Hz), 7.63 (ddd, 1 H, J = 0.6, 1.5, 8.2 Hz), 7.43 (ddd, 1 H, J = 1.5, 6.8, 8.0 Hz), 2.87-2.79 (m, 2 H), 2.02-1.39 (m, 18 H). Anal. Calcd for $C_{19}H_{26}N_2O$: C, 76.47; H, 8.78; N, 9.39. Found: C, 76.50; H, 8.79; N, 9.39.

Azacycloheptano[2,1-*b*]-4(3*H*)-quinazolinone (2d): mp 95-97 °C; IR ($CHCl_3$) 1680, 1615, 1600 cm^{-1}; ^1H NMR δ 8.27 (ddd, 1 H, J = 0.6, 1.6, 8.0 Hz), 7.73 (ddd, 1 H, J = 1.6, 6.8, 8.2 Hz), 7.62 (ddd, 1 H, J = 0.6, 1.4, 8.2 Hz), 7.44 (ddd, 1 H, J = 1.4, 6.8, 8.0 Hz), 4.43-4.38 (m, 2 H), 3.11-3.06 (m, 2 H), 1.86 (br s, 6 H). Anal. Calcd for $C_{13}H_{14}N_2O$: C, 72.87; H, 6.59; N, 13.07. Found: C, 72.83; H, 6.85; N, 12.86.

Synthesis of Cyclic Diamines 3 by Reductive Ring Opening of 2. General Procedure. To a stirred solution of quinazolinone **2** (1.00 mmol) in anhydrous THF (10 mL) was added a solution of BH_3·THF (10 mL of 1.0 M solution in THF, 10.0 mmol) under nitrogen. Stirring was continued for 2 h at room temperature, and then the mixture was heated at reflux for several hours, during which time the reaction was monitored by TLC. The mixture was cooled to room temperature, and water (ca. 5 mL) was added drop-by-drop to the stirred mixture. After 50% aqueous NaOH (ca. 2 mL) was added, stirring was continued for 1 h. The mixture was then extracted with dichloromethane (5 × 30 mL). The combined extracts were dried (Na_2SO_4) and were evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (ethyl acetate/hexane, 1:6-1:2) to afford cyclic diamine **3**.

1,5-Diazabenzo[*b*]cyclododecane (3a): colorless oil; IR ($CHCl_3$) 3280, 1610, 1590 cm^{-1}; ^1H NMR δ 7.21-7.12 (m, 1 H), 6.99-6.94 (m, 1 H), 6.63-6.54 (m, 2 H), 3.96 (s, 2 H), 3.16-3.11 (m, 2 H), 2.64-2.59 (m, 2 H), 1.85-1.37 (m, 12 H, 5 CH_2 and 2 NH). Anal. Calcd for $C_{14}H_{22}N_2$: C, 77.01; H, 10.16; N, 12.83. Found: C, 76.92; H, 10.14; N, 12.67.

1,5-Diazabenzo[*b*]cyclotridecane (3b): colorless solid; mp 38.0-39.5 °C; IR ($CHCl_3$) 3300, 1610, 1585 cm^{-1}; ^1H NMR δ 7.22-7.14 (m, 1 H), 7.04-6.99 (m, 1 H), 6.64-6.57 (m, 2 H), 3.80 (s, 2 H), 3.11-3.06 (m, 2 H), 2.72-2.67 (m, 2 H), 1.74-1.33 (m, 14 H, 6 CH_2 and 2 NH). Anal. Calcd for $C_{15}H_{24}N_2$: C, 77.53; H, 10.41; N, 12.06. Found: C, 77.24; H, 10.40; N, 12.36.

1,5-Diazabenzo[*b*]cycloheptadecane (3c): colorless oil; IR ($CHCl_3$) 3300, 1605, 1585 cm^{-1}; ^1H NMR δ 7.22-7.13 (m, 1 H), 7.04-7.00 (m, 1 H), 6.65-6.57 (m, 2 H), 3.77 (s, 2 H), 3.14 (t, 2 H, J = 5.9 Hz), 2.66 (t, 2 H, J = 5.8 Hz), 1.71-1.33 (m, 20 H, 9 CH_2 and 2 NH). Anal. Calcd for $C_{19}H_{32}N_2$: C, 79.11; H, 11.18; N, 9.71. Found: C, 79.30; H, 10.90; N, 9.52.

1,5-Diazabenzo[*b*]cycloundecane (3d): colorless oil; IR ($CHCl_3$) 3250, 1595 cm^{-1}; ^1H NMR δ 7.19-7.10 (m, 1 H), 7.00-6.88 (m, 1 H), 6.70-6.56 (m, 2 H), 4.04 (s, 2 H), 3.5-2.8 (br s, 2 H, 2 NH), 3.37-3.31 (m, 2 H), 2.66-2.60 (m, 2 H), 1.72-1.28 (m, 8 H). Anal. Calcd for $C_{13}H_{20}N_2$: C, 76.42; H, 9.87; N, 13.71. Found: C, 76.55; H, 9.60; N, 13.52.

***N*-(*o*-Aminobenzyl)azepane (7)**: colorless oil; IR ($CHCl_3$) 3440, 3280, 1615, 1600 cm^{-1}; ^1H NMR δ 7.13-6.94 (m, 2 H), 6.70-6.62 (m, 2 H), 4.94 (br s, 2 H, 2 NH), 3.60 (s, 2 H), 2.58 (br s, 4 H), 1.59 (br s, 8 H). Anal. Calcd for $C_{13}H_{20}N_2$: C, 76.42; H, 9.87; N, 13.71. Found: C, 76.44; H, 9.61; N, 13.82.

