Solubilization of Thiazolobenzimidazole Using a Combination of pH Adjustment and Complexation with 2-Hydroxypropyl-β-Cyclodextrin

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The thiazolobenzimidazole 1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-*a*]benzimidazole, TBI, is an experimental drug for the treatment of AIDS which exhibits a low water solubility (11 μg/mL) and is therefore difficult to administer in an injectable solution dosage form at a target solution concentration of 10 mg/mL. The compound has a single ionizable functional group and exhibits an increase in solubility with decreasing pH consistent with a pK_a of 3.55, but the maximum solubility attainable by pH adjustment has been shown to be only 0.4 mg/mL (at pH 2). TBI has been found to form inclusion complexes in either its neutral or its protonated form with 2-hydroxypropyl-β-cyclodextrin (HPCD). The equilibrium constants for 1:1 complex formation were found to be 81 and 1033 M^{-1} for the protonated and neutral species, respectively. Although the formation of protonated complex is less favored in comparison to the neutral complex, the contribution of this species to the overall solubility of TBI predominates at low pH. Thus, using a combined approach of pH adjustment and complexation with HPCD, a solubility enhancement of 3 orders of magnitude is possible. NMR proton spectroscopy and molecular modeling studies, conducted to understand the orientation of TBI in the complex and the effect of protonation, are described.

KEY WORDS: solubilization; complexation; 2-hydroxypropyl-β-cyclodextrin; cyclodextrins; NMR proton spectroscopy; computer simulation; molecular dynamics; AIDS chemotherapy.

INTRODUCTION

A frequently encountered difficulty in the formulation of new drug candidates in injectable dosage forms is the limited solubility of many such agents in aqueous solutions. Although a variety of methods has been employed for solubilization of such compounds (1,2), there continues to be a need for novel approaches for solubilizing water-insoluble drugs for their development as parenteral solutions.

The thiazolobenzimidazole (TBI) depicted in Fig. 1 is an experimental agent under consideration for clinical testing by the National Cancer Institute for the treatment of AIDS. The compound has a very low water solubility (11 μg/mL), a factor of 10^3 below the target solution concentration of 10 mg/mL. Although TBI possesses an ionizable functionality, its pK_a (= 3.55) is too low to provide adequate solubilization within a physiologically acceptable range (pH >2) by simple pH adjustment. Attempts to solubilize TBI using other classical approaches such as cosolvent solubilization, incorporation into the oil phase of lipid emulsions, and complexation using chemically modified cyclodextrins were unsuccessful. An examination of the structure of TBI suggested, however, that it might be possible for this drug to form inclusion complexes with cyclodextrins such as 2-hydroxypropyl-β-cyclodextrin (HPCD) in either its neutral or its protonated form, since it appeared possible for the aromatic portions of the molecular to be included in the cyclodextrin cavity while allowing the protonated imidazolyl portion to reside outside the cavity in a largely aqueous environment. This paper describes both experimental studies and molecular dynamics simulations that were conducted to explore a combined approach of pH adjustment coupled with complexation using HPCD to achieve the desired solubility enhancement of ≥3 orders of magnitude.

MATERIALS AND METHODS

TBI [1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-*a*]benzimidazole; NSC No. 625487] was supplied by the National Cancer Institute (Bethesda, MD). 2-Hydroxypropyl-β-cyclodextrin (Molecusol), having an average molecular weight of 1540 and therefore an average degree of substitution of seven 2-hydroxypropyl residues per molecule, was a gift from Pharmatec, Inc. (Alachua, FL). All other compounds were reagent grade from commercial sources and used without further purification.

Solubility Determinations

Solubility studies were carried out in 0.01 ionic strength buffers (3) varying in pH from 2 to 8 and in aqueous solutions of HPCD ranging from 7 to 40% at various pH values and ionic strengths. An amount of sample well in excess of its estimated solubility was suspended in 1–2 mL of the solvent in a 4-mL glass vial sealed with a Teflon-lined cap. The samples were rotated in a water bath (Haake A81, Berlin, Germany) or oven (Shel Oven Lab., Sheldon Manufacturing Inc., Cornelius, OR) maintained at 25 ± 0.5°C for periods of time ranging from 1 to 3 days to ensure equilibrium. All determinations were done in duplicate or triplicate. The equilibrium pH of each solution was measured (pH M82, Radiometer America, Cleveland, OH) and the samples were filtered using 0.45-μm filters (Acro LC 3S or Acro LC 3A, Gelman Sciences, Ann Arbor, MI), suitably diluted, and analyzed by HPLC.

HPLC Analyses

TBI solubilities were determined by high-performance liquid chromatography (HPLC) using a reverse-phase C_{18} column (Brownlee OD224, Applied Biosystems Inc., San Jose, CA, or Supelcosil LC-18-S, Supelco Inc., Bellefonte, PA) with a mobile phase of 70% methanol and 30% ammonium acetate buffer (0.01 *M*).

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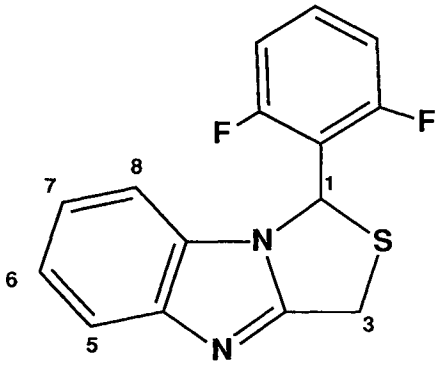


Fig. 1. Structure of thiazolobenzimidazole (TBI).

