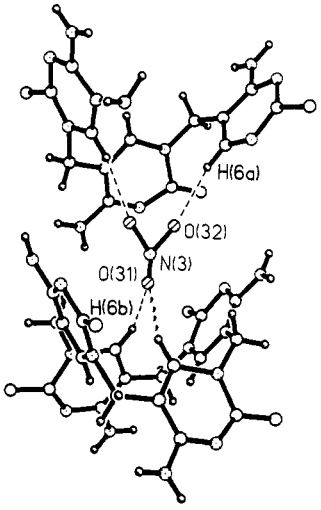

Figure 3. A side view of a nitrate ion interacting with two [16-pyrimidinium crown-4]$^{4+}$ cations. In this view, the front pyrimidinium ring of the top tetramer has been delected so that the nitrate ion can be seen.

The [16-pyrimidinium crown-4]$^{4+}$ cation (Figure 1) is cyclic wherein four 2,4,5-trisubstituted pyrimidine units are joined by methylene bridges linking the C(5) of one pyrimidine to N(1) of the next. Quaternization of the endocyclic N(1) atoms results in an overall 4+ charge for the tetramer. Bond distances and bond angles within each pyrimidinium unit are in agreement with those observed for protonated thiamin.[7] Alternating pyrimidiniums are related by a crystallographic 2-fold axis perpendicular to the mean plane of the macrocycle. Opposing pairs of hydrogen atoms, bound to the C(6) atoms (all of which are nearly coplanar), define the size of the cavity, which is no larger than 1.3 Å in diameter, too small to accommodate any anion.

The macrocycle adopts a boat conformation (Figures 2 and 3), with chloride or nitrate ions held in a pincher-like grip by opposing pyrimidinium rings. In the chloride salt the two nonequivalent chloride anions interact with the tetramer through four C(6)-H···Cl$^-$ hydrogen bonds and through four pyrimidinium ring···Cl$^-$ electrostatic contacts: C(16)···Cl(1) = 3.695 (8) Å, C(26)···Cl(2) = 3.787 (8) Å, H(C16)···Cl(1) = 2.65 Å, and H(C26)···Cl(2) = 2.77 Å; perpendicular distances from the Cl ion to the pyrimidine ring = 3.297 (5) Å for Cl(1)···pyrimidine ring II and 3.438 (5) Å for Cl(2)···pyrimidine ring I. The dihedral angles between the pyrimidinium rings are 73.5°, 81.5°, 120.2°, and 128.4° for planes I and II (I/II; Figure 2), I/II′, I/I′, and II/II′, respectively. The exposed side of Cl(1) is hydrogen-bonded to two water molecules and two NH_2 groups of neighboring tetramers, while Cl(2) is hydrogen-bonded to four waters. A solvated chloride anion Cl(3) and the other two water molecules act as hydrogen-bonding spacers between neighboring tetramers.

The nitrate structure is similar, with a nitrate ion wedged between opposite pyrimidinium rings, with the N(3) and O(31) atoms located on the 2-fold axis. This guest nitrate ion interacts with two tetramers, one above and one below. The closest contact between guest and host occurs between the central oxygen atom, O(31), and both of the C(6) protons of the "lower" rings: O(31)-C(6) 3.3 Å, O(31)-H(16) 2.5 Å, O(31)-H(6)-C(6) angle = 142°. The O(32) lateral oxygens form electrostatic contacts of 3.4 Å with the exocyclic N(41′). The same nitrate acts as a guest of the "upper" tetramer with a closest contact O(32)-C(6) 3.2 Å, O(32)-H(6) 2.3 Å, O(32)-H(6)-C(6) angle = 164°. The dihedral angle between the two pyrimidinium planes of the upper tetramer, which enclose the O(32) oxygens, is 129°, while the corresponding angle in the lower tetramer, which encloses O(31), is 125°. The dihedral angles between adjacent pyrimidinium rings are 74° and 97°. Of the three remaining nitrate ions per tetramer, the N(1) nitrate is located in a general position accounting for two nitrates, while the other, N(2), is found on a 2-fold axis and is disordered.

