Journal of Inclusion Phenomena and Macrocyclic Chemistry (2005) 51: 87–91

Chiral Recognition of Propranolol with β-Cyclodextrin in the Presence of 1- and 2-Butanol

A. YVETTE GLENN**, CHANEL A. FORTIER, IVY V. JACK, XIAOFENG ZHU and ISIAH M. WARNER*

Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803

(Received: 10 February 2004; in final form: 2 November 2004)

Key words: butanol, β-cyclodextrin, chiral, propranolol

Abstract

The purpose of this work is to investigate the chiral recognition characteristics of β-cyclodextrin with two propranolol enantiomers in the presence of organic additives. Steady-state fluorescence measurements of propranolol/β-cyclodextrin (β-CD) complexes were performed for solutions containing either 1- or 2-butanol. For each 2-butanol isomer solution, the interactions were assessed by comparing the changes in the fluorescence of (*R*)-(+)- propranolol *versus* (*S*)-(−)-propranolol as a function of CD concentration. A similar comparison study was done for the propranolol enantiomers in the presence of 1-butanol. The intensity changes for propranolol are relatively small upon addition of β-CD in the presence of the butanol alcohol. However, the present work shows that the interaction of (*R*)-(+)-propranolol with β-CD is influenced by the chirality of 2-butanol in contrast to (*S*)-(−)-propranolol.

Introduction

Cyclodextrins (CDs) are cyclic sugars that are capable of forming inclusion complexes with select-size therapeutical drugs [1–3]. This phenomenon is important to the pharmaceutical industry since it often improves the stability, solubility, and bioavailability of the complexed drug. It is also well established that the fluorescence properties of some drug molecules can be monitored upon inclusion of the drug inside the CD cavity. For example, Shuang *et al.* [4] examined the fluorescence behavior of the drug hesperidin inside several CDs. The fluorescence intensity of hesperidin increased in the CD media which was attributed to the protection of the hesperidin excited state inside the CD cavity. Sbai *et al.* [5] studied the complexation of ellipticine, an antitumor agent, with several CDs by use of fluorescence spectroscopy. The association constants for the ellipticine/CD complexes were found to be pH dependent. Another use of CDs in the area of pharmaceutics has been to differentiate between chiral drugs that can be included inside the CD cavity. For example, Tomasella *et al.* [6] compared the spectroscopic properties of the quinine and quinidine isomers upon their inclusion inside β-CD. The authors found that adding select alcohols to the β-CD solutions induced a similar enhancements in the fluorescence intensity for these isomers. The larger intensity enhancements were observed for quinidine. The difference in the intensity enhancement of quinidine *versus* quinine increases with increasing spatial volume of the alcohol.

The β-adrenergic blockers are therapeutical agents that inhibit the effect of catecholamines at β-adrenergic sites. One example of a common β-adrenergic blocker is propranolol, which has been prescribed to treat hypertension, convulsions, anxiety, and angina pectoris. Propranolol is an aryl-substituted alkylamine, containing a naphthalene moiety which comprises its fluorophore and an alkyl side chain which has one chiral center (see Figure 1 compound structure). The use of routine clinical methods in correlating theurapeutic effects of β-adrenergic blockers with serum levels in a quantitative manner has not been entirely feasible. However, Rekhi *et al.* [7] developed a simple fluorimetric method that has proved satisfactory in estimating serum levels of propranolol in patients. El-Ries *et al.* [8] used spectrophotometry for direct determination of propranolol and metoprolol tartrate. Muñoz de la Peña *et al.* [9] developed a more accurate and sensitive technique for the detection and analysis of these mixtures, whereby first-derivative synchronous spectrofluorimetry is performed for simultaneous determination of both drugs. These methods demonstrate the potential use of fluorescence in further probing propranolol solutions.

* Author for correspondence. E-mail.: iwarner@lsu.edu

** Present Address: Eli Lilly and Company, Indianapolis, IN 46285.

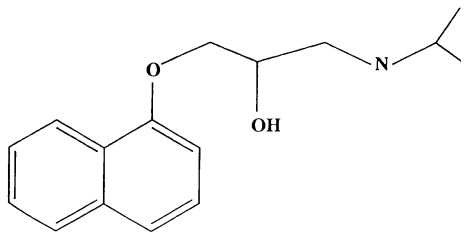

Figure 1. Structure of propranolol.

In this manuscript, an examination of the interactions of (R)-(+)- and (S)-(−)-propranolol with β-CD is reported. These interactions are assessed through the addition of select chiral and achiral alcohols. Steady-state fluorescence measurements are used to characterize these interactions. An attempt is made to evaluate the binding constants and stoichiometry of these complexes using double-reciprocal plots.

