Journal of Inclusion Phenomena **6** (1988), 599–603.
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Deuterium Relaxation Time of α-D-Tryptophan Included in Cyclodextrin Host Molecules

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(Received: 5 January 1988; In final form: 23 May 1988)

Abstract. Deuterium relaxation times of *d*- and *l*-α-D-tryptophan included in β-cyclodextrin derivatives were directly measured by deuterium NMR spectroscopy. The results showed that the molecular motion of the tryptophan molecule was strongly restricted even in the cavity of unmodified β-cyclodextrin and the additional recognition groupings – ammonium and carboxylate – on β-cyclodextrin did not affect the molecular motion of tryptophan, though the association constants were significantly enhanced.

Key words. Cyclodextrin, inclusion, deuterium relaxation, receptor.

1. Introduction

Recent rapid developments in host–guest chemistry make it possible to investigate profoundly the multiple molecular recognition due to the noncovalent interactions between host and guest molecules [1]. In a previous paper, we found that β-cyclodextrin having amino and carboxylate moieties as additional recognition groupings showed significant enhancement of the association constants for tryptophan compared with parent β-cyclodextrin [2]. The detailed thermodynamic analysis of these amino acid recognitions indicated that a relatively large entropy loss accompanied this inclusion recognition process as compared with the unmodified β-cyclodextrin case. Since such an entropy loss caused appreciable canceling of the favorable enthalpy change for the complex formation, it is important for the design and synthesis of the more improved host molecules to clarify the origin of such entropy loss. Although there are several possible mechanisms which can explain this entropy loss during the inclusion process, e.g.: (a) the loss of degrees of freedom of the guest molecules; (b) the loss of degrees of freedom of the host molecules; and (c) the change of the hydration around host and/or guest molecules, etc., a definite conclusion has not yet been obtained. The main difficulty for these problems lies in the fact that little data on the dynamic aspects of these inclusion complexes are available [3], especially concerning the modified cyclodextrins.

In the present paper, we wish to report on the dynamic molecular motion of tryptophan included in β-cyclodextrin derivatives **2X** and **2Y** as obtained from deuterium NMR spectroscopy [3b, 4].

2. Experimental

β-Cyclodextrin was purchased from Ando Chemical Co. and used after recrystallization from water. The host molecules **2** were prepared by the method described before

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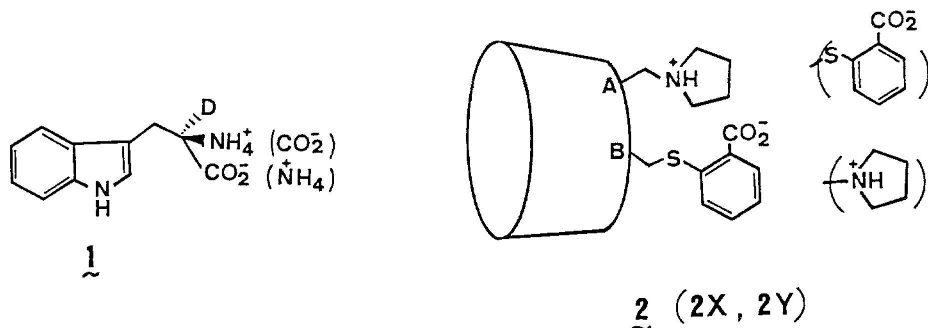

Chart 1. α-D-Tryptophans and β-cyclodextrin hosts.

[2]. Since the absolute configuration of the present isomeric amino acid receptors, **2**, 6*A*(*B*)-1-pyrrolidinyl-6*B*(*A*)-((*o*-carboxyphenyl)thio)-6*A*,6*B*-dideoxy-β-cyclodextrin, were not determined, tentative abbreviations 2**X** and **2Y** were used for these compounds. The guest molecules, *d*- and *l*-α-D-tryptophan, **1**, were prepared and optically resolved by the reported method [5]. The association constants of β-cyclodextrin **2X** and **2Y** were determined by the competitive binding with 8-anilino-1-naphthalenesulfonic acid sodium salt (ANS) in pH 8.9 borate buffer by using a Shimazu RF-503A fluorescence spectrometer thermostated at 12.0 ± 0.1°C [2]. The deuterium NMR spectra were recorded on a JEOL GX-400 NMR spectrometer equipped with a JOEL NM-G40T10 multi-nuclear probe, also thermostated at 12°C.

