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Inclusion Complexes of α- and β-Cyclodextrin with α-Lipoic Acid

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Abstract. A UV spectroscopic study has been performed in neutral aqueous solution to give the complex stability constants. Data analyses assuming 1 : 1 stoichiometry were successfully applied to both of the host–guest combinations employed, where 1 : 1 host–guest complex formations were observed at lower concentration of cyclodextrins (CDs). X-ray powder diffraction and IR spectroscopy measurements also demonstrated that inclusion complexes were formed in the solid state. Furthermore, thermogravimetry and DTA were used to investigate the thermal properties of these complexes. The differential thermal analysis, as well as temperature variation experiments below 100 °C, indicated that after complexing the 1,2-thiolane moiety of α-lipoic acid (LP) penetrated into the cavity of the CD and the S–S linkage was protected against heat.

Key words: Complexation, coenzyme, α-lipoic acid, cyclodextrins.

1. Introduction

Cyclodextrins have been applied successfully in enzyme mimetic chemistry [1]. An effective enzyme modeling system could be provided when a coenzyme structure is introduced [2]. All of the investigation results suggested that the reactivity of the coenzyme was influenced by the solvent effect, metal ions, micelles and controlled by the host molecule. It is worth noting the great increase of reaction rate achieved by the application of coenzyme chemistry in organic chemistry. Organic chemists are fascinated by the chemical function of the coenzyme as the object of biomimic chemistry. α-Lipoic acid, a coenzyme of the acetone acid dehydrogenase system, is absolutely necessary for the oxidative decarboxylation of α-ketone acids. In addition, α-lipoic acid displays lipotropic activity and decreases cholesterol levels. It is also easily reduced to the form having an SH group, which enables it to remove the toxic effect of As and Hg on many enzymes containing the SH group. Nevertheless, α-lipoic acid is very unstable to light, heat and alkali. The aim of this work is to study the formation of complexes as well as the influence of cyclodextrins on the stability of α-lipoic acid by the methods of UV and IR spectroscopy, X-ray powder diffraction and differential thermal analysis.

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2. Experimental

2.1. Materials

DL-α-Lipoic acid was used as supplied. *Anal. calcd.:* for $C_8H_{14}O_2S$, C 46.91, H 6.86. *Found:* C 46.57, H 6.84.

α-, β-CD were prepared in our laboratory and recrystallized from water. The purity of the obtained CDs was confirmed by NMR, mass, IR, X-ray powder diffraction spectra and the crystalline form of the complexes of CDs with iodine. FAB-MS: m/z 973 $(M + H)^+$ for α-CD and 1135 $(M + H)^+$ for β-CD, respectively.

2.2. Apparatus

X-ray powder diffraction spectra were recorded on a Geigerfler D/max-RB X-Ray Diffractometer. DTA and TG traces were recorded on a 4.1 Differential precise thermobalance (Beijing Optical Instrument Plant). IR and UV spectra were measured by Nicolet 10DX and Shimadzu UV-240 spectrometers respectively.

3. Results and Discussion

3.1. Structure Analysis

Solid complexes were prepared by mixing equal molar amounts of CD and LP in aqueous solution and were stored at 5 °C (LP was dissolved in a small amount of ethanol; β-CD was dissolved in a saturated solution and α-CD in a 2.4% solution, respectively). The precipitated solids were collected by filtration. The elemental analysis data of the complexes are: *Anal. calcd.:* for α-CD·LP·$3H_2O$, C 42.86, H 6.49. *Found:* C 42.96, H 6.32. β-CD·LP·$6H_2O$. *Anal. calcd.:* C 41.44, H 6.63. *Found:* C 41.34, H 6.13. The X-ray diffraction diagrams of the complexes were significantly different from those of CD and LP alone (see Figure 1 and Table I). The location of the peaks on the diffractogram are specific to a crystalline structure. Their location is comparable, but not their intensity, which is characteristic of the crystal sizes. The inclusion complexes show a crystalline structure that is actually different from those of α-, β-CD themselves as well as LP alone. Some peaks of the pure substances (2θ = 10.7, 12.6, 19.7 for β-CD; 9.5, 12.30, for α-CD; and 8, 17.8, 23.9, 32.3 for LP) cannot be found among the peaks of their complexes. The disappearance of both specific peaks of CDs and LP fully confirmed that complexes of CDs were formed with LP. The microcrystal structure of α-CD·LP and β-CD·LP was totally different and the 10.8 peak was considered as the specific peak of α-CD·LP and the 18.1 peak was of β-CD·LP, respectively. α-CD·LP seems to present a purer crystal structure than β-CD·LP. The results suggest that the crystal lattice of the complexes were different from CD and LP themselves. The IR spectra of α-lipoicacid and the complexes are also different. The carbonyl stretching bands shifted from 1690 cm^{-1} to 1705 cm^{-1} and 1715 cm^{-1} when complexed by α-CD and β-CD, respectively. This means that the microenvironment of the

