Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1931–1933

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Binding of Cocaine to Cyclodextrins

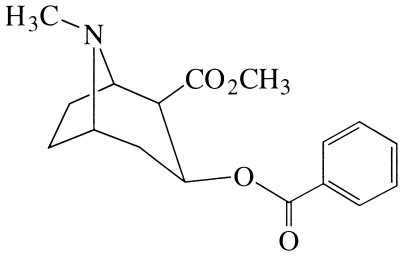
Nasri Nesnas, Jihong Lou and Ronald Breslow*

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

Received 2 May 2000; accepted 19 June 2000

Abstract—Cocaine binds into β-cyclodextrin, but not detectably into α- or γ-cyclodextrin, in water solution. NMR studies indicate the geometry of the complex, which is confirmed by molecular mechanics calculations and binding studies on cocaine analogues and cyclodextrin dimers.

Cocaine (**1**) is a potent drug of abuse for which there are currently no successful inhibitors. We have been studying artificial enzymes that can hydrolyze esters,[1] and have commenced studies on the synthesis of such catalysts for the hydrolysis of cocaine. Since hydrolysis of either ester group of **1** removes its biological activity,[2] such a catalyst could be helpful in treating cocaine addiction and overdose if it were effective in vivo. This is the impetus for the syntheses of antibodies that catalyze such a hydrolysis.[3,4]



1 Cocaine (base)

As a first step, we have examined the binding of cocaine into the cyclodextrins, which are convenient units for binding hydrophobic substrates. We find that indeed cocaine binds with reasonable affinity to β-cyclodextrin (β-CD, cycloheptaamylose (Fig. 1)) (**2**) in water solution, at least an order of magnitude more strongly than it binds to α-cyclodextrin or γ-cyclodextrin, and that NMR studies indicate a geometry of binding that is consistent with the predictions from molecular mechanics calculations. This information can guide the design of effective catalysts for the hydrolysis of cocaine.

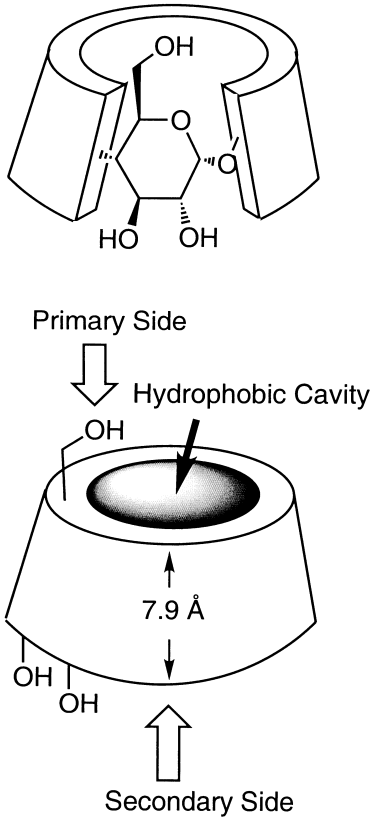


Figure 1. β-Cyclodextrin (**2**), in two representations.

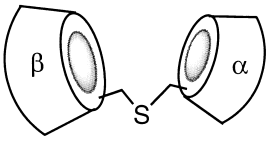
All binding studies except those specifically described as using NMR were conducted by calorimetric titration, using a MicroCal OMEGA instrument. More concentrated solutions of the cyclodextrins and their derivatives were

*Corresponding author. Tel.: +1-212-854-2170; fax: +1-212-854-2755; e-mail: rb33@columbia.edu

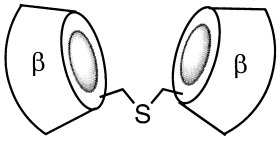
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00371-1

titrated into a dilute cocaine solution. Control runs showed that there was no detectable heat change when the cocaine was omitted from the titration experiment, so (as we have seen in other work) there was no heat effect from dilution of the cyclodextrin solution. We found that cocaine binds to β-CD **2** at pH 7.4 in unbuffered water at 25 °C in a one-to-one complex with a binding constant of $570\pm18\ M^{-1}$, $K_d = 1.75$ mM.

