Journal of Thermal Analysis, Vol. 37 (1991) 2497-2506

PHYSICOCHEMICAL CHARACTERIZATION OF CHOLESTEROL-BETA CYCLODEXTRIN INCLUSION COMPLEXES

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(Received May 27, 1991)

Study and characterization of molecular complexes between cholesterol and beta cyclodextrin has been done using X-ray diffraction, thermogravimetric analysis (TG), differential scanning calorimetry (DSC) and nuclear magnetic resonance spectroscopy (^{13}C NMR). Whatever the value of the molar ratio cholesterol/βCD used during the preparation, the same compound is always obtained. Corresponding to a molar ratio 1/3 (cholesterol/-βCD), this compound is a stable hydrate which, contrary to βCD, contains at room temperature a large amount of molecules of water. It can be dehydrated under low pressure but the thermal degradation occurs at 200°C (250°C for βCD). This implies that cholesterol is strongly bounded to βCD.

Introduction

Cyclodextrins are typical host molecules which can include a great variety of foreign molecules to form crystalline inclusion complexes [1–3]. The study of hydratation of beta cyclodextrin (βCD) was previously reported [4]. The first preparation of an inclusion complex between cholesterol and βCD was pulished by Schlenk [5], although a convincing proof of the existence of the complex was not given. Recently Claudy *et al.* [6] and Fridrich *et al.* [7] have established the existence of βCD/cholesterol inclusion complexes. A detailed study of the physico-chemical properties of this com-

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pound formed by a guest molecule (cholesterol) and a host molecule (βCD) was then undertaken.

Experimental

Beta cyclodextrin (cyclomaltoheptose) was obtained from Aldrich Chemical Co (lot n° 2801211). Cholesterol was purchased from Sigma Chemical Co (lot n° 107F-7070), and *n* hexane was from S.D.S.

The products were stored and weighted in a glove box filled with argon and equipped with a recirculation of the gas on molecular sieves and MnO to have a moisture and oxygen free atmosphere.

Preparation of the inclusion compound

Known amounts of cholesterol and βCD were allowed to react using two different procedures. In both cases, the mass of water contained in βCD was taken into account to compute the molar ratio βCD/cholesterol.

A) Cholesterol (1g) was dissolved in 25 ml of *n*-hexane. The solution was stirred and slowly warmed up to 55°C. A solution of βCD in water (10% weight) was prepared in a known molar ratio with respect to cholesterol and warmed at 65°C. The solutions were then allowed to react. A white microcrystalline powder was immediately formed. After 3 hours the solid was filtered (40 μm pore size). The powder was washed with water and hexane and dried at 40°C for 18 hours. After grinding, the product was stored at room temperature.

B) A known mass of βCD was mixed with the same mass of water, and cholesterol was slowly added under stirring until a homogeneous part was obtained. It was poured into a decantation flask with 100 ml of water and warmed at 50°C. After 12 hours, a white solid was separated, dried at 60°C during 4 hours and stored at room temperature. The product was dried under vacuum (1 Pa) at 120°C during 24 hours.

Analysis

X-ray diffraction

X-ray diffraction patterns were recorded using a Philips PW 1700 generator equipped with a horizontal axis goniometer. The K_α radiation of copper was used.

^{13}C NMR Spectroscopy

The ^{13}C NMR spectra were recorded at 62.89 MHz on a Bruker AM 250 NMR spectrometer under proton-broad-band decoupling conditions. The other experimental conditions were optimized to get the greatest accuracy for quantitative measurements while not increasing too much the spectrometer time used. It was found that an interpulse delay (acquisition time + relaxation delay) of 2.54 gave the best results in that respect. The solutions studied were prepared in hexadeuterated N,N-dimethylformamide, the concentration being usually of 100 $mgml^{-1}$. The temperature was 297 K. The chemical shifts are expressed in parts per million with respect to tetramethylsilane, using the substitution method. The spectra of pure cholesterol and pure βCD were obtained using the same conditions to provide reference spectra. To determine the molar ratio of cholesterol and βCD in the complexes, the caracteristic signal at 103.2 ppm (corresponding to 7 C of βCD) and at 121.0 ppm (corresponding to 1 C of cholesterol) were used. Both signals being chosen as they are well isolated from the other signals of the spectrum (Fig. 1), thus ensuring a better accuracy of the results. The calculations were performed using electronic integration of peak areas. A line broadening of 1 Hz was applied prior to Fourier transformation. The number of scans needed to achieve a good signal to noise ratio (and therefore reliable molar ratios for the complexes) was between 20000 and 50000 scans.

