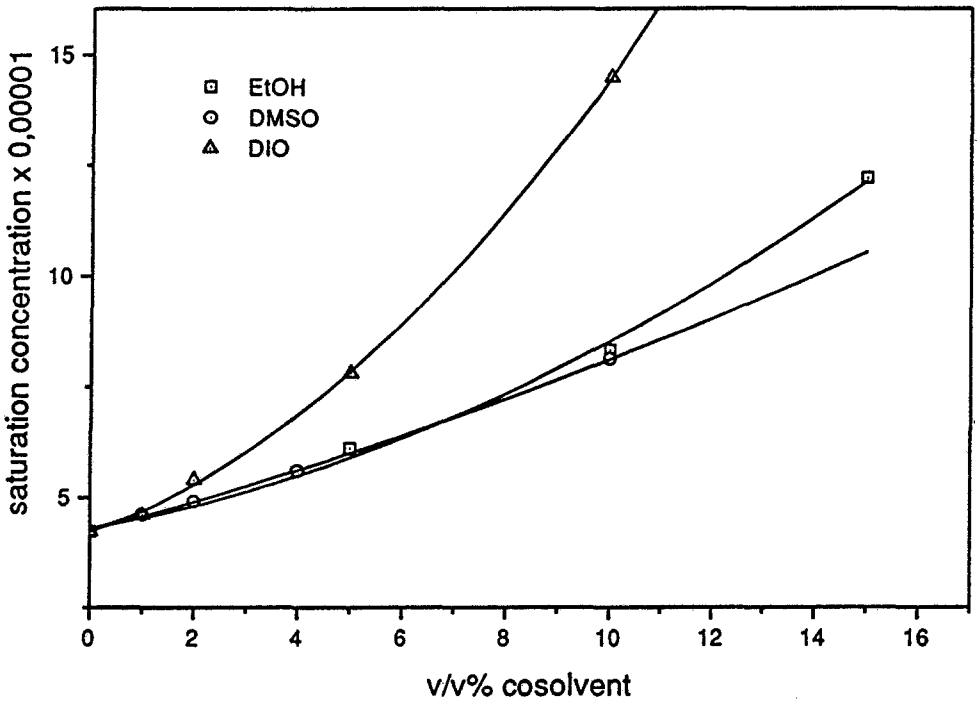


Fig. 1. Solubility of triflumizole in aqueous solution: dependence on the concentration of organic cosolvents.

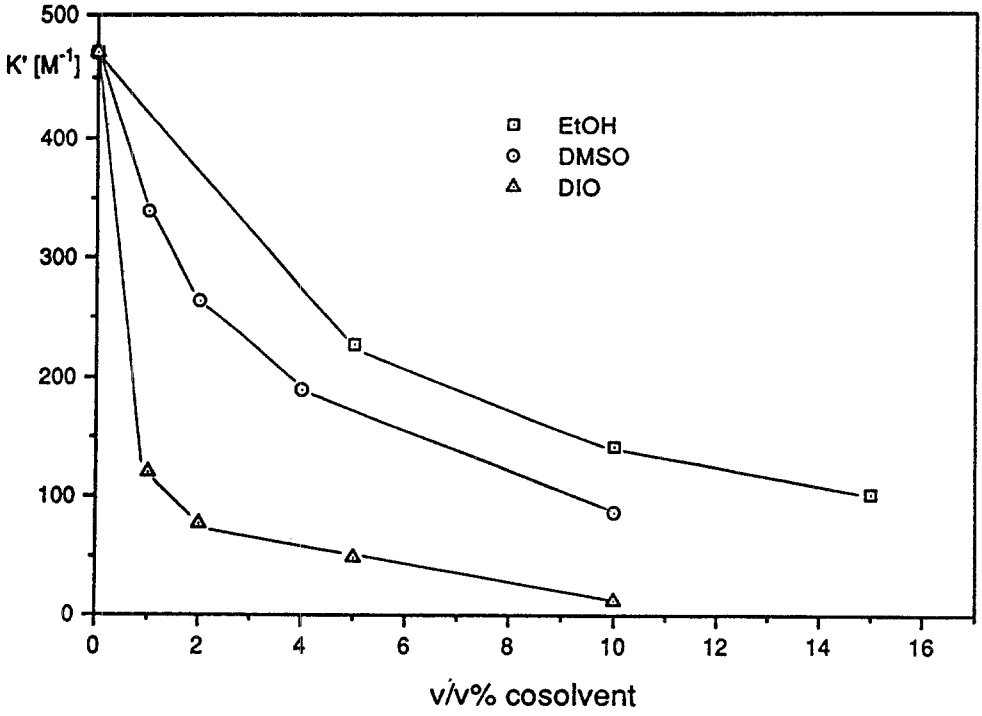


Fig. 2. One step equilibrium constant K' of the association of triflumizole with β-CD: dependence on the concentration of organic cosolvents.

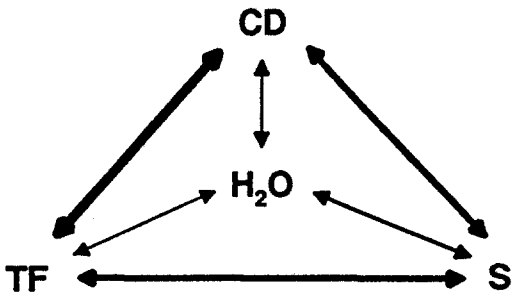
association complex is destabilized in the presence of organic cosolvents, which is recognized by the decrease of the measured association constant K' with increasing cosolvent concentration. The corresponding data are given in Table I and Figure 2. The decrease of K' is significantly larger for DIO addition than for the other cosolvents.

