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The Change of the Structure of Amylose During the Inclusion of 2-Naphthol in Sealed-heating Process

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Abstract

When a mixture of linear amylose (AS200) and 2-naphthol was heated in a glass ampoule above 60 °C, an inclusion compound was formed. The inclusion formation accompanied the structural change of AS200 from a 6_1-helix to a 7_1-helix. From the UV determination, one 2-naphthol molecule could be included per one 7_1-helical turn of amylose. It was found that the heating temperature, heating time and water content in the physical mixture were important parameters affecting the formation of inclusion compounds during sealed-heating.

Introduction

Amylose, a linear polysaccharide consisting of α-1,4-linked D-(+)-glucopyranoses, has been obtained by separation and purification from native starch. The three crystalline forms are referred to as A-, B- and V-amylose [1–11]. Native amylose has a double helix structure in the crystal, and is classified into the A- or B-amylose [1, 2]. V-amylose, a single helix structure, has been found when amylose formed inclusion compound with a variety of organic compounds [3–11].

We have developed the sealed-heating method for the preparation of solid inclusion compounds of cyclodextrin [12–15]. We have already reported that complexation was affected by the vapour pressure of the guest compound, the crystallinity of the cyclodextrin and the water content during the sealed-heating. In the previous paper, we dealt with amylose, and demonstrated that host amylose had a 7_1- and 8_1-helix structure in the sealed-heated samples depending on the amount of salicylic acid loaded [4].

In the present study, the effects of the amount of guest, the heating temperature and the water content of the systems on inclusion compound formation by the sealed-heating method were investigated by using 2-naphthol (2-NPL) as a guest.

Experimental

Materials

Amylose of molecular weight 220,000 (AS200) was used. AS200 which has no α-1,6 branches in the structure was enzymatically synthesized by Nakano Vinegar Co.,Ltd (Aichi, Japan). The particle size of AS200 was controlled between 42.5 and 150 μm by sieving. 2-Naphthol (2-NPL; Nacalai Tesque, Japan) of reagent grade was ground with a mortar and pestle before use.

Sample preparation and sealed-heating procedure

A physical mixture was prepared at various mixing ratios of AS200 and 2-NPL in glass vials using a vortex mixer for 5 min. Pretreatment of AS200 was performed by drying at 100 °C for 3 h in a vacuum or by humidifying at 22% relative humidity at 25 °C for one day. The water contents of the mixtures with dried and with humidified AS200 were estimated as 1.0 ± 0.1% and 5.0 ± 0.1%, respectively, by the Karl Fischer method using an Aquacounter: AQ-5 (Hiranuma, Japan). The physical mixtures of 1.0 ± 0.1% and 5.0 ± 0.1% water content were defined as a physical mixture of a lower and higher water content, respectively. Each physical mixture (about 250 mg) was sealed in a glass ampoule (2 mL) and heated in the gas chromatograph oven at various temperatures for a definite time. The temperature around the glass ampoule in the oven was monitored by a thermocouple.

Powder X-ray diffraction

The powder X-ray diffractograms were measured on a Rigaku Miniflex diffractometer (Cu-Kα, voltage 30 kV, current 15 mA, scanning speed 4 degrees min^{-1}).

Determination of 2-NPL

In order to determine the amount of 2-NPL included, the samples were dispersed in phosphate buffer (pH = 6.8) or *n*-hexane, and the suspensions were sonicated for 30 min. After the filtration through a membrane filter (1.0 μm pore

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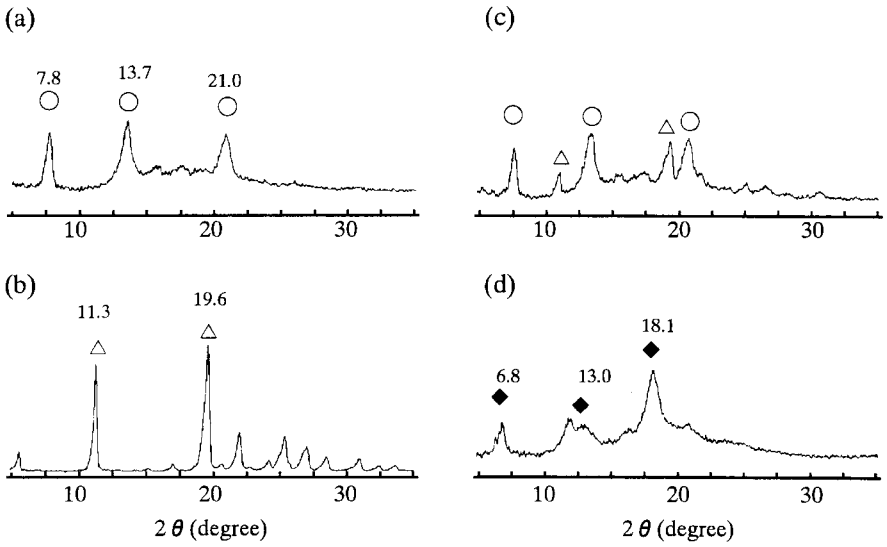