Experimental

Apparatus

Steady-state fluorescence measurements were performed on a Spex Model F2T211 spectrofluorimeter equipped with a thermostated cell housing which was maintained at 20.0 ± 0.1 °C. Samples were measured in a 1-cm^2 quartz cuvette by use of excitation and emission slit widths of 5.1 and 3.4 nm, respectively. Emission spectra were obtained in the region of 300–500 nm with an excitation wavelength of 288 nm.

Materials

The (R)-(+)-and (S)-(−)-propranolol hydrocholoride (98%), and (R)-(−)- and (S)-(+)-2-butanol (99%) were purchased from Sigma–Aldrich Corporation (St. Louis, MO USA). The 1-butanol (ACS grade) was obtained from EM Science which is now called EMD Chemicals (Gibbstown, NJ). The β-CD was supplied by American Maize Products (Hammond, IN). All chemicals were used as received.

Method

Sample preparation

A 1.0×10^{-4} M stock solution of propranolol hydrochloride was prepared by dissolving 0.0026 g of the pure enantiomer in 100 ml of deionized water. A 1.0×10^{-2} M stock solution of β-CD was also prepared by adding 1.135 g of β-CD to a 100-ml flask and diluting to the mark with deionized water. A 100-μl aliquot of the propranolol hydrochloride stock solution and the appropriate amount of the aqueous CD stock solution were added to 10-ml flasks. Appropriate amounts of the alcohol were also added to these flasks, and the contents diluted to the mark with deionized water to give 1.0×10^{-6} M propranolol hydrochloride, 1.0×10^{-3} to 8.0×10^{-3} M β-CD, and 0.05 M of the alcohol. The samples were sonicated for 10 min and allowed to stand overnight before analysis.

Results and discussion

Influence of cyclodextrin concentration

Molecules which are partially or fully included inside the CD cavity often exhibit an increase in their fluorescence intensity [10–12]. The increase is generally attributed to the isolation of these molecules from the bulk aqueous solvent, thus shielding their excited singlet states from quenching and/or other nonradiative deactivation processes. Addition of β-CD to an aqueous solution of (R)-(+)-propranolol produces an increase in the overall fluorescence intensity of the drug molecule. These results suggest that (R)-(+)-propranolol interacts with the interior of β-CD. The naphthyl moiety of propranolol comprises its fluorophore which indicates that this portion of the molecule is included in the CD cavity. A similar phenomenon is observed in the (S)-(−)-propranolol solutions. The intensity increases with increasing β-CD concentration are relatively weak in these solutions. Figure 2 shows that the differences in the intensity changes as a function of β-CD concentration between (R)-(+)- and (S)-(−)-propranolol are very small. Although the difference in the intensity changes found is small, β-cyclodextrin has the chiral recognition to two propranolol enantiomers, as shown that the propranolol enantiomers were successfully separated when β-cyclodextrin was employed as a stationary phase in chromatography [13, 14]. Alternatively, molecular modeling of the chiral recognition of propranolol enantiomers by β-cyclodextrin has also been explored [15].

The addition of alcohols to aqueous CD/guest solutions has been shown to strengthen the association of the guest molecule with the CD [16–21]. A number of

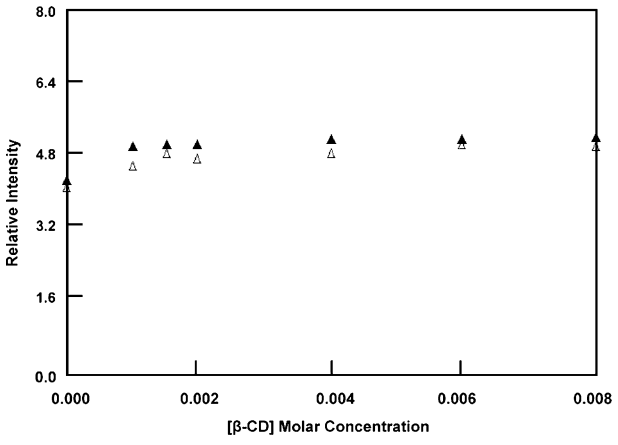

Figure 2. Plot of fluorescence intensity as a function of β-CD concentration for aqueous solutions of (▲) (R)-(+)-propranolol and (Δ) (S)-(−)-propranolol.

researchers have reported that the increased association is due to the presence of a ternary complex between the CD, the guest, and the alcohol. The extent of the increased association is governed by proper size and volume matching of the alcohol and the remaining space in the cavity after complexation with the guest molecule occurs. Previous work in our laboratory [22, 23] has shown that the chirality of the alcohol can also influence the binding of a polycyclic aromatic hydrocarbon (e.g., pyrene) with the CD. In the study reported here, an attempt to influence the association of each propranolol enantiomer with β-CD was made by adding chiral and achiral alcohols to these solutions.