One reason for the strong solvent dependence of the association constant might be the competition between the inclusion of triflumizole and cosolvent molecule (S) into the interior of β-CD, with distinct equilibrium constants K_C and K_S, respectively:

$$[TF] + [CD] \rightleftarrows [TFCD] \qquad K_C = \frac{c_{TFCD}}{c_{TF} \cdot c_{CD}}$$

$$[S] + [CD] \rightleftarrows [SCD] \qquad K_S = \frac{c_{SCD}}{c_S \cdot c_{CD}}$$

c_{TF}, c_S and c_{SCD} are the equilibrium concentrations of triflumizole, of the cosolvent, and the cosolvent/β-CD inclusion complexes, respectively. Since there is experimental evidence that the complexation process is mainly driven by hydrophobic interactions [10], the association constant K_S is evidently much smaller than K_C, but the higher cosolvent concentration leads to formation of remarkable amounts



Scheme 1.

of cosolvent/β-CD inclusion complexes. The association constants of organic molecules with β-CD have been estimated recently [1, 2]. Table I also lists the equilibrium constants K_2', which were calculated according to the two-step reaction scheme given above, using the experimentally derived optical densities. Data for K_s are taken from Ref. [2]. The K_2' values obtained reproduce the general tendency that the equilibrium constants decrease on increasing cosolvent concentration, but they do not reproduce the experimental K' values quantitatively. The largest difference between K' and K_2' is found for DIO, the most hydrophobic cosolvent, which also causes the largest increase of solubility. It might either be necessary to assume further association steps or an increased affinity of triflumizole to hydrophobic organic molecules (Scheme 1) and consequently some preferential solvation effects.

The assumption of different affinities between an organic or an aqueous solvation shell and triflumizole is supported by the nonlinear dependence of the electronic absorption spectra on the solvent composition. Triflumizole shows two absorption bands and their maxima in aqueous solution are found at $\lambda_{max} = 293.8$ nm and $\lambda_{max} = 234.5$ nm with extinction coefficients $\epsilon = 4050$ dm^3 mol^{-1} cm^{-1}, and $\epsilon = 25800$ dm^3 mol^{-1} cm^{-1}, respectively. Association with β-CD shifts both bands bathochromically ($\lambda_{max} = 236.2$ nm, $\lambda_{max} = 299$ nm) and decreases their extinction coefficients slightly ($\epsilon = 25400$ dm^3 mol^{-1} cm^{-1}, $\epsilon = 400$ dm^3 mol^{-1} cm^{-1}) (Table II).

The shift of the absorption spectrum of triflumizole upon inclusion in β-CD is evidently caused by the change of the environment, as it parallels similar spectral shifts found for solutions in DIO or EtOH. In Figure 3 the absorption band maxima of triflumizole are plotted against the concentrations of EtOH, DIO and DMSO in their binary mixtures with water.

The bathochromic shift in pure organic solvents relative to aqueous solutions is larger than for the CD complex. Interactions with the environment are thus more efficiently altered when water is replaced by an organic solvent, than by inclusion in the CD cavity. Complexed triflumizole molecules are still exposed to the aqueous environment. Nevertheless, the shift upon complexation indicates that the aromatic chromophore responsible for the UV absorption is mainly included in

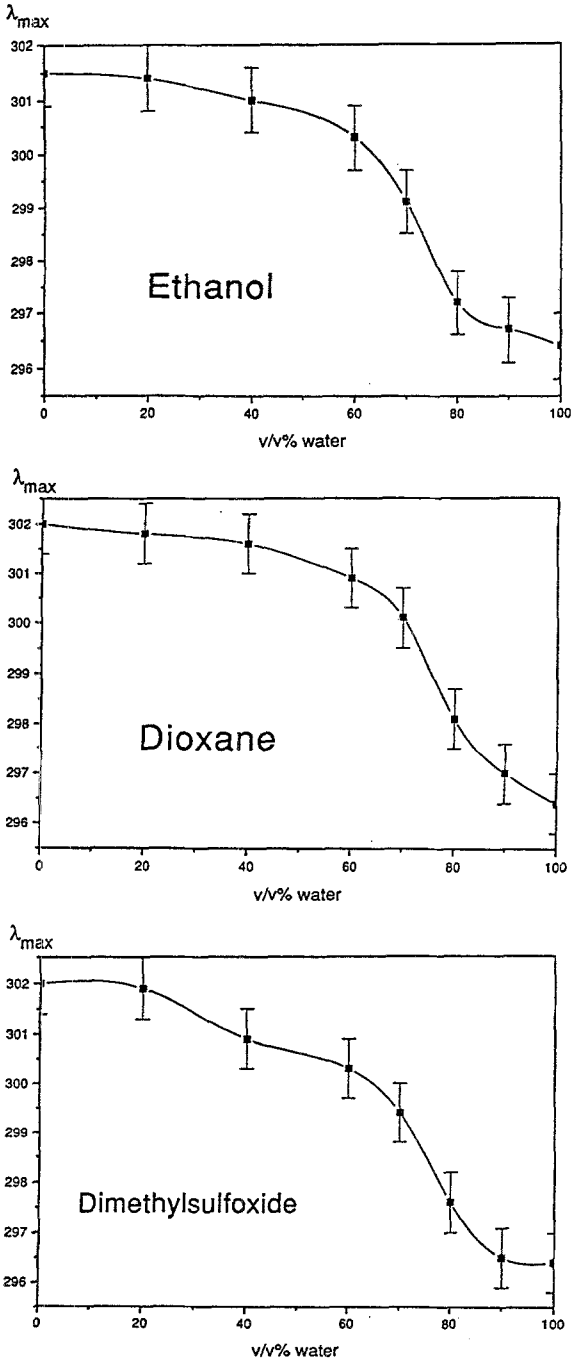


Fig. 3. Long wavelength maxima of the electronic absorption spectrum of triflumizole in binary mixtures of EtOH (a), DIO (b), and DMSO (c) with water (wavelengths given in nm).