Figure 1. Change in the powder X-ray diffraction (XRD) patterns of AS200: 2-NPL (7 glucose units: 2-NPL = 1 : 1) system by the sealed-heating process. (a) AS200 (6_1-helix amylose), (b) 2-NPL. (c) physical mixture (PM), (d) sealed-heated (SH) sample at 100 °C for 1 h.

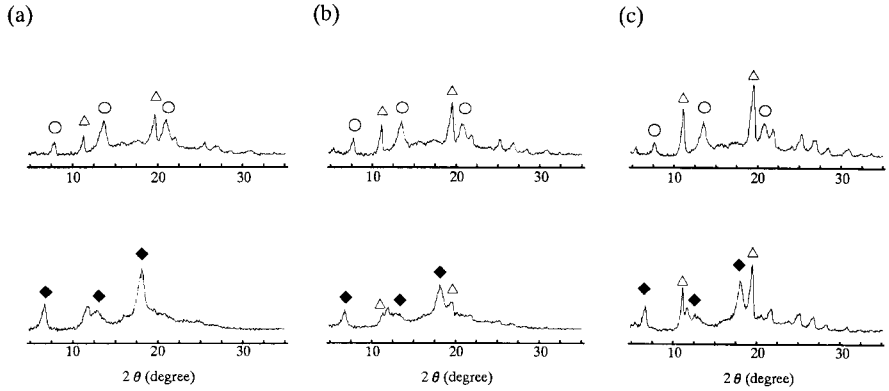

Figure 2. Powder XRD patterns of physical mixtures (upper parts) and sealed-heated samples (lower parts) of (a) 1 : 1 : 5, (b) 1 : 2, (c) 1 : 4 (7 glucose units: 2-NPL) (the sealed heating was carried out at 100 °C for 1 hr.)

size), the 2-NPL concentration in each solution was determined spectrophotometrically at 273 nm, the absorption maximun of 2-NPL, using a UV-visible recording spectrometer: UV-160 (Shimadzu, Japan).

Results and discussion

Inclusion compound formation by sealed-heating process

Figure 1 (a) and (b) shows the powder X-ray diffraction (XRD) patterns of AS200 and 2-NPL crystals, respectively. AS200 showed rather broad diffraction peaks at 2 θ = 7.8, 13.7 and 21.0° due to the 6_1-helix structure of V-amylose (indicated by open circles), while 2-NPL showed characteristic diffraction peaks at 2θ = 11.3 and 19.6° (indicated by open triangles). Sealed-heating of AS200 alone showed no change in the XRD pattern (data not shown). Figure 1 (c) and (d) shows the powder XRD patterns of the physical mixture of AS200-2-NPL (7 glucose units: 2-NPL = 1 : 1) before and after sealed-heating at 100 °C for 1 h, respectively. The diffractogram of the physical mixture was a superimposed pattern of the two components, 2-NPL and the 6_1-helix structure of amylose. After heating the physical mixture at 100 °C for 1 h, powder XRD peaks of the 6_1-helix structure of AS200 and of the 2-NPL crystals disappeared. On the other hand, new diffraction peaks appeared at 2θ = 6.8, 13.0 and 18.1° (indicated by closed diamonds). Since 7_1-helix amylose shows characteristic peaks at the same diffraction angles [4–5], complexation between AS200 and 2-NPL occurred together with the helical structural change from the 6_1- to the 7_1-helix.

The physical mixtures at various mixing ratios (7 glucose units: 2-NPL = 1 : 1.5, 1 : 2 and 1 : 4) were sealed-heated at 100 °C for 1 h and the XRD changes are shown in Figure 2. In the 1 : 1.5 system, powder XRD peaks of the 2-NPL crystal disappeared after sealed-heating, accompanying the change from the 6_1- to the 7_1-helix structure of amylose. In the cases of the 1 : 2 and 1 : 4 systems, however, XRD peaks due to 7_1-helix amylose and XRD peaks due to 2-NPL crystals were observed after the heat treatment. These results suggested that the amylose helix type of sealed-heated samples were not influenced by the load of the guest compounds in the AS200: 2-NPL system.

