Study of the Interaction Between β-Cyclodextrin and Chlorhexidine

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INTRODUCTION

Chlorhexidine, 1,6-bis-[N'(4-chlorophenyl)-N^5-biguanido] hexane was first described as an antiseptic agent by Davies et al. [1] in 1954 and has been widely used in the treatment of oral diseases [2]. One of its dosage forms is a mouth rinsing solution, Peridex (Procter & Gamble, Cincinnati, Ohio) containing 0.12% chlorhexidine digluconate. Side effects, such as bitter taste, irritation and tooth-staining have been associated with such a highly concentrated solution. In the presence of β-cyclodextrin, the above side effects were greatly reduced, while its antiseptic effectiveness remained the same [3]. β-Cyclodextrin forms inclusion complexes with many drug molecules [4]. The present study examines the interaction between chlorhexidine and β-cyclodextrin.

MATERIALS AND METHODS

Materials

Chlorhexidine diacetate and β-cyclodextrin were obtained from Sigma (St. Louis, MO) and were used as purchased. Deuterium oxide was obtained from Cambridge Isotope Laboratories.

^{1}H-NMR Method

Solutions containing 10 mM β-cyclodextrin and 2.5, 5.0, 10 or 20 mM chlorhexidine diacetate in deuterium oxide were prepared. ^{1}H-NMR spectra were obtained on a QE-300 NMR spectrometer (General Electric; Syracuse, NY) at room temperature using HDO as the internal reference. Repeated scans of the same sample showed the reproducibility of H-chemical shift measurements to be within 0.002 ppm.

UV Method

Solutions containing 50 μM chlorhexidine diacetate and 0 to 10 mM β-cyclodextrin in water were prepared. UV absorption measurements and uv spectrum scanning were performed on a Perkin-Elmer Lambda 5, UV/VIS spectrophotometer (Perkin-Elmer; Norwalk, CT) at room temperature. The uv absorption of each sample was corrected for a general medium effect as determined using an equivalent weight/volume concentration of β-cyclodextrin.

Microcalorimetry Method

Solutions of chlorhexidine diacetate and β-cylodextrin in water were simultaneously delivered, by a perisaltic pump, into the mixing chamber in a LKB Flow Microcalorimeter, Model 2107-127 (LKB Biochrom LTD.; Cambridge, England) which measures the heat flux. The concentration of either β-cyclodextrin or chlorhexidine diacetate changed from 0 to 5.0 mM (after mixing), while the concentration of the other was fixed at 2.5 mM (after mixing). All reactions were carried out in doubly distilled water at 25°C. The heat of dilution for each sample was measured and taken into account when the heat of complexation was calculated.

RESULTS

^{1}H-NMR study

The assignment of the proton chemical shifts was made by referring to the literature for β-cyclodextrin [5] or to the standard spectrum of 1-(p-chlorophenyl)-5-isopropylbiguanide hydrochloride [6] for chlorhexidine, respectively. Chemical shifts of the aromatic protons in chlorhexidine were changed by 0.090 ppm for $H_{2',6'}$ and -0.055 ppm for $H_{3',5'}$, respectively, in the presence of β-cyclodextrin. The chemical shift change Δδ of the protons in β-cyclodextrin induced by chlorhexidine was plotted against the molar ratio of chlorhexidine over β-cyclodextrin, r, as shown in Figure 1. A positive value of Δδ would indicate an upfield shift.

UV study

The uv spectrum of chlorhexidine diacetate in water was altered in the presence of β-cyclodextrin. Both peaks at 253.2nm and 230.4nm were shifted to 258.3nm and 228.7nm, respectively, in the presence of 10mM β-cyclodextrin. The intensity of the peak at 253nm increased while the intensity of the peak at 230nm decreased. The interaction between chlorhexidine as a substrate (S), and β-cyclodextrin as a ligand (L), can be expressed by the following equilibria (see discussion below):

$$S + L \rightleftharpoons SL \quad (1)$$

$$SL + L \rightleftharpoons SL_2 \quad (2)$$

if the total concentration of chlorhexidine (S_t) was much smaller than the total concentration of β-cyclodextrin (L_t), then according to Connors [7], the uv absorption difference (ΔA) at any wave length between solutions of chlorhexidine with and without β-cyclodextrin can be expressed as a function of S_t and L_t.