Cyclic pyrimidinium oligomers could serve as polycationic receptors for anions[8] and provide a multitude of novel structures with properties of wide significance. While we do not yet understand the parameters that govern the formation of the two products we have isolated from the oligomerization of thiamin, namely, [24-pyrimidinium crown-6]$^{6+}$ and [16-pyrimidinium crown-4]$^{4+}$, studies of the capability of these cyclized polymers to capture other biologically important polyatomic anions such as HPO_4^{2-} or HCO_3^-, in addition to monoatomic halide anions like F^-, Br^-, or I^-, are under way.

Registry No. Thiamine chloride hydrochloride, 67-03-8; [16-pyridinium crown-4]$^{4+}$ chloride salt, 135108-17-7; thiamine nitrate, 532-43-4; [16-pyridinium crown-4]$^{4+}$ nitrate salt, 135108-19-9.

Supplementary Material Available: Expermental details of the structure determination, listings of crystal data and summary of data collection, for [16-pyrimidinium crown-4]$Cl_4 \cdot 5.5H_2O$ and [16-pyrimidinium crown-4]$(NO_3)_4$, atomic parameters and anisotropic thermal parameters for [16-pyrimidinium crown-4]-$Cl_4 \cdot 5.5H_2O$, and bond lengths and angles and hydrogen bonds and other short contacts, atomic coordinates and equivalent isotropic displacement coefficients, anisotropic displacement coefficients, H atom coordinates and isotropic parameters, and a figure depicting the crystal packing for [16-pyrimidinium crown-4]$(NO_3)_4$ (12 pages); listing of observed and calculated structure factor amplitudes for [16-pyrimidinium crown-4]$Cl_4 \cdot 5.5H_2O$ and [16-pyrimidinium crown-4]$(NO_3)_4$ (24 pages). Ordering information is given on any current masthead page.

(5) For [16-pyrimidinium crown-4]$(NO_3)_4$: Anal. Calcd for $C_{24}N_{16}H_{32}O_{12}$: C, 39.13; N, 30.42; H, 4.38. Found: C, 39.18; N, 30.24; H, 4.39. For [16-pyrimidinium crown-4]$Cl_4 \cdot 5.5H_2O$: Anal. Calcd for $C_{24}H_{43}Cl_4O_{4.5}N_{12}$: C, 39.52; H. 5.94; N, 23.04. Found: C, 39.83; H, 5.87; N, 23.11.

(6) For [16-pyrimidinium crown-4]$(NO_3)_4$: ^{1}H NMR (300 MHz, DMSO) δ 9.76, 1 H NH_2, 9.00 1 H NH_2, 7.21 1 H C(6)-H, 5.08 2 H C(35)H_2, 2.62 3 H (C21)H_3, all singlets; ^{13}C NMR (D_2O) δ 165 C(2), 163 C(4), 143 C(6), 116 C(5), 52 C(35), 22 C(21); FAB MS calcd for cation 488, obsd 485 (loss of 3H$^+$); UV λ_{max} = 257 nm, log ϵ = 4.63.

(7) Cramer, R. E.; Maynard, R. B.; Ibers, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 76-81.

(8) Indeed, the cyclic pyrimidinium hexamer[3] incorporates a HgI_4^{2-} anion.

γ-Cyclodextrin Template Method for Controlling Stereochemistry of Bimolecular Interactions and Reactions

Akihiko Ueno*

Department of Bioengineering
Faculty of Bioscience and Biotechnology
Tokyo Institute of Technology
4259 Nagatsuta, Midori-ku, Yokohama 227, Japan

Fumio Moriwaki, Yoshiko Iwama, Iwao Suzuki, Tetsuo Osa, Tomihisa Ohta, and Shigeo Nozoe

Pharmaceutical Institute, Tohoku University
Aobayama, Sendai 980, Japan

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γ-Cyclodextrin, which is a cyclic compound composed of eight D-glucose residues joined by α-1,4-linkages, can include two molecular species in its large cavity.[1] Because of this unique property, γ-cyclodextrin can be used as a molecular flask or vessel, in which interactions and reactions between two guest molecules are facilitated.[1-3] Since guest species in γ-cyclodextrin are still

(1) For references on 1:2 host-guest complexation of γ-cyclodextrin, see: (a) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *Tetrahedron* **1987**, *43*, 1571. (b) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *J. Am. Chem. Soc.* **1988**, *110*, 4323.

