Journal of Molecular Structure 1060 (2014) 138–141

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

A ^{1}H NMR titration study on the binding constants for D- and L-tryptophan inclusion complexes with 6-*O*-α-D-glucosyl-β-cyclodextrin. Formation of 1:1 and 2:1 (host:guest) complexes

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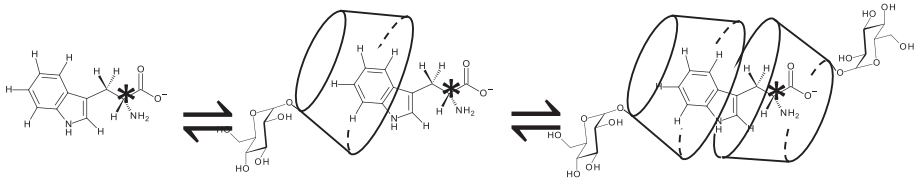
HIGHLIGHTS

- 1:1 and 2:1 (host:guest) complexes of D-, L-tryptophan with G1-β-CD are formed.
- ^{1}H NMR titration method revealed the two step inclusion process.
- The 1st inclusion is on indole-ring, and the 2nd is on the β-carbon of tryptophan.
- The 2nd inclusion shows stronger chiral recognition for the enantiomers than the 1st.

GRAPHICAL ABSTRACT

Two-step inclusion between 6-O-α-D-glucosyl-β-cyclodextrin and D-, L-tryptophan is found. The second host includes chiral-center of the guests (asterisk, *).



ARTICLE INFO

Article history:
Received 24 October 2013
Received in revised form 10 December 2013
Accepted 12 December 2013
Available online 21 December 2013

Keywords:
Tryptophan
6-*O*-α-D-Glucosyl-β-cyclodextrin
Binding constant
2:1 Inclusion complex

ABSTRACT

A ^{1}H NMR titration study revealed that 6-*O*-α-D-glucosyl-β-cyclodextrin (G1-β-CD) forms 1:1 and 2:1 (host:guest) inclusion complexes with D- and L-tryptophan in alkaline D_2O solutions (pD 11.0). The binding constants (K_1's) for the 1:1 complexes of D-isomer at 298 K (59 mol^{-1} dm^3) were virtually equal to that of L-isomer (54 mol^{-1} dm^3). On the other hand, the K_2 values for 2:1 complexes of D-isomer (42 mol^{-1} dm^3) were larger than that of L-counterpart (12 mol^{-1} dm^3). These facts suggest that the first CD molecule includes the indole ring moiety of tryptophan, followed by inclusion with the second CD molecule in the vicinity of chiral center, α-carbon of the guest, to result in the difference in K_2's for two enantiomers. Two-dimensional NMR measurement (Rotating-frame nuclear Overhauser Effect SpectroscopY, ROESY) supported this interpretation.

1. Introduction

The addition of cyclodextrins (CDs) to aqueous protein solutions causes various changes in the properties of proteins [1,2]. For example, we reported that thermal stabilities of proteins are lowered by the addition of CDs to aqueous protein solutions [3–5]. The refolding reactions of thermally denatured proteins are partially hindered by CDs [4,5]. We also found that the H-D exchange rate for the peptide bonds of lysozyme is accelerated by CDs in an aqueous solution [6]. These peculiar effects of CDs on the conformation of proteins are concerned with the inclusion of the side chains of amino acid residues by CDs. Among CDs, 6-*O*-α-D-glucosyl-β-cyclodextrin (G1-β-CD) caused larger changes than the other CDs such as α- and γ-CD. G1-β-CD was used in the place of parent β-CD, since the former is more water-soluble than the latter. The fact that G1-β-CD gives the most effective influence on the conformational changes of proteins suggests that the size of hydrophobic cavity of G1-β-CD fits the side chains of aromatic amino acids, such as tryptophan, phenylalanine, and tyrosine [7]. Inclusion of the indole moiety of tryptophan by G1-β-CD was confirmed by our ^{1}H NMR study on the system of lysozyme [8]. However, it was unknown how strong G1-β-CD interacts with tryptophan, though interactions between parent β-CD and tryptophan have been evaluated by means of potentiometric and spectrophotometric techniques at various pH's [9–13]. In the present study, we tried to determine the binding constants for complexation of G1-β-CD with D- and L-tryptophan in D_2O at 298 K by means of ^{1}H NMR titration technique. The method is