$$\Delta A = S_t \frac{\Delta\alpha_{11}K_{11}L_t + \Delta\alpha_{11}K_{11}K_{12}L_t^2}{1 + K_{11}L_t + K_{11}K_{12}L_t^2} \cdots\cdots\cdots \quad (3)$$

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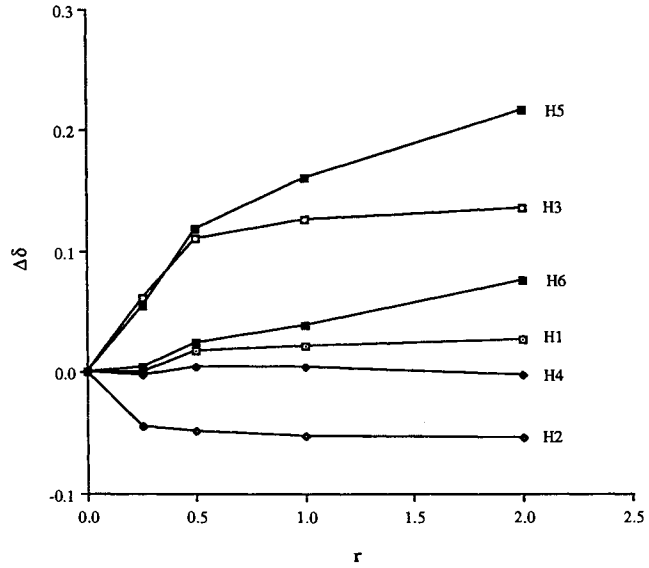

Figure 1. Chemical shifts of β-cyclodextrin protons as a function of the molar ratios of chlorhexidine to β-cyclodextrin in D_2O at room temperature.

where K_{11} and K_{12} are the stability constants for complexation steps 1 and 2, respectively; $\Delta\alpha_{11}$ and $\Delta\alpha_{12}$ are the uv absorptivity differences between 1:1 complex (SL) and pure chlorhexidine, and between 1:2 complex (SL_2) and pure chlorhexidine, respectively. The experimentally measured ΔA values at 240nm were plotted against L_t in Figure 2. The stability constants K_{11} and K_{12} were obtained by curve fitting according to equation (3), using RS1 (BBN Softwear Products Corporation; Cambridge, MS). They were 287±21 (5) for K_{11}, 268±23 (5) for K_{12}, 5.43±0.56(5) for $\Delta\alpha_{11}$ and 7.8±0.23(5) for $\Delta\alpha_{12}$, respectively. Using the obtained parameters, K_{11}, K_{12}, $\Delta\alpha_{11}$ and $\Delta\alpha_{12}$, theoretical ΔA values can be calculated as a function of L_t (equation (3)), and they were also plotted in Figure 2.

Microcalorimetry study

Based on equilibria (1) and (2),

$$K_{11} = \frac{[SL]}{[S] * [L]} \tag{4}$$

$$K_{11}K_{12} = \frac{[SL_2]}{[S] * [L]^2} \tag{5}$$

$$S_t = [S] + [SL] + [SL_2] \tag{6}$$

$$L_t = [L] + [SL] + 2[SL_2] \tag{7}$$

where [S], [L], [SL] and $[SL_2]$ are the concentrations of free chlorhexidine, free β-cyclodextrin, 1:1 complex, SL, and 1:2 complex, SL_2, respectively, in the system. The heat flux (P) measured by microcalorimeter can be expressed as:

$$P = R\{\Delta H_{11}[SL] + (\Delta H_{11} + \Delta H_{12})[SL_2]\} \tag{8}$$

where ΔH_{11} and ΔH_{12} are enthalpy changes for the interactions expressed by equations (1) and (2), respectively, and R is the total flow rate. By combining equations (4)–(8), the following equations can be derived:

$$P = RS_t \frac{\Delta H_{11}K_{11}[L] + (\Delta H_{11} + \Delta H_{12})K_{11}K_{12}[L]^2}{1 + K_{11}[L] + K_{11}K_{12}[L]^2} \cdot \cdot \tag{9}$$

$$L_t = [L] + S_t \frac{K_{11}[L] + 2K_{11}K_{12}[L]^2}{1 + K_{11}[L] + K_{11}K_{12}[L]^2} \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \tag{10}$$

