ORIGINAL ARTICLE

Characterization of the inclusion complex of *β*-cyclodextrin with sorbic acid in the solid state and in aqueous solution

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Abstract Crystal structure of *β*-cyclodextrin (*β*-CD) complexes with sorbic acid, usually as food preservative, has been determined by single-crystal X-ray diffraction at 113 K. The space group of *β*-cyclodextrin-sorbic acid complex is P1 with unit cell dimensions of a = 15.284(3) Å, b = 15.402(3) Å, c = 17.981(4) Å, α = 99.67(3)°, β = 112.83(3)°, γ = 102.48(3)° and Z = 1. The result indicates that the *β*-CD molecules form head-to-head dimers which pack in the intermediate mode. Each dimer contains two guest molecules whose methyl groups are located at the dimer interface while the carboxyl groups protrude from the *β*-CD primary faces. Water molecules (25.5) are distributed outside the cyclodextrin cavity over 31 sites. Furthermore, nuclear magnetic resonance spectroscopy (^{1}H NMR) has been employed to investigate the inclusion behavior between the host *β*-CD and guest sorbic acid in aqueous solution. The results obtained enabled us to structurally characterize the *β*-CD inclusion complex with sorbic acid.

Introduction

α-, *β*-, *γ*-Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six, seven, eight D-glucose units linked by

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α-(1 → 4) glycosidic bonds [1]. As illustrated in Fig. 1, they resemble truncated, hollowed cones in which all glucoses adopt the commonly observed $^4C_1$-chair conformation and are oriented syn. The hydroxyl groups render the CD hydrophilic outside; in contrast, their central cavities are hydrophobic because they are coated with hydrogen atoms from the C3–H and C5–H methine groups, the C6–H_2 methylene groups, and the glycosidic oxygens (O4) [2]. Due to this special property, CDs are widely utilized in food and pharmaceutical industries to encapsulate compounds that are sensitive to the environment, have a low solubility in water and high volatility [3–6]. Therefore, more favorable properties of CD inclusion complexes can be obtained.

Sorbic acid (2,4-hexadienoic acid) and its potassium salt, potassium sorbate are one of the safest and most efficient, versatile food preservatives used today, since they are highly effective inhibitors of most common microorganisms that can cause food spoilage. Although sorbic acid and its derivatives are widely used in a variety of applications in the food and beverage industry [7, 8], they are harmful at higher than permitted safety levels. In the report of Kuo and Hsieh, they determined sorbic acid in food products by cyclodextrin-modified capillary electrophoresis with multi wave length detection [9]. Sorbic acid forming complexes with the CDs were efficiently loaded and their release could be sustained. And proper use of these inclusion complexes for industrial applications requires a complete knowledge of their structure. Hence, the work presented here, uses the method of X-ray diffraction to explain the reason of inclusion complex by the determination of crystal structure in the solid state as well as ^{1}H NMR spectroscopy in the aqueous solution. Using NMR, it is possible to confirm the inclusion phenomenon, while analysis of the crystal structure leads to a more precise characterization of the complex.
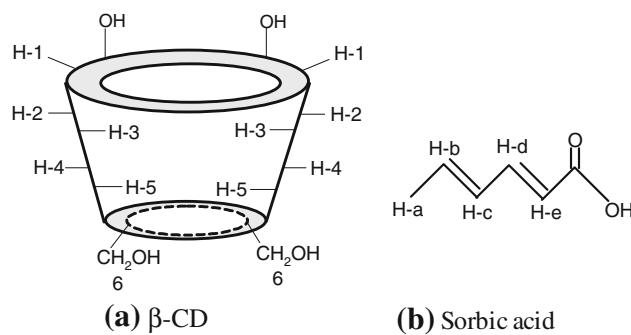
Fig. 1 Chemical structure and atomic numbering of β-CD and sorbic acid

Experimental

Preparation of inclusion complex of β-CD with sorbic acid

Sorbic acid and β-CD (both purchased from Kewei Company of Tianjin University, PR China) in a 1:1 molar ratio were fully dissolved separately in ethanol and water, then two solutions were mixed by magnetic stirrer and stirred at 70 °C for 2 h. After filtration (0.22 μm filter) at the same temperature, the solution was cooled to room temperature and allowed to evaporate spontaneously. After 7 days, triclinic colourless crystals were separated, and then washed with water to remove any remaining β-CD. Non-included guest was removed by washing with ethanol. Water used was doubly distilled and deionized.