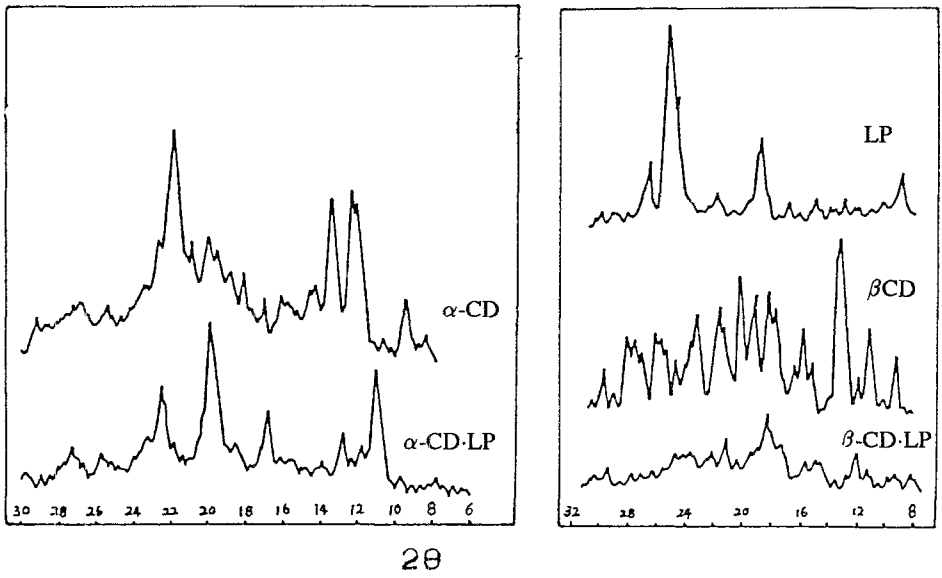

Fig. 1. X-ray powder diffractograms of the complexes.

TABLE I. 2θ values of complexes.

Complex	2θ
LP	8, 17.8, 23.3, 23.9, 32.3
α-CD	9.5, 12, 12.3, 13.4, 19.6, 20, 21, 22
α-CD·LP	10.8, 12.8, 16.9, 19.7
βCD	8.9, 10.7, 12.6, 15.4, 17.6, 18.9, 19.7, 21.4, 22.8
β-CD·LP	8.1, 9.0,11, 14.7, 15.5, 17.7, 18.1, 18.4, 21

carbonyl was different. The UV spectrum of α-lipoic acid in aqueous solution was also affected by the presence of cyclodextrins. The intensity of the absorption maximum (λ_{max} = 333) decreased with the increase of cyclodextrin concentration (concentrations were 1×10^{-3}, 0–7.5 $\times$ 10^{-3} and 0–3.5 $\times$ 10^{-3} M for LP, α-CD and β-CD, respectively). A minimum point of the absorbance vs molar ratio (CD/LP) plot at about 1 for both α-CD and β-CD suggested that the stoichiometry of the complexes was 1 : 1. The stability constants were calculated according to the Benesi–Hildebrand procedure by plotting $1/\Delta A$ vs 1/[CD]:

$$1/\Delta A = K_d/\Delta\varepsilon[CD]\}[LP]_0 + 1/\Delta\varepsilon[LP]_0 \quad (1)$$

where ΔA denotes the absorbance difference with and without CDs at λ_{max} 333 nm. $[LP]_0$ and [CD] are the initial concentrations of LP and CD, respectively. The stability constants, log K_a, calculated by dividing the intercept and slope of the straight line (see Figure 2) was 3.34 for α-CD and 3.95 for β-CD, respectively.