The experimentally measured P values were plotted against S_t in Figure 3 when L_t was fixed. Since P is related to S_t through [L] which is not measurable, it is necessary to calculate [L] from equation (10) using the equilibrium constants obtained from the uv study. By rearranging equation (9), the following equation can be derived:

$$\frac{P(1 + K_{11}[L] + K_{11}K_{12}[L]^2)}{RS_tK_{11}[L]} = \Delta H_{11} + (\Delta H_{11} + \Delta H_{12})K_{12}[L] \cdot \cdot \cdot \cdot \cdot \tag{11}$$

According to equation (11), the left hand side of the equation (represented by Y) is linearly related to K_{12}[L]

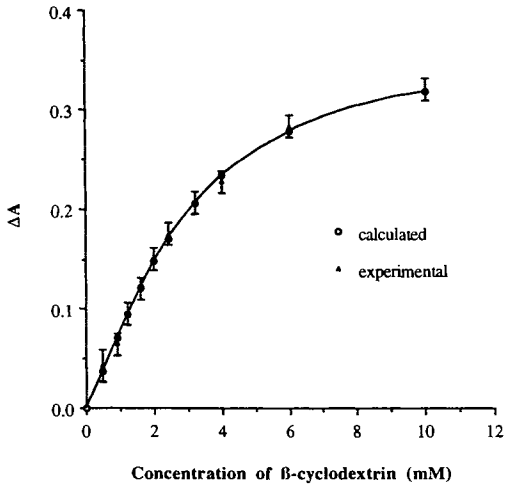

Figure 2. UV absorption changes (ΔA) of chlorhexidine diacetate solution (0.05mM), at 240nm, as a function of β-cyclodextrin concentration at room temperature.

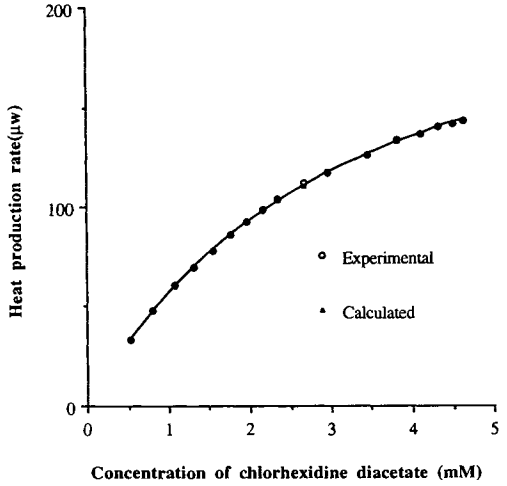

Figure 3. Heat production rate as a function of chlorhexidine concentration while β-cyclodextrin concentration is held constant at 2.5mM at 25°C.

(represented by X). A plot of the experimental data indeed show the above relationship (Figure 4). From the slope and intercept of these straight lines, ΔH_{11} and ΔH_{12} can be calculated, and then the free energy changes (ΔG) and entropy changes (ΔS) can also be calculated according to equations (12) and (13):

$$\Delta Gi = -RTLnKi \quad (12)$$

$$\Delta Si = (\Delta Hi - \Delta Gi)/T \quad (13)$$

where R is molar gas constant (8.314 J $mol^{-1}K^{-1}$) and T is the absolute temperature in K. The obtained parameters are $\Delta H_{11}(KJM^{-1}) = -24.6\pm1.9(8)$; $\Delta H_{12}(KJM^{-1}) = -35.6\pm6.6(8)$; $\Delta G_{11}(KJM^{-1}) = -14$; $\Delta G_{12}(KJM^{-1}) = -13.9$; $\Delta S_{11}(JM^{-1}K^{-1}) = -36$, and $\Delta S_{12}(JM^{-1}K^{-1}) = -73$. The theoretical P values calculated using the obtained parameters were plotted against S_t in Figure 3.

DISCUSSION

Chlorhexidine is a symmetrical molecule with two p-chloro-phenyl substituted biguanide groups, which provide four sites of protonation. Two apparent pKa's have been reported [8] for chlorhexidine: $pKa_1 = 10.3$, for the first two protonations, and $pKa_2 = 2.2$, for the last two protonations. Between pH 4–8, the chlorhexidine dictation is the major species in solution. The pH values of chlorhexidine diacetate solutions in water with concentrations ranging from 0.5mM to 20mM were within the range of 6.90 to 7.23, so it was not necessary to use a buffer to control the pH during the experiments.