3. Results and Discussion

The greatest advantage of deuterium quadrupole relaxation for the investigation of molecular motion is that the relaxation time obtained for the deuterium nucleus may be directly related to the motional correlation time without considering other relaxation mechanisms such as dipole-dipole and spin-rotational interactions [4]. Since the relaxation times of **1** in the presence of the hosts are observed as the average of those for the free and complexed species, it is necessary in order to obtain the relaxation time of pure complexed **1** to determine the association constants of hosts with **1** which provide the complexed fractions of **1** under the given condition. The association constants of β-cyclodextrin **2X** and **2Y** with tryptophans determined by the competitive binding with ANS in pH 8.9 borate buffer at 12°C, are shown in Table I.

Table I. Association constants (K) between tryptophan and cyclodextrin hosts.

	K (M^{-1})		
Guest	β-Cyclodextrin	**2X**	**2Y**
l-Trp	7.9 ± 1.3	62.4 ± 2.3	49.1 ± 2.4
d-Trp	9.0 ± 1.2	53.4 ± 2.1	50.1 ± 2.3

[a] pH 8.9, borate buffer at 12°C.

Under the present condition, the association constants for **2X** and **2Y**, as expected, are 5.3–8.0 times larger than that for parent β-cyclodextrin, due to the additional Coulombic interactions between ammonium-carboxylate charges of host and guest molecules, though the selectivities of the present hosts for *d*- and *l*-tryptophan are not large in all cases. The deuterium quadrupolar relaxation times (T_q) of **1** were obtained from the line width of the deuterium NMR spectra ($\nu_{1/2}$) by using Eq. 1 [4].

$$T_q^{\text{obs}} = 1/(\pi \cdot \nu_{1/2}) \quad (1)$$

As shown in Fig. 1, the significant line broadening due to the restriction of the molecular motion was observed in the presence of the host molecule. In order to obtain the relaxation time of the pure complex state (T_q^{complex}), the observed T_q is corrected by using Eq. 2, assuming the small rate constant for dissociation compared with the present relaxation process of the complexed species [6],

$$1/T_q^{\text{obs}} = \alpha/T_q^{\text{complex}} + (1-\alpha)/T_q^{\text{free}} \quad (2)$$

where T_q^{free} is the relaxation time of free tryptophan and α is the fraction of the complex species which is calculated from the association constant shown in Table I. The deuterium quadrupolar relaxation times thus obtained are directly related to the molecular rotational correlation time, τ_q, of the tryptophan molecule when pseudoisotropic motion is assumed [3b] (Eq. 3),

$$1/T_q = \tfrac{3}{8}(2\pi e^2 qQ/h)^2 \tau_q \quad (3)$$

where *e*, *q*, *Q* and *h* are the electronic charge, a component of the operator electronic quadrupole moment, a scalar nuclear quadrupole moment and Planck's constant, respectively [7]. The values of T_q^{complex} and the corresponding τ_q are summarized in Table II. As