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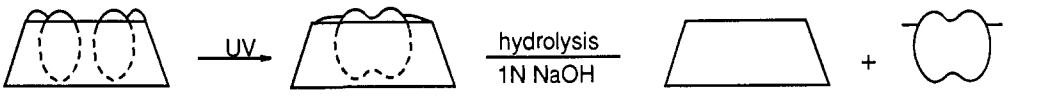
Scheme I



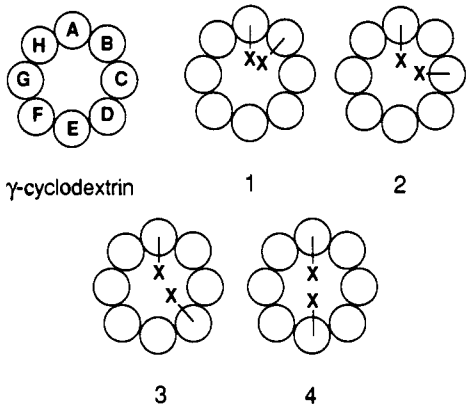
regioisomer (4) γ-cyclodextrin photodimer (6)

Table I. Relative Yields of Photodimers of 1-Anthracenecarboxylic Acid

starting material	solvent[a]	relative yield (%) 5	6	7	8
1	10% EG	0	3	91	6
1	MeOH	0	2	64	34
2	10% EG	8	9	80	3
2	MeOH	1	2	46	51
3	10% EG	6	92	2	0
3	MeOH	41	55	1	3
3	10% EG (*l*-borneol)[b]	32	51	9	8
4	10% EG	11	89	0	0
4	MeOH	41	55	1	3
1-AnCOOH[c]	MeOH	48	28	4	20

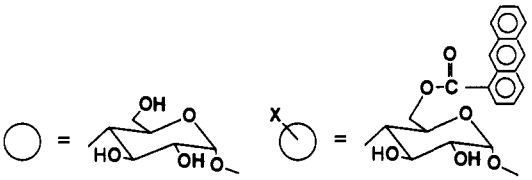
[a] 10% EG/10% ethylene glycol aqueous solution. [b] Photodimerization was performed in the presence of *l*-borneol (2 mM). [c] 1-Anthracenecarboxylic acid.

allowed to move and rotate, any stereochemical control cannot be expected for their bimolecular events. However, stereospecific interactions and reactions between two guest species would be attained if they are covalently linked to AB, AC, AD, and AE glucose units of γ-cyclodextrin. To substantiate this idea, we have now investigated photodimerization of 1-anthracenecarboxylate in regioisomers of 6A,6X-bis(anthracenyl-1-carbonyl)-γ-cyclodextrins (**1**–**4** for X = B–E). As hoped, the photodimerization proceeds stereospecifically, giving expected photodimers among four possible products (**5**–**8**).

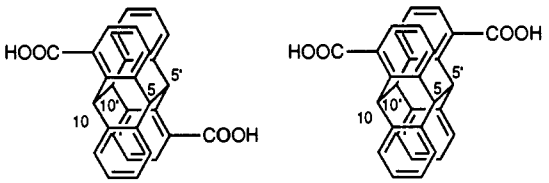


γ-cyclodextrin 1 2

3 4

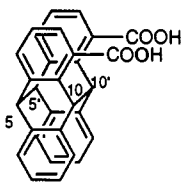


Regioisomers **1**–**4** were prepared by reactions of sodium 1-anthracenecarboxylate and 6A,6X-bis(2-naphthylsulfonyl)-γ-cyclodextrins in dimethyl sulfoxide at 80 °C for 5 h.[3] The products were purified on HPLC through a reversed-phase column and characterized by IR, UV, ^{1}H NMR, FAB MS spectra, and elemental analysis. Irradiation of the regioisomers in a 10% ethylene glycol aqueous solution or methanol (5.0×10^{-5} M) was performed with a 500-W xenon lamp with a cutoff filter for isolating UV light greater than 300 nm. In all cases, the irradiation was continued until the absorption at 365 nm decreased to steady values. The yields of photodimerization were estimated to be more than 90% from the absorption decreases. The irradiated samples were treated with an alkaline solution to hydrolyze the ester linkages and then neutralized with a hydrochloric acid solution. This reaction sequence is shown in Scheme I. The products obtained from each regioisomer were analyzed on HPLC through a reversed-phase column (ODS-120A) with a mixture of acetonitrile and buffer solution (0.2 M Et_3N–H_3PO_4, pH 3). The HPLC peaks of the photodimers **5**, **6**, **7**, and **8** were eluted in this