1,2-Dihydroazacyclooctano[2,1-*b*]-4(3*H*)-quinazolinone (6). To a solution of $NaBH_3CN$ (80 mg, 1.27 mmol) in ethanol was added quinazolinone **2a** (100 mg, 0.44 mmol) and bromocresol green indicator (2 mg). The mixture was stirred under acidic conditions to bromocresol green for 2 days at room temperature. A solution of 1 M ethanolic HCl was added as necessary to maintain acidity. The mixture was then diluted with water and was extracted with dichloromethane (3 × 50 mL). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give the crude product, which was purified by preparative TLC (Merck aluminum oxide 60PF_{254}, type E, development with $CHCl_3$) to afford dihydroquinazolinone **6** as a colorless solid (96 mg, 95%): mp 160-163 °C; IR ($CHCl_3$) 1640, 1615 cm^{-1}; ^1H NMR δ 7.90 (dd, 1 H, J = 1.6, 7.8 Hz), 7.27 (ddd, 1 H, J = 1.6, 7.4, 8.0 Hz), 6.84 (ddd, 1 H, J = 1.2, 7.4, 7.8 Hz), 6.62 (dd, 1 H, J = 1.2, 8.0 Hz), 4.92 (ddd, 1 H, J = 1.6, 2.8, 7.0 Hz), 4.31 (ddd, 1 H, J = 4.4, 5.2, 14.2 Hz), 4.11 (br s, 1 H, NH), 3.02 (ddd, 1 H, J = 3.4, 10.0, 14.2 Hz), 2.15-1.38 (m, 10 H); high-resolution EIMS calcd for $C_{14}H_{18}N_2O$ 230.1420, found 230.1409.

Registry No. **1a**, 673-66-5; **1b**, 935-30-8; **1c**, 947-04-6; **1d**, 105-60-2; **2a**, 58314-97-9; **2b**, 60811-55-4; **2c**, 131043-45-3; **2d**, 4425-23-4; **3a**, 131043-46-4; **3b**, 131043-47-5; **3c**, 131043-48-6; **3d**, 131043-49-7; **4a**, 131043-41-9; **4b**, 131043-42-0; **4c**, 131043-43-1; **4d**, 131043-44-2; **5**, 31162-13-7; **6**, 131043-50-0; **7**, 19668-00-9.

Molecular Recognition: α-Cyclodextrin and Penicillin V Inclusion Complexation

Zhihong Helena Qi,[1a] Vivien Mak,[1a] Luis Diaz,[1b] David M. Grant,[1b] and Ching-jer Chang*[,1a]

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Received July 27, 1990

Specific molecular recognition between α-cyclodextrin (1) and a β-lactam antibiotic, penicillin V (2), was systematically determined. A stable 1:1 inclusion complex was established in the solid state, gaseous phase, and solution. The distinct structure of this inclusion complex was rigorously elucidated by FT-IR, FAB-MS, CP/MAS solid-state ^{13}C NMR, and 500-MHz ^1H NMR. Based on strictly determined ^1H NMR data, a time-averaged conformation of the α-CD-penicillin V inclusion complex was proposed, which was supported by CPK model studies and the intermolecular NOE results. Moreover, α-cyclodextrin exhibited significant catalytic activity toward the hydrolysis of penicillin V in weakly alkaline solution. These findings imply that the initial molecular recognition and the concomitant molecular association are essential in a biomimetic process.

Introduction

Cyclodextrins (cycloamyloses, CD) are naturally occurring cyclic oligosaccharides composed of 6-8 α-(1→4)-linked D-glucosyl residues. The shape of a CD molecule is a toroidal, hollow, truncated cone with primary and secondary hydroxyl groups crowning the narrower and the wider rims, respectively. The ability of CDs to admit a variety of guest molecules into their hydrophobic cavities without any covalent bond being formed[2,3] has rendered them very useful models for studying topochemistry and

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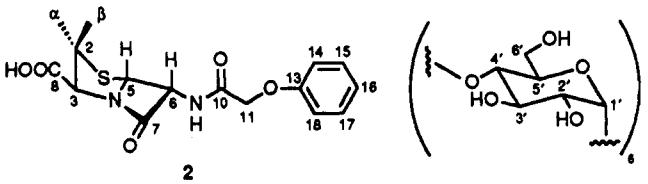
0022-3263/91/1956-1537$02.50/0

catalytic mechanisms of enzymes.[4] Penicillins are a major class of widely used β-lactam antibiotics. The resistance developed by pathogenic bacteria to the action of penicillins is largely ascribed to the cleavage of the β-lactam ring[5] by penicillinases (class A β-lactamases), which have been demonstrated to be nonclassic "serine proteases".[6-8] To design and formulate more effective antibiotic agents, it is essential to understand the catalytic and inhibitory mechanisms of β-lactamases, but the structure of an enzyme–substrate (or inhibitor) complex is too complicated. Hence a biomimetic study of a relevant model system is of great significance. Several model systems employing carbohydrates were investigated in order to explore the mechanism of action of penicillinase,[9] among them β-CD (CD with seven glucose units) was shown to be specific in accelerating hydrolysis of a series of penicillins under mildly alkaline solution.[10] In acidic condition, however, β-CD exhibited retardation effect on degradation of some penicillins.[11] Since no detailed structural information was available from those previous reports, the study of molecular recognition and interaction between penicillins and CDs has been difficult. Therefore, we sought to undertake a systematic investigation of the host–guest recognition of α-CD (CD with six glucose units, 1) and penicillin V (phenoxymethyl penicillin, 2). Presented here are our findings of the formation and structure elucidation of a defined 1:1 inclusion complex, as well as preliminary kinetic results of α-CD-catalyzed hydrolysis of penicillin V.