X-ray diffraction

A single crystal was mounted in a capillary tube in the presence of a small amount of mother liquor. X-ray diffraction experiment was carried out using a SMART CCD (Bruker) with Mo K_α radiation ($\lambda = 0.71073$ Å) operating at 50 kV, 30 mA. A total of 21,304 reflections were measured in the θ-range 2.15°–25.02°. Data reduction was carried out with the program SAINT, including semi-empirical absorption correction with SADABS.

The crystal structure was determined by direct methods and refined using full-matrix least-squares based on F^2 with the program SHELX, based on 1,814 parameters and 140 restraints. The refinement converged at $R1 = 0.0700$ and 0.0737 (β-CD-sorbic acid complex) for observed ($F^2 > 2r(F^2)$) and all reflections. Water molecules were located on a difference Fourier map. All non-hydrogen atoms were refined anisotropically. Except for those hydrogen atoms attached to the water molecules, the all of other hydrogen atoms were added in ideal positions and refined as riding models (further details, see Table 1).

Table 1 Crystallographic data and structure refinement

Empirical formula	$2C_{42}H_{70}O_{35} \cdot 2C_6H_8O_2 \cdot 25.5H_2O$
Formula weight	2,982.76
Temperature (K)	113 (2)
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	$P1$
a (Å)	15.284 (3)
b (Å)	15.402 (3)
c (Å)	17.981 (4)
α (°)	99.67 (3)
β (°)	112.83 (3)
γ (°)	102.48 (3)
V (Å^3)	3,656.7 (13)
Z	1
Calculated density (mg/m^3)	1.341
Absorption coefficient (mm^{-1})	0.122
F (000)	1,578
Crystal size (mm^3)	0.16 × 0.14 × 0.10
θ range for data collection (°)	2.15–25.02
Limiting indices	$-18 \leq h \leq 15, -18 \leq k \leq 18, -21 \leq l \leq 21$
Reflections collected/unique	21,304/14,962
R (int)	0.0272
Data/restraints/parameters	14,962/140/1,868
Goodness-of-fit on F^2	1.024

NMR studies

Proton nuclear magnetic resonance studies were performed at room temperature in deuterium oxide solution (D_2O) with a Varian INOVA 500 MHz devices. Tetramethylsilane was used as reference and no correction was made for susceptibility of the capillary.

Results and discussion

Crystal structure

Overall description of the complex structure

The inclusion complex of β-CD with sorbic acid is determined by X-ray diffraction as $2C_{42}H_{70}O_{35} \cdot 2C_6H_8O_2 \cdot 25.5H_2O$ in the triclinic space group $P1$, with $a = 15.284(3)$, $b = 15.402(3)$, $c = 17.981(4)$ Å, $\alpha = 99.67(3)°$, $\beta = 112.83(3)°$, $\gamma = 102.48(3)°$ and $Z = 1$. The detail data are shown in Table 1.