NMR Proton Spectroscopy

The 1D and 2D proton spectra were obtained on a Varian Unity-500 NMR spectrometer at 25°C. The spectra were referenced to DSS in D_2O (99.9 atom% D, Cambridge Isotope Laboratories) and TMS in 1,4-dioxane-d_8 (99.5 atom% D, Aldrich Chemical Co., Milwaukee, WI). Both α-D-glucose (Sigma Chemical Co., St. Louis, MO) and 2-hydroxypropyl-β-cyclodextrin were exchanged for deuterium before use. Samples for NMR analysis were prepared by dissolving 600 mg of exchanged α-D-glucose or HPCD and 5 mg of TBI into 3 mL of D_2O. NMR analyses of the neutral complexes were carried out without further treatment. NMR spectra of the protonated complexes were generated in samples at p*D* values ≤2.0, adjusted by adding a small amount of concentrated DCl. Based on the equilibrium binding constants determined in this study (cf. Table III), the above conditions should have resulted in >90% of the drug being in complexed form.

Computer Simulations of Binding of TBI with HPCD

Constant-volume, constant-temperature molecular dynamics (MD) simulations of the inclusion complexes were performed on the DISCOVER (Version 2.7) molecular dynamics simulation program (Biosym. Technologies, San Diego, CA). Energy minimizations were conducted for three systems, all of which assumed 1 molecule of protonated TBI, 1 molecule of β-cyclodextrin (CD), and 749 water molecules. β-Cyclodextrin was used in the simulations rather than 2-hydroxypropyl-β-cyclodextrin because the incorporation of seven 2-hydroxypropyl residues into the β-cyclodextrin molecules resulted in too many additional conformational possibilities. The systems simulated were (i) TBI and CD separated from each other by a distance greater than the nonbonded cutoff distance (>8.5 Å); (ii) TBI complexed with CD with its imidazole ring inserted into the CD cavity; and (iii) TBI complexed with CD with its phenyl ring inserted into the CD cavity. The structure of β-cyclodextrin was constructed from single glucopyranose units. Adjusting the torsion angles about the oxygen atom connecting the monomers and repeated energy minimization resulted in a stable seven-membered torus-shaped ring similar to the crystal structure reported by Lindner and Saenger (4). Each molecular system was contained in a box size of 25.5 × 25.5 × 37.0 Å with periodic boundary conditions (density = 1.03 g/cm^3). The step size was 1 fsec. To start the simulations, different seed numbers were used for initial Maxwellian velocity distribution for each system. TBI was inserted into the wide end of the CD torus using molecular graphics. In order to remove this initial bias, the complex was subjected to simulated annealing; the temperature of the complex was increased at a rate of 50 K/100 steps from 50 to 1000 K. After an additional 2 psec of simulation at 1000 K, the temperature was decreased back to 300 K. Simulations were continued and the coordinates were saved every 0.1 psec for analysis.

RESULTS AND DISCUSSION

TBI Solubility in Various Solvent Systems

Preliminary solubility studies employed a variety of classical cosolvent systems and a lipid emulsion in an attempt to identify systems providing a solubility of ≥10 mg/mL. These solubility data are listed in Table I. TBI is relatively lipophilic, with a soybean oil/water partition coefficient of approximately 10^3 (from the solubility ratios) and a soybean oil solubility of 15 mg/mL. However, the highest concentration achievable in a lipid emulsion (Liposyn II, 20%) was found to be only 3 mg/mL. Relatively high percentages of the cosolvents dimethyl sulfoxide (DMSO), propylene glycol, and *N*-methyl-2-pyrrolidinone were required to reach solubilities in excess of 10 mg/mL, and therefore such systems would be physiologically unacceptable for intravenous administration. Of the cosolvents, *N*-methyl-2-pyrrolidinone is the most lipophilic and therefore provided higher solubilization at a lower percentage of organic component, but a percentage greater than 50% was necessary to achieve the target concentration.

Table 1. Solubility of TBI in Various Solvent Systems at 25°C

Solvent	Solubility (mg/mL)	CV (%)	*n*
Water	0.011	22	3
Dimethylsulfoxide (DMSO)	>50[a]		
70% DMSO/30% water	7.6	2.4	2
50% DMSO/50% water	1.11	0	2
30% DMSO/70% water	0.21	5.5	3
70% DMSO/30% 0.01 *M* HCl	7.6		1
50% DMSO/50% 0.01 *M* HCl	1.28	0.3	2
30% DMSO/70% 0.01 *M* HCl	0.87	0	2
Propylene glycol (PG)	>12[a]		
50% PG/50% water	0.75		1
40% PG/60% water	0.22	4.8	4
33% PG 67% water	0.15	5.6	2
10% PG/90% water	0.021	1.1	2
50% PG/50% 0.01 *M* HCl	1.77	4.4	2
N-Methylpyrrolidinone (NMP)			
70% NMP/30% water	>40[a]		
50% NMP/50% water	7.9	1.8	2
40% NMP/60% water	3.1	0.6	2
30% NMP/70% water	1.2	0.5	2
Soybean oil	15	8	5
Liposyn II 20%	3.1	2	4
Liposyn II 10%	1.8	9	5

[a] Visual estimate.