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Results and Discussions

The α-CD–penicillin V inclusion complex was prepared by crystallization method from H_2O/MeOH solution and was shown to be an 1:1 host–guest complex by ^1H NMR integration. This molar composition of the complex remained constant after repeated crystallization. The FT-IR spectrum of this inclusion complex showed significant broadening and decrease in the intensities of aromatic stretching frequencies of the guest molecule in comparison with penicillin V itself and its adsorption complex with α-CD, indicating a strong intermolecular interaction between the phenyl moiety of penicillin V and α-CD. Hence, it was speculated that α-CD formed a stable host–guest complex by including the phenyl ring of penicillin V into its cavity. The structure of the 1:1 α-CD–penicillin V complex in the solid state was further studied by CP/MAS solid-state ^{13}C NMR (Figure 1). The spectral analysis was based on our previous ^{13}C NMR study of penicillins in solution.[12] Although no significant chemical shift changes were observed, there was a remarkable line broadening effect on the carbon signals of the phenyl ring of penicillin V in the complex, suggestive of the changes of spin–spin relaxation time arising from the more restricted molecular motion in the inclusion structure. These data unambiguously indicate that in the solid-state complex, the phenyl ring of penicillin V is included in the α-CD cavity.

Recently, Ashton et al.[13] reported the use of fast atom bombardment mass spectrometry (FAB-MS) for direct

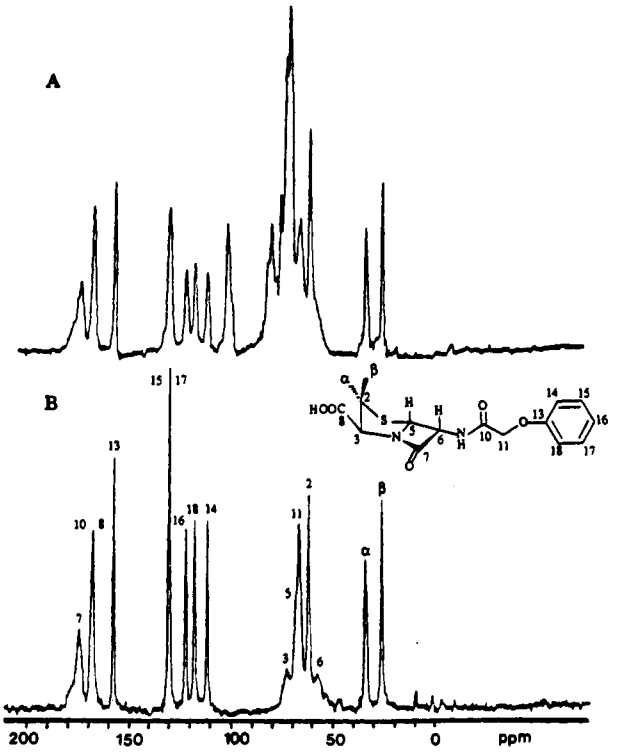

Figure 1. 25-MHz CP/MAS solid-state ^{13}C NMR spectra of (A) α-CD–penicillin V complex (1:1) and (B) penicillin V (the numbers represent the carbon chemical shift assignment for penicillin V). The change of spin–spin relaxation time in the phenyl ring carbons of penicillin V is revealed by significant line broadening effect in reference to the two geminal methyl carbons (α and β).

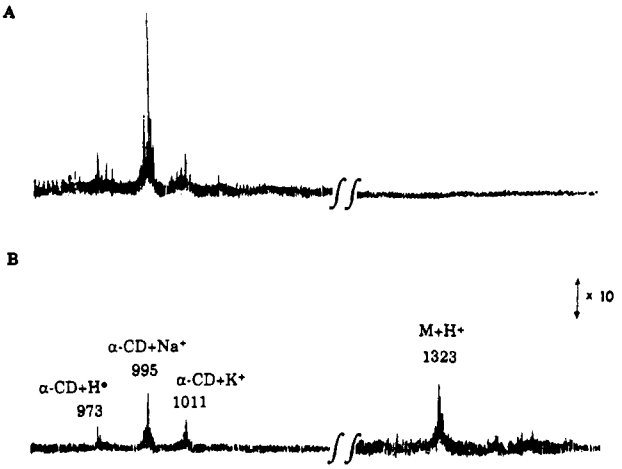

Figure 2. FAB mass spectra of (A) α-CD–penicillin V adsorption complex and (B) α-CD–penicillin V inclusion complex.