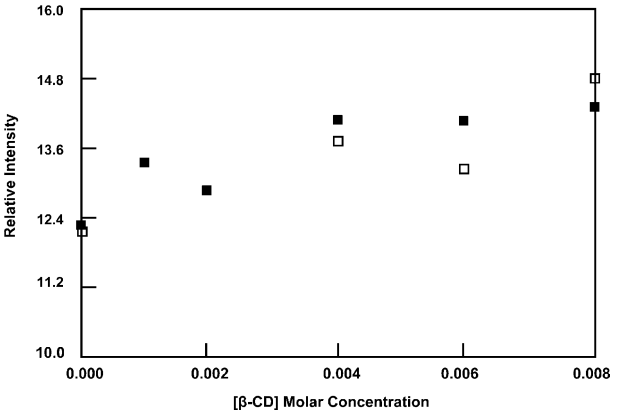

Figure 4. Plot of fluorescence intensity of (S)-(−)-propranolol as a function of β-CD concentration in the presence of (■) (R)-(−)-2-butanol and (□) (S)-(+)-2-butanol.

Addition of chiral alcohols

Figure 3 depicts the effect of varying the β-CD concentration on the overall intensity of (R)-(+)-propranolol in the presence of (R)-(−)- and (S)-(+)-2-butanol. The values for the (S)-(+)-2-butanol solutions were normalized so that a more direct comparison of the intensity changes between these alcohol solutions is indicated. Interestingly, the fluorescence intensity of (R)-(+)-propranolol increases in the presence of 2-butanol with respect to the intensity in the absence of 2-butanol for 0 M β-CD (see Figure 2). This observation suggests that 2-butanol alone influences the fluorescence of (R)-(+)-propranolol. An increase in the intensity with increasing concentration of β-CD is observed in these solutions. However, it is useful to note that the fluorescence intensity increase appears to be more dramatic for the (R)-(−)-2-butanol solutions. The relative intensity increases from 12.6 for 0 M β-CD to 14.6 for 8.0×10^{-3} M β-CD in the presence of (R)-(−)-2-butanol. In contrast, the relative intensity increases from 12.6 to 13.9 in the presence of (S)-(+)-2-butanol. Figure 4 shows that the relative intensities for (S)-(−)-propranolol appear less consistent with increasing β-CD concentration than the intensities for (R)-(+)-propranolol (see Figure 3). Also, there is a lesser difference in the relative intensities of the corresponding β-CD solutions for the two alcohols in the S-(−)-propranolol solutions. Hence, from these results, it appears that the interaction of (R)-(+)-propranolol with β-CD is influenced by the chirality of 2-butanol whereas the (S)-(−)-propranolol/β-CD complex is not significantly influenced by the alcohol chirality.

Addition of 1-butanol

The position of the hydroxyl group along the alkyl chain of the alcohol can be crucial to the optimal organization of the alcohol with respect to the void volume in the CD cavity. For example, Muñoz de la Peña, *et al*. [22] reported a stronger association between β-CD and pyrene with the addition of 2-propanol than with the addition of 1-propanol. Zung *et al*. [23] reported a similar phenomenon for the γ-CD/pyrene complex. To evaluate the importance of stereochemistry in the interaction of the modifier with CD complexes, the

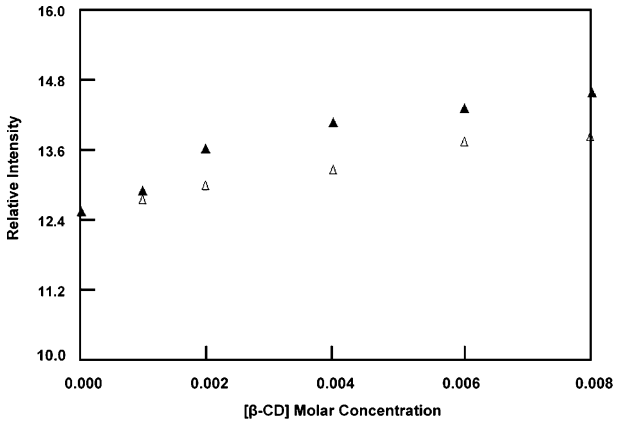

Figure 3. Plot of fluorescence intensity of (R)-(+)-propranolol as a function of β-CD concentration in the presence of (▲) (R)-(−)-2-butanol and (Δ) (S)-(+)-2-butanol.