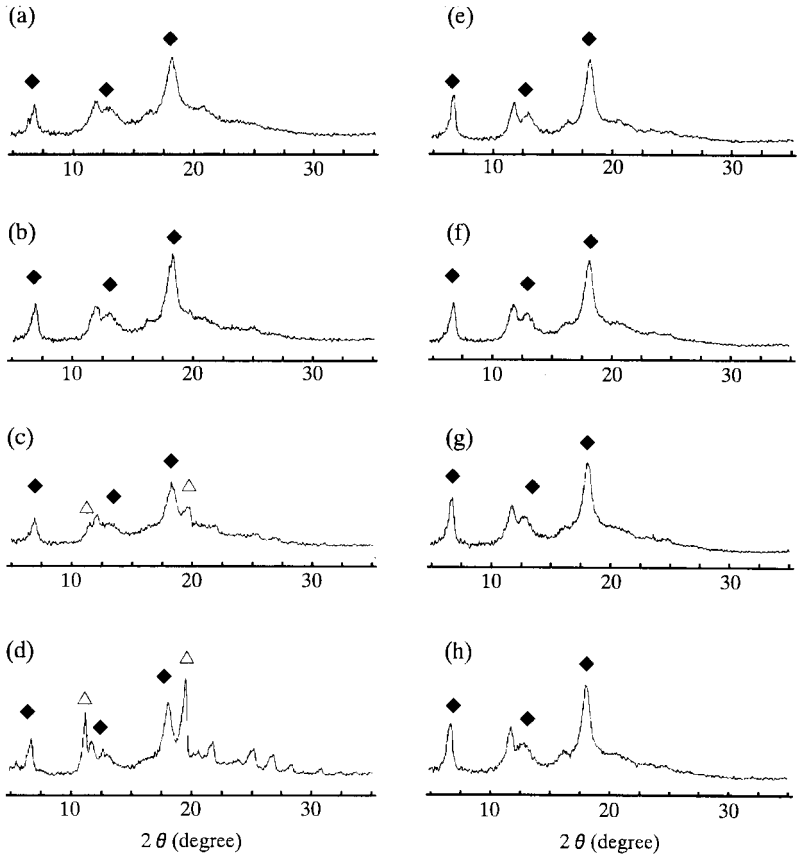

Figure 3. Change in the powder XRD patterns of sealed-heated samples before and after washing with *n*-hexane (a) SH sample of 1 : 1 (7 glucose units: 2-NPL) system, (b) SH sample of 1 : 1.5 system, (c) SH sample of 1 : 2 system, (d) SH sample of 1 : 4 system, (e) after washing sample (a) with *n*-hexane, (f) after washing sample (b) with *n*-hexane, (g) after washing sample (c) with *n*-hexane, (h) after washing (d) sample with *n*-hexane.