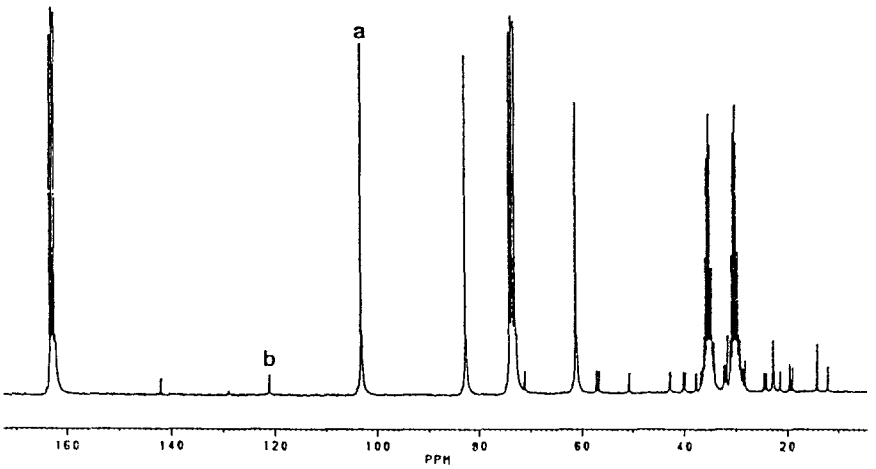

Fig. 1 ^{13}C NMR spectrum of a βCD/cholesterol complex, signal a: βCD, signal b: cholesterol

Gravimetry and ionic chromatography

After dissociation of the complex at 60°C in a propanol-water (50/50) solution during 30 min, the amount of βCD was determined by means of a Dionex apparatus with amperometric detection (P.A.D.) following the procedure described by Koizumi *et al.* [9]. Cholesterol was extracted with hexane and quantified by gravimetric analysis.

Thermal analysis

Samples of anhydrous and hydrate forms were subjected to thermal analysis by means of two techniques.

TG

A microbalance MTB 10 (SETARAM France) was used. It was controlled by an HP86 deskcomputer. Experiments were performed with a heating rate of 2 deg·min^{-1} under argon flow or at low pressure (1 Pa) and analysis of evolved gas. The sample (10 to 20 mg) was placed into a glassy pan. To avoid any escape of particles during experiment, a cover made of fritted glass was used.

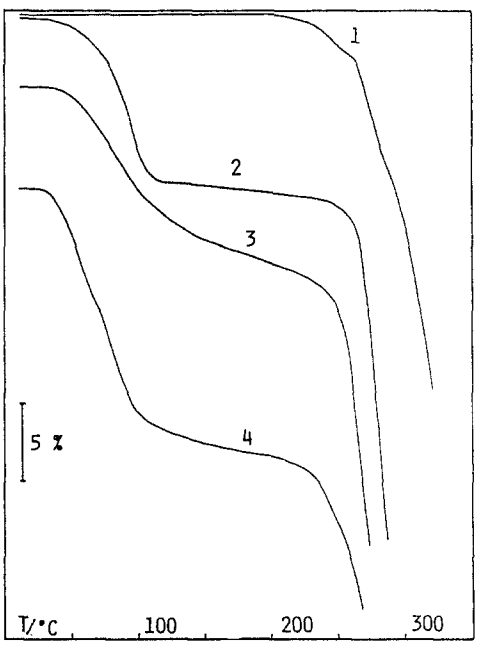

Fig. 2 TG curves of cholesterol (1), hydrated βCD (2) and hydrated inclusion complexes 2/1 (3) and 3/1 (4) molar ratio