The solubilities of TBI in aqueous solutions varying in pH, HPCD concentration, and ionic strength are shown in Table II. A 30-fold increase in solubility was achieved with pH adjustment to 2.0, the lower limit of the acceptable range for parenteral products (5). Figure 2 shows the pH–solubility profile for TBI in the absence of HPCD. The solid line in Figure 2 represents the best fit of the data by nonlinear least-squares regression analysis assuming that the solid phase is the neutral compound. This analysis yielded estimates for the intrinsic solubility of 9.26 μg/mL and a pK_a of 3.55.

Increases in TBI solubility were observed in systems containing HPCD, with the magnitude of increase dependent on the HPCD concentration, pH, and, to some extent, ionic strength. At neutral pH, a plot of TBI solubility versus HPCD concentration (not shown) was approximately linear, suggesting that the complexes were primarily 1:1. (Computer analyses of the pH 7 data indicated that a model which allowed for the formation of both 1:1 and 1:2 complexes gave slightly better fits to the data but could not be shown to be statistically superior to a model which assumed only 1:1 complexes. The value of the equilibrium constant for binding of a second HPCD molecule to a previously formed 1:1 complex was approximately 0.1% of the value for $K_{1:1}$, indicating significant steric repulsion for the formation of 1:2 complexes. Therefore, 1:2 complexes were ignored in the final data treatment.) The increases in solubility attained by pH adjustment coupled with HPCD complexation were synergistic (i.e., greater than predicted from a simple linear combination of their individual effects).

Table II. TBI Solubility in Aqueous Solutions Varying in pH, Percentage HPCD, and Ionic Strength

HPCD (%, w/v)	pH	Ionic strength	Solubility ± SD (*n*) (mg/mL)
0	2.03	0.011	0.40 ± 0.02 (2)
0	3.00	0.010	0.050[c]
0	3.03	0.010	0.041 ± 0.009 (2)
0	3.65	0.010	0.020[c]
0	3.98	0.010	0.014 ± 0 (2)
0	5.94	0.010	0.008[c]
0	6.99	0.010	0.009[c]
0	7.96	0.010	0.011[c]
7	2.30	0.014	1.51[c]
7	7.0[a]	0.000	0.41[c]
13	7.0[a]	0.000	0.84[c]
20	2.10	0.024	5.2[c]
20	2.20	0.018	3.42[c]
20	7.0[a]	0.000	1.18[c]
26	7.0[a]	0.000	1.89[c]
30	2.00	0.031	7.75[c]
40	7.0[a]	0.000	2.79[c]
40	2.10	0.038	10.3[c]
40	2.70	0.020	5.28[c]
40	2.04	0.044	12.1[c]
40	2.05	0.065[b]	12.43[c]
40	2.04	0.099[b]	13.52[c]
40	2.025	0.149[b]	13.56[c]

[a] pH was not adjusted; assumed to be 7.0 in regression analyses.
[b] Ionic strength was adjusted with 1 *M* KCl.
[c] Single determination.

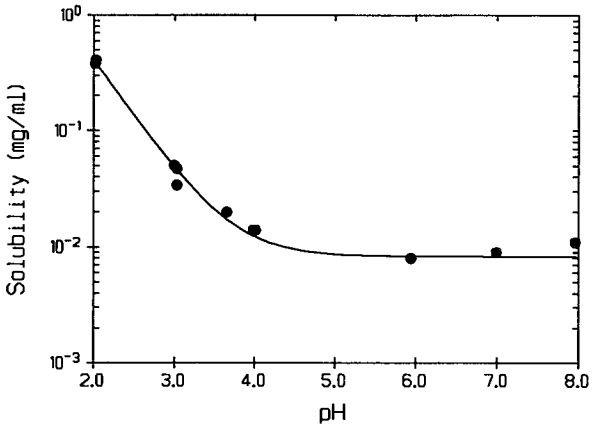


Fig. 2. pH–solubility profile for TBI.

A mathematical model was developed to describe the solubility of TBI over a pH range of 2–7 in aqueous solutions varying in HPCD concentration. The model takes into account the ionization of TBI and the fact that both the protonated and the neutral species form 1:1 complexes with HPCD with different binding constants, as depicted in the following scheme.

$$\begin{array}{ccc} \text{TBIH}^+ & \overset{K_p}{\underset{\text{HPCD}}{\rightleftharpoons}} & \text{Protonated 1:1 complex} \\ K_a \uparrow\downarrow & & \uparrow\downarrow \\ \text{TBI} & \overset{K_n}{\underset{\text{HPCD}}{\rightleftharpoons}} & \text{Neutral 1:1 complex} \end{array}$$

The various *K* values represent equilibrium constants for formation of the various species in solution.

