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Inclusion complexation of doxorubicin and daunorubicin with cyclodextrins*

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Abstract: The interaction of α-, β- and γ-cyclodextrins with the anthracycline antibiotics doxorubicin and daunorubicin was investigated by LC, circular dichroism (CD) and absorption spectroscopy. All studies were performed in aqueous media at different temperatures and pH values. The anthracyclines complex only with γ-cyclodextrin. Lineweaver–Burk and Scott's plots were used to calculate the stability constants of the anthracycline-γ-cyclodextrin inclusion complexes.

Keywords: *Anthracyclines; cyclodextrins; inclusion complexes; HPLC; circular dichroism; absorption spectroscopy.*

Introduction

Cyclodextrins (CyD) are cyclic, non-reducing oligosaccharides. Three different natural CyD are known: α-, β- and γ-CyD consisting of six, seven and eight glucose units, respectively. The structure of γ-CyD is shown in Fig. 1(a). The inside of the molecule forms a cavity, while hydroxyl groups are located on the outer surface. This means that the outside is hydrophilic and the inside of the cavity is relatively hydrophobic. Because of their unique shape and physicochemical properties, CyDs are capable of forming inclusion complexes with a variety of hydrophobic drug molecules by taking up a whole molecule, or some part of it, into the cavity [1, 2]. The stability of the formed complex depends on how well the guest molecule fits into the CyD cavity and the strength of the mostly hydrophobic inter-

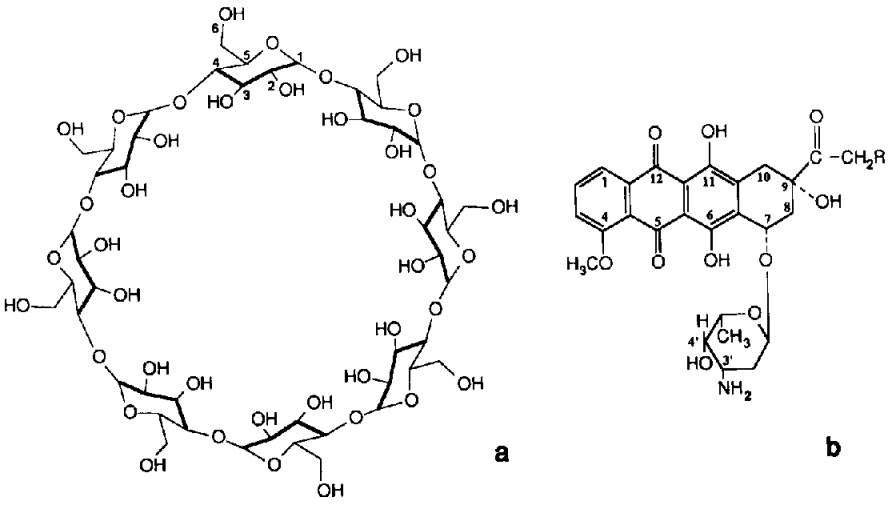

Figure 1
Chemical structures of γ-CyD (a) and Dx (R=OH) or Dr (R=H) (b).

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actions between the guest and host molecules. Encapsulation may cause changes in properties of the guest, such as solubility, chemical stability and bioavailability [3, 4].

In aqueous solutions, γ-CyD forms inclusion complexes with anthracyclines [5] which are potent antitumour antibiotics. The structure of these cytostatics consists of a tetracyclic anthraquinoid aglycone, glycosidically linked to an amino-sugar (Fig. 1b). In present cancer chemotherapy, doxorubicin (Dx) and daunorubicin (Dr) are the two most frequently used members of the anthracycline group [6]. In aqueous media the anthracycline antibiotics are chemically unstable [7, 8] and it has been shown that complexation in acidic solutions with γ-CyD increases the chemical stability of the anthracyclines [5]. From this stability study, performed with an LC method, complex stability constants of anthracycline–γ-CyD complexes have been obtained.

