BIOORGANIC CHEMISTRY **25,** 155–162 (1997)
ARTICLE NO. BH971062

# Molecular Recognition Study of a Supramolecular System

## XI. Chiral Recognition of Aliphatic Amino Acids by Natural and Modified $\alpha$-Cyclodextrins in Acidic Aqueous Solution

Yu Liu,[1] Bao-Hang Han, Ai-Di Qi,[2] and Rong-Ti Chen

*Department of Chemistry, Nankai University, Tianjin 300071, People's Republic of China*

*Received January 10, 1997*

The competitive inclusion method was used to determine the stability constants (log $K_a$) for complexation of natural $\alpha$-cyclodextrin (**1**) with aliphatic amino acids in acidic solution. The stability constants (log $K_a$) for the complexation of modified $\alpha$-cyclodextrin, mono-[6-(1-pyridinio)-6-deoxy]-$\alpha$-cyclodextrin (**2**), with these biological molecules were measured using the differential spectra method. Both natural $\alpha$-cyclodextrin (**1**) and chemically modified $\alpha$-cyclodextrin (**2**) can recognize not only the size of guest amino acid molecules and the length of their hydrophobic side chains, but also the chirality of enantiotopic L/D-amino acids. The longer the hydrophobic side chain borne on an amino acid, the more stable the complex formed by $\alpha$-cyclodextrin hosts (**1**) and (**2**). Host compounds (**1**) and (**2**) preferably include L-amino acids, which would benefit from discrimination of L/D-amino acids. Introduction of a positively charged 1-pyridinio moiety to C-6 of $\alpha$-cyclodextrin apparently enlarges the inclusion ability and enantioselectivity by electrostatic interaction. Comparing the data obtained in acidic medium with those formerly measured in pH 7.20 phosphate buffer solution, a result can be given: when the pH of the medium increases, the complexation ability and selectivity of modified $\alpha$-cyclodextrin (**2**) for most amino acids examined here are slightly enhanced, showing the highest enantioselectivity up to 10.3 for L/D-serine. 

## INTRODUCTION

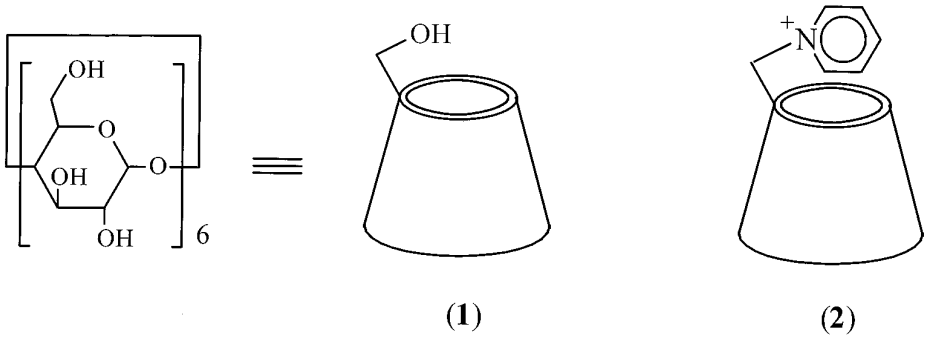
Due to the importance of amino acids as basic components of protein, which is indispensable in the natural living body, molecular recognition of amino acids and their derivatives has been one of the more attractive topics of host–guest chemistry, or supramolecular chemistry (*1*, *2*). Amino acids (guests), possessing amino, carboxyl, and hydrophobic $\alpha$-R groups can form complexes with other molecules (hosts) by various interaction modes, such as hydrophobic interaction, electrostatic interaction, van der Waals interaction, and hydrogen bonding. Many artificial receptors, such as crown ether, porphyrin, cage ligand, and organometallic complexes, have been synthesized to investigate the role of these interactions (*2*).

However, there are few studies on molecular recognition of amino acid by cyclodextrins, a class of natural receptors. Cyclodextrins are cyclic oligosaccharides con-

[1] To whom correspondence should be addressed.
[2] On leave from Tianjin College of Traditional Chinese Medicine.