When solid TBI (neutral form) is in excess, the total solubility, *S*, is given by the following equation:

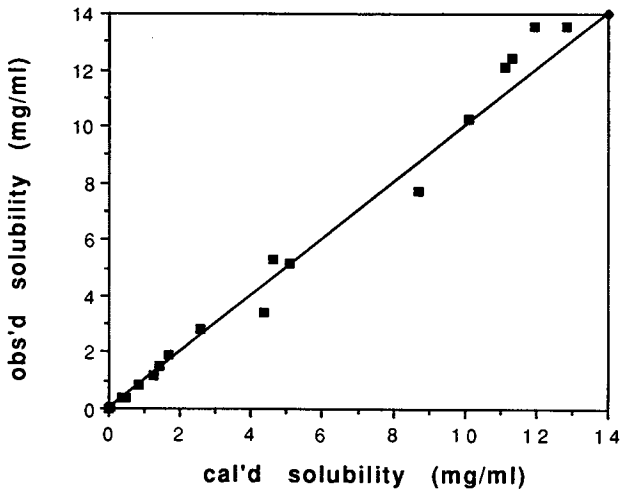


Fig. 3. Observed versus calculated solubilities [Eq. (1)] of TBI in solutions varying in pH, HPCD concentration, and ionic strength.

Table III. Parameters Describing the Solubility of TBI in Aqueous Solutions Obtained from Regression Analysis [See Eq. (1)]

Parameter	Estimate	SD
S_o	3.3×10^{-5} M	2.2×10^{-6}
K_a	2.8×10^{-4} M	3.1×10^{-5}
K_n	1033 M^{-1}	93
K_p	81 M^{-1}	8.5

$$S = [TBIH^+] + [TBI] + [\text{protonated complex}] + [\text{neutral complex}] \quad (1)$$

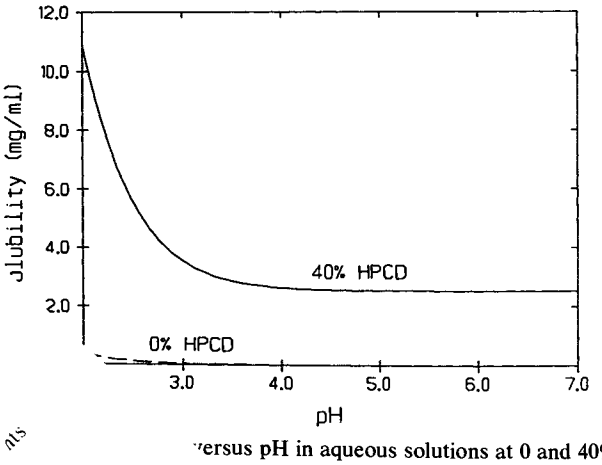
where

$$[TBI] = S_o$$
$$[TBIH^+] = [TBI][H^+]/\gamma K_a = S_o H^+/\gamma K_a$$
$$[\text{protonated complex}] = K_p[HPCD]_f[H^+]S_o/\gamma K_a$$
$$[\text{neutral complex}] = K_n[HPCD]_f S_o$$
$$[HPCD]_f = [HPCD]_{total}/(1 + K_p[H^+]S_o/\gamma K_a + K_n S_o)$$

Activity coefficients, γ, were adjusted for ionic strength (log $\gamma = -0.509 \cdot \sqrt{I}$).

Equation (1), which describes the solubility of the thiazolobenzimidazole over a wide range of pH values and concentrations of HPCD complexing agent, was fitted by nonlinear least-squares regression analysis to the solubility data in Table II to obtain estimates for the parameters S_o, K_a, K_p, and K_n. Figure 3, showing the experimental solubilities versus model calculated solubilities, supports the suitability of the model for describing TBI solubility over a wide range of experimental conditions. Values of the parameters and estimates of their error are shown in Table III.

Figure 4, which displays a plot of TBI solubility versus pH in the absence of added HPCD and in the presence of 40% HPCD, clearly shows the advantage of combining pH adjustment with inclusion complex formation. Even though the complex formation constant between HPCD and TBI in its protonated form was found to be nearly 13-fold smaller than the binding constant between HPCD and the neutral form of TBI, the highest solubilities were achieved in acidic solutions where the predominant species in solution was the protonated complex. This occurred because the concentration of protonated TBI, which serves as the driving force for complex formation, far exceeds that of the neutral species at pH values well below the pK_a. In 40% HPCD it is possible to achieve a solution concentration of 10 mg/mL by adjusting the solution pH to 2–2.1. The model described above proved to be particularly useful in determining which combinations of pH and HPCD concentration are necessary to achieve a given solubility. The model was used to generate a series of predicted isosolubility lines shown in Fig. 5 to serve as a guide in selecting an appropriate combination of pH and complexing agent for the preparation of 2, 5, and 10 mg/mL solutions. A solution concentration of 10 mg/mL was the highest possible concentration attainable within the constraints of [HPCD] ≤ 40% and pH >2. TBI concentrations of 2 or 5 mg/mL could be obtained at a higher pH and/or a lower [HPCD].



...ersus pH in aqueous solutions at 0 and 40% ...PCD concentration.