DSC

Differential Scanning Calorimetry measurements were carried out using a Mettler TA2000B apparatus controlled by an HP85 computer. The apparatus was calibrated for temperature and enthalpy by melting high purity metals or compounds [8]. The instrument was flushed with argon (51 h^{-1}) which was chosen because of its density and low thermal conductivity. 10 to 15 mg samples were transfered into 40 μl aluminium crucibles which were sealed and weighted. The cover had calibrated orifices to allow for vaporization of water when hydrates were studied. All experiments were performed with a heating rate of 5 deg·min^{-1}.

Results and discussion

Molar ratio of the inclusion compound

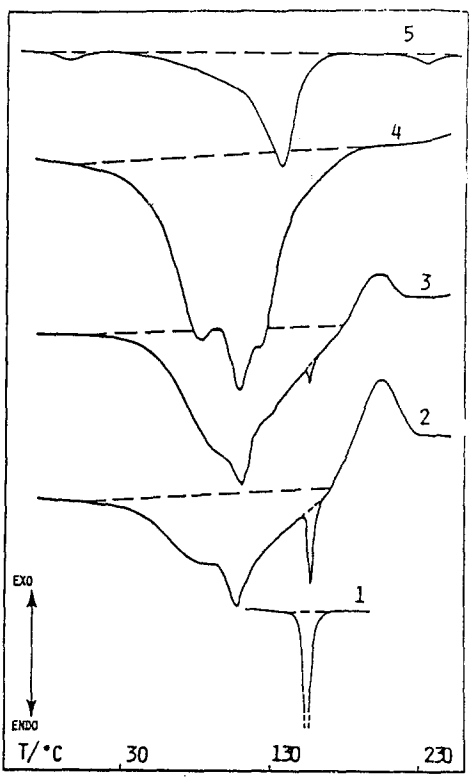

Fig. 3 DSC curves of cholesterol (1), hydrated βCD (5) and hydrated inclusion complexes 1/1 (2), 2/1 (3) and 3/1 (4) molar ratio

Table 1 Analysis results of the inclusion complexes

Procedure	Initial molar ratio βCD/chol.	Integration ^{13}C NMR		HPIC βCD and gravimetric cholesterol analysis	Loss of mass H_2O weight /%
		1	2		
A	1/1	3.15	3.34	3.21	7.4
	2/1	3.10	3.04	3.50	10.4
	3/1	3.01	3.22	3.16	16.9
B	1/1				5.2
	2/1				10.0

Analysis results are summarized in Table 1. We have found that the molar ratio is approximatively 3/1. The most accurate value was obtained with the NMR method which does not require any special preparation of the sample.

Thermoanalytic methods (DSC, TG and X-ray diffraction) have shown that when using the two differents procedures (*A* or *B*), the same final compound was obtained.

Hydrated compounds

Amounts of water contained in the sample were determined by thermogravimetric analysis (TG) under argon. Typical curves of cholesterol, βCD and inclusion complexes (initial molar ratio 2/1 and 3/1) are given in Fig. 2 (TG) and Fig. 3 (DSC). The thermal behaviour of each product can be described as follow:

– Cholesterol: the melting is observed at 149°C (enthalpy of melting: 22.9 kJ·mol^{-1}). A loss of weight is obtained starting at 200°C and due to the vaporization of the product.