We have found previously that a CD dimer can be very effective in binding cholesterol, with both CD units enclosing part of the molecule.[5] Thus we have examined the possibility that cocaine could also bind even more strongly into a CD dimer if one of the CDs bound the benzoate group and the other bound the tropane ring. We synthesized the CD dimer **3** in which an α-CD (cyclohexaamylose) and a β-CD are linked on their primary faces at C-6. We first reacted benzyl mercaptan with purified α-CD-6-tosylate and then cleaved the benzyl group with Na in liquid NH_3. We then reacted the resulting α-CD-6-thiol with β-CD-6-iodide, and purified the resulting **3** by reverse-phase column chromatography. We examined the binding of cocaine to **3**, and found $K = 1091\pm30\ M^{-1}$, a little higher than for **2**. Thus the additional α-CD in **3** does not add a lot to the free energy of binding into β-CD alone.



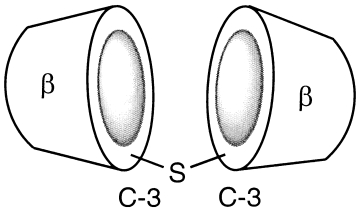
3



4

Additional evidence came from our study of the β-CD dimer **4**,[5] also linked on the primary faces at C-6. We found, from the relative amounts of the two components at the inflection point (this is calculated by the standard computer program supplied with the instrument), that **two** cocaine molecules bound to **4**, with identical binding constants of $800\pm30\ M^{-1}$, only slightly higher than those of simple β-CD itself. Thus in **4** there is no cooperative binding of a single cocaine by the two CDs. Instead, apparently, the two cocaines bind into the secondary faces of the CDs in **4**, avoiding cooperative interaction.

We also examined the binding of cocaine into the CD dimer **5** linked at the C-3 positions on the secondary face.[6] We now found that only **one** cocaine bound, and with an observed binding constant of only $310\pm90\ M^{-1}$. If a correction is applied for the symmetry of **5**, the value is half that indicated. Thus cocaine indeed binds only to the secondary side of CD, as the contrast in behavior of CD dimers **4** and **5** reveals. The lower binding in dimer **5** relative to simple β-CD indicates that there is no useful cooperative binding of cocaine between the two CDs in **5**, but that binding into one cavity is interfered with by the presence of the second CD. This probably reflects some partial binding of one CD into the cavity of the other in **5** itself in the absence of cocaine, overwhelming a small advantage that might result from double binding of cocaine into the two cavities in **5**.



5

We also examined a β-CD dimer linked on the secondary face in which one of the CDs has a glucose unit that has been converted to a derivative of altrose with inversion at C-2 and C-3 (from ring opening of the 2,3-mannoepoxide). We know that such a CD has an indent into the cavity, which generally leads to low binding constants.[7] This dimer bound one cocaine molecule, and with a binding constant of only $65\pm6\ M^{-1}$. Apparently there is again some interference from the binding of one (inverted) CD into the cavity of the other, decreasing the affinity for cocaine as in **5**. The decreased affinity is not compensated, as it was in part in **5**, by some extra binding of cocaine into the (inverted) CD.

We considered that the entire cocaine molecule might be able to bind into γ-CD, which with eight glucose units has a larger cavity than β-CD. No binding of cocaine into γ-CD could be detected, nor was cocaine binding into α-CD detectable within the sensitivity of the method. Both must have binding constants less than $60\ M^{-1}$.