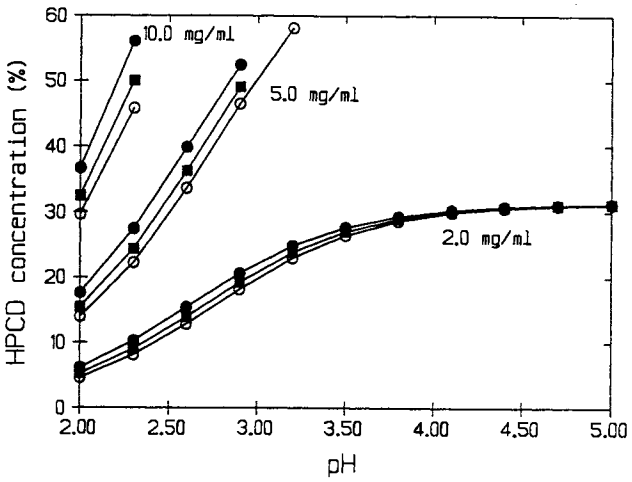


Fig. 5. Isosolubility curves for TBI versus pH and HPCD concentration at different ionic strengths. (⊙) I = 0.15; (■) I = 0.07; (●) I = 0.01.

NMR Proton Spectroscopy

Complexes of both unprotonated TBI in neutral solutions and protonated TBI at pD = 1.88–2.00 were analyzed by NMR spectroscopy. The chemical shift assignments of TBI in aqueous solutions were made with the aid of two-dimensional chemical shift-correlated spectroscopy (COSY) and found to be consistent with the structural formula and

Table IV. Chemical Shifts and Proton Assignments for Protonated TBI in Aqueous Solutions in the Presence or Absence of 200 mg/mL Hydroxypropyl-β-Cyclodextrin at 25°C

Chemical shift δ (ppm)			
0 mg/mL HPCD	200 mg/mL HPCD	Δδ (ppm)[a]	Assignment
7.09	7.05	−0.04	3′-H and 5′-H
7.53	7.50	−0.03	4′-H
7.35	6.99	−0.36	5-H
7.47	7.43	−0.06	6-H
7.60	7.50	−0.10	7-H
7.82	7.73	−0.09	8-H

[a] Difference in chemical shift in the presence and absence of 200 mg/mL HPCD.

Table V. Chemical Shifts and Proton Assignments for Unprotonated TBI in Aqueous Solutions in the Presence or Absence of 200 mg/mL Hydroxypropyl-β-Cyclodextrin at 25°C

Chemical shift δ (ppm)			
0 mg/mL HPCD	200 mg/mL HPCD	Δδ (ppm)[a]	Assignment
7.02	6.99	−0.03	3′-H and 5′-H
7.44	7.41	−0.03	4′-H
7.14	6.81	−0.33	5-H
7.21	7.15	−0.06	6-H
7.32	7.21	−0.11	7-H
7.69	7.58	−0.11	8-H

[a] Difference in chemical shift in the presence and absence of 200 mg/mL HPCD.

with previous assignments in DMSO-d_6 (6). The results for protons of interest are compiled in Tables IV and V. Figures 6 and 7 show the COSY spectra of protonated TBI in the absence and presence of 200 mg/mL HPCD in aqueous solution, respectively. The cross-peaks in the spectra indicate the couplings between adjacent protons. The complexation between HPCD and protonated TBI is clearly demonstrated by the shift of the corresponding diagonal peaks. The doublets at δ 7.82 in Fig. 6 and at δ 7.73 in Fig. 7 have cross-peaks with the triplets at δ 7.60 and δ 7.50, respectively, which in turn cross-correlate with the up-field triplets at δ 7.47 and δ 7.43, respectively. These latter peaks couple with the doublets at δ 7.35 and δ 6.99. Likewise, the triplets at δ 7.09 in Fig. 6 and at δ 7.05 in Fig. 7 cross-correlate with the corresponding peaks at δ 7.53 and δ 7.50. Thus, all the resonances of interest are unambiguously assigned.

The nonspecific solvent effect of HPCD on the chemical shifts of various protons in TBI was approximated at p*D* = 1.88 by comparing the chemical shifts of protonated TBI in solution with those in a 200 mg/mL solution of α-D-glucose. Small changes in chemical shift (≈±0.01 ppm) were generally observed. At neutral p*D*, the solubility of TBI in water in the absence of HPCD was so low that its proton peaks could be detected only in the absence of 200 mg/mL α-D-glucose due to the limited dynamic range. Based on the results in acidic solutions, however, the solvent effect in neutral solution was assumed to also be small.

As shown in Tables IV and V, while complex formation induced small changes (≈−0.03 ppm) in the chemical shifts of protons at positions 3′, 4′, and 5′ in protonated and unprotonated TBI, large up-field shifts were generally observed for protons at positions 5, 6, 7, and 8 and, particularly, at 5-H. Proton 5-H in unprotonated TBI not only was shifted considerably up-field upon complexation, but was