5

6



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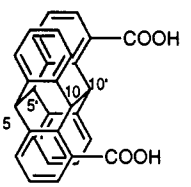

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order. The gross structures of these photodimers were determined by ^{1}H NMR (500 MHz) experiments (HH- and HC-COSY, NOESY, DQ-COSY, COLOC, and HMBC) with their methyl esters. The esters of **5** and **6** exhibit two doublet signals for H-5 and H-10′ and for H-5′ and H-10, while those of **7** and **8** exhibit singlet signals for H-10 and H-10′ and for H-5 and H-5′, and the positions of the carbonyls were finally determined by the analysis of the spectra taken with shift reagent, Eu(tfc)$_3$.

Table I shows relative yields of **5**-**8**. Photodimerization of 1-anthracenecarboxylic acid in methanol gave **5**–**8** with the ratio of 48:28:4:20, but the ratio markedly changed for the reactions in the regioisomers. In a 10% ethylene glycol aqueous solution, cis-head–head photodimer **7** was predominantly formed from **1** and **2**, whereas trans-head–head photodimer **6** was formed from **3** and **4**. This result was exactly what we had expected since the anthracene moieties in **1** and **2** must be oriented so as to form **7**, whereas those of **3** and **4** oriented so as to form **6** in the intramolecular complexes. It is noteworthy that **7**, which is difficult to be formed by photodimerization of 1-anthracenecarboxylic acid in methanol (yield 4%) due to the steric repulsion between two substituents, is preferentially formed from **1** and **2**. Irradiation of the regioisomers in methanol gave the photodimers with lower selectivities as shown by the increased relative yields of **8** and **5** from **1** and **2** and from **3** and **4**, respectively. This result is consistent with the fact that complexation of cyclodextrins occurs only in aqueous solution, and the anthracene moieties are unlikely to be tightly included into the cavities in methanol. When *l*-borneol was present as a guest for **3** in a 10% ethylene glycol aqueous solution, the selectivity became worse showing a decreased relative yield of **6** (51%) and an increased one of **5** (32%). This decrease in the selectivity corresponds to the fact that the host **3** changes the location of the anthracene moieties from the interior to the

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outside of the cavity in accommodation of the guest.[4]

There is a possibility for an asymmetric reaction to occur in the chiral frameworks of **1-4**. In order to examine this point, we have measured the CD spectrum of **6** in methanol. The sample of **6** was isolated from the reaction mixture of **3**. The CD spectrum exhibits peaks at 230 and 283 nm and a trough at 250 nm with the values of molecular ellipticity, $[\theta]$, of 18800, 5360, and -19300 deg cm^2 dmol^{-1}, respectively, demonstrating that the reaction product is optically active.

Stereochemical control of bimolecular reactions are known to be attained in some crystals.[5] The results shown here demonstrate that similar control can be achieved even in solution by using γ-cyclodextrin template with the advantage of facility in regulating the orientation of the reactive species as desired. Another aspect of this method is that it may assist the study on the physical nature of bimolecular interactions by providing a pair of the species placed with different orientations.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan and in part by a Grant for International Joint Research Project from the NEDO, Japan.

(4) This type of complexation is suggested from the CD data that **3** decreases CD intensities in the anthracene transition regions upon addition of *l*-borneol. The analysis of the CD variations gave 2000 M^{-1} as the binding constant, so 80% of **3** should exist as the complex at 2 mM of *l*-borneol.

