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Short Communication

A microcalorimetric investigation of the binding of cinnarizine to cyclodextrins

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Introduction

Earlier investigations have shown that cyclodextrins complex with cinnarizine (CN) [1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)-piperazine] [1], a poorly soluble drug which increases cerebral blood flow. Complexation of the drug with β-cyclodextrin (β-CyD) increases the solubility and dissolution rate but does not affect the bioavailability, compared with that of the drug alone, following oral administration to dogs [2]. On the other hand, the bioavailability of CN on oral administration of the CN–β-CyD complex was enhanced by the simultaneous administration of DL-phenylalanine (DL-Phe) which competes for the β-CyD molecule in aqueous solution [3]. These differences in behaviour are attributable to the large stability constant of the CN–β-CyD complex in aqueous solution [2, 3]. However, the explanation of replacing the CN with DL-Phe in the GI tract is in conflict with the values of the stability constants of CN–β-CyD (6.2×10^3 M^{-2} in pure water) and DL-Phe–β-CyD complex (1.0×10^3 M^{-1} at pH 11.3). It was of interest to investigate the thermodynamics of the interaction between the drug and the cyclodextrins in acidic solution.

Experimental

Materials

The CN was a gift of Esai Co., Ltd (Tokyo, Japan) and was used without further purification. The α-, β- and γ-cyclodextrins were obtained from Nippon Shokuhin Kako Co., Ltd. All other chemicals and solvents used were of analytical reagent grade and deionized water was used throughout the study.

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Method

All measurements were made in an LKB Flow Microcalorimeter Model 2107-121 (LKB Bromma, Sweden). Full details of the experimental method and methods of calculation of the thermodymamic parameters are given in an earlier paper [4]. The titrations were performed at a constant drug concentration. An accurately known total flow rate (approximately 26 ml h^{-1}) of the HCl solution (pH 2.0) at 25°C was used. Solubility studies were carried out by the method of Higuchi and Connors [5]. An excess amount of drug was added to HCl–KCl buffer solutions (pH 2.0) containing various concentrations of β-CyD and the solutions were then shaken at 25 ± 0.5°C. After equilibrium was attained, an aliquot was filtered through a 0.45-μm membrane filter. A portion of each sample was diluted and analysed spectrophotometrically. The apparent stability constants (K') were calculated on the basis of 1:1 and 1:2 stoichoimetry from the phase diagrams obtained [6].

Results and Discussion

Figure 1 shows the titration curves of heat flux as a function of CyD concentration. From Fig. 1 it can be seen that more than one site contributes to the heat evolved in the interaction of CN with α-CyD. No stability constants have been estimated from this data; the interaction between CN and α-CyD shows the Type A diagram of solubility method.

The interaction of CN with γ-CyD gave small exothermic reactions but the fluxes were too small for quantitative interpretation. The literature reports a 1:1 complex for γ-CyD and CN in water [1].

It is evident that plateau values of heat flux in the CN–β-CyD system can be estimated with considerable reliability, thus enabling the stability constant (K) and the heat of reaction per mole of complex (ΔH) to be computed, assuming a 1:1 interaction. The good fit of the theoretical line with the experimental data indicate the formation of only the 1:1 complex under these experimental conditions. Figure 2 shows the phase solubility diagram of the CN–β-CyD system.

Although the phase solubility method had shown evidence of a 1:2 complex (with a stability constant of 39 M^{-1}) as well as the 1:1 complex, this finding was not confirmed in the present investigation. This is not surprising since the stability constant is low; it is

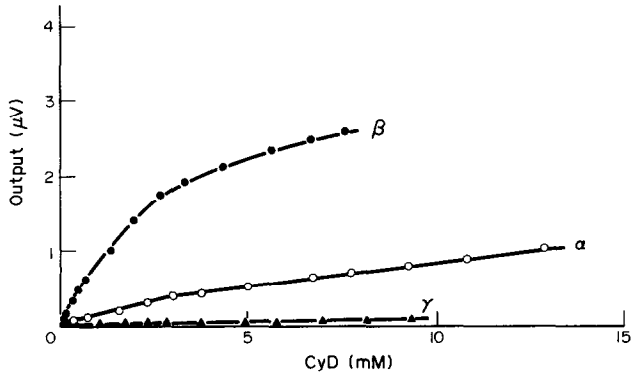

Figure 1
Voltage output of the calorimeter for the reaction between CN and cyclodextrins as a function of CyD concentration at pH 2.0 and 25°C. Voltage output × calibration constant^{-1} × flow rate = observed ΔH(J) [4].

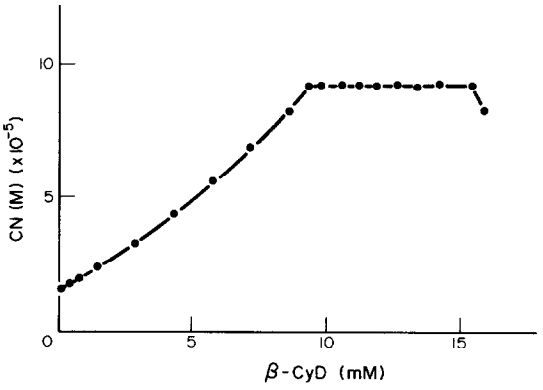

Figure 2
Solubility of CN as a function of β-CyD concentration in HCl–KCl buffer (pH 2.0) at 25°C.

Table 1
Stability constants and derived thermodynamic parameters for the interaction between CN and β-CyD at 25°C

K_{sol}* ($mol\,l^{-1}$)	K_{cal}† ($mol\,l^{-1}$)	ΔH ($J\,mol^{-1}$)	ΔG ($J\,mol^{-1}$)	ΔS ($J\,mol^{-1}\,K^{-1}$)
406.1 (1:1) 39.7 (1:2)	322 ± 17	−16,306	−24,251	+26.7

* K_{sol} was obtained by the solubility method.
† K_{cal} was obtained by flow microcalorimetry.

probable that the heat of reaction is also small. Table 1 shows the stability constants obtained by calorimetry and by the solubility method.

The value of the stability constant obtained by calorimetry is in excellent agreement with that obtained by the solubility method. The derived thermodynamic parameters are also shown in Table 1; Gibbs free energy is represented by $\Delta G = -RT\ln K$, where K in M^{-1} is corrected to a unitless mole fraction scale by use of the approximation ($1000\,d_w/18.02$) or 55.34, where d_w is the density of water at 25°C. Entropy is then obtained from $\Delta G = \Delta H - T\cdot\Delta S$.

The data suggest that the β-CyD cavity allows significant penetration of CN. In comparison with that of the CN–β-CyD system in pure water, the stability constant with CN in the ionized state is significantly reduced in a similar manner to that reported for the barbiturates [7, 8]; the reaction is accomplished, however, by a large evolution of heat. This reduction in the stability constant with change of pH is suggested as being responsible for the ability of DL-Phe to replace CN in the GI tract, so explaining the data previously reported [1].

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