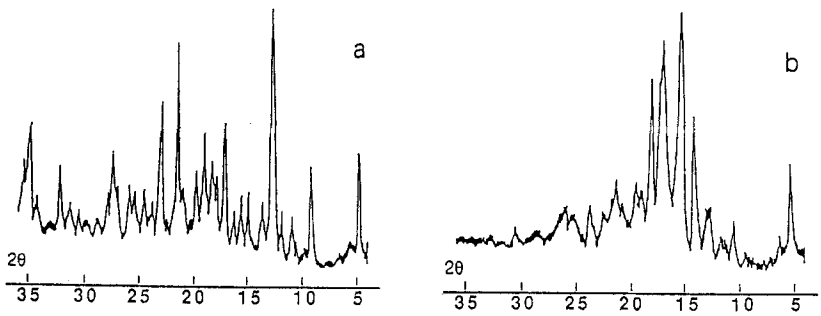

Fig. 4 X-ray diffractograms of cholesterol (*b*) and βCD (*a*)

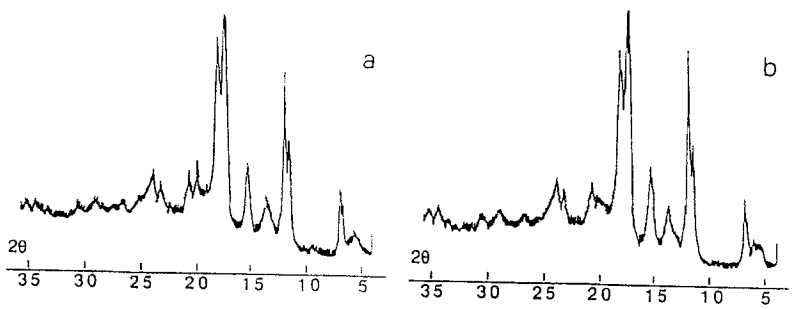

Fig. 5 X-ray diffractograms of hydrated inclusion complexes 3/1 (*a*) and 2/1 (*b*) molar ratio

– βCD: the dehydration occurs in the range 30–100°C with a loss of weight of 15% due to the vaporization of 11 molecules of water. Decomposition begins at 230°C.

– Inclusion complexes: the dehydration is obtained in the same temperature range as for βCD but a slight continuous loss of weight is observed during heating up to the decomposition near 200°C. The inclusion complexes, prepared with different molar ratio βCD/cholesterol value have the same thermal behaviour. The loss of weight only differs (Table 1) as does the endothermal effect of dehydration observed with DSC (Fig. 3). In some experiments, traces of free cholesterol have been found (Fig. 3) by the melting peak and the exothermal effect which appears immediately after melting. According to Fridrich *et al.* [7], this fact suggest the formation of an inclusion complex as reported by Nakai *et al.* [10]. An additional washing with hexane is carried out to remove free cholesterol.

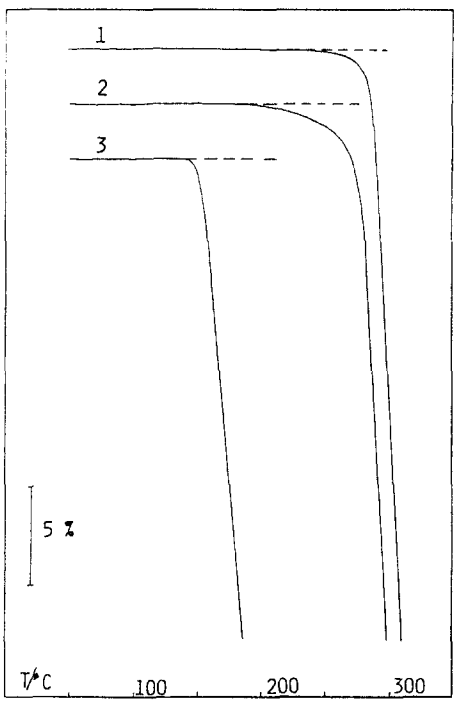

Fig. 6 TG curves of anhydrous products, βCD (1), 3/1 molar ratio (2), cholesterol (3)

The X-ray diffraction patterns of cholesterol, βCD and hydrated complexes are given in Figs 4 and 5. It is obvious that inclusion complexes have the same X-ray diffraction pattern. Slight differences can be attributed to

the crystallinity of the products. No trace of βCD or cholesterol were identified in the diffractograms.

Anhydrous compounds

The curves $\Delta P/P = f(T)$ obtained under low pressure (1 Pa) are summarized in Fig. 6.

– Cholesterol: the onset of vaporization is observed at the melting temperature.