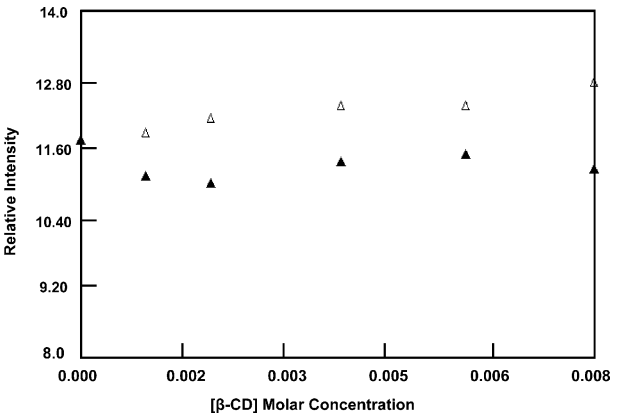

Figure 5. Plot of fluorescence intensity as a function of β-CD concentration for (▲) (R)-(+)- propranolol and (Δ) (S)-(−)-propranolol in the presence of 1-butanol.

influence of 1-butanol on the association of propranolol with β-CD was also examined. Figure 5 presents the observations as a result of varying the concentration of β-CD on the relative intensity of (R)-(+)- and (S)-(−)-propranolol in the presence of 1-butanol. Note that the relative intensities of (R)-(+)- and (S)-(−)-propranolol are the same in the presence of 1-butanol at 0 M β-CD. However, a noticeable difference in the intensity of these enantiomers occurs in the presence of β-CD. The intensity of (S)-(−)-propranolol increases as a function of β-CD concentration in these solutions. In contrast, the intensity of (R)-(+)-propranolol decreases upon addition of 1.0×10^{-3} M or 2.0×10^{-3} M β-CD and then appears to level off with further addition of β-CD. Thus, it appears that chiral recognition of these enantiomers with β-CD is enhanced in the presence of 1-butanol. Again, note from Figure 2 the small differences in the intensity changes between (R)-(+)- and (S)-(−)-propranolol as a function of β-CD concentration in the absence of alcohol.

Complex stoichiometry

The stoichiometry of propranolol with β-CD is important for assessing their interaction in solution. Some methods have been used for this purpose, including the Job plot [24] and the double reciprocal plots [25]. To further assess the association in this work, we attempted to determine the stoichiometry and the binding constants between β-CD and propranolol by use of the double-reciprocal plots. Unfortunately, the small changes in the propranolol fluorescence with increasing β-CD concentration yielded poor linear fits. Although these findings can depict a mixed stoichiometric ratio [26] for the β-CD/propranolol complex, it is possible that the alkylammonium side chain hinders effective inclusion of the naphthyl ring inside the β-CD cavity. This results in weak interactions between β-CD and propranolol [24, 25].

The intensity increases of (R)-(+)- and (S)-(−)-propranolol with increasing β-CD concentration are more pronounced in the presence of 2-butanol than in the absence of alcohol. These results would suggest some degree of interaction between the alcohol and the β-CD/propranolol complex. This observation is in good agreement with the results observed for the CD/pyrene complexes, where the authors attributed the enhanced intensity increases (i.e., more pronounced I/III ratio decreases) to conclusion of the alcohol and formation of the ternary complex. A plausible explanation for the low degree of alcohol association in the propranolol solutions is that the alcohol associates preferably with functional groups on the propranolol side chain. In addition, the bulky side chain hinders organization of the alcohol within the cavity. It is interesting to note a noticeable difference in the intensity of (R)-(+)-propranolol and decreases for between (R)-(−)- and (S)-(+)-2-butanol solutions. However, the intensity differences for (S)-(−)propranolol in the presence of the 2-butanol isomers appear rather inconsistent. These results would suggest that the interaction of (R)-propranolol with β-CD is influenced by the chirality in the alcohol.

Conclusions

Chiral recognition of individual enantiomers requires three-dimensional attachment with the chiral selecting agent. In the case of CDs, this involves the following requirements: (1) inclusion of the enantiomer, (2) tight fit of the complexed portion of the enantiomer, and (3) interaction of the chiral center of the enantiomer with the secondary hydroxyls of the CD cavity. In the present work, the change in the fluorescence intensity of propranolol upon addition of β-CD in the presence of alcohol suggests a change in the microenvironment around propranolol which implies its inclusion within the β-CD cavity. The intensity increases for (S)-(−)-propranolol and decreases for (R)-(+)-propranolol as a function of β-CD concentration, suggesting interaction of the propranolol chiral center with the chiral recognition groups on the CD cavity. However, a tight fit of propranolol with β-CD is not apparent since the fluorescence intensity changes are very weak as a function of CD concentration.