In the present study anthracycline–γ-CyD complexation has been examined by LC, circular dichroism (CD) and absorption spectroscopy. Changes in chemical stability, Cotton effects and absorption properties, respectively, have been used to obtain anthracycline–γ-CyD complex stability constants.

Experimental

Chemicals

Dx was a gift from Dr S. Penco (Farmitalia, Milan, Italy). Dr was kindly provided by Rhône-Poulenc Nederland BV (Amstelveen, The Netherlands). The CyDs came from Nihon Shokukin Co. Ltd (Tokyo, Japan) and were used as received. All other chemicals were of analytical grade and deionized water was filtered through a Milli-Q Water Purification System (Millipore, Bedford, MA, USA) before use.

Glassware

Since anthracyclines have a great tendency to adsorb to glass in neutral and alkaline solutions [9], glassware containing these solutions were silanized before use, with trichloormethylsilane in toluene (3%, v/v) and subsequent rinsing with methanol.

Circular dichroism

CD spectra were obtained with a Dichrograph III (Jobin Yvon, Longjumeau, France). Sensitivity calibration has been performed frequently with isoandosteron. The bandwidth was programmed automatically, the sensitivity 2×10^{-6} deg. nm^{-1}.

Spectra were obtained at 25 and 50°C. The following aqueous solutions were used: pH 3, perchloric acid; pH 7, 0.01 M phosphate buffer; and pH 10, 0.01 M carbonate buffer. A constant ionic strength of 0.3 was maintained for each solution by addition of sodium chloride. The anthracycline concentration was 7×10^{-5} M throughout the study, the α- and β-CyD concentrations were both 4×10^{-3} M, whilst the γ-CyD concentration was varied from 3.9×10^{-3} to 1.2×10^{-4} M.

Absorption spectroscopy

UV–vis absorption spectra were recorded on a Perkin Elmer Lambda 5 UV–vis Spectrophotometer (Perkin Elmer, Oak Brook, IL, USA). Spectra have been measured at 25°C and pH 3.7 and 10, respectively, using the same buffer solutions as described under *Circular dichroism*. The anthracycline concentration was 3.5×10^{-5} M, the α- and β-CyD concentrations were 2.5×10^{-2} and 1.3×10^{-2} M, respectively, and the γ-CyD was varied from 2.5×10^{-2} to 3.9×10^{-4} M.

Liquid chromatography

The LC conditions and apparatus as well as the circumstances of the kinetic experiments were described earlier [5]. Only pH and temperature of the degradation media differ, these were 1.5 and 25°C, respectively.

Results and Discussion

Circular dichroism

Neither α- nor β-CyD had any significant effect on the CD spectra of Dx and Dr (curve 1, Fig. 2). However γ-CyD showed an effect on the CD spectrum of both anthracyclines. This is illustrated for Dx in Fig. 2. At all pH values studied this effect was in the same order of magnitude.

These results suggest that the cavity sizes of α- and β-CyD are not wide enough to include any part of the anthracycline molecule, while γ-CyD appears to be large enough to include the anthraquinoid part of the molecule. This part of the anthracycline molecule is responsible for the minimum at 540 nm and the maximum at 450 nm in the CD spectra of Dx and Dr [10].

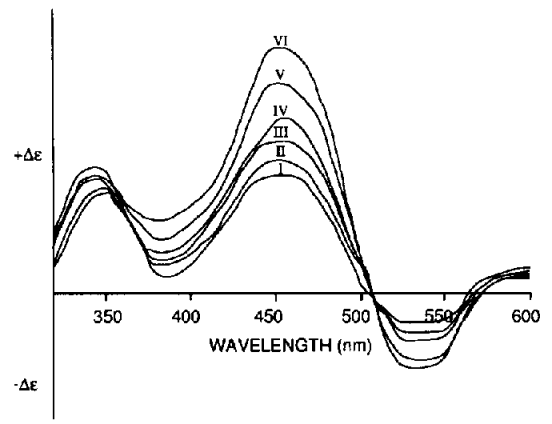

Figure 2
Influence of γ-CyD on the circular dichroism spectrum of Dx at pH 7 and 25°C; [Dx] = 7.78×10^{-5}; [γ-CyD]: I = 0, II = 1.20×10^{-4}, III = 2.41×10^{-4}, IV = 4.81×10^{-4}, V = 9.62×10^{-4}, VI = 1.92×10^{-3} M.