TABLE II. Electronic absorption maxima of triflumizole in different environments. (Wavelengths in nm, extinction coefficient ϵ in dm^3 mol^{-1} cm^{-1}).

	λ_{max}	ϵ
Water	234.5	25800
	293.8	4050
β-CD inclusion complex in	236.2	25400
water	299	4000
Ethanol	237.4	25500
	301.5	4900
Dioxane	240.6	25300
	301	5200

the host. There is no difference in the band maximum between protic and aprotic organic solvents (EtOH and DIO) and the shift of the absorption band occurs largely at water concentrations higher than 70% in both binary mixtures. EtOH or DIO have, therefore, similar interactions with the chromophore of TF, which differ essentially from interactions with an aqueous environment.

4. Molecular Modeling of the Complexation Reaction between Triflumizole and β-CD

All calculations were performed with the program MolDoc [11], developed in our group. As subroutines it contains Allinger's MM3(92) force field [12], as well as the program MSEED [13] for solvent accessible surface area evaluation. The parameter set of MM3(92) has been extended following Ref. [14] to handle TF. The program offers the possibility of exploring the conformational space according to the Metropolis Monte Carlo Method [15], with the option of simulated annealing [16]. Solvation can be taken into account by a continuum model (for detailed description see below).

Previous calculations on the geometry of β-CD have shown that the flexibility of this host compound allows a large number of low energy conformations [17–23]. In solution, the molecules are highly non-symmetric; i.e. they generally do not have the shape of a circular, truncated cone [17]. Although it might be assumed that the conformational space in complexes is reduced due to restricted internal motion of the glucosidic subunits, it is evident that a large number of different possible geometries of the association complex exists, as the guest might occupy different positions and orientations.

4.1. ASSOCIATION PATHWAYS

Energetically favourable locations of the guest within the CD cavity were found by the following method, which is similar to that described by Jaime *et al.* [24]: simulations were started from the crystallographic host geometry [22, 23], which was minimized within the MM3 force field, applying the matrix diagonalization method. The geometry of the guest molecule TF was also minimized. The host-to-guest distance was defined as the distance between the mean position of the glucosidic oxygens of β-CD and the center of mass of the guest molecule. A positive distance indicates a guest location near the side of the secondary, 2- and 3-hydroxyl groups, and a negative value means that complexation proceeds from the primary hydroxyl side (6-hydroxyl groups). Docking was started from a fixed distance of 10 Å, where the total energy is near to the summed energy of the two separated molecules. The guest was then pushed in 1 Å increments through the CD cavity and the geometry was minimized after each step. The resulting host–guest distance was determined and the next move was started from this minimum geometry. This leads to a sequence of local minimum positions along one possible complexation path. This procedure was performed for various starting geometries, i.e. the aromatic or the aliphatic moiety pointing toward the macrocycle. Pathways where the heterocycle faces CD were not calculated, as penetration of the cavity is sterically not possible. The topology of the resulting state sequence depends intrinsically on the initial relative orientation of the two molecules, as shown by two examples given in Figure 4, curves A and B.

The resulting energies of the minimum geometries should be compared to 500 kJ/mol, the energy for the separated host–guest pair (the minimum energy obtained for β-CD was 295 kJ/mol and that of TF 205 kJ/mol), and should identify sterically feasible complex geometries. The binding energies arise from nonbonded (Coulomb and Van der Waals) interaction terms. Hydrogen bonding is described in the force field used, but contributions to the interaction energies arising from the polarizability of atoms and bonds are not included, however. Important effects arise from solvent interactions which will be discussed in some detail below.

The two curves plotted in Figure 4 indicate the formation of two structurally different complexes: in (A) the aliphatic side chain is imbedded in the CD cavity and in (B) the aryl ring is mainly complexed. The calculations demonstrate the formation of energetically stable complexes for the two functional moieties considered, all of nearly the same energy but at different host–guest distances. As these reaction pathways represent a sequence of different local minima, the results indicate the conformational flexibility of such complexes. Although distinct regions of low energy complexes are found, more favourable complex geometries might exist. The density of local minimum geometries with comparable energies are not recorded by the complexation coordinates shown. Furthermore, it is not evident that all these geometries are easily accessible by a self-assembling process or whether energetic barriers prevent the formation of such structures.