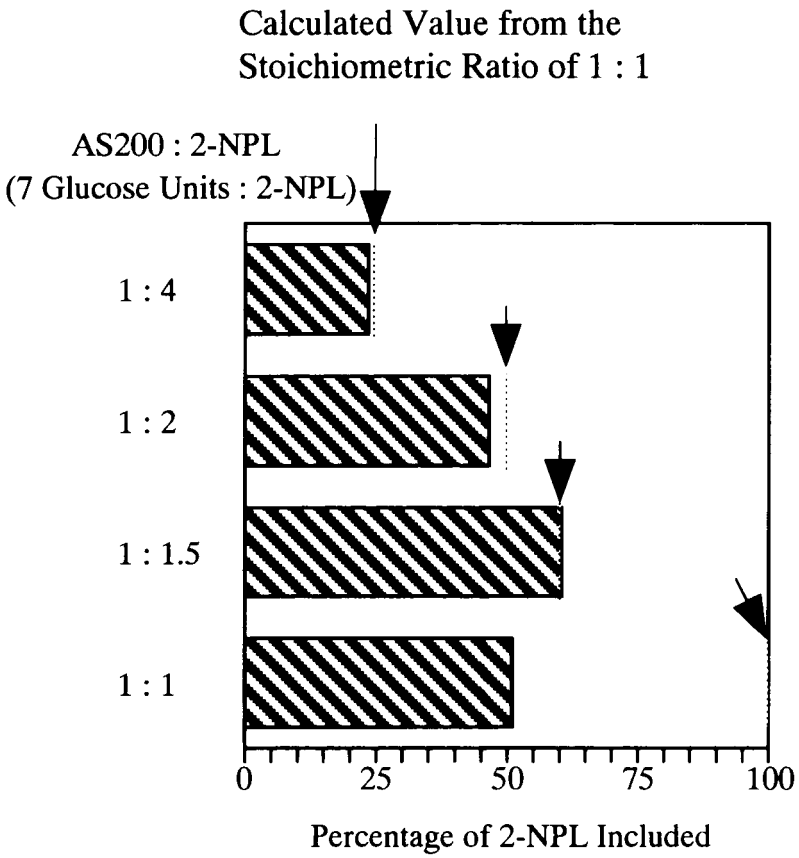

Figure 4. Fraction of 2-NPL included in sealed-heated samples with AS200 (the sealed-heating was carried out at 100 °C for 1 h.).

Table 1. Solvent extraction of 2-NPL from sealed-heated samples with AS200

Interaction mode of 2-NPL with AS200	Solvent	
	Phosphate buffer	*n*-Hexane
No interaction	○	○
Interacting	○	×

○: extracted; ×: not extracted.

Figure 3 shows the powder XRD patterns of sealed-heated samples before and after washing with *n*-hexane. No significant changes after washing with *n*-hexane were found for the 1 : 1 and 1 : 1.5 systems. In the cases of the 1 : 2 and 1 : 4 systems, however, the XRD peaks at 19.6° due to 2-NPL crystals disappeared and only the XRD peaks due to the 7_1-helix structure were observed after washing with *n*-hexane. To determine the stoichiometry, each sealed-heated sample was dispersed in phosphate buffer (pH = 6.8) or *n*-hexane, and the amounts of 2-NPL dissolved were assayed by using UV spectroscopy. Since phosphate buffer dissolved not only 2-NPL but also AS200, the 2-NPL amount found in phosphate buffer was considered to be the total 2-NPL amount in the samples. From the results shown in Figure 3, it was assumed that *n*-hexane could dissolve only 2-NPL which was not included in AS200. The above assumptions are summarized in Table 1. Figure 4 illustrates the percentage of 2-NPL included in each sealed-heated sample. In the systems of mixing ratios of 1 : 4, 1 : 2, and 1 : 1.5 (7 glucose units: 2-NPL), the percentage of 2-NPL included was evaluated as 23.2, 46.5 and 60.5%, respectively. These values were in fair agreement with the calculated values on the assumption that one 2-NPL molecule was included in one 7_1-helical turn of amylose. In the 1 : 1 system, however, the percentage of the 2-NPL included was evaluated as 49.5%. This value corresponded to one 2-NPL molecule in two helical turns of 7_1-helix amylose (shown by arrows in Figure 4). When the 1 : 1 physical mixture was sealed-heated at 120 °C for 1 h and 6 h, the percentage of 2-NPL included was enhanced to 66.0% and 79.9%, respectively. The binding ratio of the inclusion compound between AS200 and 2-NPL approached to the ratio of 1 : 1 (one 7_1-helical turn: 2-NPL) by the using of accelerated heating conditions. These results suggested that the load of 2-NPL in the physical mixture affected the inclusion ratio.

Effect of heating temperature and water content on inclusion behavior

Nakai *et al.* reported that the heating temperature and the water content of cyclodextrin affected the inclusion compound formation in cyclodextrin-guest systems during the sealed-heating process [12]. To investigate the effect of the heating temperature and water content in the physical mixture on the inclusion compound formation of amylose, the physical mixtures of higher and lower water content were prepared and were sealed-heated at 40, 60, 80, 100 and 120 °C. Figure 5 shows powder XRD patterns of the physical mixtures