Two crystallographically independent β-CD molecules are in head-to-head orientation forming a dimeric channel which contains two independent guest molecules (a, b), as shown in Fig. 2. The β-CD monomers assemble through O3–H···O3 intermolecular hydrogen bonds to form the
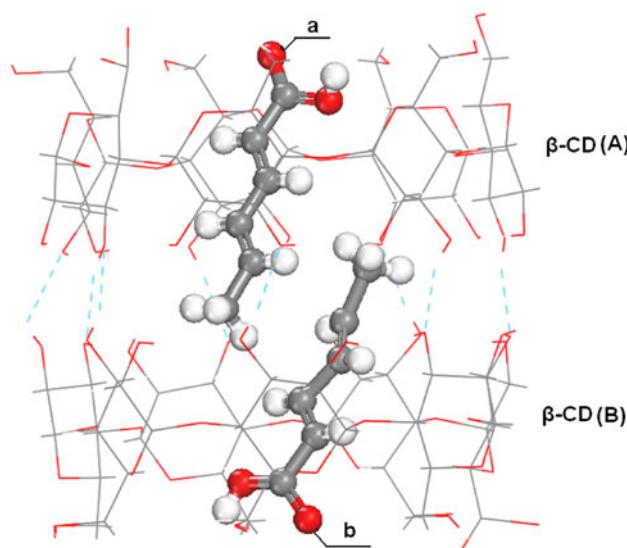
Fig. 2 The head-to-head dimer of β-CD-sorbic acid complex. Solvent water molecules are omitted for clarity

β-CD dimer, which is stacked parallelly along c-axis, as frequently observed in the inclusion complexes of β-CD.

The guest molecule adopts essentially planar structure, and the conformation of it is orientated with the hydrophilic carboxyl groups at the primary face of the host and with the hydrophobic methyl residues at the dimer interface.

Characteristics of the β-CD and guest in the dimer

In the macrocyclic structure of β-CD, each glucose residue adopts $^4C_1$ chair conformation, and has an approximate

7-fold-axis of symmetry. The geometrical data of β-CD molecules are listed in Table 1.

The secondary hydroxyl groups between neighboring glucose residues form intramolecular hydrogen bonds, which may maintain the annular shape of the β-CD. O2(n)···O3(n + 1)bonds distances are in the range 2.696–2.838 Å and average values for two β-CDs in the dimer are 2.787 Å (A), 2.777 Å (B), respectively.

In the complex, each β-CD ring has a round structure that is characterized by a heptagon composed of seven O4 atoms with a side length of 4.373 Å for β-CD (A) and 4.356 Å for β-CD (B). These O4 atoms are coplanar within 0.043 and 0.058 Å for β-CD (A), (B), respectively. The O4 (n − 1)···O4 (n)···O4 (n + 1) angles, varying from 123.522° to 133.504° for β-CD(A)and 124.875° to 130.296° for β-CD(B), are within 0.02° from the regular heptagon of 128.571°. Meanwhile, the O4 planes of two β-CDs, in the dimer, are almost parallel with an angle of 0.97°. Overall, the geometrical parameters indicate that upon complex formation, the guest molecule has slightly distorted the macrocyclic conformation of the β-CD.

A convenient measure to describe the orientation of the C6–O6 bond of each glucose residue is torsion angle O5n–C5n–C6n–O6n, as shown in Table 2. Gn denotes the nth glucosidic residue of the β-CD. For β-CD(A), five of the seven primary hydroxyl groups exhibit the (+)-gauche orientation pointing inwards of the β-CD cavity, as shown by the torsion angle of O5n–C5n–C6n–O6n in the range of 52.515° to 66.224°. One of them (O24–H) has a (−)-gauche orientation pointing outwards, as indicated by the corresponding torsion angle of −63.470°. The last one is disordered at two sites, among which one points outwards

Table 2 Geometrical parameters of inclusion complex of β-CD with sorbic acid (distance in Å and angles in°)

	G1	G2	G3	G4	G5	G6	G7
Residue A							
D[a]	4.406	4.315	4.490	4.307	4.324	4.520	4.248
φ[b]	125.558	127.948	132.552	127.662	123.552	133.504	129.140
d[c]	−0.043	0.022	0.004	−0.017	0.019	−0.027	0.042
D[d]	2.799	2.838	2.696	2.769	2.799	2.790	2.821
Torsion angle O5n–C5n–C6n–O6n	65.643	72.553 (−62.509)	65.117	52.515	−63.470	66.224	65.535
Residue B							
D[a]	4.415	4.374	4.289	4.460	4.286	4.367	4.304
φ[b]	130.296	124.875	130.118	129.883	127.277	128.242	129.223
d[c]	−0.034	−0.009	0.058	−0.045	−0.006	0.021	0.015
D[d]	2.787	2.799	2.793	2.781	2.800	2.719	2.762
Torsion angle O5n–C5n–C6n–O6n	57.339	63.010	67.535	66.615	67.249	−62.966 (60.684)	66.528

