Study on the supramolecular inclusion complex of β-cyclodextrin with retinoic acid

Yanling Zhang, Kaijun Liao, Weisheng Liu & Xueyi Ma*
National Laboratory of Applied Organic Chemistry,
Department of Chemistry, Lanzhou University,
Lanzhou 730000, P R China
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Inclusion compound of retinoic acid with β-cyclodextrin has been prepared by coprecipitating method and the structure of resulting product studied by elemental analysis, phase solubility diagram, differential scanning calorimetry (DSC) analysis, FTIR and NMR spectroscopy as well as X-ray diffractionmetry. Results indicate that the formed supramolecule self-assembles in aqueous solution in the molar ratio 2:1 (host: guest).

The importance of vitamin A for human health has been emphasized in recent studies1, while its derivative, retinoic acid (RA) has been widely used as a therapeutic agent to treat several types of skin disease and cancer2.3. However the application of retinoic acid is limited due to its poor water solubility, unstability and side affects on the human body.

Cyclodextrins (CDS) are macrocyclic oligosaccharides built up from 6, 7, or 8 glucopyranose units called α, β, and γ-CD, respectively. CDs have been widely used for various purposes because of their remarkable property of forming inclusion complexes with a variety of molecules4-6. A large number of studies in the field of pharmaceuticals show that β-CD can improve the water solubility of poorly soluble drugs, reduce the toxicity and increase the dissolution rate7-8.

The behaviour of retinoic acid with β-CD inclusion complex in aqueous medium has been reported9-10. The present work reports the structure of the retinoic acid/β-CD complexes deduced by element analysis, DSC analysis, phase solubility diagram, FTIR and NMR spectroscopy as well as X-ray diffractionmetry.

Experimental

β-CD (99.8% Suzhou Weijing Co. China) was purified by recrystallization from water. Retinoic acid and other commercially available reagents were used without further purification.

Elemental analysis was done on Carlo Erba 1106 instrument. Infrared spectra were recorded with Nicolet 170SX FT-IR spectrometer, using the KBr disc method. DSC was recorded with a DSC-7 instrument. Powder X-ray patterns were obtained using a Rigaku D/max-2400 diffractometer (Japan), with Cu-kα radiation, voltage 40 kV, current 40 mA, DS/ss10, RS 0.15 mm at a scanning speed of 8°/min. NMR spectra were recorded with a Bruker AM-400 NMR spectrometer.

The mixture of RA and β-CD was made by two methods: (1) simply mixing the two molecules in molar ratio of 1:2 in a ceramic mortar, (2) Coprecipitating in which RA (0.33 mmol, 0.1002 g) was dissolved in 10 ml ether. To this was added 40 ml aqueous solution of β-CD (0.75 mmol, 0.9461 g). Then the mixture was stirred in nitrogen atmosphere at 40°C for 8h. The pale yellow complex product was collected and washed with ether and water.

Results and discussion

The phase-solubility diagram was recorded according to Higuchi et al.11,12. For this purpose, aqueous solutions of β-CD with concentrations of 0, 1, 2, 3, 4, 5, 6, 7×10−3 M were prepared. Excess amounts of RA were added to each solution of β-CD. Next, the solutions were agitated for 10 min with ultrasonantor at 20°C, centrifuged and carefully filtered. The 5 ml samples of filtrates were diluted to 10 ml with methanol. Their absorptions were measured by UV spectrophotometry after appropriate dilution with methanol at 326nm. Figure 1 shows the equilibrium phase solubility diagram for the RA/β-CD complex in water. The isotherm is an A3 type solubility curve and shows that a soluble complex (2:1) is formed13.

Thermal studies of macromolecules can predict a product’s performance in terms of its stiffness, toughness or stability. Melting point, phase transition, pyrolysis, and curing temperatures can be accurately measured. The inclusion complex of RA/β-CD was examined by differential scanning calorimetry and provided a quantitative and qualitative estimation of the solid state reactions (Fig. 2). Results show that in the case of a mixture, each component behaves independently, i.e., RA melted and decomposed below 180°C, β-CD melted and decomposed around 300°C. In the inclusion compound, there was no peak
around 180°C. It is stable up to 300°C and decomposed above the melting point of β-CD component. These results show that RA is tightly held in the β-CD cavity. The disappearance of the peak for water in the β-CD cavity at 100°C indicates that water in the β-CD cavity was extended by guest molecular while the inclusion complex of RA with β-CD was being formed.

FTIR analysis provides much information about inclusion complex in powder or microcrystalline states. Figure 3 shows the infrared spectra of RA, of the mixture of RA and β-CD at 1:2 molar ratio, as well as that of the complex obtained by coprecipitating. In the IR of the inclusion compound, the band due to RA shifted to a lower wave number and broadened. The asymmetrical in-plane (–CH₃, 1440 cm⁻¹), and symmetrical in-plane (–CH (CH₃)₂, 1357, 1346 cm⁻¹) bendings observed for pure RA are shifted to 1412 cm⁻¹ and 1368, 1340 cm⁻¹ respectively in the complex. These data indicate that the vibrating and bending of guest molecular (RA) was restricted due to the conformation of an inclusion complex

and all methyl groups in RA molecular were inserted into the cavity of β-CD.

According to the molecular model, the depth of β-CD is about 7Å, and the length of RA molecular chain is about 15Å. In the complex, β-CD molecules are almost closely packed from end-to-end of RA molecular chain. The proposed structure of RA/β-CD inclusion complex is shown in Fig. 4. The chemical shifts of β-CD protons in the presence of RA obtained at 400 MHz in DMSO-d₆ are shown in Table 1. The chemical shifts of protons H-3, H-5 which are oriented towards the interior of β-CD cavity show noteworthy upfield shifts, while the chemical shifts of H-1, H-2 and H-4 protons are unaffected as a result of complexation. These observations clearly prove the formation of the inclusion complex and are consistent with the
reasoning of Demarco and Thakkar\textsuperscript{10} that the screening environment can be sensed only by the hydrogens on the inner surface (H-3 and H-5), but not by the hydrogens on the outer surface, if inclusion occurs.

The changing resonance of C$_2$-OH and C$_3$-OH protons shows the interaction created between β-CD molecules to be oriented head-to-head. The results are consistent with the structure proposed above.

X-ray diffraction patterns of β-CD, RA, the inclusion complex and the mixture of β-CD and RA are shown in Fig. 5. The diffraction pattern of the mixture is the simple superimposition of signals of the two components, while that of the inclusion complex is different from those of pure RA and β-CD. Many new peaks appear and the peaks of inclusion complex become weaker or smaller demonstrating the amorphous character of the inclusion complex\textsuperscript{17}.

Inclusion complex can be formed with different molar ratio (host:guest). The conformation of inclusion complex of RA with β-CD was determined by elemental analysis \text{C}_{62}\text{H}_{98}\text{O}_{37}. Calc. C: 46.7, H: 6.73; Found: C: 46.91; H: 6.78. (containing 5H$_2$O). The results show retinoic acid formed only axial inclusion complexes with 1:2 stoichiometry with β-CD.

In order to further confirm the structure of the RA/β-CD inclusion complex, X-ray powder diffraction patterns were analyzed using the qualitative analysis processing (Rint 2000 software). The measured X-ray powder diffraction is shown in Fig. 6.

The main diffraction peaks are similar to those of the β-CD polymer complex\textsuperscript{14,18}. The results show that the inclusion is isomorphous with a channel-type structure.

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References