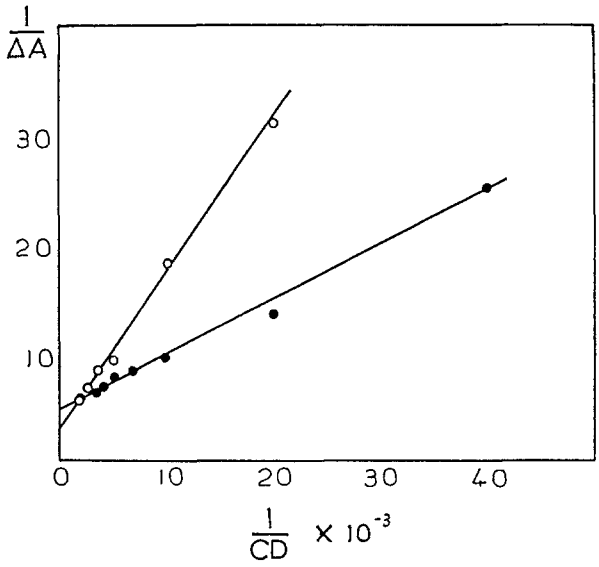

Fig. 2. Absorbance change of LP upon complexation with CDs. *Conc.*: of LP: 0.2 nM, α-CD (○), β-CD (●).

The absorbance change on addition of CDs, indicates the S—S moiety of LP was included in the cavity, and the microenvironment of the lone electron pair at the S atom was affected by the hydrophobic cavity.

3.2. Thermostability of the Complexes

DTA and TG data of the complexes are listed in Table II and Figure 3. It has been established that β-CD decomposes in three phases starting at about 280 °C with the last at about 524 °C. The first endothermic peak on the DTA probably indicates a break in the β-CD ring, the last two endothermic peaks being the carbonization of the β-CD. The TG and DTA curves of α-CD are similar to that of β-CD (Figure 3a). As shown in Figure 3b, the endothermic peak at 53 °C for LP indicates a phase change. The abrupt TG decrease and the DTG endothermic peaks indicate the break in the ring and the carbonization of α-lipoic acid, while the DTA curves of the complexes have no endothermic peak corresponding to the phase change. This indicates that the complexes obtained were pure substances and no uncomplexed LP had adhered to the complex surface (Figure 3d). Compared with LP, the complexes of α-, β-CD decomposed at a higher temperature (Figure 3c). The heating of these complexes was studied below 100 °C. The half-life of LP and its complex with β-CD was 5.2 h and 73 h, respectively (see Figure 4). These results demonstrate that after complexing the thermal stability of LP increases significantly.

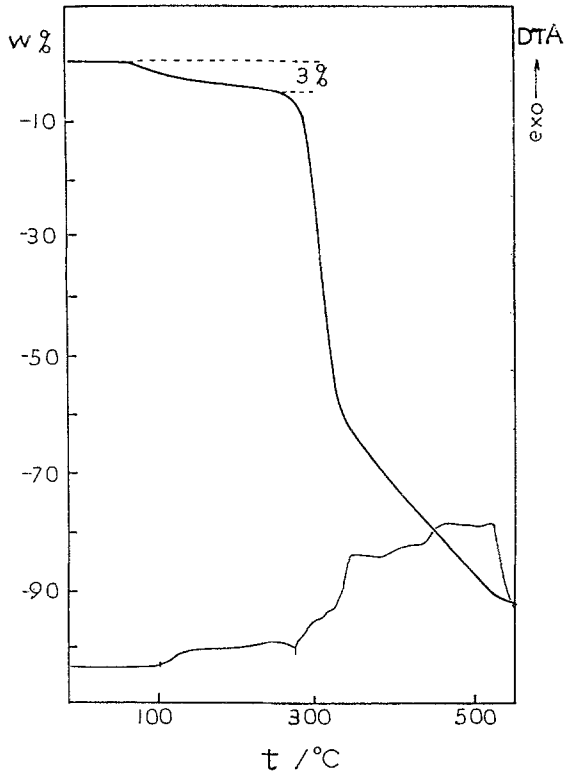

Fig. 3a. TG (—) and DTA (—) of β-CD.