[a] Distance between atoms O4n···O4(n + 1)

[b] Angles between atoms O4(n − 1)···O4n···O4(n + 1)

[c] Deviations (Å) from the least-squares optimum plane of the seven O4n atoms

[d] Intramolecular hydrogen-bond distance between O3n···O2(n + 1)

from the β-CD cavity, and the other points inwards with the occupation of 0.542 (O9) and 0.458 (O9′), respectively. For β-CD(B), 6 primary hydroxyl groups point inwards of the β-CD cavity, and the (O64–H) is disordered at two sites, among which one points outwards from the β-CD cavity, and the other one points inwards with the occupation of 0.559 (O64) and 0.401 (O64′), respectively.

Two guest molecules reside within the dimer cavity in similar manner with the carboxyl groups protruding from the primary ends of β-CDs. Their plane are inclined about 66.81° and 62.13° with respect to the O4 plane of β-CD (A), β-CD (B), respectively.

Network of O–H···O hydrogen bonds and the role of water molecules

Owing to the existence of O2, O3, O6 hydroxyl groups in glucoses and two hydroxyl groups in water molecules, which can act simultaneously as hydrogen bonding donor or as acceptor, so a large number of O–H···O hydrogen bonds have been found in the cyclodextrin hydrate crystal structure. In the peracylated β-CDs, the macromolecular structure is stabilized by inter and intramolecular O–H···O hydrogen bonds between adjacent acylglucose units, which is a common feature found in carbohydrate crystal structure with a high density of acceptor O atoms [10]. In this work, the hydrogen-bond parameters d (H···A), d (D···A) and ∠DHA, are shown in the Table 3.

Hydrogen bonds among different β-CDs play two different roles. One of them forms interaction between two β-CD monomers in the dimer through O3 (n)–H···O3 (m), including O8–H8, O18–H18, O23–H23, O33–H33, O38–H38, O43–H43, O47–H47, O58–H58. The other contributes to the stabilization of the crystal packing rather than to the stability of the complex, such as O4–H4 and O14–H14. Thus the H-bonding inside and outside β-CD strengthen the association of the macrocycles within and between the columns.

All 25.5 water molecules are located outside the cyclodextrin cavity over 31 sites. Yet it is difficult to find that some water molecules exist as mediators, which play an important role in crystal stabilization. Every water molecule has no option but to form O–H···O (W) interaction with another O–H of the β-CD or the guest molecule.

Hence, it can be seen that sorbic acid molecule is held in the cavity primarily by hydrophobic forces and weak O–H···O (W) interaction.

Crystal packing

The overall shape and packing of the β-CD macrocycle in crystal lattices are affected by the size and character of the respective guest molecules. According to Mentzafos and

Table 3 Intermolecular hydrogen bonds (distance in Å and angles in°, D-donor atom, A-acceptor atom, H-hydrogen atom)

