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Crystal structure determination of the β-cyclodextrin–*p*-aminobenzoic acid inclusion complex from powder X-ray diffraction data

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ABSTRACT

The 1:1 inclusion complex of β-cyclodextrin and *p*-aminobenzoic acid was prepared and characterized by TG-DTA. The crystal structure of the complex was solved directly from powder X-ray diffraction data using the direct space approach and refined using Rietveld refinement techniques. The complex crystallizes in monoclinic $P2_1$ space group, with unit cell parameters a = 20.7890 Å, b = 10.2084 Å, c = 15.1091 Å, β = 110.825°, V = 2997 Å^3. The amino group is located at the wide side of the β-cyclodextrin cavity, forming hydrogen bonds with β-cyclodextrin, and the carboxyl group is located at the narrow side. The crystallographic data obtained from powder diffraction data were compared with the single crystallographic data, and the result shows that solving crystal structure of cyclodextrins inclusion complexes of such complexity is accessible to powder diffractionists to some extent.

Traditionally, crystal structure of cyclodextrin (CD) inclusion complexes can be determined from single crystal X-ray diffraction techniques,[1–4] which are the most straightforward tool for elucidating crystal and molecular structures. However, it is difficult and time consuming to prepare single crystals of CD inclusion complexes and the crystals are easily dehydrated.[5] Herein, starting from the methodological point of view, we wish to develop an easy and efficient way to solve the crystal structure of CD inclusion complexes. The crystal structure of the β-CD–*p*-aminobenzoic acid inclusion complex was directly determined from powder X-ray diffraction data using the direct space approach, and the result was compared with the single crystallographic data of the β-CD–*p*-aminobenzoic acid inclusion complex, which was determined from single crystal diffraction data by Zhang et al.[6]

TG-DTA analysis: Thermal analysis can be used as a routine method for the recognition of inclusion complexation. TG-DTA analysis was adopted to characterize the formation and the phase purity of the inclusion complex and to explore the crystallization water content in the inclusion complex. The TG and DTA curves for β-CD, *p*-aminobenzoic acid, the inclusion complex, and the 1:1 physical mixture are illustrated in Figure 1.

Two endothermic peaks are observed in the thermal decomposition of inclusion complex (Fig. 1a). The first peak between 70 and 107 °C corresponds to the dehydration of the complex, with a 12.4% mass loss in this stage, which indicates that the complex contains 10 mol of crystallization water. There is no further mass loss in the temperature range of 110–300 °C, which indicates that the *p*-aminobenzoic acid gains additional thermal stability after inclusion complexation into the β-CD cavity, because the melting point of *p*-aminobenzoic acid is ~189 °C (Fig. 1b). The second stage is related to the liberation of the small molecule and the degradation of the β-CD structure.

In the thermal decomposition of β-CD (Fig. 1b), a small endothermic peak was observed at 225 °C without any weight loss; this represents a physical process and is attributed to the reversible transformation of β-CD.[7] In the thermal decomposition of 1:1 mechanical mixture (Fig. 1a), there is a gradual mass loss between 185 and 300 °C corresponding to the melt of *p*-aminobenzoic acid. Thermal analysis proves the formation of the β-CD–*p*-aminobenzoic acid inclusion complex and confirms the inclusion complex is of a single phase. The final stoichiometry is determined by combining thermal analysis and elemental analysis, and the result is $C_{42}H_{70}O_{35}\cdot C_7H_7NO_2\cdot 10H_2O$. However, Zhang reported that the β-CD–*p*-aminobenzoic acid inclusion complex contained five crystallization water molecules in each asymmetric unit. Because the amount of the crystallization water in CDs can be affected by ambient conditions,[8] it is reliable to analyze the amount of the crystallization water by using thermal method for complicated polyhydrates. Thermal methods can also be used to determine the content of crystallized water in single crystal X-ray diffraction techniques as additional evidence. To find the origin of the different crystallization water in the above mentioned complexes, the crystallization water content needs further study. An ideal approach for study of the crystallization water is to prepare the single crystals and powder crystals of the studied complex and to collect the

