Pergamon

Tetrahedron Letters, Vol. 38, No. 35, pp. 6171-6172, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01427-5

Very Strong Binding of Lithocholic Acid to β-Cyclodextrin

Zhiwei Yang and Ronald Breslow*

Department of Chemistry, Columbia University, New York, New York 10027

Abstract: Lithocholic acid binds to β-cyclodextrin in water with a binding constant exceeding one million M^{-1}, much higher than that of other substrates. The A,B ring cis fusion permits better filling of the cyclodextrin cavity.

β-Cyclodextrin (cycloheptaamylose, β-CD) binds many hydrophobic species into its cavity in water solution, with binding constants dependent on the fit of the substrate into the cavity and the hydrophobicity of the substrate. The best substrates examined so far, such as p-*tert*-butylphenol, have binding constants of the order of 10^4 M^{-1}. Cholesterol also binds into β-CD with an association constant[1] of $2x10^4$ M^{-1}, although we found[2] that it binds into a sulfur linked β-CD **dimer** with an association constant of $5x10^6$ M^{-1}.

We were interested in the relative affinities to β-CD of steroids with an AB *trans* fusion and those with an AB *cis* fusion. In order to use titration calorimetry to determine the binding constants[3] we examined 5α-cholanic acid 3β-ol (**1**), which is *trans* AB fused, and its isomer lithocholic acid (**2**), which is *cis* AB fused, in water solution at pH 9 with dilute aqueous DMSO at 25 °C. As the results in Table 1 show, the *trans* isomer **1** had a 10^4 M^{-1} binding constant in water with 10% by volume DMSO. However, the *cis* isomer lithocholic acid **2** bound 20 times more strongly in that medium, and when the DMSO concentration was lowered to 1% the binding constant for **2** exceeded 10^6 M^{-1}. As far as we know, this is the highest known binding constant of any substrate into simple β-CD. Compound **3** gives results similar to **2**. All titrations showed 1:1 binding, and none of the compounds examined here showed appreciable binding into α-cyclodextrin.

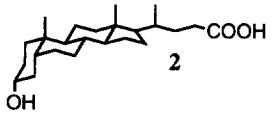
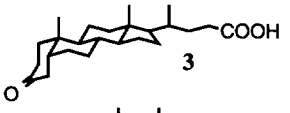
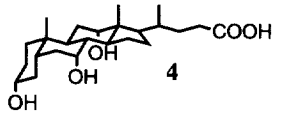
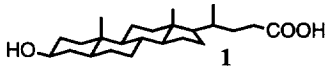
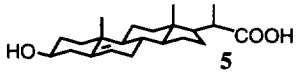
As the data in Table 1 show, the apparent binding constant of β-CD for lithocholic acid in 10% DMSO solution at 0.5 mM is 17% of the value at 0.1 mM, which is 72% of the value at 0.05 mM. This trend must reflect self association of the lithocholic acid when it is not in the β-CD cavity. Thus the true binding constant of monomeric lithocholic acid, and without DMSO, is even higher than the best value in the table.

Molecular modeling explained these results. Monte Carlo conformational searches of the complexes were performed using MacroModel and the Amber force field with water solvation included. The conformation of β-CD was allowed to relax in the initial calculations and in the global minimum energy structures. After searching for 1000 steps for each complex, only 10 unique conformations within 10 kcal/mol of the global minimum were found for lithocholic acid-β-CD complex. All these conformations have their A rings at the secondary face of β-CD, while the B, C, and part of the D ring thread through the cavity (cf. Figure 1). The A,B *cis* junction of lithocholic acid permits ring A to better fill the widened area of the β-CD cavity at the secondary face and to bury much of **2**. As expected, the additional hydroxyl groups in cholic acid (**3**) prevent binding.

Similar calculations were done on **1**. The calculations predict an energy advantage on binding **2** rather than **1** to β-CD, but by 5.2 kcal/mol, which is larger than the observed value. In **1**, in contrast to **2**, the AB *trans* junction prevents effective filling of the secondary region of the β-CD cavity and exposes more of **1** to solvent.

Acknowledgment: This work has been supported by the NIH.

Table 1 : Apparent binding constants of steroids to β-cyclodextrin in water/DMSO at 25 °C

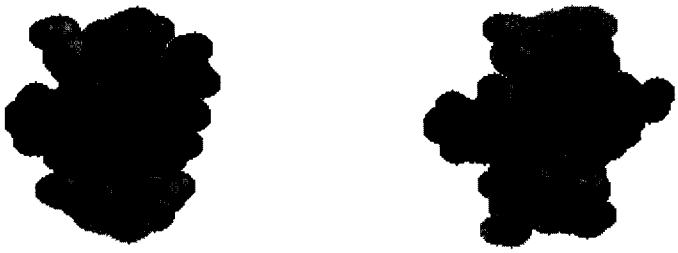
Steroid	K (M^{-1})	$-\Delta H°$ (kcal/mol)
 2	(1.17 ± 0.10) x 10^{6} [a]	8.6 ± 0.1
	(2.01 ± 0.16) x 10^{5} [b]	9.6 ± 0.1
	(2.77 ± 0.30) x 10^{5} [c]	10.2 ± 0.2
	(4.83 ± 0.84) x 10^{4} [d]	14.1 ± 0.5
 3	(2.40 ± 0.26) x 10^{5} [b]	8.74 ± 0.2
 4	not measurable[b]	
 1	(1.07 ± 0.30) x 10^{4} [b]	9.1 ± 2.1
 5	(1.67 ± 0.19) x 10^{3} [b]	27.4 ± 2.0

(a). 2.0 mM β-CD, 0.05 mM steroid, 0.2 M pH 9.0 $NaHCO_3$-Na_2CO_3 buffer, with 1% DMSO.

(b). 2.0 mM β-CD, 0.10 mM steroid, 0.2 M pH 9.0 $NaHCO_3$-Na_2CO_3 buffer, with 10% DMSO.

(c). 2.0 mM β-CD, 0.05 mM steroid, 0.2 M pH 9.0 $NaHCO_3$-Na_2CO_3 buffer, with 10% DMSO.

(d). 5.0 mM β-CD, 0.5 mM steroid, 0.2 M pH 9.0 $NaHCO_3$-Na_2CO_3 buffer, with 10% DMSO.



(a) (b)

Figure 1. Binding of **2** (a) and of **1** (b) into β-CD (50% shown).

REFERENCES

1. Frilink, H. W.; Eissens, A. C.; Hefting, N. R.; Poelstra, K.; Lerk, C. F.; Meijer, D. K. *Pharm Res.* **1991**, *9*, 1.
2. Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1993**, *115*, 9353.
3. Breslow; R.; Zhang, B. *J. Am. Chem. Soc.* **1996**, *118*, 8495.

(*Received in USA* 9 *June* 1997; *revised* 8 *July* 1997; *accepted* 10 *July* 1997)