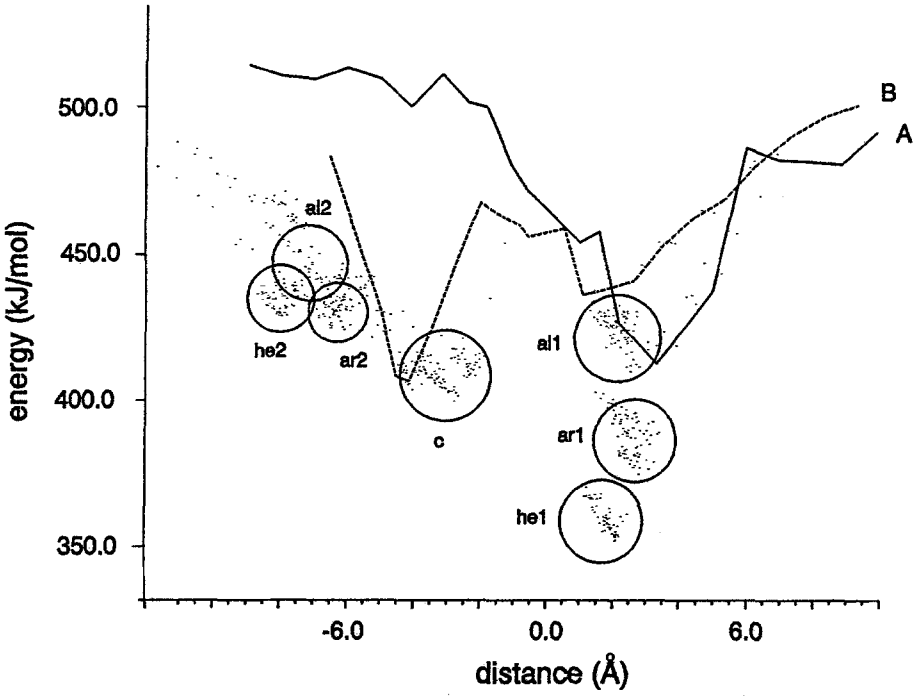


Fig. 4. • Calculated reaction paths moving triflumizole through the β-CD cavity starting from the secondary hydroxyl side. (A) the aliphatic residue inside the cavity; (B) the aromatic residue inside the cavity. • Monte Carlo simulations of the association reaction between triflumizole and β-CD. Circles indicate locally stable minimum regions: (1) Starting from the primary hydroxyl side, (2) Starting from the secondary hyroxyl side, (C) Starting from a complex geometry, with triflumizole positioned in the center of CD. al, ar, he indicate the functional moieties imbedded in the cavity (aliphatic, aromatic and heterocyclic residue).

4.2. Monte Carlo docking

Some limitations occuring in the simulations described above were eliminated by applying a dynamic Monte Carlo docking algorithm: starting from a host–guest distance of 7 Å or −12 Å and a given mutual orientation, the distance and relative orientation of the guest was changed stochastically within narrow limits: the guest was translated along a randomly chosen direction by a randomly chosen step size between 0 and 0.5 Å, and stochastically rotated within the range of 0° to 5°. The resulting structure was then minimized and was accepted according to the Metropolis criterion [15] at a temperature of 290 K. This should yield a Boltzmann distribution of the accepted geometries. Seven runs for different starting geometries, i.e. starting from the secondary and primary hydroxyl side of the host and one of the three moieties of triflumizole pointing toward the macrocycle, as well as one run starting from a central position, were performed and 100 accepted conformations were collected for each case. The potential energies of these configurations were then plotted against the host–guest distance as shown in Figure 4.

All of these runs show clearly defined regions of attraction: after a short, first molecular recognition phase of the complexation reaction, all structures are found within locally stable minimum regions, defined by their host–guest distance. The circles indicate the regions for complexes obtained from different starting geometries: al1, ar1 and he1 refer to structures started with the aliphatic chain, the aromatic ring and the heterocycle pointing towards the secondary hydroxyl side of the macrocycle, al2, ar2 and he2 are the respective geometries starting from the primary hydroxyl side. The relative orientation of the functional groups of the guest did not change significantly during the complexation reaction. The structural regions represent conformations, which are well defined by the starting structure. Thus al1 represents the space of conformations where the aliphatic side chain is included in the cavity: these structures match the respective minima found by the calculations pushing the molecule through the cavity (Curve A, Figure 4). However, additional low energy regions (ar1 and he1) are found, which did not show up before. Structure he1, where the heterocycle is imbedded in the macrocycle, gives the most favourable structures found. Some representative geometries are displayed in Figure 5a.

Starting from the other side of the ring gives three different regions with similar energies. The guest, however, is located quite far out of the cavity. Not all of these minima conform to the minima of the complexation coordinates described above (e.g. Curve B, Figure 4). Because of the lack of a tight fit, attractive forces between the host and guest molecules are much smaller on the primary hydroxyl side of the CD ring. A better complexation could only be found after significantly longer simulations. Maximum inclusion (host–guest distance of 0 Å) is energetically unfavourable, most likely because of steric hindrance. In a physical system, however, hydrophobic interactions could recruit additional forces to push the guest molecule deeper into the cavity.

The minimum in Curve B (Figure 4), where the aromatic moiety is imbedded in CD, was reproduced in this case, the Monte Carlo run was started from the center of the cavity, i.e. at an initial distance of -1 Å and the aromatic ring pointing toward the secondary, both other moieties towards the primary hydroxyl side of CD. The energy of the complexes drops quickly and the result matches now the minimum of Curve B. Complex geometries like al1, ar1, he1 and al2, ar2, he2, in which one structural subunit is complexed, are easily obtained by this procedure and so could be realized in solution.

Structures with optimal complexation are obtained in runs started from an initial host–guest distance of 0 Å (Region C, Fig. 4). This results in a considerable distortion of the macrocyclic ring system, as is shown in Figure 5b. Their formation might therefore be additionally hindered by energetic barriers, which should most probably also slow down the kinetics of their formation in solution.