Which part of cocaine is binding into the β-CD cavity in water? Some evidence comes from our examination of cocaine relatives—tropine (**6**), tropinone (**7**), tropine benzoate (**8**), and tropacocaine (**9**). We found no evidence for the binding of tropine **6** or tropinone **7** to β-CD, even though our past work had shown that [2.2.2]-bicyclic systems and [2.2.1]-bicyclic systems bind well into β-CD.[8] Apparently the charged nitrogen in cocaine at pH 7.4 is enough to block binding of the tropane unit. However, tropacocaine **9** bound into β-CD with a constant of $500\pm60\ M^{-1}$, essentially as strong as the binding of cocaine, and tropine benzoate **8** has a binding constant of $1120\pm56\ M^{-1}$. These data indicate that it is the benzoate group that is the principal binding unit, with a little help from some additional binding by carbons of the tropane unit.

^{1}H NMR studies helped clarify the picture further. We examined the NMR shifts of various protons of cocaine as it was titrated with β-CD at 25 °C. From this the binding constant was obtained, $500\pm130\ M^{-1}$, essentially the same as that from calorimetric titration. More to the point, the shifts of the cocaine protons indicate which parts of the molecule are bound into the β-CD cavity.

We found that the largest shift, upfield by 0.173 ppm with 10 mM β-CD, was seen for the methyl ester protons, suggesting that the methyl group binds into the β-CD cavity. The upfield shift probably indicates that the methyl group binds into the cavity on the shielding face of the bound phenyl ring, to help fill up the cavity, as our NOE and modeling studies also show (vide infra).

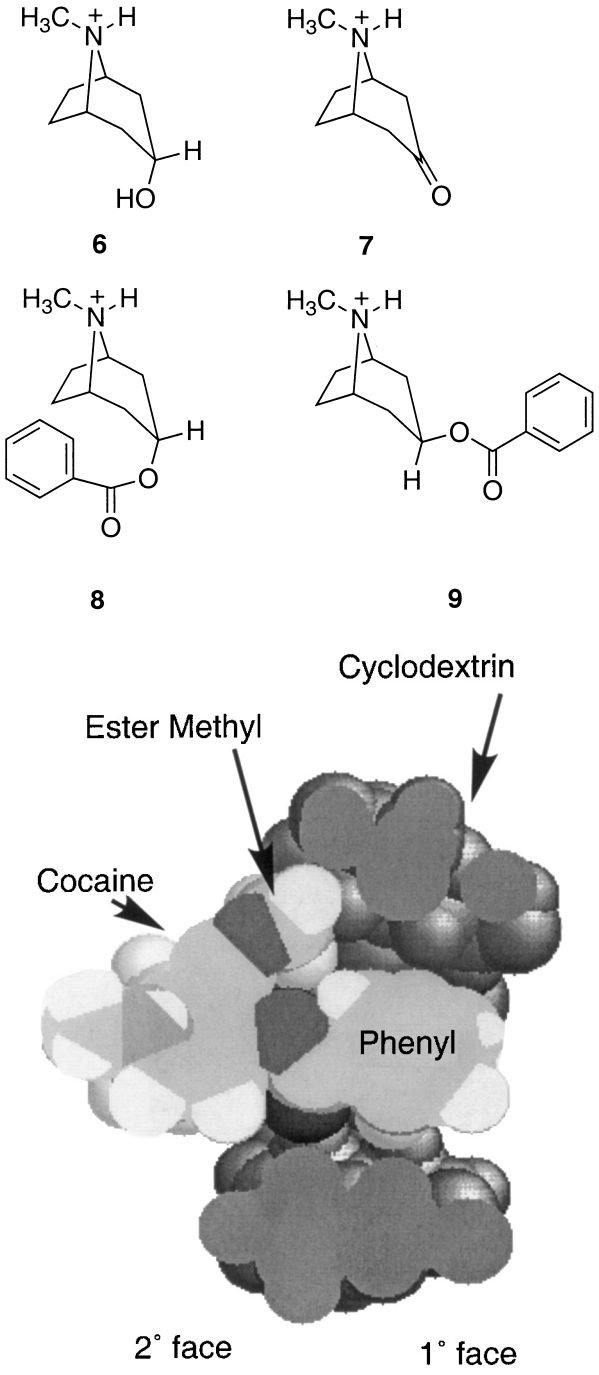


Figure 2. A 50% slice through the MacroModel best picture of the complex of cocaine (light shade) with β-cyclodextrin (dark shade).