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Isotopically Enhanced Infrared Spectroscopy: A Novel Method for Examining Secondary Structure at Specific Sites in Conformationally Heterogeneous Peptides

Lema Tadesse, Ramina Nazarbaghi, and Lee Walters*

The Scripps Research Institute
Department of Molecular Biology
MB-15, 10666 North Torrey Pines Road
La Jolla, California 92037

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Detailed mapping of secondary structure(s) in flexible peptides and proteins is an important goal of biological chemistry. This knowledge is relevant to the study of protein folding pathways and peptide-macromolecular interactions as diverse as hormone-receptor, antigen-antibody, and substrate-enzyme binding. Vibrational transitions occur very rapidly compared to the time periods needed for interconversion of different secondary structures in peptides. For this reason, Fourier transform infrared (FTIR) spectroscopy has the potential to quantitate populations of peptide conformers in solution[1] and is not subject to the problem of conformational averaging associated with the relatively long time scales of NMR spectroscopy.[2] However, FTIR is subject to an important limitation that also restricts the application of circular dichroism spectroscopy to peptide mapping; i.e., neither can analyze the conformation(s) of amino acids at specific positions. We now report a novel approach in which FTIR, enhanced by site-specific isotopic labels,[3-15] is used to examine secondary structure(s) of amino acid residues at *specific* locations in solutions of conformationally heterogeneous peptides.

The stretching frequencies of peptide backbone carbonyl groups are sensitive indicators of local conformation.[1,16,17] In the data presented below, ^{13}C-labeled carbonyl groups are incorporated into a series of synthetic peptides. Replacing ^{12}C with ^{13}C is expected to reduce the stretching frequency of an isolated carbonyl oscillator by ~37 cm^{-1}.[18] Thus, when the spectra of labeled and natural abundance molecules are compared, the transitions originating at the *labeled sites* can be detected and evaluated.

Peptides and native proteins may have different IR transitions associated with the same class of secondary structure(s).[1] Most experimental work correlating secondary structure(s) with the frequencies of IR transitions has been done for native proteins.[16,17] The isotopic approach described in this report will be a valuable tool for systematically correlating IR absorption bands and secondary structure(s) in relatively unconstrained, dynamic peptide conformers. For illustrative purposes, in this initial demonstration, we have used the secondary structure assignments commonly utilized in the interpretation of spectra of native proteins.[16,17]

Peptide **1** was designed to be water soluble and monomeric and to contain two distinct conformational regions, containing mostly alanine and glycine residues, respectively. In peptide **2**, five alanines are ^{13}C-1 enriched; in peptide **3**, five glycines are similarly labeled (labeled residues are underlined).[19]

peptide **1** AEAE AAAAA EAEWEGE GGGGG EGEG

peptide **2** AEAE AAAAA EAEWEGE GGGGG EGEG

peptide **3** AEAE AAAAA EAEWEGE GGGGG EGEG

The peptide **1** amide I spectrum is shown in Figure 1a. A broad band centered at 1645 cm^{-1} indicates backbone disorder, and a peak at 1621 cm^{-1} indicates a β-strand extended chain conformation.[16,17] A small shoulder at 1687 cm^{-1} is consistent with reverse turn (or possibly β-strand) conformations.[16,17] Peaks at 1564 and 1707 cm^{-1} reflect ionized and protonated carboxylate groups, respectively.[20]

There are prominent differences in the spectra of peptides **1**, **2**, and **3**, due entirely to the presence of ^{13}C in the labeled peptides. In the peptide **2** spectrum, Figure 1b, there is a large decrease in the 1621-cm^{-1} β-strand extended chain peak, and a concurrent increase in area in the ~1575-1595-cm^{-1} region (arrow A in the figure), reflecting an isotopic shift of ~36 cm^{-1}. The reverse turn shoulder at 1687 cm^{-1} is less distinct, with its area shifted into the 1645-cm^{-1} band. These isotopic shifts are easily seen in a *difference spectrum*, obtained by simple algebraic subtraction of the two spectra. The [(peptide **2**) - (peptide **1**)] difference spectrum, Figure 1c, shows distinct negative peaks at 1621 and 1687 cm^{-1} and a positive peak centered at ~1584 cm^{-1}. Therefore it is possible to conclude that the alanines in positions 5-9 of peptide **1** are ordered in a β-strand extended chain conformation with some degree of reverse turn present.

* To whom correspondence should be addressed.

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(18) In many molecules the carbonyl stretch can be accurately modeled as a simple harmonic oscillator. The frequency of harmonic motion $\nu = 1/2\pi(\sqrt{k/\mu})$ in which k is the bond force constant and μ is the reduced mass of the bonded atoms [$\mu = (m_1)(m_2)/(m_1 + m_2)$ where m_1 and m_2 represent the masses of the oxygen and carbon].
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