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Table I. 500-MHz ^{1}H NMR Chemical Shifts of 0.01 M Penicillin V before and after Complexation with Equal Moles of α-CD

proton	penicillin V (ppm)	α-CD–penicillin V (ppm)[a]	$\Delta\delta_{int}$ (ppm)[b]	$\Delta\delta_{int}$ (Hz)
15, 17	7.3600	7.4020	0.0420	21.0
16	7.0621	7.0954	0.0333	16.6
14, 18	6.9920	7.0550	0.0630	31.5
11a	4.7250	4.7355	0.0105	5.3
11b	4.7182	4.7392	0.0210	10.5
6	5.4890	5.5023	0.0133	6.7
5	5.5341	5.5474	0.0133	6.7
3	4.1963	4.2060	0.0097	4.9
2-β	1.4810	1.5020	0.0210	10.5
2-α	1.4510	1.4594	0.0084	4.2

[a] Chemical shifts of penicillin V in the fully complexed state; calculated[20b] from the observed values for solution ([α-CD]/[penicillin V] = 0.01 M/0.01 M), using the predetermined K_d. [b] Intrinsic chemical shift displacement, defined as difference between the free and fully complexed state.

detection of complex formation between organometallic compounds and methylated CDs using *m*-nitrobenzyl alcohol matrix. However, their attempts to observe adducts formation between parent CDs and neutral or cationic guests proved fruitless. Using dithiothreitol/dithioerythritol (3:1) as a matrix, we obtained the FAB-MS spectrum of the α-CD–penicillin V inclusion complex (Figure 2) with a distinct peak at *m/z* 1323. This protonated molecular ion is completely absent in the FAB-MS spectrum of the adsorption complex between α-CD and penicillin V, indicating the existence of a stable inclusion complex in the gaseous phase. This result clearly demonstrated that FAB-MS would be a unique approach to studying the molecular structures of inclusion complexes. It seems likely that the previous failure to detect the inclusion adducts with neutral or cationic guests by FAB-MS[13] may be attributed to the matrix effect, resulting probably from a direct competition between the guest and the matrix (*m*-nitrobenzyl alcohol).

On the basis of the proposed inclusion structure in the solid state, the formation and structure of α-CD–penicillin V inclusion complex in weakly acidic (pD 5.4) solution was intensively studied by 500-MHz ^{1}H NMR spectroscopy. Proton spectra of each α-CD–penicillin V system consisted of only one set of resonance, suggesting that the chemical exchange of the complexation peocess

$$\alpha\text{-CD} + \text{PV} \rightleftharpoons \alpha\text{-CD–PV}$$

was a rapid equilibrium compared with the NMR time scale, where α-CD, PV, and α-CD–PV are host guest, and complex between them, respectively. The phenyl resonances showed noticeably larger downfield shifts than other protons of the penicillin V molecule upon addition of α-CD (Table I). This phenomenon indicated that, in analogy to many of previously reported cases,[14,15] the phenyl ring of the guest is selectively inserted into the α-CD cavity, driven primarily by hydrophobic interactions. Moreover, the magnitude of the shifts increases as a function of an increasing ratio of [α-CD]/[PV], exhibiting a type of "saturation" curvature (Figure 3). A modified Hildebrand–Benesi plot[16] of the phenyl proton shift data in the form of [α-CD]/Δδ vs [α-CD] (Figure 4) gave excellent linear line fits, supporting the 1:1 complex formation assumption. The dissociation constant of this inclusion complex, K_d, was calculated to be 1.9×10^{-2} M by a least-squares fitting procedure (supplementary material) from the plots.[17,18]

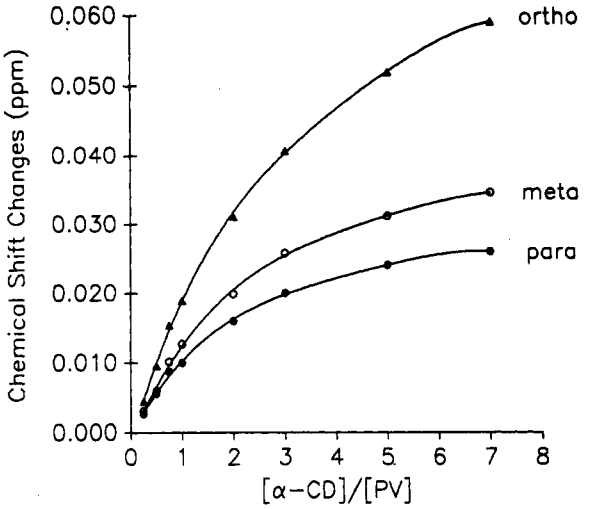

Figure 3. A plot of the α-CD-induced chemical shift changes of the penicillin V phenyl protons as a function of the molar ratio of [α-CD]/[PV].

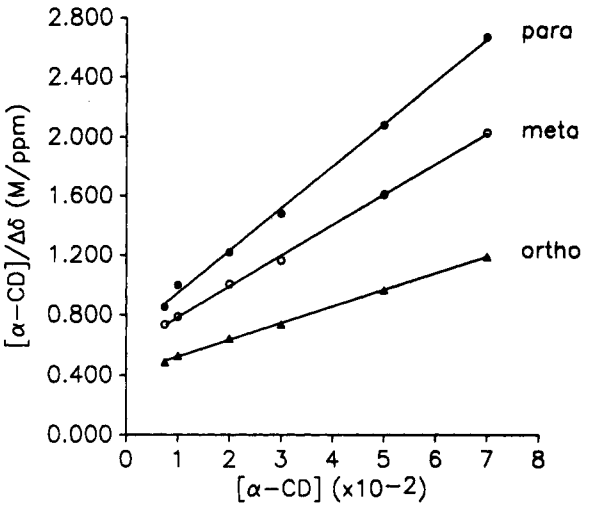

Figure 4. A modified Hildebrand–Benisi plot of [α-CD]/Δδ vs [α-CD]. The dissociation constant, K_d, was obtained graphically (supplementary material) and is a mean value calculated from all three types of phenyl protons.