Acknowledgements

Funding for this research was provided by a grant from the National Science Foundation. The authors would also like to acknowledge G.A. Reed (American Maize Products) for kindly providing the β-CD used in this study.

References

1. A. Mucci, L. Schenetti, M.A. Vandelli, B. Ruozi, G. Salvioli, and F. Forni: *Supramol. Chem.* **12**, 427–433 (2001).
2. R.E. Galian, A.V. Veglia, and R.H. de Rossi: *Analyst* **123**, 1587–1591 (1998).
3. P. Bortolus, G. Marconi, S. Monti, B. Mayer, G. Kohler, and G. Grabner: *Chem-Eur J.* **6**, 1578–1591 (2000).
4. S. Shuang, S. Guo, L. Li, and M. Cai: *Anal. Lett.* **31**, 1357–1366 (1998).
5. M. Sbai, S.A. Lyazidi, D.A. Lerner, B. del Castillo, and M.A. Martin: *Analyst* **121**, 1561–1564 (1996).
6. F.P. Tomasella, Z. Pan, and L.J.C. Love: *Supramol. Chem.* **1**, 25 (1992).
7. G.S. Rekhi, S.S. Jambhekar, P.F. Souney, and D.A. Williams: *J. Pharmaceut. Biomed.* **13**, 1499–1505 (1995).
8. M.A. El-Ries, F.M. Abou Attia, and S.A. Ibrahim: *J. Pharmaceut. Biomed.* **24**, 179–187 (2000).
9. A. Muñoz de la Peña, F. Salinas, and M.S. Duran: *Anal. Chim. Acta* **255**, 317 (1991).
10. B.D. Wagner and G.J. McManus: *Anal. Biochem.* **317**, 233–239 (2003).
11. Y. Liu, Y. Song, H. Wang, H.Y. Zhang, T. Wada, and Y. Inoue: *J. Org. Chem.* **68**, 3687–3690 (2003).
12. S.M.Z. Al-Kindy, F.E.O. Suliman, and A.A. Al-Hamadi: *Anal. Sci.* **17**, 539–543 (2001).

13. M.W. Matchett, S.K. Branch, and T.M. Jefferies: *Chirality* **8**, 126–130 (1996).
14. L.Y. Tang, C.E. Silverman, and J.A. Blackwell: *J. Chromatogr. A* **829**, 301–307, (1998).
15. H. Kim, K. Jeong, S. Lee, and S.H. Jung: *B. Kor. Chem. Soc.* **24**, 95–98 (2003).
16. H.R. Zhang, S.Y. Guo, L. Li, and M.Y. Cai: *Aanl. Chim. Acta* **463**, 135–142 (2002).
17. C.H. Evans, M. Partyka, and J. Van Stam: *J. Incl. Phenom. Macro.* 38, 381–396 (2000).
18. S. Hamai: *J. Phys. Chem. B* **101**, 1707–1712 (1997).
19. V.C. Anigbogu, A. Muñoz de la Peña, T.T. Ndou, and I.M. Warner: *Anal. Chem.* **64**, 484 (1992).
20. N. Husain, V.C. Anigbogu, M.R. Cohen, and I.M. Warner, *J. Chromatogr.* **635**, 211 (1993).
21. J.M. Schuette, A.Y. Will, R.A. Agbaria, and I.M. Warner, *Appl. Spectros.* **48**, 581 (1994).
22. A. Muñoz de la Peña, T.T. Ndou, J.B. Zung, K.L. Greene, D.H. Live, and I.M. Warner: *J. Am. Chem. Soc.* **113**, 1572 (1991).
23. J.B. Zung, A. Muñoz de la Peña, T.T. Ndou, and I.M. Warner, *J. Phys. Chem.* **95**, 6701 (1991).
24. A.F. Casy: *Trends Anal. Chem.* **12**, 185 (1993).
25. H. Mwakibete, D.M. Bloor, and E. Wyn-Jones: *J. Incl. Phen. Mol. Recog. Chem.* **10**, 497 (1991).
26. V.K. Smith, T.T. Ndou, and I.M. Warner: *J. Phys. Chem.* **98**, 8627 (1994).