In most of the structures only parts of the guest molecule are imbedded in the CD cavity. This could enable the formation of complexes with more than one host molecule. Therefore, a Monte Carlo simulation of the formation of 1 : 2

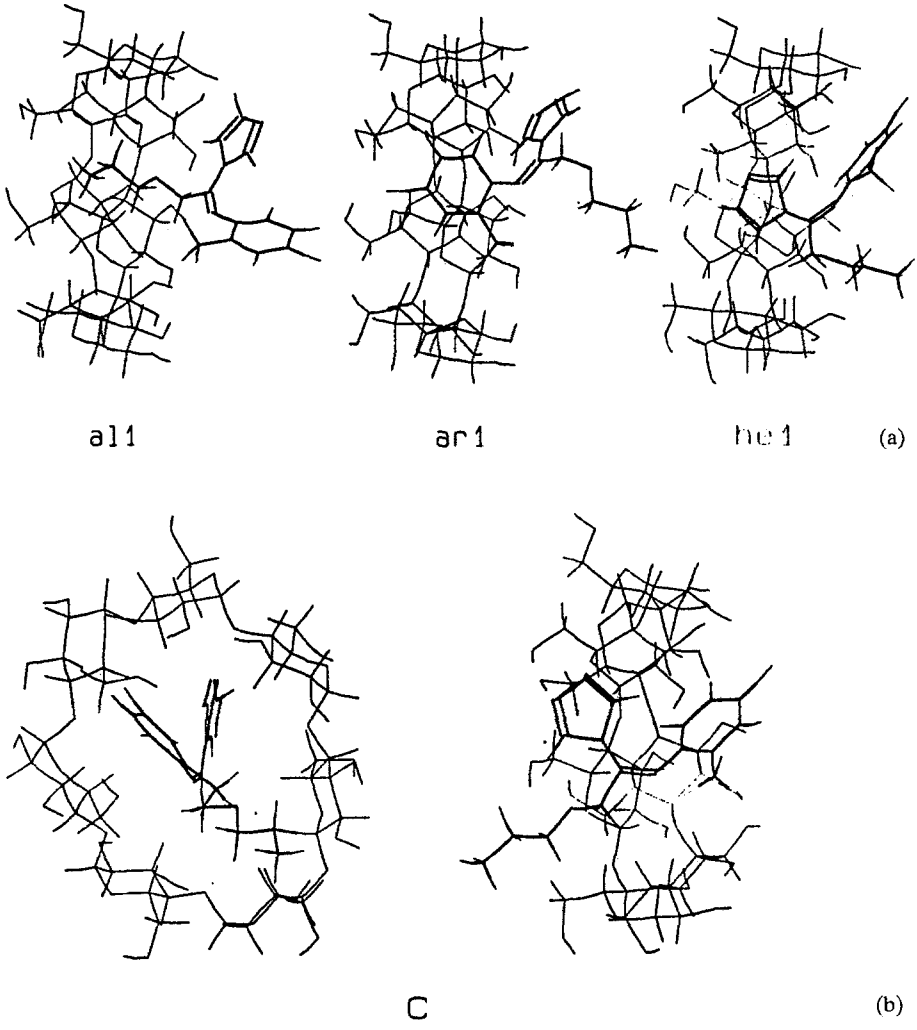


Fig. 5. (a) Calculated structures of inclusion complexes between triflumizole and β-CD from the Regions all, arl and hel (Figure 4), respectively. (b) Distortion of the macrocycle for optimum complexation. The structure is taken from Region C (Figure 4).

guest-to-host complexes was perfomed. Experimental evidence for 1 : 2 complex formation between α-naphthol and α-CD has been presented previously and is in agreement with higher order complexation, as suggested by theoretical calculations [25]. Depending on the relative orientation of the two CD molecules and the guest, 12 possible types of complexes can be considered. Due to steric strains, a simultaneous inclusion of the heterocycle and another moiety is energetically not feasible. Therefore, only complexes where the aliphatic and aromatic moieties are embedded in the CD cavities were considered: the interaction of two macrocycles by their 2- and 3-OH groups, i.e. the secondary hydroxyl sides oriented towards

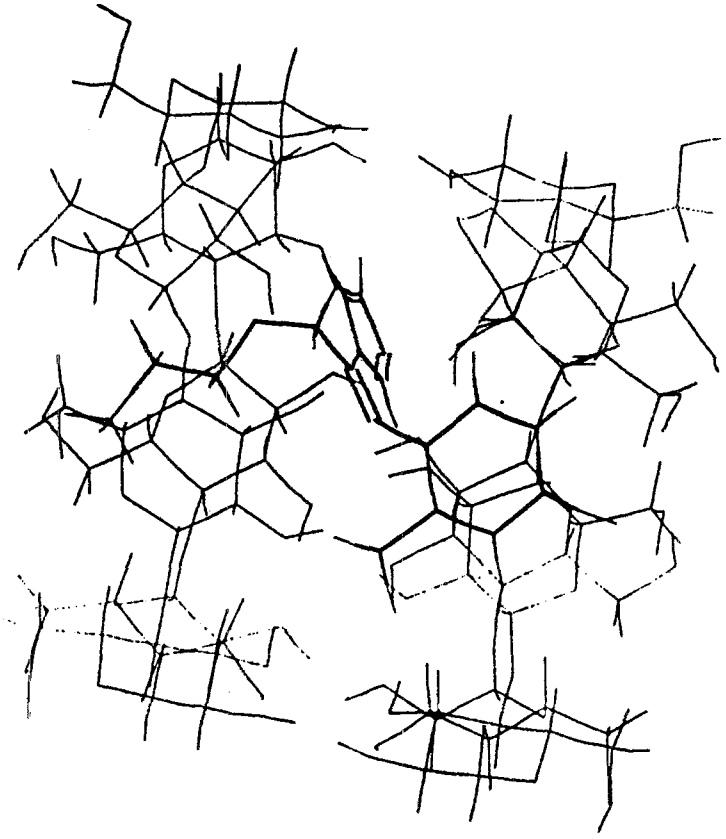


Fig. 6. Most stable geometry proposed for 1 : 2 guest-to-host complexes of triflumizole with β-CD.

each other, leads to considerably more stable complexes than association of the two primary hydroxyl sides or complexation of two CD molecules in the same orientation.

Simulations were performed for all three possible orientations of the host. The most stable structure was obtained when the secondary hydroxyl sides interact and the guest is completely included in this large cavity. An appropriate geometry is shown in Figure 6. The stability of these complexes arises from both the interaction between the two hosts and between the guest and hosts, which gain the binding energy mainly from attractive van der Waals forces. The formation of such stable higher aggregates thus seems feasible.