On the other hand, presence of penicillin V in α-CD solution resulted in significant upfield shifts of the H-3′ and H-5′ resonances of α-CD. These chemical shift changes for H-3′ and H-5′, protons which are located on the inner surface of the α-CD torus, have been interpreted in terms of the magnetic anisotropy effect arising from the aromatic ring of the guest molecules.[14,15] The magnitude of the intrinsic chemical shift displacements of H-3′ and H-5′ is known to be essential to the delineation of the solution structure. Direct spin simulations were thus employed to determine the precise coupling constants and chemical shifts (Table II), because of the highly second-order nature of some of α-CD resonances which resulted in severe signal overlap (Figures 5 and 6). The 500-MHz ^{1}H NMR spectrum of α-CD contains only one set of non-

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Table II. 500-MHz ^{1}H NMR Computer-Simulated Spectral Data for α-CD before and after Complexation with Penicillin V

protons	α-CD	α-CD–penicillin V
	Chemical Shift (ppm)	
1′	5.0240	5.0146
2′	3.6032	3.5934
3′	3.9519	3.9273
4′	3.5549	3.5451
5′	3.8105	3.8805
6′a	3.8800	3.8743
6′b	3.8349	3.8299
	Coupling Constant (Hz)	
J_{12}	3.5	3.5
J_{15}	−0.4	−0.5
J_{23}	10.1	10.2
J_{34}	8.5	8.5
J_{45}	10.1	10.1
J_{46a}	−0.3	−0.3
J_{46b}	−0.7	−0.7
J_{56a}	1.9	1.9
J_{56b}	4.4	4.5
J_{6a6b}	−11.6	−11.4

Table III. Observed (Δδ) and Intrinsic ($\Delta\delta_{int}$) Chemical Shift Changes of H-3′ and H-5′ of α-CD upon Addition of Penicillin V

resonances	H-3′	H-5′
Δδ (ppm)[a]	−0.025	−0.010
$\Delta\delta_{int}$ (ppm)[b]	−0.083	−0.033

[a] Observed for the solution ([α-CD]/[penicillin V] = 0.01 M/0.01 M); negative values indicate upfield shifts. Resonances were assigned with the aid of computer spin simulation using the RACCOON program. [b] Calculated from the Δδ values based on the dissociation constant K_d.[20b]

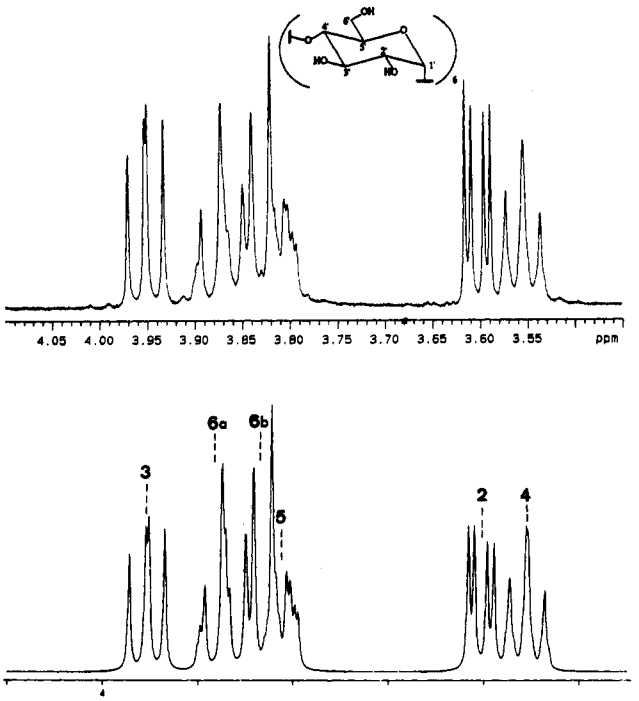

Figure 5. 500-MHz proton NMR spectrum of 0.01 M α-CD (top) and its computer-simulated spectrum (bottom). The RACCOON spin simulation program was used.

equivalent resonances, suggesting that all six glucose units have identical conformations on the NMR time scale and that the molecule has hexagonal symmetry. The fact that $\Delta\delta_{(H3')} > \Delta\delta_{(H5')}$ strongly manifests that the inclusion process takes place by approaching of the guest molecule to the α-CD cavity from the broader, secondary hydroxyl

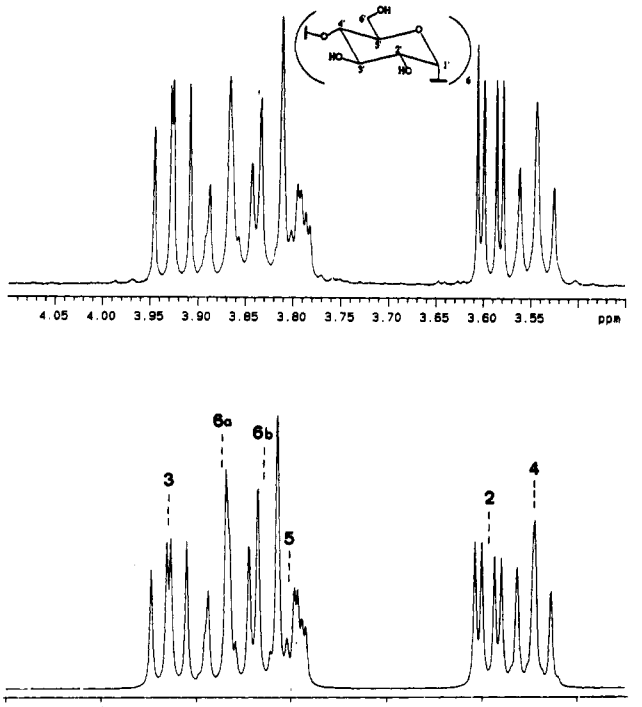

Figure 6. 500-MHz proton NMR spectrum of 0.001 M α-CD with equal moles of penicillin V (top) and its computer-simulated spectrum (bottom). The RACCOON spin simulation program was used.