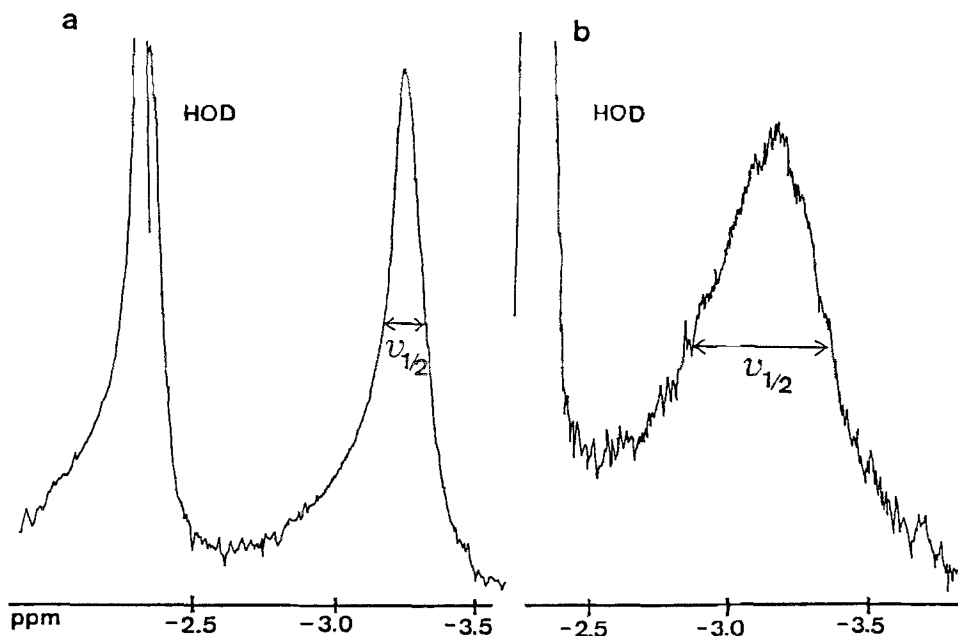

Fig. 1. Deuterium NMR spectra (61.4 MHz) of α-D-tryptophan at pH 8.9, 12°C. (a) [Trp] = 1×10^{-2} M without host. (b) [Trp] = 1×10^{-2} M with [**2X**] = 1.9×10^{-2} M. The chemical shift was measured from $CDCl_3$

Table II. Deuterium relaxation times (T_q) and correlation times (τ_q) of α-D-Tryptophan included in cyclodextrin hosts.[a]

		T_q(ms)[b] [τ_q(ps)][c]		
		Host		
Guest	Free	β-Cyclodextrin	**2X**	**2Y**
l-Trp	30 ± 1 [77 ± 1]	4.4 ± 0.6 [529 ± 82]	4.3 ± 0.2 [541 ± 26]	4.9 ± 0.2 [475 ± 20]
d-Trp	–	4.2 ± 0.6 [554 ± 91]	4.9 ± 0.2 [475 ± 20]	4.8 ± 0.2 [485 ± 21]

[a] pH 8.9, borate buffer at 12°C.
[b] The values of T_q^{complex} in Eq. 2.
[c] Obtained from Eq. 3 by using T_q^{complex}.

is clearly seen in the molecular rotational correlation time of each host–guest couple, the molecular motion of tryptophan slows down by a factor of 6.2–7.2 upon inclusion in β-cyclodextrin, **2X** and **2Y**. The differences between the slow-down factors for these host molecules, however, are quite small in spite of large differences of their association constants for tryptophan. Since the overall correlation times of β-cyclodextrin complexes have been estimated to be 400–700 ps [8], the present results indicate that the rotational freedom of complexed tryptophan is almost lost even in the cavity of unmodified β-cyclodextrin and the molecular motions of tryptophan and host molecules are strongly coupled. Since there is no appreciable difference between the molecular motions of tryptophan included in β-cyclodextrin and **2**, the large (unfavorable) entropy loss observed for the complexation of **2** with tryptophan [2] may be attributed to the more unfavorable change of the hydration during the complexation and/or the loss of the motional freedom of the additional recognition groups of **2** compared with unmodified β-cyclodextrin. It, however, should be noted that the present guests, **1**, were labelled by deuterium only at the α position of tryptophan and not at the indole ring. Therefore, it is still unclear whether the indole ring rotates in the cavity of β-cyclodextrin independently from the motion of the α-carbon of **1**. In order to elucidate the relationship between the binding and recognition abilities of modified cyclodextrins and the motional restrictions of guests, further detailed analyses of the dynamic molecular motions of the cyclodextrin complexes by using deuterium NMR spectroscopy are now in progress.

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