4.3. Monte Carlo docking considering solvation

Solvent effects have not been taken into account in the calculations presented above, although it is obvious that the difference in the free energy of solvation between solvated host and guest molecules and of the solvated complex influences

the complexation constant. Hydrophobic triflumizole has a larger affinity to the hydrophobic CD cavity than to polar water. One can therefore assume that important contributions to the complexation process arise from hydrophobic interactions.

A simple way to describe solute–solvent interaction without considering water molecules explicitly is the continuum approximation [26–29]. It is one or two orders of magnitude faster in computation time than a discrete solvent description and takes entropic effects implicitly into account, as the free energy of solvation is calculated. This is done by a summation over the contributions A_i of individual atoms to the solvent accessible surface area:

$$\Delta G_{\text{solv}} = \sum_i \sigma_i \cdot A_i.$$

The atomic solvation parameters (ASPs) σ_i (kJ/mol Å^2) represent the change of the free energy of transfer between different solvents, normalized onto the solvent accessible surface area, and are obtained experimentally from thermodynamic data [26,27,29]. For the present calculations, the hydrophobic contributions to the solvation energy were considered, since the hydrophobic effect seems to be a driving force of the complexation [10]. This method proved to be suitable for the calculation of CD inclusion complexes [29]. Hydrophobic atoms have positive ASPs, and these are all carbon atoms, according to Wesson and Eisenberg's [30] parameter set used. Therefore, the hydrophobic contribution to the solvation energy results as:

$$\Delta G_{\text{HI}} = \sum_j \sigma_c \cdot A_j^c$$

σ_c are the ASPs (+0.05 kJ/mol Å^2 for all carbons) and A_j^c the corresponding hydrophobic solvent accessible surface areas. They were calculated using a probe radius of 1.4 Å, equal to the value taken for evaluating the ASPs [30]. The Metropolis criterion, depending on both conformational energy and hydrophobic contribution to the solvation energy, can be written as [31]:

$$P(\Delta E_{\text{conf}}, \Delta G_{\text{HI}}) = \exp\left(-\frac{\Delta E_{\text{conf}}}{RT}\right) \cdot \exp\left(-\frac{\Delta G_{\text{HI}}}{RT}\right).$$

Two comparable runs, simulating the inclusion of the aliphatic side chain of triflumizole from the secondary hydroxyl side were performed under identical conditions: one considering only ΔE_{conf} in the Metropolis criterion (Figure 7) and another taking both ΔE_{conf} and ΔG_{HI} into account (Figure 8).

After an initial decrease of energy and distance, describing the early phase of molecular recognition and adaptation of the complex structure, an energy plateau of 443 kJ mol^{-1} is reached in both cases. However, host–guest distance and solvation energy reach a considerably smaller value when the solvation energy term is