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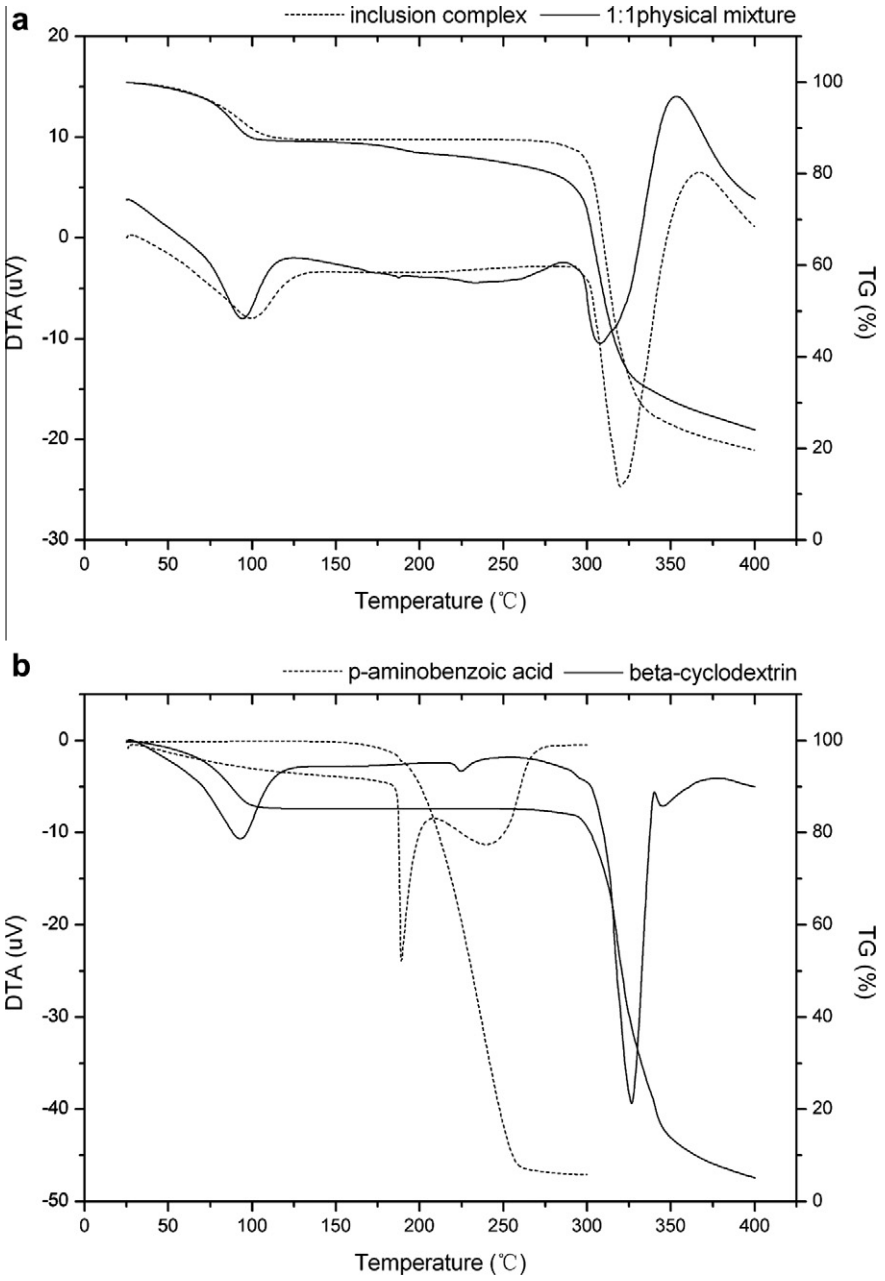

Figure 1. TG and DTA curves of (a) inclusion complex of and 1:1 physical mixture; (b) *p*-aminobenzoic acid and β-CD.

diffraction data in the same experimental conditions. Moreover, the combined use of temperature variable X-ray diffraction and thermal analysis may be applied to study the crystallization water content of such complicated polyhydrates if the corresponding single crystals could not been obtained.

Crystal structure determination: During the Powder Solve and Rietveld refinement stage, each glucose unit, together with its secondary O atoms, was defined as a separate rigid-body and rotation and translation were allowed relative only to glycosidic plane. Because the primary units of the β-CD molecule are more flexible, they were not included in the rigid-body definition. After the Rietveld refinement, comparison between the measured X-ray diffraction pattern and the calculated pattern for the fully refined structure is shown in Figure 2. The final *Rwp* and *Rp* of the Rietveld refinement were 7.84% and 5.94%, respectively, indicating the reliability of the refinement.[9] The crystallographic data are summarized in Table 1.