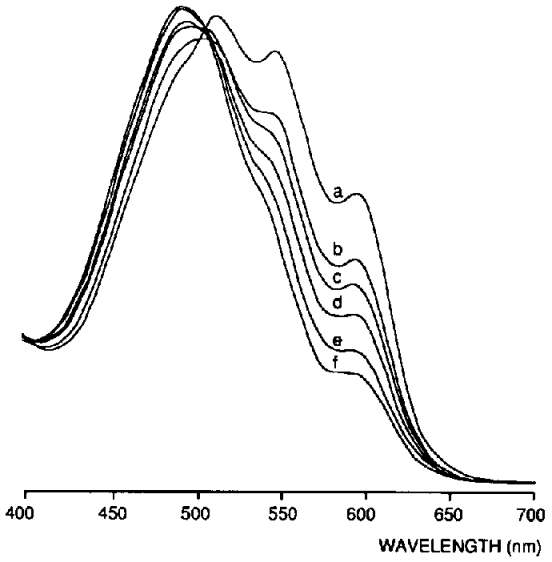

Figure 3
Influence of γ-CyD on the absorption spectrum of Dr at pH 10 and 25°C; [Dr] = 3.53×10^{-5} M; [γ-CyD]: a = 0, b = 3.86×10^{-4}; c = 7.71×10^{-4}; d = 1.54×10^{-3}; e = 3.08×10^{-3}; f = 6.17×10^{-3} M.

Absorption spectroscopy

Figure 3 shows the influence of γ-CyD on the absorption spectrum of Dr, and the same effects were also observed for Dx. These effects, however, were not observed in acidic and neutral media. The spectra of both anthracyclines in alkaline solutions shift, with increasing γ-CyD concentration towards the spectra obtained in acidic and neutral media. This indicated that the phenolic hydroxylgroups (C6 and/or C11, Fig. 1), with a pK_a value of around 10 [7, 8], undergo less deprotonation in the presence of γ-CyD. Thus, the pK_a value of the phenolic group increases on addition of γ-CyD. This result also suggests that the aglycone moiety of the anthracycline is included in the γ-CyD cavity.

The absorption measurements were also performed with α-and β-CyD. Over the whole pH range both CyDs did not cause any significant change compared with the spectra of the free Dx or Dr (for Dr curve a, Fig. 3). The location of the maxima as well as the molar extinctions of the compounds did not alter. This confirmed the supposition that the cavity size of α- and β-CyD, in comparison with γ-CyD, is too small to include the lipophilic aglycone parts of Dx and Dr.

Liquid chromatography

The degradation of Dx and Dr in the absence and presence of various CyDs has been investigated at 25°C and pH 1.5 as described earlier [5]. α- and β-CyD did not affect the observed rate constant, k_{obs}. In the presence of γ-CyD a decrease in k_{obs} could be noticed, in accordance with earlier results. Stability constants, K_s, of the anthracycline-γ-CyD complexes have been calculated, using Lineweaver–Burk plots [5, 11]. The resulting values for K_s are listed in Table 1.

Determination of the stability constants

The complexation of an anthracycline with γ-CyD is illustrated in the following scheme:

$$\text{anthracycline} + \gamma\text{-CyD} \overset{K_s}{\rightleftarrows} \text{anthracycline-}\gamma\text{-CyD},$$

where K_s represents the stability constant of the inclusion complex, as defined by equation (1):

$$K_s = \frac{[\text{anthracycline–}\gamma\text{-CyD}]}{[\text{anthracycline}]\,[\gamma\text{-CyD}]}. \qquad (1)$$

Apart from degradation studies, stability constants can also be determined when suitable ellipticity or absorptivity changes of anthracyclines in the presence of different CyD concentrations occur. These changes were measured at appropriate CD (λ = 450 nm) and absorption (λ = 580 nm) bands, respectively.