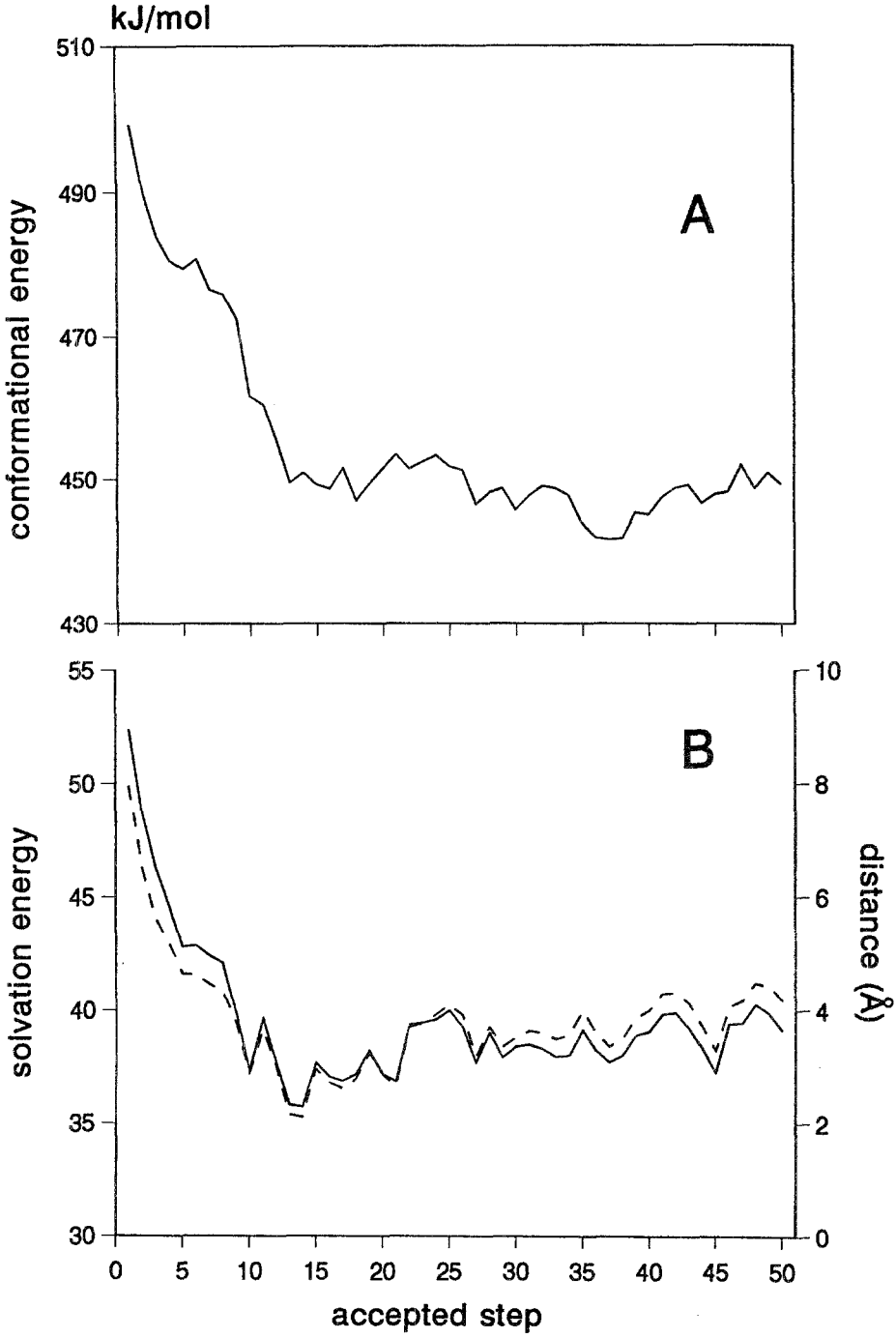


Fig. 7. Potential energies (A), as well as solvation energies (B, full line) and host-guest distances (B, dashed line) during a Monte Carlo run simulating the complexation of triflumizole and β-CD. The solvation energy was not considered in the Metropolis criterion.

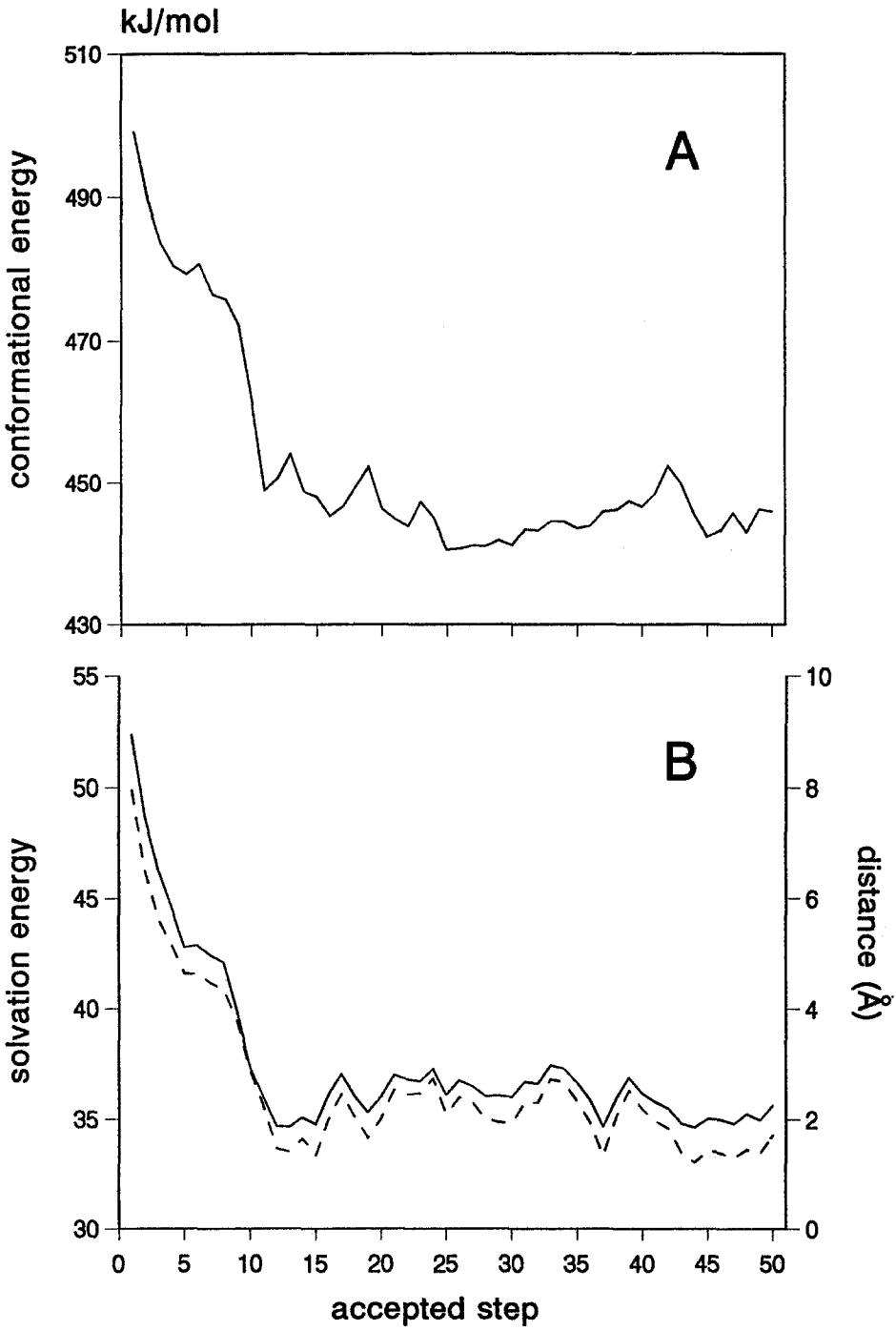


Fig. 8. Energies (A: conformational, B: solvation) and host–guest distances (dashed line) from the complexation of triflumizole and β-CD obtained from a Monte Carlo run considering both conformational energy and solvation energy terms in the Metropolis criterion.

included in the Metropolis criterion. Moreover, their fluctuation between successive iteration steps is smaller. Solvation thus stabilizes the complex and reduces its flexibility. A comparison of the experimentally measured and theoretically predicted induced circular dichroism of aromatic guests included in β-CD proved the reliability of complex structures, obtained by this cominimization of conformational energy and contributions to the solvation energy, arising from the hydrophobic solvent accessible surface [29].