From the crystallographic data, we find that the crystal system and the space group are consistent with those of single crystal. We take the results of single crystal as the accurate values, and use the relative error to represent the differences between the results of this study and the single crystal. Thus, comparing powder data with single data, the relative errors of length *a*, *b*, *c* and angle β are 0.60%, 0.23%, 0.66%, and 0.096%, respectively. These differences may result from the different diffraction conditions, the different crystallization conditions, and the different data processing algorithms in our software. The three-dimensional single crystal diffraction data are compressed into one dimension in the powder diffraction pattern, which is also a reason for these differences. Furthermore, in the diffraction experiment, the transmission mode may be adopted to improve the resolution of the diffraction data, which can further improve the accuracy of the structure determination.

Geometry of the inclusion complex: As shown in Figure 3(a), the asymmetric unit consists of one β-CD, one *p*-aminobenzoic acid,

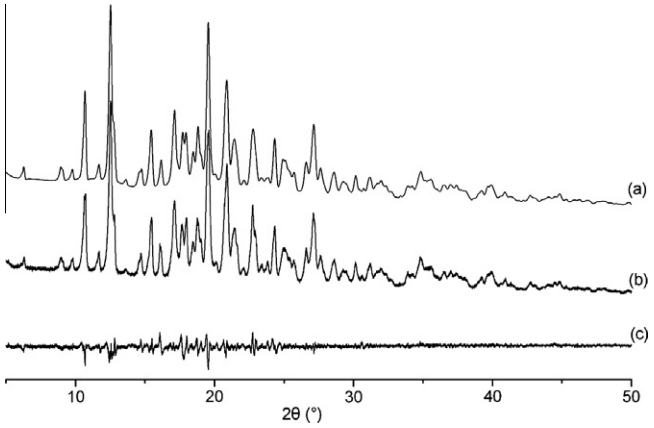

Figure 2. Comparison between the measured XRD pattern and the calculated pattern (a) measured pattern; (b) calculated pattern; (c) difference between them.

Table 1
Crystallographic data

Chemical formula	$C_{42}H_{70}O_{35}{\cdot}C_7H_7NO_2{\cdot}10H_2O$
Formula weight	1452
Crystal system	Monoclinic
Space group	$P2_1$
a (Å)	20.7890
b (Å)	10.2084
c (Å)	15.1091
β (°)	110.825
Volume (Å^3)	2997
Z	2

and 10 water molecules. All glucose residues are in the usual $^4C_1$ chair conformation, and the overall β-CD molecule has an approximate sevenfold axis. The structure of the studied inclusion complex obtained in Zhang's study is shown in Figure 3(b). The geometric parameters for the β-CD molecule are listed in Table 2.

From Table 2 we can see that the conformation of β-CD molecule is almost the same as that of the single crystal, which shows that the conformation of host molecule can be correctly determined from powder diffraction data. The conformation and the position relationship of the guest molecule are described as follows. The *p*-aminobenzoic acid molecule is embedded inside the β-CD cavity. The amino group is located at the wide side of the cavity, forming hydrogen bonds with β-CD, and the carboxyl group is located at the narrow side. Its molecule plane makes an angle of 50.08° with the O4 plane made by the seven O4n atoms of the β-CD, and the mass center of the aromatic ring is 1.108 Å away from the O4 plane center to the O6 side of β-CD. The orientation of the guest molecule is consistent with the single crystal, but the position and the angle with O4 plane are somewhat different from the single crystal data. As the guest molecule was docked to β-CD with Auto Dock (Fig. 4), which can only find an approximate binding site for the two molecules. Utilizing more accurate docking techniques or further optimizing the docking result may improve this situation. Nevertheless, the crystal structure determination from the powder diffraction data for CD inclusion complexes of such complexity is accessible to a certain extent. Solving the crystal structure of the β-CD–*p*-aminobenzoic acid inclusion complex is a benefit of the powerful model building tools, and it will become more accurate and common with the development of model building tools.

The intermolecular hydrogen bonds of β-CD with the guest molecule and water molecules are listed in Table 3. The guest is held in the cavity mainly by hydrogen bonds and van der Waals contacts. All the crystallization water molecules are located outside the β-CD cavity. Water molecules form extensive hydrogen bonding networks in the crystal structure. The host molecules are stacked along the crystallographic *b*-axis in cage type mode.