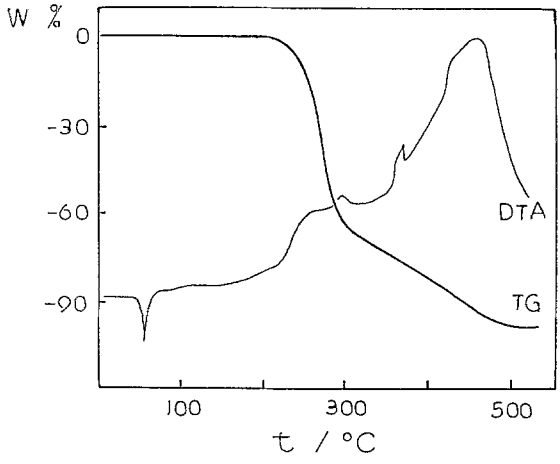

Fig. 3b. TG (—) and DTA (—) of LP.

3.3. Influence of CDs on the Surface Feature of LP Aqueous Solution

Otagiri and coworkers [3] have investigated the effects of CDs on the hemolysis induced with chlorpromazine (CPZ). The shape changes induced with CPZ in

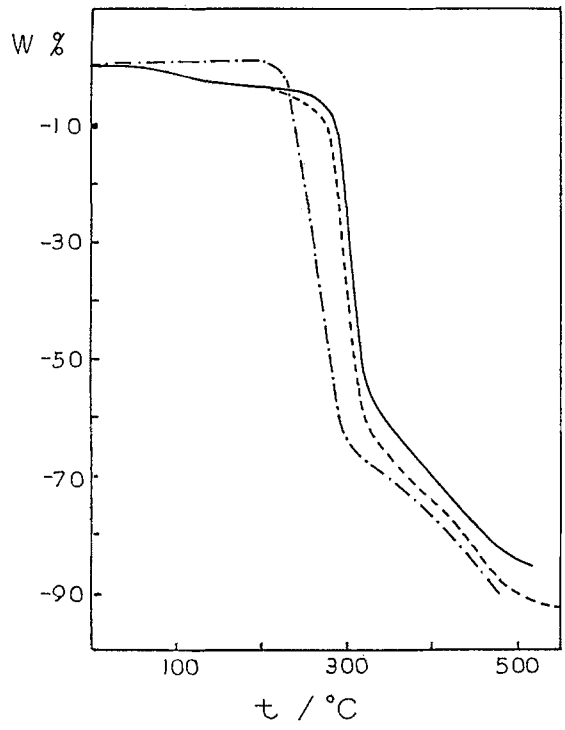

Fig. 3c. TG curves of α-CD·LP (- - -), β-CD·LP (—), LP (– · – · –).

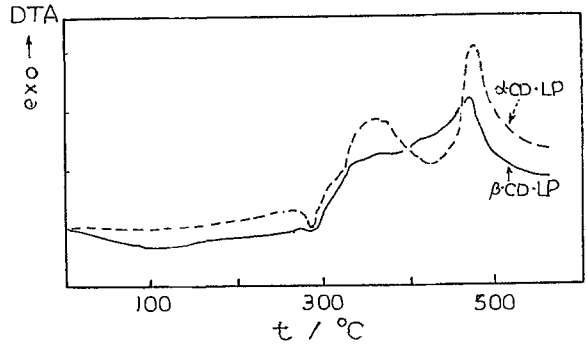

Fig. 3d. DTA curves of α-CD·LP (- - - -) and β-CD·LP (—).