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http://dx.doi.org/10.1016/j.molstruc.2013.12.020

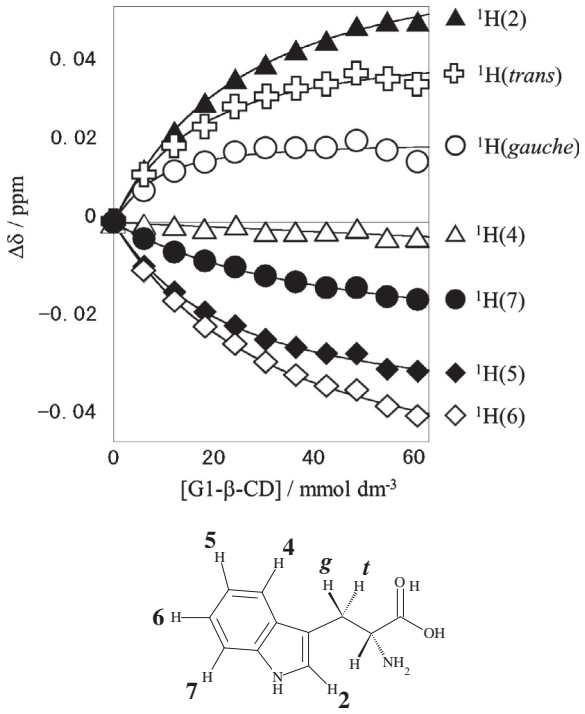

Fig. 1. The plot of $\Delta\delta$'s for ^{1}H signals of D-tryptophan (5.9 mmol dm^{-3}) with increasing concentration of G1-β-CD at pD 11.0 and 298 K. The solid line curves show fitting curves on an assumption of simple 1:1 complexation.

favorable for the accurate determination of binding constants of CD inclusion complexes, since many NMR signals used for numerical analysis are simultaneously available [14]. To begin with the investigation, the deuterium ion exponent (pD) of the D_2O solution was adjusted to be 11.0, at which it is anticipated that the binding constants are significantly larger and more reliable than those at neutral or acidic pD [9–13]. Furthermore, the spatial arrangement of the inclusion complexes was examined by two-dimensional NMR measurement (Rotating-frame nuclear Overhauser Effect SpectroscopY, ROESY) to support the NMR titration measurements.

2. Results and discussion

Eleven solutions of D- or L-tryptophan with different concentrations of G1-β-CD were prepared in a buffer D_2O solution at pD 11.0, as described in Experimental. The ^{1}H NMR spectra of the samples showed that the signals of ^{1}H bound to α-carbon of tryptophan overlap with the signals of G1-β-CD ^{1}H's, and changes ($\Delta\delta$) in chemical shift (δ) of the α-carbon ^{1}H with increasing concentration of G1-β-CD could not be followed. However, signals due to the other ^{1}H's of D-tryptophan, including *gauche*- and *trans*-^{1}H's bound to β-carbon and the indole ^{1}H's, were separated from the G1-β-CD signals. Fig. 1 shows $\Delta\delta$ for D-tryptophan with elevating concentration (c_{host}) of G1-β-CD at pD 11.0 and 298 K (the numbering for ^{1}H's of tryptophan is depicted below the figure). The relationships between $\Delta\delta$'s and c_{host} were analyzed by the curve-fitting method [15], upon an assumption of simple 1:1 complexation of G1-β-CD