1. Experimental

1.1. Materials and methods

β-CD was purchased from Tianjin Bodi Chemical Holding Co. Ltd, China, and re-crystallized twice from distilled water prior to use. *p*-Aminobenzoic acid was purchased from Chengdu Kelong Chemical Reagent Co. Ltd, China and used as received.

Powder X-ray diffraction data were performed on an X' Pert Pro MRD (PANalytical) diffractometer. Elemental analysis was conducted by the EURO EA300 elemental analyzer. TG-DTA analysis was performed on an EXSTAR 6000 analyzer, using a heating rate of 10 °C/min in flowing air atmosphere, with a temperature range of 25–400 °C. All the structure solution work was performed on Materials Studio 4.0 (Accelrys) in the State Key Laboratory of Polymer Materials Engineering (Sichuan University, China).

1.2. Synthesis of the inclusion compound

β-CD (5 g) was dissolved in water (50 mL) at 55 °C, while *p*-aminobenzoic acid (0.7 g) was dissolved in ethanol, and then the two solutions were mixed and stirred at 50–55 °C for 4 h. The mixture was filtered at this temperature, then cooled to 5 °C, and kept for 12 h. The resulting suspension was filtered and the white polycrystalline powder was washed by ethanol and air dried. Anal. Calcd for $C_{42}H_{70}O_{35}{\cdot}C_7H_7NO_2{\cdot}10H_2O$: C, 40.50; H,6.68; N, 0.96. Found: C, 40.12; H, 6.88; N, 0.95.

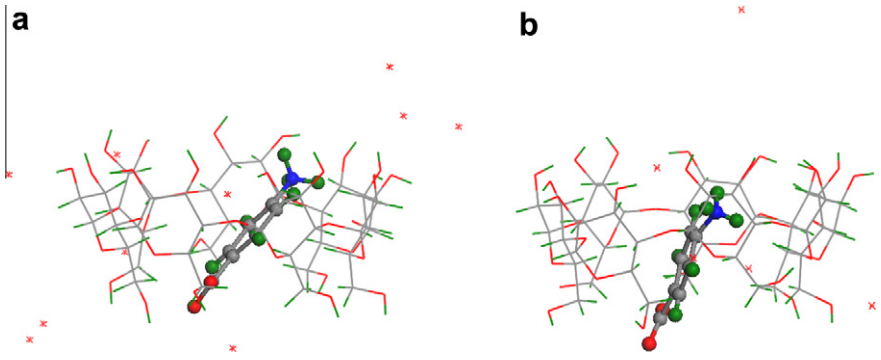

Figure 3. Structures of the inclusion complex determined from (a) powder X-ray diffraction and (b) single X-ray diffraction.

Table 2
Geometrical parameters for the β-CD molecule

	Residue						
	1	**2**	**3**	**4**	**5**	**6**	**7**
D^a (Å)	−0.197	−0.049	0.273	−0.114	−0.195	0.191	0.092
D^b (Å)	2.891	2.785	2.770	2.858	2.842	2.943	2.862
D^c (Å)	4.436	4.440	4.237	4.341	4.475	4.387	4.287
D^d (Å)	5.113	4.848	5.049	5.173	4.904	4.979	5.147
φ (°)	126.09	132.31	127.61	125.22	131.47	128.33	127.22
Torsion angle 1 (°)	50.17	48.38	59.07	59.21	55.55	179.36	61.65
Torsion angle 2 (°)	−67.26	−70.92	−60.95	−60.78	−67.17	61.57	−58.09

D^a = deviation of O4n atom from the least-squares optimum plane of the seven O4n atoms; D^b = distance between atoms O3n···O2(n + 1); D^c = distance between atoms O4n···O4(n + 1); D^d = distance between the centroid of seven O4 atoms and each individual O4 atom; φ = angle between O4(n − 1)···O4n···O4(n + 1); torsion angle 1: C4n–C5n–C6n–O6n; Torsion angle 2: O5n–C5n–C6n–O6n.