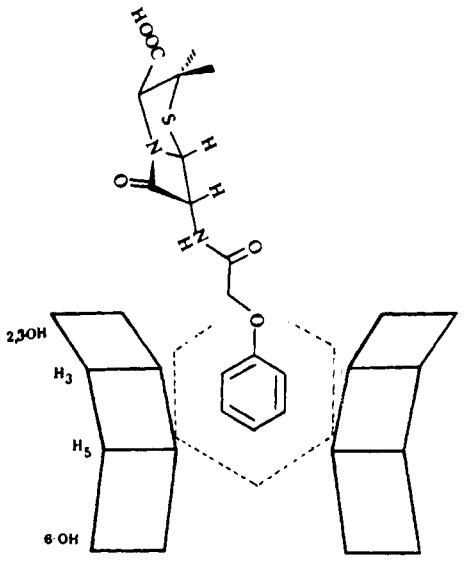

Figure 7. Proposed host–guest geometry of α-CD-penicillin V complex in weakly acidic solution (pD 5.4). The molecular size and shape were drawn with the aid of CPK model. The dotted line around the phenyl ring of penicillin V represents the van der Waals radius boundary of aromatic protons. The α-CD cavity depth measured is 7.3 Å. The cavity diameter is the average of the distances between H3′–H3′ (6 Å), and O4′–O4′ (located toward the inner surface of the cavity at a height of in between H-3′ and H-5′, 6.2 Å) at the plane of H-3′, and the distances between H-5′ (4 Å) and O4′–O4′ (6.2 Å) at the plane of H-5′.

group side.[19] From the phenyl ring induced intrinsic chemical shift displacement of H-3′ and H-5′ (Table III), the depth of the penicillin V insertion into the α-CD cavity was semiquantitatively estimated according to the Johnson–Bovey theory.[15,20]

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Table IV. Intermolecular Nuclear Overhauser Effect Data

α-CD proton irradiated	penicillin V protons enhanced (%)		
	meta[a]	ortho[b]	para[c]
H-5′	6.1	5.5	2.4
H-3′	5.3	6.2	1.4

[a] Protons 15 and 17. [b] Protons 14 and 18. [c] Proton 16.

The time-averaged conformation of the α-CD-penicillin V inclusion complex is depicted as shown in Figure 7, where the center of the phenyl ring of penicillin V is located at 1.0 ± 0.3 Å inside the α-CD cavity relative to the plane comprised of the H-3′ protons. This probable structure was supported by the Corey–Pauling–Koltun (CPK) model study. The limited size of the hydrophobic α-CD cavity prohibits the inclusion of the thiazolidine fragment. While molecular sizes of the phenyl ring and α-CD cavity permit such a snug binding, the proposed "induced-fit" type complexation process[21b] provides a better interpretation of this conformation. Further support of this proposed structure came from our intermolecular ^{1}H NOE data. Selective irradiation of either H-3′ or H-5′ of α-CD gave rise to nuclear Overhauser enhancements to both meta and ortho protons of penicillin V (Table IV), implying that the protons on the phenyl ring of the guest molecule underwent comparable degree of intermolecular dipolar relaxation inside the α-CD cavity. In comparison, *p*-nitrophenolate was found by previous 1D[14] and 2D[22] NOE results to penetrate only partially into the α-CD cavity. These examples have shown that intermolecular NOE data can provide direct and important information about a stable host-guest inclusion complex structure in solution. Nevertheless, such studies remain scarce, and earlier reports were primarily limited to α-CD–nitrophenol (nitrophenolate) systems. This situation may well arise from difficulties in finding optimal experimental conditions,[14] as well as obtaining consistant results. By employing difference NOE method[23] and repeating the same measurement with separately prepared samples, we were able to obtain unambiguous intermolecular NOE results (Figure 8).

To explore the probability of employing α-CD as a biomimetic model of penicillinase, we investigated the influence of added α-CD on the hydrolysis rate of penicillin V. In weakly acidic solution (pH 5.0), no obvious rate enhancement or retardation of penicillin V degradation was observed. Under mildly alkaline condition (pH 10.0), however, excess α-CD exhibited significant catalytic effect on penicillin V degradation (Figure 9). The pseudo-first-order rate constant ratio in the presence and absence of α-CD (k_{obs}/k_{un} = 19.8) revealed about 20-fold rate increase at α-CD:PV molar ratio of 50:1, whereas the added excess (molar ratio 300:1) amount of monomeric analogue of α-CD, methyl α-D-glucopyranoside, only gave rise to slight rate enhancement toward the hydrolysis of penicillin V (k_{obs}/k_{un} = 1.6). Furthermore, ^{1}H NMR studies showed no marked changes of penicillin V chemical shifts upon addition of methyl α-D-glucopyranoside, quite contrary to the situation when α-CD was present. This preliminary result demonstrates that it is the cyclic structure of α-CD that is responsible for its catalytic activity, and it also implies the great importance of molecular recognition in the α-CD biomimetic function.