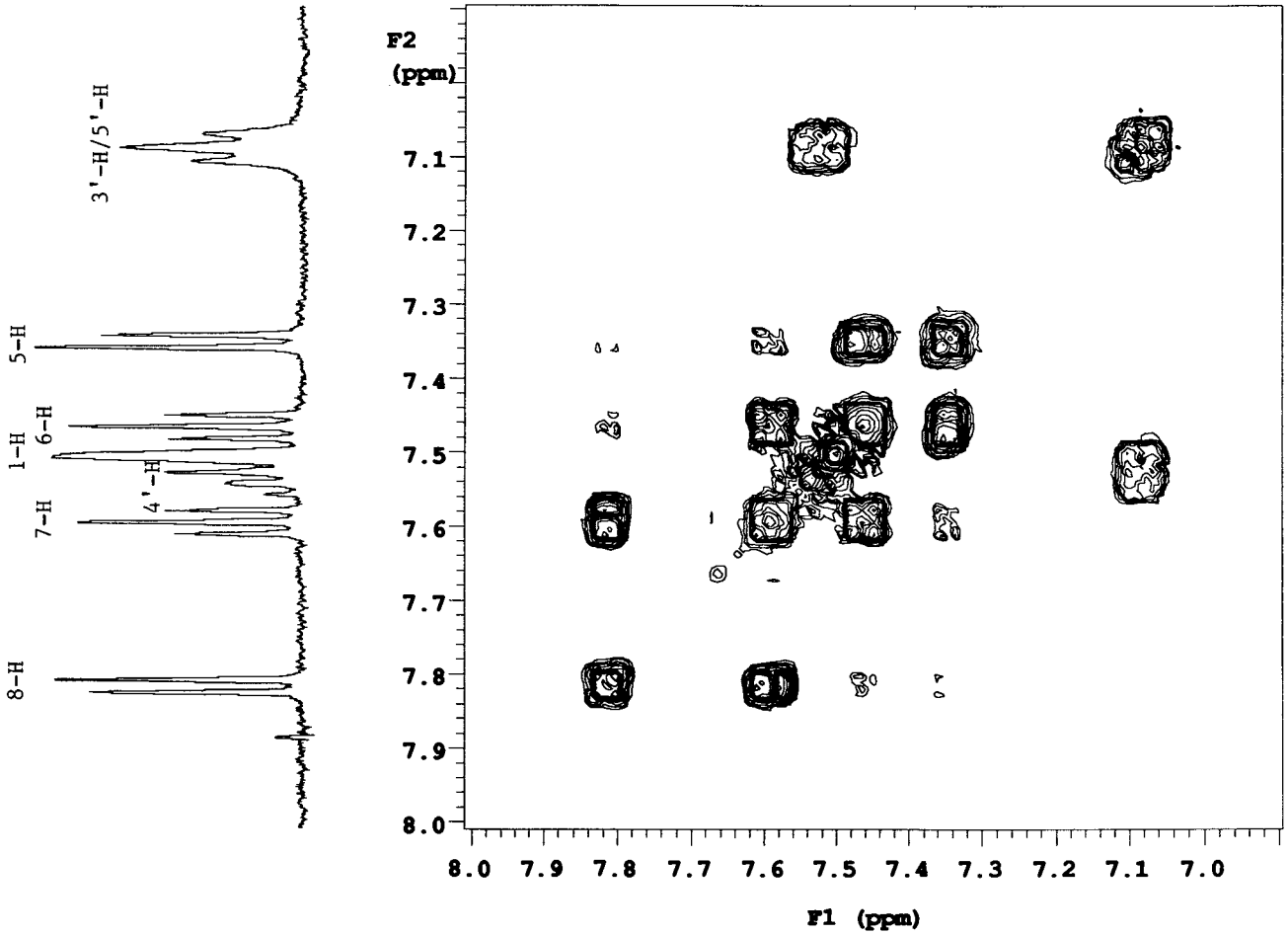


Fig. 6. COSY contour plot in the range of 6.9–8.0 ppm for TBI in D_2O at p*D* = 2.00 and 25°C. The 1D spectrum and the proton assignments are shown along the F2 axis.