β-cyclodextrin is a cyclic compound composed of seven glucopyranoside units. Its conformation can be regarded as a truncated cone with the H3's and H5's directed toward its interior, and the H1's, H2's, H4's and H6's located on its exterior [4]. The results from the NMR study showed that H3 and H5 have greater chemical shift changes induced by chlorhexidine than the other protons in β-cyclodextrin (Figure 1), indicating that the guest molecules is within the cone cavity. Furthermore, the magnitudes of $\Delta\delta$'s for H3 and H5 suggest a shielding effect from aromatic π electrons and

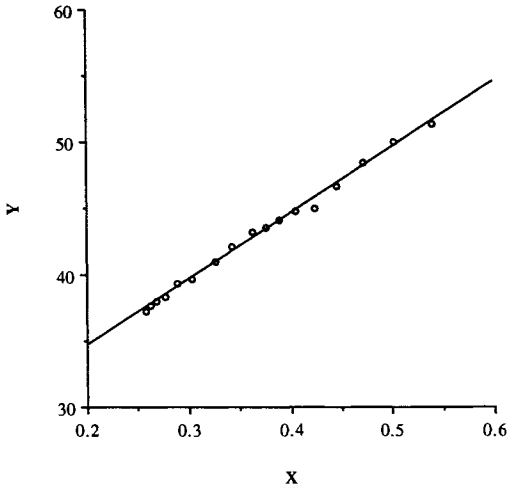

Figure 4. Linearized plot of the results from microcalorimetry study in figure 3. The concentration of β-cyclodextrin is held constant.

therefore the presence of an aromatic ring of the chlorhexidine molecule in the cavity [5]. This is supported by the observation that the chemical shifts of the aromatic protons in chlorhexidine are changed by β-cyclodextrin (see Result section). It is also shown from the NMR study that $\Delta\delta$ of H5 is greater than that of H3, indicating that β-cyclodextrin interacts with chlorhexidine from its primary hydroxyl side. The fact that $\Delta\delta$'s of H6 and H2 have opposite signs indicates that chlorhexidine molecule penetrates only half way into the β-cyclodextrin cavity due to the hydrophilic biguanide group adjacent to the phenyl ring, so that H6, located right above the phenyl ring, experiences the shielding effect of the aromatic π electrons (with an upfield shift), while H2, located on the side of the phenyl ring, experiences the deshielding effect (with a downfield shift).

Alteration of the UV spectrum of chlorhexidine by β-cyclodextrin is another indication that the phenyl ring is in the β-cyclodextrin cavity in the complexes, for the phenyl rings are the chromophore moieties in the chlorhexidine molecule.

Since there are two phenyl rings in the chlorhexidine molecule, the stoichiometry of the complexes should be one chlorhexidine molecule : one or two β-cyclodextrin molecules.

The similarity of K_{11} and K_{12} obtained by uv (see Result section) suggested that the two chloro-phenyl groups in chlorhexidine are sufficiently separated from each other that the complexation of the first phenyl group with β-cyclodextrin has little effect on the ability of the second phenyl group to interact with another β-cyclodextrin molecule.

The thermodynamic parameters for complex formation are usually obtained from measurements made at various temperatures and van't Hoff plots. However, they usually lack precision [9; 10; 11; 12]. Microcalorimetry has been successfully used to study the complexation between β-cyclodextrin and various pharmaceutical agents [13; 14]. An advantage of the microcalorimetry technique is that the heat is measured directly at a fixed temperature, e.g. 25°C, so that less variance is introduced into the final results. The favorable free energy changes for 1:1 and 1:2 complexes (see Result section), are composed of favorable enthalpy changes and unfavorable entropy changes. The ΔH is more favorable and the ΔS is less favorable for the 1:2 complex than for the 1:1 complex.

The entropy change may come mainly from two sources. First, it arises from the loss of the translational and rotational degrees of freedom due to the association of the two molecules, giving rise to a negative entropy change. Second, it comes from the change in the ordering of the solvent molecules. Szejtli [15] suggested that the solvent ordering decreases upon complex formation, due to the displacement of the highly ordered water molecules in the cavity of the β-cyclodextrin molecule and those surrounding the hydrophobic portions of the guest molecule that enter the β-cyclodextrin cavity. As a result, a positive entropy change is produced. The overall entropy change is the net result of the two opposite effects. The negative entropy change observed for the chlorhexidine-β-cyclodextrin system indicates that the effect from a reduction of the translational and rotational degrees of freedom is greater than that of the solvent disordering.