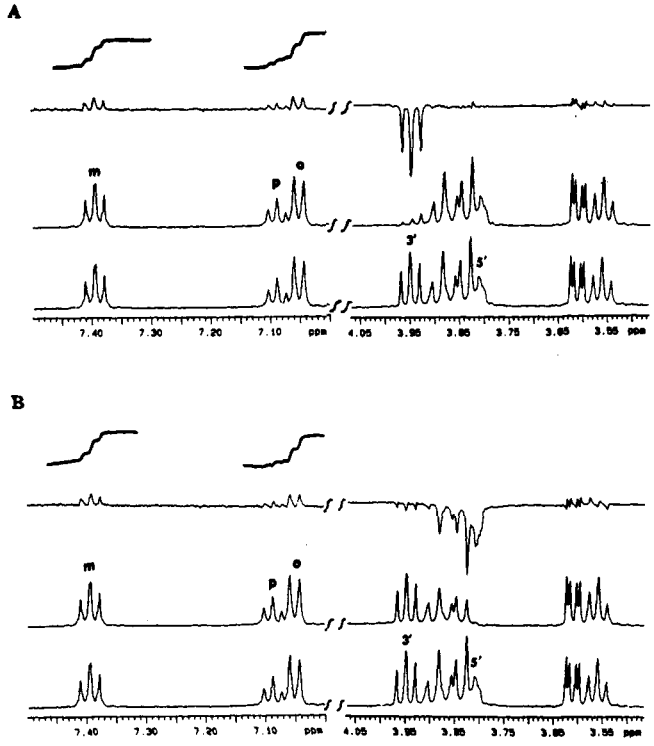

Figure 8. A 500-MHz proton homonuclear difference NOE experiment. The intermolecular nuclear Overhauser enhancement of the phenyl (meta, para, ortho) protons of penicillin V upon irradiation of H-3′ (A) and H-5′ (B) of α-CD are shown in difference spectra. In each set of spectra, at the bottom is a reference where the irradiating rf was set far from any resonances of sample. The difference spectrum (top of each set) was obtained by subtraction of the reference from the middle spectrum where H-3′ or H-5′ of α-CD was subjected to irradiation, respectively.

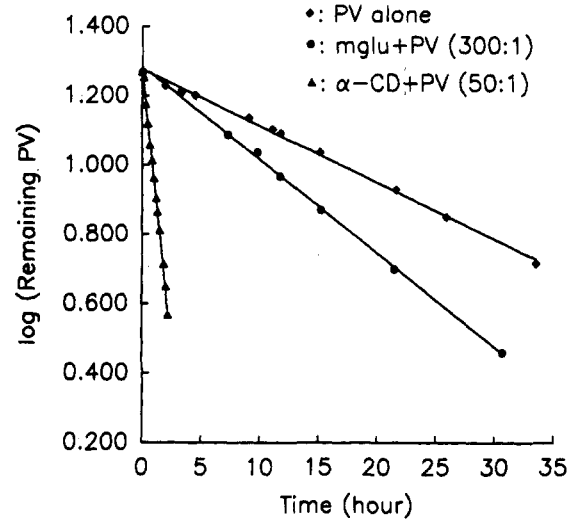

Figure 9. Pseudo-first-order degradation of penicillin V (3×10^{-4} M) in the presence of 1.5×10^{-2} M α-CD or 9×10^{-2} M methyl α-D-glucopyranoside (mglu).

Conclusions

In summary, penicillin V forms a stable 1:1 inclusion complex with the hydrophobic cavity of α-CD in the gas-

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eous phase and in solid state. The complexation also occurs in solution, and the structural elucidation of this complex provides an insight into our understanding of the host–guest molecular recognition, a basis for enzyme–substrate interaction. The kinetic studies show that α-CD can simulate the catalytic activity of β-lactamase in the alkaline hydrolysis of penicillin V. Therefore, α-CD may serve as a suitable biomimetic model. Further elaboration of α-CD as a β-lactamase mimic is currently in progress.

Experimental Section

The following materials were used: α-CD (Sigma Chemical Co.), penicillin V (Sigma Chemical Co.), methyl α-D-glucopyranoside (Aldrich Chemical Co.). Their purities were examined by ^1H and ^{13}C NMR measurements and used without further purification. Aqueous solutions were made with doubly distilled water and reagent-grade buffers. D_2O was purchased from Aldrich Chemical Co.

IR spectra were measured as KBr disks using a Perkin-Elmer Model 1600 FT-IR spectrometer. All IR spectra were recorded at room temperature from 4000 to 600 cm^{-1}. FAB mass spectral results were obtained with a Kratos MS-50 sector mass spectrometer utilizing 3:1 DTT/DTE (dithiothreitol/dithioerythritol) as the ionization matrix. An accelerating voltage of 8 kv was used for the experiments. Melting points were recorded on a Fisher-Johns melting point apparatus and uncorrected.