– βCD: the decomposition starts at 250°C and already at 200°C for the inclusion complex. In the temperature range 200–250°C, there is dissociation of the complexes.

These results are confirmed by those obtained with DSC measurements shown Fig. 7.

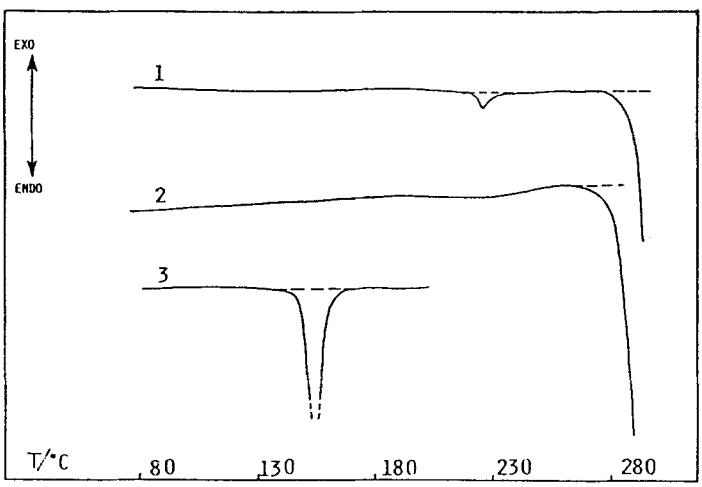

Fig. 7 DSC curves of anhydrous products, βCD (1), 3/1 molar ratio (2), cholesterol (3)

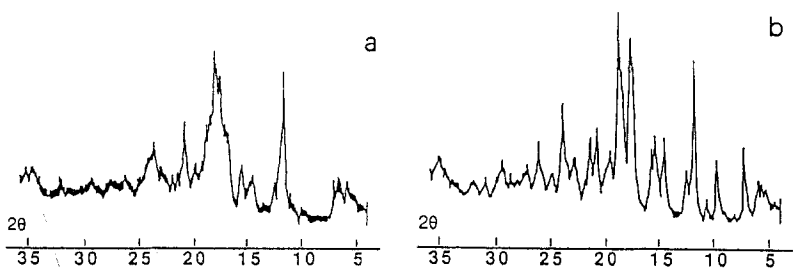

Fig. 8 X-ray diffractograms of anhydrous inclusion complexes, 3/1 (*a*) and 2/1 (*b*) molar ratio

As previously stated, the X-ray diffractograms of the anhydrous products are identical (Fig. 8), but different of the hydrated products and free βCD and cholesterol.

Conclusion

All the results of the experiments carried out by means of various analytical techniques such as DSC, TG, X-ray diffraction and ^{13}C NMR spectroscopy demonstrate that cholesterol and βCD form inclusion compounds. In spite of using different molar ratio or synthesis, the only compound obtained corresponds to the molar ratio 3/1 (βCD/cholesterol). At room temperature, it contains 17% weight of water corresponding approximatively to 42 molecules of water. Under low pressure anhydrous complex can be made. Thermal degradation of the complex begins at 200°C (250°C for βCD). This fact confirms that cholesterol and βCD are strongly bounded.

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Zusammenfassung — Mittels Röntgendiffraktion, TG, DSC und ^{13}C-NMR-Spektroskopie wurden Molekülkomplexe zwischen Cholesterol und Betacyclodextrin untersucht und charakterisiert. Unabhängig von dem bei der Herstellung angewendeten Cholesterol/ßCD-Verhältnis wurde immer die gleiche Verbindung erhalten. Bei einem Molverhältnis 1/3 (Cholesterol/ßCD) ist diese Verbindung ein stabiles Hydrat, das im Gegensatz zu ßCD bei Raumtemperatur eine große Anzahl von Wassermolekülen enthält. Es kann in Vakuum dehydratiert werden, die thermische Zersetzung erfolgt bei 200°C (250°C für ßCD). Dies setzt voraus, daß Cholesterol fest an ßCD gebunden ist.