0045-2068/97 $25.00




(1) (2)

SCHEME 1

sisting of six, seven, or eight $\alpha(1 \rightarrow 4)$-linked glucopyranose units ($\alpha$-, $\beta$-, or $\gamma$-cyclodextrin, respectively) (*3*). The oligosaccharide ring forms a torus, whose openings are not identical; the primary hydroxy groups on C-6 of the glucose residues lie on the narrow side, while the wider opening contains the secondary hydroxyl groups on C-2 and C-3. The inner surface of the toroidal cavity is hydrophobic; conversely, the outer surface is hydrophilic. The strong binding affinity of cyclodextrins for hydrophobic molecules in aqueous media makes them naturally occurring receptors for organic molecules inorganic molecules, and biological molecule substrates. Therefore, many studies have concentrated on unmodified cyclodextrins, especially $\beta$-cyclodextrin, for reasons of cost and also because its cavity has the right size to include a wide variety of aromatic and aliphatic substrates (*4–12*). Surprisingly, few studies on molecular recognition of biological molecules, such as nucleotides, nucleosides (*13*), and amino acids (*14–17*), have been attempted with cyclodextrins, and there has never been any systematic study. However, we have recently reported chiral recognition of aromatic amino acids by binuclear Cu(II) complexes with cyclodextrins (*18*). Modified cyclodextrins bearing pyridinio groups have also been synthesized to examine the thermodynamics of molecular recognition of aliphatic amino acids, showing significant results (*19*). Because both natural cyclodextrins and aliphatic amino acids have no absorption, it is difficult to examine their complexation using either general spectrophotometric titration or calorimetric titration, due in the latter case to the much smaller enthalpy change of the complexation process. In this study, we have determined the complexation stability of $\alpha$-cyclodextrin (**1**) with some aliphatic amino acids, using the competitive inclusion method (*20*). The molecular recognition of these biological guests by modified $\alpha$-cyclodextrin (***2***) (see Scheme I) bearing a pyridinio group has also been studied in the same aqueous medium, employing a differential spectra technique for evaluating the effect caused by the pyridinio group (*18*). The weak noncovalent forces contributing to formation of a supramolecular system, i.e., hydrophobic, van der Waals, and hydrogen bonding interactions, are discussed in terms of the size/shape fit relationship.

## EXPERIMENTAL

### *Materials*

$\alpha$-Cyclodextrin (***1***) of analytical reagent grade was purchased from Makarai and dried overnight *in vacuo* at 110°C. Commercially available amino acids (Institute of Biochemistry, CAS) were chromatographically pure and used without further purification. Methyl orange of analytical reagent grade was used without further purification. Sulfuric acid was of the best commercial quality available. Pyridine of analytical reagent grade was dried over calcium hydride and was distilled in the presence of fresh calcium hydride powder just before use. Mono-[6-(1-pyridinio)-6-deoxy]-$\alpha$-cyclodextrin (**2**) was prepared from mono-(6-*O*-mesitylenesulfonyl)-$\alpha$-cyclodextrin (*21*), which was obtained from sulfonylation of $\alpha$-cyclodextrin with mesitylenesulfonyl chloride (*22*), according to the procedure described by Matsui *et al.*

### *Apparatus*

The spectrophotometric titrations were performed, using the competitive inclusion method and the differential spectra technique, on a Shimadzu UV-240 ultraviolet–visible spectrophotometer, with a constant-temperature bath attached to maintain the cells at a constant temperature by circulating water.

### *Method*

The differential spectra method has been described elsewhere (*19*). The measurement of the stability constant for complexation of $\alpha$-cyclodextrin (**1**) with aliphatic amino acids using the competitive inclusion method is as follows.

