THE BINDING OF PYRENE AND OTHER PROBES TO CD POLYMERS

T. C. WERNER, J. L. IANNACONE AND M. N. AMOO
Department of Chemistry
Union College
Schenectady, NY 12308
U. S. A.

ABSTRACT

The binding interactions of commercial epichlorohydrin (EP)-linked CD polymers (CDPs) with pyrene and p-substituted phenols are compared with those of the respective CD monomers for these probes. Shifts in pK_a for the phenols are consistent with a more open site on the CDPs than the CDs. The relative affinity for pyrene exhibited by β-CDP is estimated by a competition experiment with pyrene / β-CD binding. Spectral studies on synthesized EP-linked sucrose polymers are employed to gain insight into the role of glyceryl-linker units in guest binding.

1. INTRODUCTION

Commercially-available water-soluble cyclodextrin polymers (CDPs), formed by linking CDs with epichlorohydrin (EP), have the general formula $[CD\text{-}(CH_2\text{-}CHOH\text{-}O)_nX]_p$, where CD is α-, β- or γ–CD; X is H or a CD; p is >1 and the average n value is 12-15. The CDPs contain about 55% of the CD monomer, and GFC data indicate a broad range of molecular weights (MW) is observed for each CDP, with prominent peaks at MW ~ 2000 (one CD/polymer chain) and MW = 9-10,000 (4-5 CDs/polymer chain).

We have reported systematic studies of the relative binding interactions and environments of three naphthalene-based fluorescence probes and pyrene with all three commercial CDP / CD combinations [1,2]. Our results are consistent with a significant role for the glyceryl linker units in the binding interaction with the CDPs. We also find evidence against the existence of cooperative binding of pyrene by two CDs on the polymers[2]. Such an interaction, called a "clam shell" arrangement, has been reported for pyrene bound to β-CD [3,4].

We report herein a continuation of our studies on the binding of spectral probes to CDs and CDPs, including the measurement of pKa's for p-nitrophenol (p-NP) and methylparaben (MPB, p-hydroxybenzoic acid, methyl ester)) bound to α-CD and α–CDP, a comparison of the relative strengths of pyrene binding to β-CD and β-CDP, and an evaluation of pyrene interaction with polymers containing the glyceryl linkers but no CD units.

Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **25**: 77–80, 1996.

2. MATERIALS AND METHODS

2.1 Materials

The commercial CDPs were obtained from Cyclolab R&D Laboratory Ltd., while the CDs were gifts from the American-Maize Products Company. All other chemicals were the highest grade available from Aldrich Chemical Company. Pyrene was recrystallized twice from ethanol, and β-CD was recrystallized from water.

2.2 Methods

Absorption spectra were obtained using a Hewlett-Packard 8452A Diode Array Spectrophotometer, while fluorescence spectra were obtained with a Perkin-Elmer Lambda 5B Spectrofluorometer. GFC data were acquired using a Perkin-Elmer HPLC equipped with a TSK G2000SW column, which is employed with an aqueous mobile phase for separation of molecules in the 500-100,000 MW range.

The pK_a's of p-NP and MPB in the presence of α-CD and α-CDP were determined from the intercepts of plots of eq.(1), where A is the absorbance at the wavelength maximum for the anion form at a given pH and A_t is the absorbance at this same wavelength at a pH where only the anion form exists.

$$pH = pK_a + Log\,[A / (A_t - A)] \qquad (1)$$

The pH was adjusted by adding small amounts of 3M HCl to solutions containing 0.010 M Na_3PO_4. The concentration of α-CD or α-CDP was 0.020 M. A clear isosbestic point was observed in all cases.

3. RESULTS AND DISCUSSION

3.1 pK_a's of p-NP and MPB bound to α-CD and α-CDP

The pK_a's for free p-NP and free MPB and when both are bound to α-CD and α–CDP are given in Table 1.

Table 1. pK_a's of p-NP and MPB when free and when bound to α-CD and α–CDP

Probe	Free	0.020 M α-CD	0.020 M α-CDP
p-NP	7.21 +/- 0.01	6.14 +/-0.04	6.34 +/- 0.02
MPB	8.50 +/- 0.01	8.13 +/- 0.02	8.44 +/- 0.01

Values for free p-NP and for p-NP bound to α-CD are in agreement with literature values [5]. The pK_a values in the presence of the polymer are intermediate between those for the free probes and those when the probes are bound to the monomer. This suggests a different, more open binding site exists for these probes in the CP polymer than in the CD monomer. A similar conclusion was ascertained for pyrene based on fluorescence lifetime data in the presence of quenchers [2]. Thus, benzene derivatives, like

naphthalenes and pyrene, appear to have distinctly different binding environments in CDs and CDPs.