Table 1
Complex stability constants of γ-CyD with Dx and Dr

Method	pH	Temperature (°C)	K_s (M^{-1}) Dx	Dr
HPLC	1.5	25	650	487
		50	200	210
Circular dichroism	1.5	25	600	560
		50	325	399
	3.0	25	600	540
		50	290	390
	7.0	25	670	775
	10.0	25	617	570
Absorption spectroscopy	10.0	25	713	653

K_s values were calculated according to Scott's equation (equation 2) [12]:

$$\frac{[\text{anthracycline}]\,[\gamma\text{-CyD}]}{d} = \frac{1}{\Delta\epsilon}\,[\gamma\text{-CyD}] + \frac{1}{K_s \cdot \Delta\epsilon}, \qquad (2)$$

where d is the change in ellipticity or absorbance of the anthracyclines by the addition of γ-CyD, and $\Delta\epsilon$ represents the difference between the molar ellipticities or absorptivities for the free and complexed drug.

The values of K_s (±SD) for the complexation of Dx with γ-CyD obtained by CD at pH 10 and by absorption at pH 7 were 670 ± 90 M^{-1} (n = 6) and 736 ± 100 M^{-1} (n = 7), respectively. All the stability constants shown in Table 1 are mean values of triplicate determinations.

In Table 1 the complex stability constants of Dx and Dr with γ-CyD are summarized. It is obvious that all stability constants are in the same order of magnitude. This, and the fact that the changes in ellipticity and absorptivity at all wavelengths are in the same order of magnitude for Dx and Dr, suggests that the mode of complexation for both anthracyclines is the same in the pH range 1.5–10.

Comparison of the values from Table 1 and those, obtained from degradation studies [5] reveals that the values of K_s are in the same order, thus CD and absorption spectroscopy are suitable analytical techniques to determine anthracycline–γ-CyD complex stability constants.

References

[1] J. Szejtli, *Cyclodextrin Technology*, pp. 79–306. Kluwer Academic, Dordrecht (1988).
[2] W. Saenger, *Angew. Chem.* **92**, 361 (1980).
[3] K. Uekama and M. Otagiri, *Crit. Rev. Ther. Drug. Carrier Syst.* **3**, 1–40 (1987).
[4] D. Duchêne, F.G. Glamot and C. Vaution, in *Cyclodextrins and their Industrial Uses* (D. Duchêne, Ed.), pp. 211–258. Editions de Sante, Paris (1987).
[5] O. Bekers, J.H. Beijnen, E.H. Groot Bramel, M. Otagiri and W.J.M. Underberg, *Pharm. Weekbl. Sci. Ed.* **10**, 207–212 (1988).
[6] F. Arcamone, *Med. Res. Rev.* **4**, 153–188 (1984).
[7] J.H. Beijnen, O.A.G.J. van der Houwen, M.C.H. Voskuilen and W.J.M. Underberg, *Int. J. Pharm.* **31**, 75–82 (1986).
[8] J.H. Beijnen, O.A.G.J. van der Houwen and W.J.M. Underberg, *Int. J. Pharm.* **32**, 123–131 (1986).
[9] E. Tomlinson and L. Malspeis, *J. Pharm. Sci.* **71**, 1121–1125 (1982).
[10] S.R. Martin, *Biopolymers* **19**, 713–721 (1980).
[11] O. Bekers, J.H. Beijnen, E.H. Groot Bramel, M. Otagiri, A. Bult and W.J.M. Underberg, *Int. J. Pharm.* **52**, 239–248 (1989).
[12] R.L. Scott, *Rec. Trav. Chim.* **75**, 787–788 (1956).

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