*Determining the disassociation constant of the $\alpha$-cyclodextrin–methyl orange complex.* When its concentration is kept constant ($10^{-5}$ mol $\cdot$ dm$^{-3}$), methyl orange characteristically absorbs at 508 nm. While the concentration of $\alpha$-cyclodextrin ($10^{-3}$ mol $\cdot$ dm$^{-3}$) is varied from low to high, the maximum absorbance of the chromophoric moiety borne by methyl orange decreases regularly, indicating formation of 1 : 1 stoichiometric complex. An excellent linear relationship is obtained from the Benesi–Hildebrand equation (*23*). The disassociation constant ($K_d = 1.05 \times 10^{-3}$ mol $\cdot$ dm$^{-3}$) of the $\alpha$-cyclodextrin–methyl orange complex can be calculated from the slope and intercept. $\Delta\varepsilon$, the difference in molar absorbance between methyl orange and the $\alpha$-cyclodextrin–methyl orange complex, can also be determined; $2.24 \times 10^4$ (mol $\cdot$ dm$^{-3}$)$^{-1}$ $\cdot$ cm$^{-1}$.

*Measurements of the complexation stability constant ($K_a$) of amino acids with $\alpha$-cyclodextrin by applying the competitive inclusion method.* While the concentrations of methyl orange and $\alpha$-cyclodextrin are being fixed, the addition of amino acids results in a regular increase in the characteristic absorbance, showing that amino acids can expel the complexed methyl orange and be included by $\alpha$-cyclodextrin. Therefore, methyl orange can be a kind of spectrometric probe for measuring the inclusion stability constants of nonaromatic amino acids with $\alpha$-cyclodextrin. Assuming that amino acids also form a 1 : 1 stoichiometric inclusion complex, we

can obtain the competitive inclusion equilibrium,

$$\mathrm{CD} + \mathrm{MO} \underset{K_d}{\rightleftharpoons} \mathrm{CD \cdot MO} \quad [1]$$

$$\mathrm{CD} + \mathrm{AA} \overset{K_a}{\rightleftharpoons} \mathrm{CD \cdot AA}, \quad [2]$$

where MO and AA represent methyl orange and amino acids, and CD · MO and CD · AA signify the corresponding CD complexes. The initial concentrations were designated $a_0$ for MO, $b_0$ for AA, and $c_0$ for CD; $c$ represents the equilibrium concentration of CD. Under the experimental condition $a_0 \ll b_0, c_0$, the stability constants ($K_a$) of amino acid complexation with $\alpha$-cyclodextrin can be calculated from (*22*)

$$K_a = \frac{c_0 - c}{c(b_0 - c_0 + c)}. \quad [3]$$

$c$ is given by

$$c = \frac{K_d \cdot \Delta A}{a_0 \cdot \Delta\varepsilon - \Delta A}, \quad [4]$$

where $\Delta A$ designates the change in absorbance of methyl orange with the addition of CD and AA. The results obtained verify the 1:1 stoichiometry of inclusion complexation as assumed above.

## RESULTS AND DISCUSSION

### *Competitive Inclusion Method*

This method is efficient in determining the stability constants of the complexes for which neither the host nor the guest displays absorption. Matsui *et al.* (*22*) determined the association constants and thermodynamic parameters for complexes of $\alpha$- and $\beta$-cyclodextrins with a variety of alcohols using the competitive inclusion method, which they called ''the spectrophotometric examination of the inhibitory effect of alcohols on the association of cyclodextrin with azo dyes.'' Connors *et al.* verified this method, which they called the ''competitive indicator method,'' by measuring stability constants for complex formation between $\alpha$-cyclodextrin and 4-nitrophenol, and found that the method gave a result consistent with other methods (*24*). However, the disadvantage of this method is that methyl orange demands an acidic condition. This makes it difficult to compare the data measured by this method with those usually measured in different media by other methods.

### *UV Spectra*

The absorbance of methyl orange remarkably decreased upon addition of $\alpha$-cyclodextrin (**1**), indicating that the chromophoric molecule was included in the cavity. However, the absorbance increased slightly in the spectrometric titration as