Solid-state ^{13}C NMR experiments were run on a Bruker CPX-100 spectrometer operating at 25.2 MHz in cross-polarization/magic angle spinning mode. ^1H NMR spectra were recorded at 20 °C on a Varian VXR-500 spectrometer with 32K computer memory operating at 500 MHz. Chemical shift was determined relative to the external standard DSS (2,2-dimethyl-2-silapentanesulfonic acid, sodium salt). The deuterated buffer (pD 5.4) was prepared by lyophilizing a potassium phosphate solution (pH 5.0, 0.2 M), followed by exchanging for deuterium twice with D_2O (99.8% D), and finally dissolving to the original volume in D_2O.[24]

Complex Formation between α-CD and Penicillin V. The inclusion complex was prepared by slowly adding a penicillin V free acid solution (0.5 mmol/5 mL of MeOH) to an α-CD solution (0.5 mmol/5 mL of water) with vigorous stirring. This mixture was filtered and kept at 5 °C overnight. The crystal materials were collected through filtration and washed with small amounts of doubly distilled water and chloroform. The well-dried complex decomposed at 204 °C. IR (KBr) showed characteristic streching frequences of penicillin V at 1780 (COOH), 1765 (lactam C=O), 1660 (side chain amide I), 1600 (aromatic ring), and 1540 (amide II) cm^{-1}. The 500-MHz ^1H NMR spectral integration showed that this was an 1:1 complex of α-CD and penicillin V (chemical shift data were listed in Table I), and this molar composition remained constant after recrystallization in H_2O/MeOH.

The adsorption complex was prepared by adding α-CD powder to an equal molar amount of penicillin V free acid dissolved in chloroform, followed by removal of chloroform under vacuum. The resulting product decomposed at 128 °C and gave a similar FT-IR spectrum as that of the physical mixture of α-CD and penicillin V.

Determination of α-CD–Penicillin V Dissociation Constant by ^1H NMR Method. The concentration of penicillin V was held constant at 0.01 M, and the α-CD concentrations were varied between 0.004 and 0.07 M. Changes in chemical shift of the phenyl protons of penicillin V vs the ratio of [α-CD]/[PV] were treated according a modified Hildebrand–Benesi equation[16-18] (supplementary material). The reported dissociation constant, K_d, was the mean value from all three types of the phenyl protons.

Determination of the Time-Averaged Geometry of α-CD–Penicillin V Complex. The probable (time-averaged) position of the guest, penicillin V, in the α-CD cavity in aqueous solution was determined by fitting the intrinsic chemical shift displacements ($\Delta\delta_{int}$) of H-3′ and H-5′ of α-CD to those reflecting the ring-current effect of the aromatic ring of the guest and was calculated using the Jonson–Bovey equation.[20a] Here $\Delta\delta_{int}$ is defined as $\delta_c - \delta_o$, while δ_c and δ_o are the chemical shifts in the fully complexed and free states, respectively. To determine the $\Delta\delta_{int}$, the ^1H NMR spectra of 0.01 M α-CD in the absence and presence of equal molar penicillin V were first measured and analyzed with the aid of the RACCOON spin simulation program, and the computer-simulated spectra were generated as the final test of the assignment for the observed chemical shifts ($\Delta\delta_{obs}$) and coupling constants (J). The values of δ_c and thus of $\Delta\delta_{int}$ were then estimated from $\Delta\delta_{obs}$ in conjunction with the predetermined K_d value.[20b] The magnitude of the ring-current effect on H-3′ or H-5′ was calculated as a function of the position of the center of the aromatic ring in the cavity. The most probable position of the included guest in the cavity is the one at which both observed $\Delta\delta_{int}$ for H-3′ and H-5′ show maximal agreement with the calculated ring-current shifts.[15]

Intermolecular NOE Experiment. Samples were prepared using pD 5.4 phosphate buffer, 10 mM penicillin V free acid, and 70 mM α-CD where about 77% penicillin V was bound to α-CD. Each sample was carefully degassed to remove paramagnetic oxygen by the alternative "freeze–pump" method[25] for at least five times. The irradiating rf was gated off during acquisition, but kept on during the long (25 s) delay between pulses. The percentage enhancements were mean values calculated from integrals of difference spectra of three repeated experiments; estimated NOE accuracy ±1.5%.

Kinetics of Penicillin V Hydrolysis. The hydrolytic reactions of penicillin V at pH 10 were monitored by following the decrease of the starting materials with HPLC, a Waters Associates system composed of two 6000 A solvent delivery units with a Model 680 solvent programmer. A commercially produced stainless steel column (Alltech Econosphere, C18, 5 μm, 250 × 4.6 mm i.d.) was used, and sample introduction into the column via a Waters U6K universal injector at ambient temperature (25 ± 0.5 °C). The HPLC mobile phase, 4:3 (v/v) 0.05 M potassium phosphate buffer: CH_3CN (pH 3.5) was filtered through 2-μm millipore filters and degassed prior to use. The rates of loss of penicillin V were measured for (a) 3×10^{-4} M penicillin V alone (k_{un}), (b) in the presence of 1.5×10^{-2} M α-CD, or (c) 9×10^{-2} M methyl α-D-glucopyranoside (k_{obs}). Each reaction was followed through at least 70% of completion, and the pseudo-first-order rate constants (k_{un} and k_{obs}) were evaluated from plots of logarithm of the peak area of penicillin V vs time. The validity of this analytical method was established by (a) preliminary hydrolysis studies monitored with both HPLC and ^1H NMR (following the cleavage of β-latam ring) gave consistently parallel results, and (b) on-line UV analysis of the penicillin V peak during HPLC chromatography with a LKB photodiode array UV detector showed that the penicillin V peak contained only a single component.

Acknowledgment. We wish to express our appreciation to the Purdue Research Foundation for the David Ross Fellowship provided to Z.H.Q. and V.M. and the National Institute of General Medical Sciences, NIH, PHS (Grant No. GM08521-29) for the financial support.

Supplementary Material Available: Equation derivation for the graphic estimation of K_d by proton NMR method (1 page). Ordering information is given on any current masthead page.

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