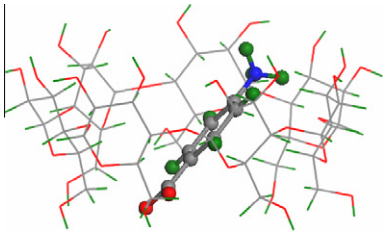

Figure 4. The structure of the complex obtained from Auto Dock.

Table 3
Hydrogen bonds of β-CD with the guest molecule and water molecules

D–H	D(H···A) (Å)	∠DHA (°)	D(D···A) (Å)	A
Between β-CD and p-aminobenzoic acid molecule				
N154–H156	2.158	139.46	3.087	O47
N154–H157	1.892	156.96	2.945	O60
N154–H155	2.325	150.40	3.337	O′47
Between β-CD and water molecules				
O146–H147	1.944	157.60	2.846	Ow173
O114–H115	1.758	115.93	2.335	Ow166
O138–H139	2.804	123.53	3.421	Ow′169
O138–H139	2.124	137.87	2.899	Ow′173
O13–H14	1.980	145.72	2.816	Ow′171
O82–H83	2.622	124.91	3.260	Ow′174
O1–H2	2.893	113.94	3.406	Ow′167
O30–H31	2.473	148.07	3.319	Ow′173
O43–H44	2.384	127.74	3.060	Ow′165

O means in the same asymmetric unit; O′ means in the different asymmetric unit; Ow means water molecule in the asymmetric.

1.3. Powder X-ray diffraction experiment

Powder X-ray diffraction measurement was performed on an X' Pert Pro MRD diffractometer using CuKα radiation (λ_1 = 0.154056 nm) with an X'celerator detection system, operating at 40 kV, 40 mA. The diffraction data were recorded at 295 K with a step size of 0.006565° (2θ) and a counting time 11.22 ms per step from 5° to 50° in 2θ.

1.4. Calculation of molecular structure and crystal structure

1.4.1. Preliminary work

Computational chemistry has been widely utilized in the CD field for the prediction of the geometry of the complexes and the estimation of binding energies.[10] The molecular structure of β-CD was extracted from the single crystal structure of β-CD hydrate clathrate obtained from the Cambridge Structural Database (Ref code: BCDEX03), and the structure of *p*-aminobenzoic acid was created by using Materials Studio 4.0. The structure of both β-CD and *p*-aminobenzoic acid were optimized and prepared for docking in Auto Dock 4.0.[11] The guest molecule has been docked to β-CD with Auto Dock 4.0 by using Lamarckian genetic algorithm. The resulting molecular structure was used as the initial molecular structure for solving the crystal structure.

1.4.2. Indexing

The pretreated powder diffraction pattern was indexed by X-CELL method.[12,13] The results and the calculated density of the candidate structures are listed in Table 4. However, the density of organic crystals is usually in the range of 1.1–1.9 g cm^{-3}. According to the calculated density, we chose candidate structures 3 and **4** to carry out Pawley refinement. Both of the *Rwp* values of the two candidate structures were 2.95%, and then we chose candidate structure **3** for the following structure determination.

1.4.3. Powder Solve and refinement

MC/SA search algorithm in Powder Solve package[14] was used to constantly adjust the conformation, position, and orientation of the trial model in the unit cell determined from the indexing step in order to maximize the agreement between the calculated and the measured diffraction data. The structural solution obtained from

Table 4
Crystal structure of inclusion complex from indexing

No.	FOM	System	*a* (Å)	*b* (Å)	*c* (Å)	*β* (°)	*Dc* (g/cm^3)	Space group
1	336	Monoclinic	21.0626	10.2306	29.9379	109.51	3.17	C2/c
2	229	Monoclinic	15.2757	39.1101	10.2900	90.42	3.14	C2/c
3	227	Monoclinic	21.1121	10.2121	15.1733	111.75	1.59	P21
4	226	Monoclinic	20.9420	10.2123	15.1730	110.55	1.59	P21
5	223	Monoclinic	20.9437	10.2255	14.9234	109.42	3.20	C2

FOM: Figure of merit.

the Powder Solve step was subsequently refined by Rietveld refinement techniques based on the measured powder X-ray diffraction pattern. In the Rietveld refinement, cell parameters, atomic fraction coordinates, thermal vibration, and preferred orientation parameters were optimized to get an optimum crystal structure.

Supplementary data

Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 798968. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK. (fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk or via: www.ccdc.cam.ac.uk).

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