3.2 Competitive Binding of Pyrene to β-CD and β-CDP

Since ambiguity exists about the nature of the pyrene binding sites on β-CDP, a binding constant can not be determined [2]. Instead, we have chosen to compare the relative strengths of pyrene / CDP and pyrene / CD binding by observing the effects of a fixed [β-CDP] on the pyrene I/III emission ratio (I = 373 nm, III = 384 nm) in the presence of increasing [β-CD]. In the absence of β-CDP, the I/III ratio decreases from 1.75 to 0.82 as [β-CD] goes from 0 to 0.010 M. The limiting value for this ratio is 0.78 at [β-CD] ~0.015 M. As a result, we can calculate the % bound pyrene from eq.(2), where I/III is the observed ratio at a given [β-CD]:

$$\% \text{ bound pyrene} = \{[1.75 - I/III] / [1.75 - 0.78]\} \times 100 \qquad (2)$$

If β-CDP is present at 0.0025 M, this ratio changes from 1.55 to 1.12 over the same [β-CD] concentration range. Thus, we can use eq.(2) to determine % bound pyrene in the presence of 0.0025 M β–CDP by replacing 1.75 in eq.(2) with 1.55. Using these relations, we find that % bound pyrene decreases from 96% in the presence of 0.010 M β-CD but no β-CDP to 56% in the presence of 0.010 M β-CD and 0.0025 M β-CDP. Since the K value for the 2:1 β-CD : pyrene complex is quite large (6.76×10^4, [6]), we can infer that binding must also be also quite strong between pyrene and β-CDP. For the above example, a 40% decrease in pyrene bound to the monomer is observed in the presence of the polymer, even though the ratio of the β-CD binding site concentration from the monomer to that from the polymer is four. Unusually favorable 2:1 β-CD : pyrene complex formation with the polymer, with two CDs coming from the same polymer chain [4], could account for this but is unlikely for two reasons. First, the limiting I / III ratio for pyrene bound to β-CDP (1.55) is much higher than that for pyrene bound to β-CD (0.78). Second, the I /III ratio for pyrene bound to β-CDP is unaffected by the addition of 2,2,3,3,3-pentafluoro-1-propanol (PFP, [2]). When PFP is added to pyrene in the presence of β-CD, binding is enhanced and the I / III ratio drops to 0.38 [6]. These results suggest a quite different binding environment exists for pyrene with β-CDP than with β-CD and supports the notion of glyceryl linker participation in the former case.

3.3 Synthesis of an EP-linked sucrose polymer

An EP-linked polymer were synthesized, using the procedure of Xu et al. [4], starting with 25% sucrose and a 10:1 initial mole ratio of EP to sucrose [25%(10:1)SP]. GFC data for this polymer are shown in Fig.1, along with GFC data for commercial β-CDP. The sucrose polymer, which has a MW distribution intermediate between the two major

components for commercial β-CDP (see Fig.1) , was prepared to see if the CD cavity is necessary for pyrene binding. The pyrene I/III ratio in water is 1.75, while the limiting values for this ratio in the presence of 25%(10:1)SP and commercial β-CDP are 1.69 and 1.55, respectively. Clearly, the CD units must also be somewhat involved, but the latter I/III ratio is much greater than the limiting ratio for pyrene bound to β-CD (0.78), thereby indicating a more open, hydrophilic site for β-CDP than for β-CD. It seems likely that bound pyrene has contact with both the cavity and glyceryl linker units in the case of the commercial CD polymer.

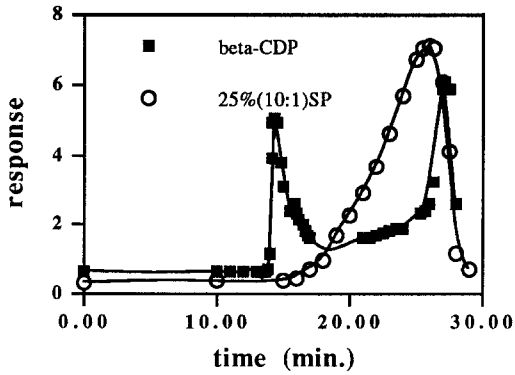

Figure 1 GFC Data

CONCLUSION

We have shown that spectral probes often show binding behavior to CDs that depend on whether the CD is monomeric or part of an EP-linked polymer. While these probes can bind very strongly to the CDPs, their environment is more open and hydrophilic than what is observed with the CD monomers, suggesting a role for the EP linker units.

ACKNOWLEDGMENTS

TCW acknowledges the support of the Petroleum Research Fund for support of this work and the American Maize-Products Company for donation of the CDs.

REFERENCES

[1] Werner, T., Warner, I., The use of naphthalene fluorescence probes to study the binding sites on cyclodextrin polymers, *J. Incl. Phen.* **18**, 385-396 (1994)

[2] Werner, T., Colwell, K., Agbaria, R., Warner, I., The binding of pyrene to cyclodextrin polymers, *J. Incl. Phen.* , in press

[3] Munoz de la Pena, A., Ndou, T., Zung, J., Warner, T., Stoichiometry and formation constants of pyrene inclusion complexes with β- and γ-cyclodextrin, *J. Phys. Chem.* **95**, 3330-3334 (1991)

[4] Xu, W., Demas, J., DeGraff, B., Whaley, M., Interactions of pyrene with cyclodextrins and polymeric cyclodextrins, *J. Chem. Phys.* **97**, 6546-6554 (1993).

[5] Connors, K., Lipari, J., Effect of cycloamyloses on apparent dissociation constants of carboxylic acids and phenols, J. *Pharm. Sci.* **65**, 379-383 (1976).

[6] Elliott, N., Ndou, T., Warner, I., Influence of fluorinated alcohols on cyclodextrin : pyrene complexation, *J. Incl. Phen.* **16**, 99-112 (1993)