D–H	d(H···A)	∠DHA	d(D···A)	A
Between sorbic acid and water molecules				
O71–H71A	2.206	121.2	2.549	W91[x, y, z]
O74–H74A	1.740	169.0	2.575	W93[x, y, z]
Between two β-CD molecules				
O4–H4	2.040	172.8	2.855	O19[x, y, z]
O8–H8	1.982	164.2	2.781	O43[x, y, z]
O14–H14	1.935	173.2	2.752	O29[x, y, z]
O18–H18	2.059	150.0	2.799	O68[x, y, z]
O23–H23	2.097	163.4	2.893	O63[x, y, z]
O33–H33	2.034	152.5	2.787	O53[x, y, z]
O38–H38	1.947	168.3	2.755	O13[x, y, z]
O43–H43	2.011	159.0	2.781	O8[x, y, z]
O47–H47	2.489	122.6	3.011	O3[x, y, z]
O57–H57	1.953	169.7	2.794	O12[x, y, z]
O58–H58	1.889	166.7	2.693	O28[x, y, z]
O59–H59	2.299	130.3	2.897	O39[x, y, z]
O67–H67	1.995	154.2	2.756	O2[x, y, z]
O69–H69	1.978	155.1	2.745	O49[x, y, z]
Between β-CD molecules and water molecules				
O17–H17	2.061	131.7	2.677	W86[x, y, z]
O19–H19	2.007	161.2	2.796	W94[x, y, z]
O29–H29	1.986	153.4	2.745	W90[x, y, z]
O32–H32	1.994	147.7	2.724	W77[x, y, z]
O34–H34	2.329	116.8	2.797	W89[x, y, z]
O39–H39	1.882	164.3	2.680	W80[x, y, z]
O44–H44	1.925	166.2	2.728	W75[x, y, z]
O47–H47	2.475	107.8	2.835	W88[x, y, z]
O49–H49	1.911	174.2	2.728	W78[x, y, z]
O52–H52	2.394	112.1	2.708	W92[x, y, z]

co-workers, four packing modes of β-CD dimeric complexes have been categorized: channel, screw-channel, chessboard and intermediate [11]. Such variety in the crystal packing has been interpreted to be caused by differences in the lateral displacement of the host molecules.

Figure 3 displays the crystal packing form of the β-CD-sorbic acid inclusion complex. Clearly, β-CDs are not exclusively packed in channel type as frequently found when the guest is linear and long, which can not be entirely engulfed in one β-CD cavity [12, 13]. By contrast, β-CDs of dimers here are packed in intermediate mode. Similar mode can be found in the complexes of β-CD with 1-adamantanecarboxylic acid [14].

NMR study

^{1}H NMR spectroscopy is the most widely used technique to gain information about inclusion modes and geometries

Fig. 3 Projections of dimers for β-CD-sorbic acid complex. **a** Projection along the *b*-axis. **b** Projection along the *c*-axis
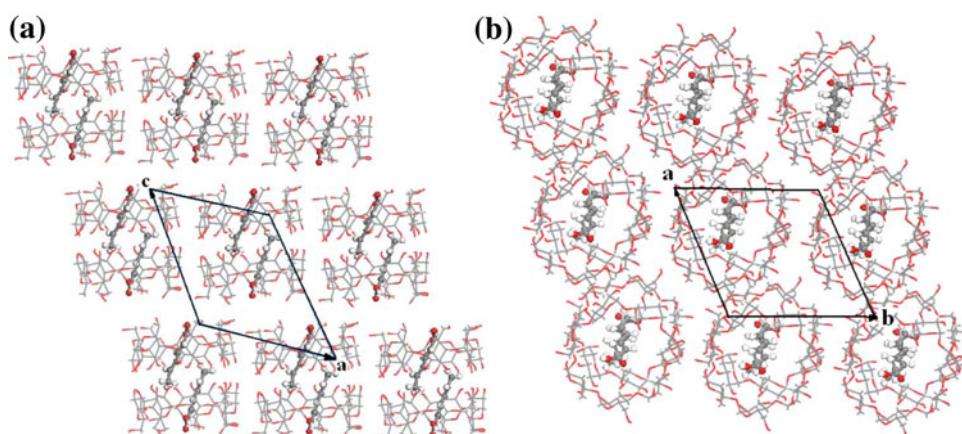

[15]. It is very helpful to provide evidence of the information of inclusion complexes in aqueous solution, since the proton environments in both host and guest molecules will be affected by their interaction, thus will be reflected by chemical shift variations of protons from both species.

For the sake of the comparison, the spectra of β-CD and sorbic acid as well as their complex are shown in Fig. 4, while the spectral characteristics of β-cyclodextrin and sorbic acid are illustrated in Table 4.