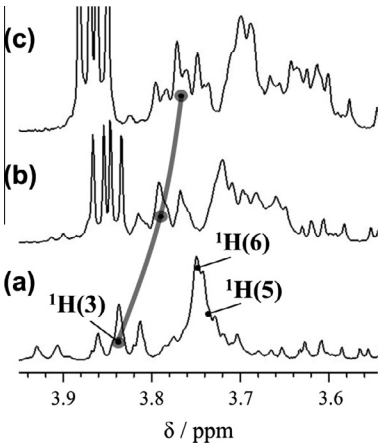

Fig. 2. The chemical shift change of ^{1}H(3) signal of G1-β-CD (2.1 mmol dm^{-3}) with increasing concentration of D-tryptophan at pD 11.0 and 298 K. [D-tryptophan] = 0.0 (a), 15.9 (b), and 31.7 (c) mmol dm^{-3}.

with D-tryptophan. The calculated curves (solid line) were fairly well-fitted to observed data, and individual binding constants (K_1), together with the difference ($\Delta\delta_c$) in δ between the fully complexed and free guest, are summarized in Table 1. The $\Delta\delta$ for the ^{1}H(4) was too small to determine K_1 accurately. Obviously, the K_1 value obtained from the ^{1}H(*gauche*) was much larger than those from the ^{1}H's of the indole moiety. The K_1 value for the ^{1}H(*trans*) was also significantly large. This discrepancy is mainly brought about by the fact that the $\Delta\delta$'s for the ^{1}H(*gauche*) and ^{1}H(*trans*) increased with the addition of G1-β-CD till c_{host} = 40 mmol dm^{-3} and then decreased in the region of $c_{host} \geqslant 50$ mmol dm^{-3}. Repeated measurements for the system gave similar results. This fact indicates that the assumption of simple 1:1 complexation is wrong for this host–guest system. It is possible that not only 1:1 but also 2:1 (host:guest) complexation occur. The possibility of existing 2:1 equilibrium as well as 1:1 one for such complexation system has been pointed out, not only for cyclodextrin but also for other inclusion complexes, e.g. inclusion of aromatic compounds by cucurbit[8]uril studied with UV–Vis. Spectroscopy [16].

In order to determine the K_1 value for the system directly, we examined an effect of the addition of D-tryptophan on the $\Delta\delta$ of the G1-β-CD ^{1}H's, where the concentration of G1-β-CD was so lower than that of D-tryptophan that the 2:1 complexation is negligible. The signals of the ^{1}H(3), ^{1}H(5), and ^{1}H(6) of G1-β-CD significantly shifted to the high-field direction with the addition of D-tryptophan (Fig. 2). The ^{1}H(3) signals were well-defined, whereas those for the ^{1}H(5) and ^{1}H(6) were ill-defined, and we carried out the curve-fitting analysis of the relationship between $\Delta\delta$ for ^{1}H(3) and the concentration (c_{guest}) of D-tryptophan upon an assumption of simple 1:1 complexation to give the K_1 value of 59 mol^{-1} dm^3. This value is somewhat smaller than that (88 mol^{-1} dm^3) obtained for a parent β-CD-D-tryptophan system by Sebestyén et al. in an aqueous solution at pH 10.5 [10]. Then, using the K_1 value of 59 mol^{-1} dm^3, we analyzed the relationships between $\Delta\delta$ and c_{host} shown in Fig. 1 on the assumption that 1:1 complexation is followed by 2:1 complexation by means of the method reported by Funasaki et al. [17] to obtain the binding con-

Table 1
The individual binding constants (K_1), the changes ($\Delta\delta c$) in δ between the complexed and free guest, and correlation coefficients (r) determined by the curve-fitting analysis of relationships between $\Delta\delta$ and c_{host} upon an assumption of simple 1:1 complexation of G1-β-CD with D-tryptophan in D_2O at pD 11.0 and 298 K.

	^{1}H(2)	^{1}H(5)	^{1}H(6)	^{1}H(7)	^{1}H(gauche)	^{1}H(trans)
K_1/mol^{-1} dm^3	47	56	37	27	217	81
$\Delta\delta c$	0.072	−0.040	−0.057	−0.026	0.021	0.046
r	0.9995	0.9983	0.9989	0.9978	0.9698	0.9955