The favorable enthalpy change can be partitioned into

three parts, solvent-solvent interaction, solvent-solute interaction and solute-solute interaction. The contribution of the solvent-solvent interaction to complex formation is mainly by the hydrophobic effect, which is believed to be a major binding force for complexation. Griffiths and Bender [16] have suggested that the water molecules associated with the cavity of the cyclodextrins are enthalpy rich because they can not have a full complement of hydrogen bonds. Based on the same argument, the water molecules surrounding the hydrophobic portions of the substrate molecule can also be considered enthalpy rich for the same reason. The inclusion of a substrate is favored by release of these high energy water molecules.

The solvent-solute interactions include all the solvation phenomena, and can either increase or decrease the binding effect. The solute-solute interaction includes van der Waals forces (electrostatic, induction, and dispersion forces), and hydrogen bonds. It usually increases the binding force of the complex.

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REFERENCES

[1] S. E. Davies, J. Francis, A. R. Martin, F. L. Rose and G. Swain. Laboratory Investigation of a New Antibacterial Agent of High Potency. *Brit. J. Pharmacol.*, 9:192–196 (1954).

[2] O. Fardal, and R. S. Turnbull. A Review of the Literature on Use of Chlorhexidine in Dentistry. *J. Am. Dent. Assoc.*, 112:863–869 (1986).

[3] A. R. Gallopo and D. M. Lynch. Dentrifrices Containing Cyclodexstrin Complexes of Bis(biguarnidohexane) Compounds. *Eur. Pat. Appl. EP 306455* (3.8.1989); *CA*, 111:180501f (1991).

[4] J. Szejtli. *Cyclodextrin Technology*, Kluwer Academic Publishers, Netherlands, pp. 186-293 (1988).

[5] P. V. Demarco, and A. L. Thakkar. Cyclohepta-amylose Inclusion Complexes. A Proton Magnetic Resonance Study. *Chem. Comm.*, 2-4 (1970).

[6] *Standard Proton NMR Spectrum*, Sadtler Research Laboratories Inc. 3992 M, 1967

[7] K. A. Connors. *Binding Constants-The measurement of Molecular Complex Stability*, John Wiley & Sons, New York, pp. 161-167 (1987).

[8] W. B. Hugo, and A. R. Longworth. *J. Pharm. Pharmaco.*, 16:655–662 (1964).

[9] M. Otagiri, T. Miyaji, K. Uekama and K. Ikada. Inclusion Complexation of Barbiturates with β-cyclodextrin in Aqueous Solution. I. Spectroscopic Study on the Mode of Interaction. *Chem. Pharm. Bull. (Tokyo)*, 24:1146-1154 (1976).

[10] T. Miyaji, Y. Kurono, K. Uekama and K. Ikeda. Simultaneous Determination of Complexation Equilibrium Constants for Conjugated Guest Species by Extended Potentiometric Tritation method: On Barbiturate-β-cyclodextin System. *Chem. Pharm. Bull (Tokyo)*, 24:1155–1159 (1976).

[11] K. Uekama, F. Hirayama, M. Otagiri, Y. Otagiri and K. Ikeda. Inclusion Complexation of β-Cyclodextrin with Some Sulfonamides in Aqueous Solution. *Chem. Pharm. Bull. (Tokyo)*, 26:1162–1167 (1978).

[12] K. Ikeda, K. Uekama and M. Otagiri. Inclusion Complexation of β-Cyclodextrin with Antiinflammatory Drugs Fenamates in Aqueous Solution. *Chem. Pharm. Bull. (Tokyo)*. 23:201–208 (1975).

[13] G. E. Hardee, M. Otagiri and J. H. Perrin. Microcalorimetric Investigations of Pharmaceutical Complex: I. Drugs and β-Cyclodextrin. *Acta. Pharm. Suec.*, 15:188–199 (1978).

[14] H. Ueda and J. H. Perrin. A Microcalorimetric Investigation of the Binding of Flurbiprofen to Cyclodextrins. *J. Pharm. & Biomed. Anal.*, 4:107–110 (1986)

[15] J. Szejtli. *Cyclodextrin Technology*, Kluwer Academic Publishers, Netherlands, pp. 107-110 (1988).

[16] D. W. Griffiths and M. L. Bender. Cycloamyloses as Catalysts. *Adv. Catal.*, 23:209-261 (1973).