TABLE II. DTA and TG data of the complexes.

Compd.	First wt. loss		Second wt. loss		Third wt. loss
	°C	(wt.%)	°C	(wt.%)	°C
LP	–	–	213–300	65	300–485
α-CD·LP	76	3	264–342	62	342–543
β-CD·LP	67–120	3	261–342	58	342–546

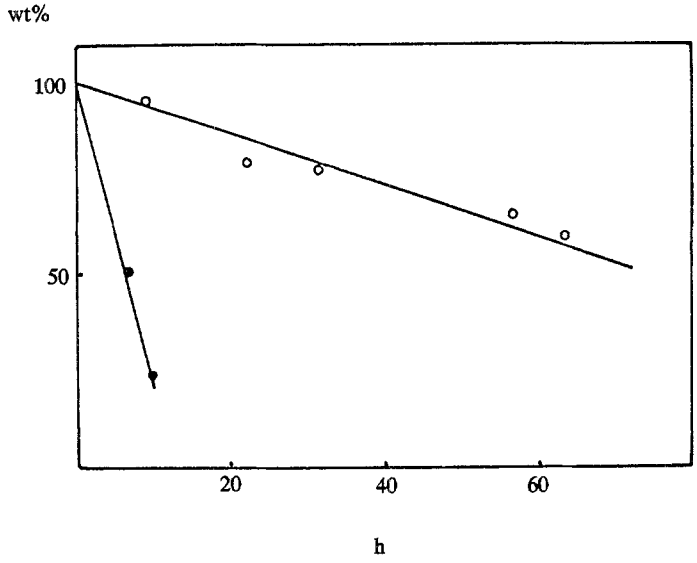

Fig. 4. Plot of heating experiments of LP(•) and its β-CD complex (○) under 100 °C.

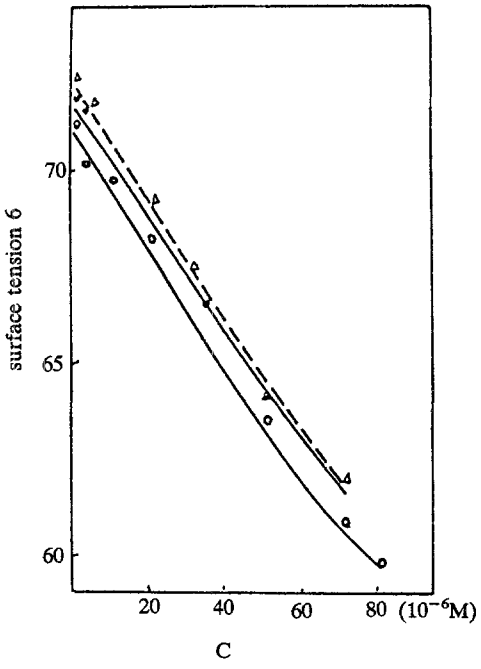

Fig. 5. Plot of σ-C curve of LP (○), α-CD LP (●) and β-CD·LP (△) in water.

isotonic solution were significantly prevented and CDs themselves have no effect on the discocytic form of erythrocytes under the experimental conditions. A good correlation was found between the stability constants and the inhibitory effect on the drug uptake into erythrocytes, indicating that the binding activity of the complexed drug was much smaller than that of the drug alone. Our experiments show that the surface tension increased on adding CD to a LP solution. As seen from Figure 5, the effect of β-CD is larger than that of α-CD. That is probably due to β-CD having a large cavity which can protect the LP molecule well. This method will enable us to investigate some derivatised α-lipoic acid drugs for injection.

4. Conclusion

Our experiments allow us to conclude that the complexes of LP with α-CD and β-CD were formed in aqeuous solution and the solid state. UV spectroscopy analysis indicated the S—S moiety penetrated the cavity of CDs. Thermal analysis and heating experiments below 100 °C showed that the thermal stability of LP was improved significantly by its inclusion in β-CD. The increase of the surface activity of LP in the included state means that the local irritation of pharmaceuticals containing the LP structure maybe improved by inclusion in CDs.

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