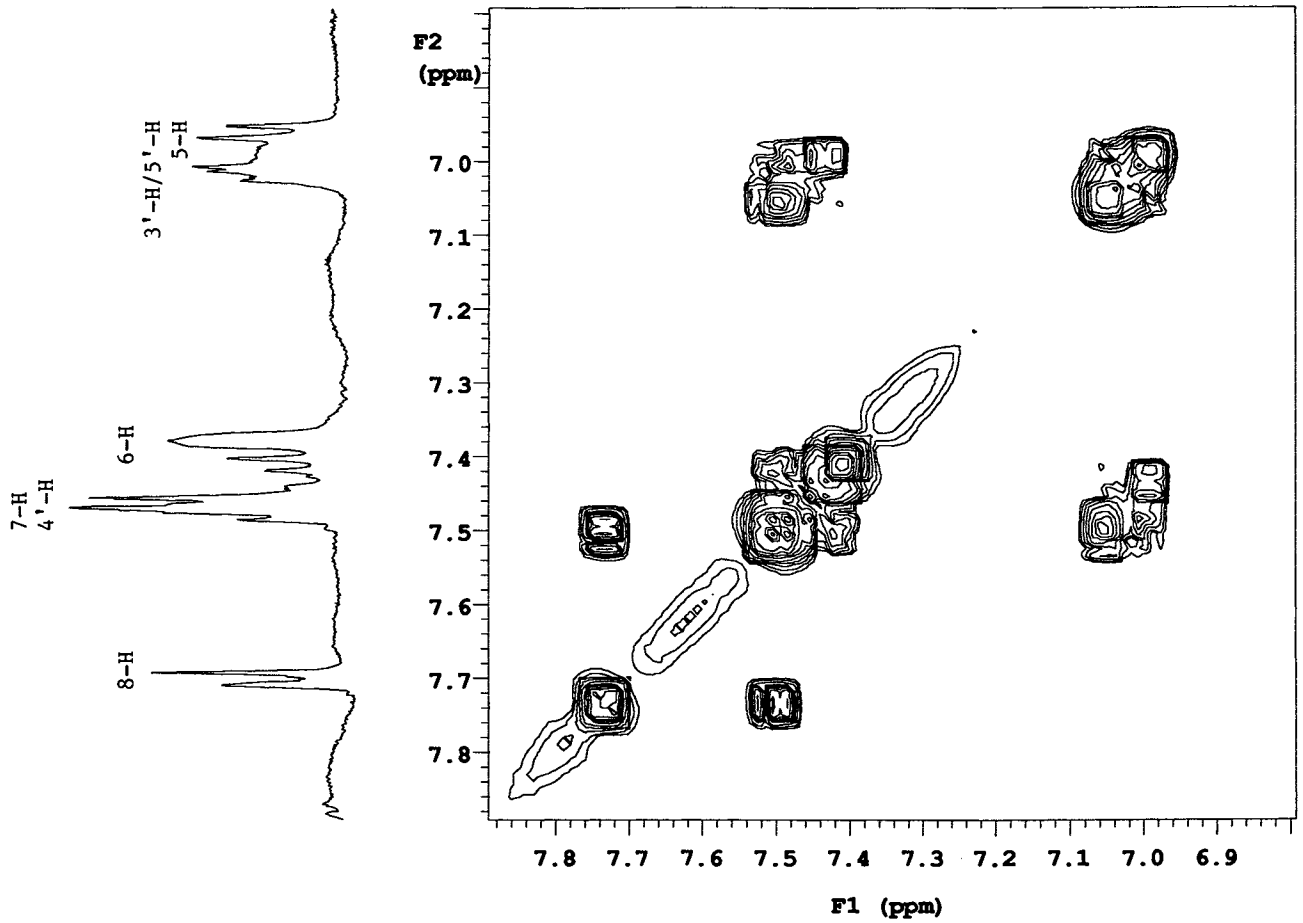


Fig. 7. COSY contour plot in the range of 6.8–7.9 ppm for TBI in D_2O at pD = 1.88 and 25°C and in the presence of 200 mg/mL HPCD. The 1D spectrum and the proton assignments are shown along the F2 axis.

split into two doublets with a separation of 40 Hz. Such a split did not occur in dioxane solvent. If a proton is located outside the cyclodextrin cavity, its chemical shift should be affected only by the changes in the solvent nature due to the presence of the relatively high concentration of HPCD. Changes in chemical shift have been shown to be minimal (≈0.01 ppm) when α-D-glucose is incorporated into solutions in place of HPCD. On the other hand, if a proton resides within the cavity of HPCD, the dramatic change in microenvironment from water to the hydrophobic interior of the cyclodextrin ring may induce a large alteration in its chemical shift. The relatively large changes in the chemical shifts of the protons at positions 5, 6, 7, and 8 suggest that the thiazolobenzimidazole ring of TBI resides within the HPCD cavity. The extent of penetration of this portion of the TBI molecule into the cavity and its orientation depend on both hydrophobic and steric interactions. The relative orientation of TBI with respect to the symmetric axis of the HPCD cavity may be tilted toward C-5 such that the proton at this position lies within the most hydrophobic region of the cavity, as the change in its chemical shift upon complexation is greatest. Alternatively, the chemical shift of the proton at C-5 may exhibit the highest sensitivity to changes in solvent microenvironment, in which case the large change in shift observed would not necessarily indicate deeper penetration into the HPCD cavity.

Table VI. Chemical Shifts and Proton Assignments for TBI in Deuterated Dioxane and Dioxane/Water Cosolvents at 25°C

Chemical shift (ppm)				
100% dioxane	Δδ[a]	3:1 dioxane:water (pD = 1.88)	Δδ[b]	Assignment
7.00	−0.02	7.09	0.00	3'H and 5'H
7.36	−0.08	7.50	−0.03	4'-H
6.91	−0.23	7.04	−0.31	5-H
7.05	−0.16	7.19	−0.28	6-H
7.14	−0.18	7.30	−0.30	7-H
7.62	−0.07	7.71	−0.11	8-H

[a] Difference in chemical shift for unprotonated TBI in deuterated dioxane and water.

[b] Difference in chemical shift for protonated TBI in deuterated 3:1 dioxane:water and water.

The interior lining of the HPCD cavity is composed of $-CH_2-$ units and glycosidic bridge oxygens. As a result, the microenvironment of the cavity, in the presence of water molecules, may be expected to be similar to that of dioxane. This is consistent with observations that UV spectral changes of some compounds in inclusion complexes are almost identical with those observed when the compounds are dissolved in dioxane (7). Based on these observations, the