We have previously reported a case in which the binding of a phenyl group (of phenylalanine) into the secondary face of β-CD was reinforced by the additional binding of a portion of a proline ring in the same peptide.[9] The proton on C-3 in cocaine (to which the benzoate group is attached) was shifted downfield by 0.063 ppm. The *ortho* and *para* benzoate protons were shifted downfield by 0.046 and 0.031 ppm, respectively, while the *meta* protons were shifted upfield by 0.046 ppm. By contrast, the *N*-methyl protons showed no shift.

ROESY and NOESY studies and 1-D decoupling studies were performed at 500 MHz on 3.4 mg cocaine hydrochloride (0.01 mmol) and 11.4 mg β-CD (0.01 mmol) in 1 mL D_2O after preliminary D_2O evaporation to remove exchangeable protons. The *ortho* benzoate protons at δ8.08 and the *meta* benzoate protons at δ7.55, but not the *para* benzoate protons at δ7.80, showed coupling through space to C-3 and C-5 β-CD protons, on the interior of the cavity. No such coupling in the complex was seen between the methyl ester protons and cyclodextrin protons, but the ester methyl group did show a weak NOE to the benzoate ortho protons. Apparently the methyl group reinforces phenyl binding on the secondary β-CD face without intruding very far into the cavity.

Molecular modeling helped confirm this picture. Using MacroModel,[10] we ran a conformational search of 1000 structures using the AMBER force field and continuum dielectric modeling of solvent water. The cocaine inserted preferentially into the secondary face of β-CD, and with the phenyl group bound and the methyl of the ester group partially bound and sitting in the shielding zone of the phenyl ring, near the *ortho* protons. The derived structure is shown in Figure 2. It is completely consistent with what our physical studies had suggested.

Conclusion

These studies make it clear that cocaine binds with reasonable affinity to β-cyclodextrin by inserting its phenyl group and the methyl of the carbomethoxy group into the secondary face of the cyclodextrin. In this structure both ester carbonyls should be accessible to catalytic groups mounted on the secondary face of the cyclodextrin. Studies of such potential catalysts will be reported elsewhere in due course.

Acknowledgement

This work has been supported by the National Institutes of Health.

References and Notes

1. (a) Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1997**, *119*, 1676. (b) Breslow, R.; Nesnas, N. *Tetrahedron Lett.* **1999**, *40*, 3335. (c) Yan, J.; Breslow, R. *Tetrahedron Lett.* **2000**, *41*, 2059.
2. (a) Mets, B.; Diaz, J.; Soo, E.; Jamdar, S. *Life Sci.* **1999**, *65*, 1317. (b) Morishima, H. O.; Whittington, R. A.; Iso, A.; Cooper, T. B. *Anesthesiology* **1999**, *90*, 1684.
3. Landry, D. W. *Sci. Am.* **1997**, *276*, 42.
4. Janda, K. *Chem. Eng. News* **1999**, *20*, 47.
5. Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1996**, *118*, 8495.
6. The dimer **5** was prepared by reacting β-CD-2,3-alloepoxide with Na_2S in DMF at 70 °C, and isolated by reverse-phase LOBAR chromatography.
7. (a) Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390. (b) Breslow, R.; Yang, Z.; Ching, R.; Trojandt, G.; Odobel, F. *J. Am. Chem. Soc.* **1998**, *120*, 3536.
8. Breslow, R.; Trainor, G.; Ueno. A. *J. Am. Chem. Soc.* **1983,** *105*, 2739, and further unpublished work.
9. Maletic, M.; Wennemers, H.; McDonald, D. Q.; Breslow, R.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1490.
10. Mohamadi, F.; Richards, N. G. J.; Guid, W. C.; Lishamp, R.; Lipton, M.; Canfield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.