5. Conclusions

Host–guest interactions cause the solubility enhancement of the fungicide triflumizole in aqueous β-CD solutions. The stability of the inclusion complex is dependent on the environment, as organic cosolvents destabilize the complex. The decrease of the association constant K' with increasing cosolvent concentration cannot be explained by a mechanism assuming only competitive inclusion of the substrate and cosolvent molecules: further association steps and the modification of the solvation shell by organic cosolvents might additionally influence the self-assembling process.

A large number of energetically similar geometries are proposed for these complexes by molecular mechanics calculations. This is due to the high flexibility of the β-CD macrocycle and also to the various association sites of the guest. Monte Carlo simulations were, therefore, performed for various starting geometries and they estimate the conformational space for probable inclusion complex structures. Higher order 2 : 1 host to guest complexation is also suggested by the calculations. Solvation effects are mimicked by cominimization of the hydrophobic contribution to the solvation energy and the conformational energy. This method shows up the restrictions imposed on the conformational space by the hydrophobic effect. This might explain the destabilization of the complexes upon addition of organic cosolvents, as they should increase hydrophobic contributions in the bulk of the solution.

Acknowledgements

We thank the *Fonds zur Förderung der wissenschaftlichen Forschung* in Austria (project P09750-CHE) and the *Forschungsförderungsfond für die gewerbliche Wirtschaft* (*projects* 8055, 9332) for financial support Ch. K. thanks the *Von Schadsche Stiftung Frankfurt* for a scholarship. Technical assistance by H. Martinek and E. Liedl is gratefully acknowledged.

References

1. S. Li and W.C. Purdy: *Chem. Rev.* **92** (1992).
2. J. Taraszewska: *J. Incl. Phenom.* **10**, 69 (1991).
3. J. Lehmann, E. Kleinpeter and J. Krechl: *J. Incl. Phenom.* **10**, 233 (1991).
4. T. Osajima, T. Deguchi and I. Sanemasa: *Bull. Chem. Soc. Jpn.* **64**, 2705 (1991).

5. O. Huber and J. Szejtli: *Proc. of the Fourth Int. Symposium on Cyclodextrins*, Kluwer Academic Publishers, Dordrecht, Boston, London, 1988.
6. S.E. Brown, J.H. Coates, C.J. Easton, S.F. Lincoln, Y. Luo and A.K.W. Stephens: *Aust. J. Chem.* **44**, 855 (1991).
7. H. Viernstein, S. Reiter and P. Wolschann: *Monatsh. Chem.* **125**, 681 (1994).
8. H.A. Benesi and J.H. Hildebrand: *J. Am. Chem. Soc.* **71**, 2703 (1949).
9. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instr.* **4**, 117 and 146 (1965).
10. Y. Kotake, E.G. Janzen: *J. Am. Chem. Soc.* **111**, 5138 (1989).
11. B. Mayer: *MolDoc* (version 2.1), 1994.
12. N.L. Allinger, Y.H. Yuh, and J.H. Lii: *J. Am. Chem. Soc.* **111**, 8551 (1989).
13. G. Perrot, B. Cheng, K.D. Gibson, J. Vila, K.A. Palmer, A. Nayeem, B. Maigret, and H.A. Scheraga: *J. Comput. Chem.* **13**, 1 (1992).
14. J.H. Lii and N.L. Allinger: *J. Comput. Chem.* **12**, 186 (1991).
15. N. Metropolis, A.W. Rosenbluth, M.B. Rosenbluth, and A.H. Teller: *J. Chem. Phys.* **21**, 1087 (1953).
16. S. Kirkpatrik, C.D. Gelatt and M.P. Vecchi: *Science* **220**, 671 (1983).
17. K.B. Lipkowitz: *J. Org. Chem.* **56**, 6357 (1991).
18. A.S. Kostense, St.P. van Helden and L.H.M. Janssen: *J. Comp. Aided Mol Design* **5**, 525 (1991).
19. D.A. Wertz, Chen-Xi Shi and C.A. Venanzi: *J. Comput. Chem.* **13**, 41 (1992).
20. W. Linert, P. Margl, and I. Lukovits: *Comput. Chem.* **16**, 61 (1992).
21. I. Tabushi: *Acc. Chem. Res.* **15**: 66 (1982).
22. K.K. Chacko and W. Saenger: *J. Am. Chem. Soc.* **103**, 1708 (1981).
23. C. Baetzel, W. Saenger, B.E. Hingerty and G.M. Brown: *J. Am. Chem. Soc.* **106**, 7545 (1984).
24. M. Fathallah, F. Fotiadu, and C. Jaime: *J. Org. Chem.* **59**, 1288 (1994).
25. H.R. Park, B. Mayer, P. Wolschann and G. Köhler: *J. Phys. Chem.* **98**, 6158 (1994).
26. D. Eisenberg and A.D. McLachlan: *Nature* **319**, 199 (1986).
27. T. Ooi, M. Oobatake, G. Nemethy and H.A. Scheraga: *Proc. Natl. Acad. Sci. USA* **84**, 3086 (1987).
28. K.B. Lipkowitz, B. Baker, and R. Larter: *J. Am. Chem. Soc.* **111**, 7750 (1989).
29. B. Mayer, G. Marconi, Ch. Klein, P. Wolschann, and G. Köhler: *J. Am. Chem. Soc.* submitted.
30. L. Wesson and D. Eisenberg: *Protein Science* **1**, 227 (1992).
31. Ch.Th. Klein, B. Mayer, G. Köhler, and P. Wolschann: *Biopolymer* submitted.