As can be seen from Table 4, it is obvious to find that all the chemical shifts of protons are downfield. In the presence of sorbic acid, H-1 and H-6 of the β-CD exhibit substantial changes, and they undergo strong shielding exceeding 0.10 ppm. The fact that the chemical shifts of them are greater than that of H-3 (0.06 ppm) and H-5 (0.07 ppm) demonstrates that the methyl group and carboxyl group of sorbic acid can not be fully included by β-CD, in other words, they protrude from the primary face

Fig. 4 500 MHz ^{1}H NMR spectra in the region 0.5–8.0 ppm for inclusion complex of sorbic acid
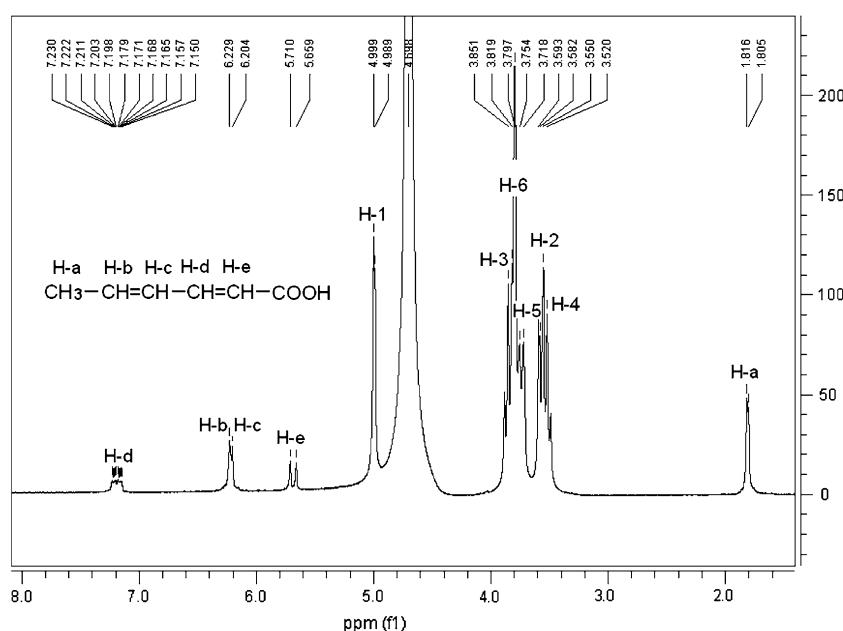
Table 4 Chemical shift data (δ in ppm) of protons in sorbic acid, β-cyclodextrin and in the complex

β-cyclodextrin/ppm						Sorbic acid/ppm				
	H1	H2	H3	H4	H5	H6	H-a	H-b	H-c	H-d	H-e
δ_{free}	4.90	3.47	3.79	3.41	3.68	3.70	1.70	6.17	6.15	7.17	5.70
$\delta_{complex}$	5.00	3.57	3.85	3.52	3.75	3.80	1.81	6.23	6.20	7.20	5.71
$\triangle\delta$	0.10	0.10	0.06	0.11	0.07	0.10	0.11	0.06	0.05	0.03	0.01

and secondary face. This result is consistent with conclusion of the crystal structure. The reason of the abnormal changes of proton H-2, H-4 is likely attributable to deshielding effect of hydroxyl groups of accumulative water molecules which are located outside the β-cyclodextrin cavity. Consequently, their chemical shifts increase greatly. As distinguished from other works [16, 17], H-3 and H-5 of our work shift downfield, that is to say, they were situated in the state of deshielding for the conjugative effect, so it is concluded that they approximate to the plane of guest. As can be seen from Fig. 2, the sorbic acid passes aslant through the cavity of β-CD.

On the other hand, the chemical shift of proton H-a of the sorbic acid, in the presence of β-CD, is downfield ($\triangle\delta = 0.11$ ppm), whereas the other protons are not affected by their surrounding environment. The unusual phenomenon above can not be interpreted owing to our deficiency in the mutual interaction between organic molecules of different structures.

In addition, according to the ^{1}H NMR spectra in Fig. 4, the area ratio of the H-a signal of the sorbic acid versus H1 signal of β-CD is 3:7 (area is not shown here), and there are seven H-1 atoms in each β-CD molecule. Therefore, it can be concluded that the stoichiometry of the complex is 1:1.

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