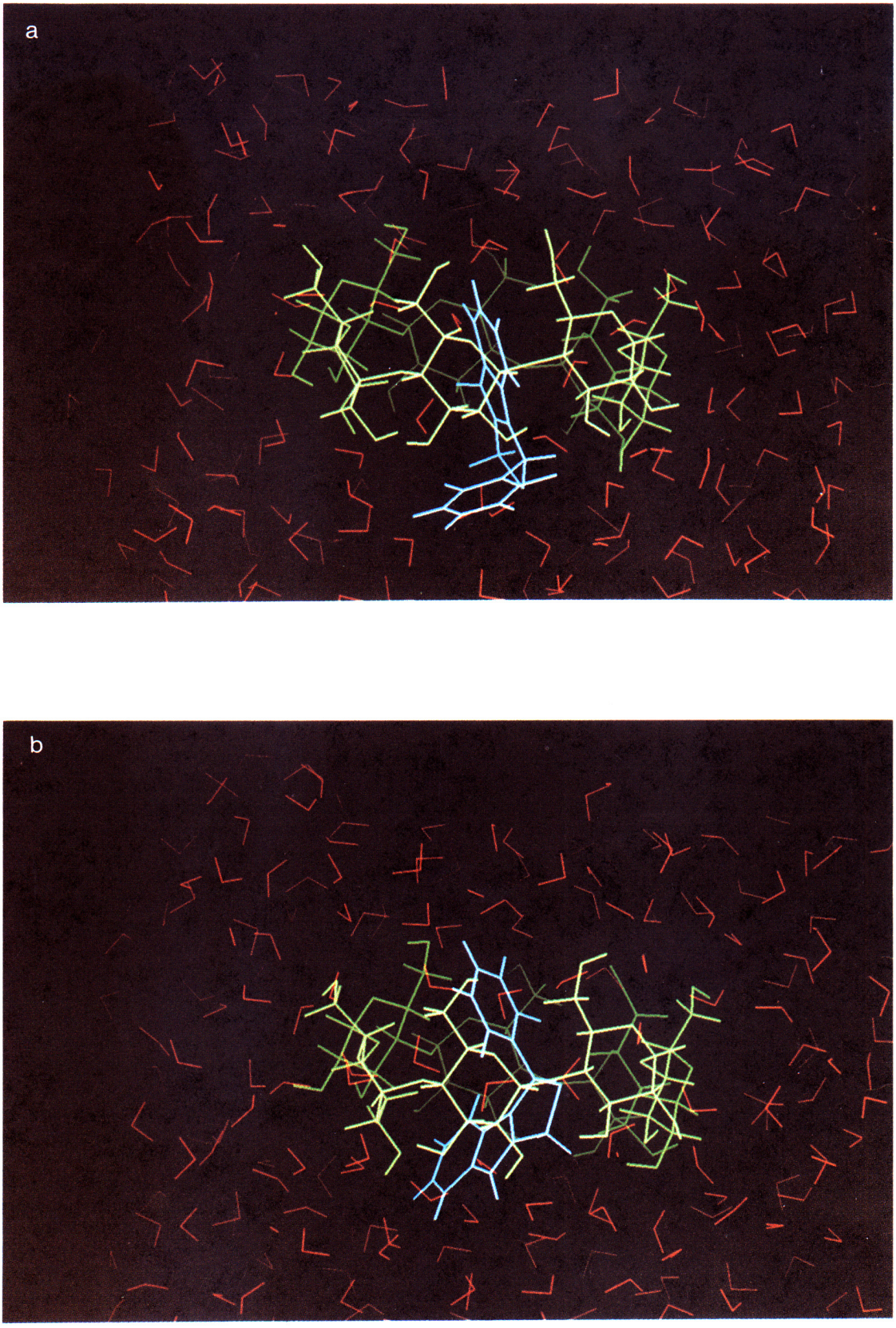


Fig. 8. (a) Structure of the TBI–cyclodextrin complex with the imidazole portion of TBI inserted into the cavity. (b) Structure of the TBI–cyclodextrin complex with the difluorophenyl ring of TBI inserted into the cavity.

medium effect of dioxane on proton chemical shifts of TBI was investigated. As illustrated in Table VI, changes in chemical shifts of protons at the 5 and 8 positions in TBI on going from water to dioxane approximately parallel those occurring in the presence of 200 mg/mL HPCD in aqueous solution, while the changes at positions 6 and 7 are larger on going from water to dioxane. The changes in chemical shifts of the protons on the phenyl ring (i.e., 3′, 4′, and 5′-H) from water to dioxane remain small. Thus, one cannot preclude the possibility that complexes may also form with the phenyl ring inserted into the cyclodextrin cavity. The changes in chemical shifts of protons in the phenyl ring upon HPCD complexation (≈0.03 ppm), though smaller than those in the thiazolobenzimidazole ring, are significantly larger than the changes expected from a nonspecific HPCD solvent effect (≈±0.01 ppm).

Molecular Dynamics Simulations

Energy minimizations were conducted for three systems, all of which assumed 1 molecule of protonated TBI, 1 molecule of β-cyclodextrin (CD), and 749 water molecules. The systems simulated were (i) TBI and CD separated from each other by a distance greater than the nonbonded cutoff distance; (ii) TBI complexed with CD with its imidazole ring inserted into the CD cavity, as shown in Fig. 8a; and (iii) TBI complexed with CD with its phenyl ring inserted into the CD cavity, as shown in Fig. 8b. Unfortunately, the overall energy differences among all three systems were quite small in comparison to the total interaction energies and not statistically significant. Moreover, these simulations did not consider relative entropies. Thus, while the molecular dynamics simulations suggested that both orientations are possible, they provided no statistically significant information regarding which orientation was favored.

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