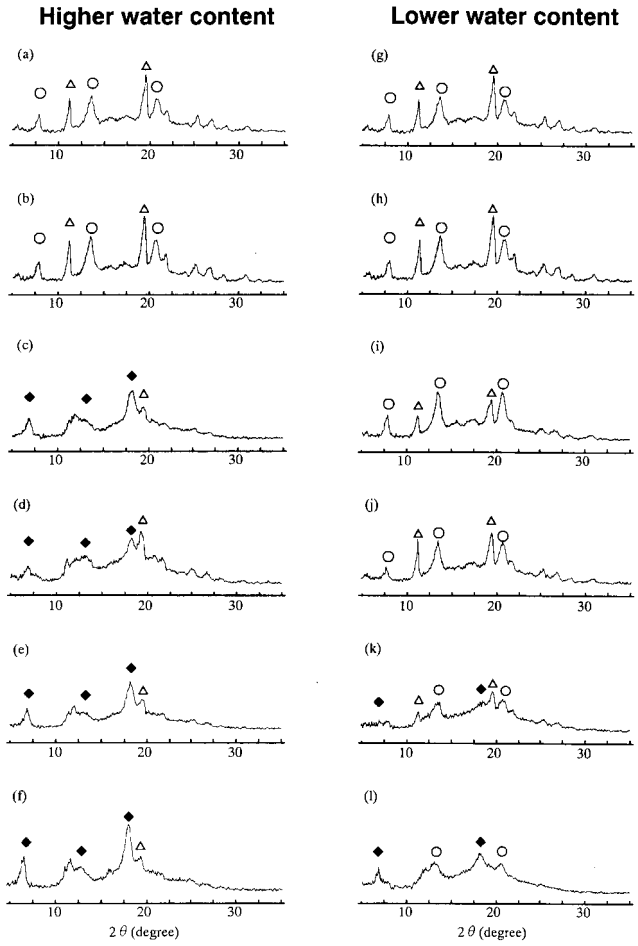

Figure 5. Effect of sealed-heating condition on the powder XRD pattern of sealed-heated samples of AS200: 2-NPL (7 glucose units: 2-NPL=1: 2) The diffraction patterns of the left hand side (from (a) to (f)) were of higher water content samples, while those of the right hand side were of lower water content samples. Heating condition: (a) physical mixture of higher water content, (b) 40 °C for 3 d, (c) 60 °C for 3 d, (d) 80 °C for 6 h, (e) 100 °C for 1 h, (f) 120 °C for 1 h, (g) physical mixture of lower water content, (h) 40 °C for 3 d, (i) 60 °C for 3 d, (j) 80 °C for 3 d, (k) 100 °C for 6 h, (l) 120 °C for 6 h.

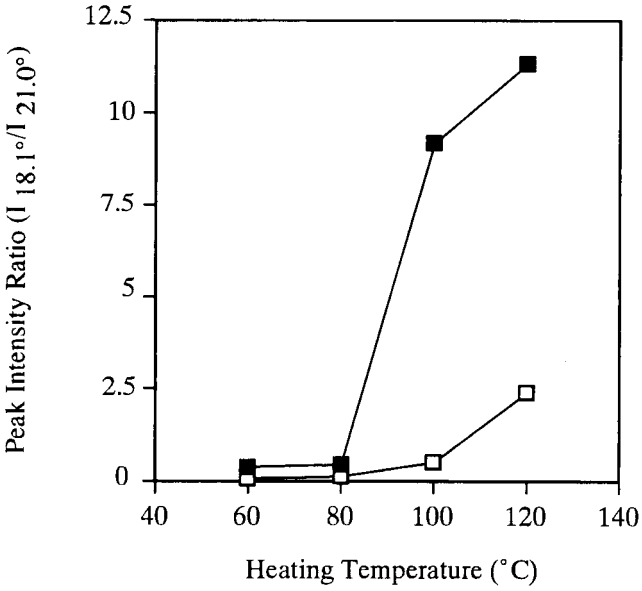

Figure 6. Effect of heating temperature on complex formation. ■: higher water content system, □: lower water content system.

of higher and lower water content after the heat treatment at various conditions. In the cases of higher water content, the sealed-heating at 40 °C did not cause the helical structure change of amylose even after 3 days of treatment. In the cases of sealed-heating at 60 and 80 °C, the powder XRD peaks due to 7_1-helical structure of amylose were observed after heating for 3 d and 6 h, respectively. When the physical mixture was heated at 100 or 120 °C, it took less than 1 h for the formation of the 7_1-helix inclusion compound. The physical mixtures of lower water content were also heated under the same condition. After the sealed-heating at 80 °C for 3 d, a 7_1-helix inclusion compound was not formed, while the sealed-heating at 100 and 120 °C for 6 h induced the inclusion compound formation.

Figure 6 shows the intensity ratio of the peak at 2θ = 18.1 ° due to the 7_1-helix structure to the 2θ = 21.0 ° peak due to the 6_1-helix structure after the sealed-heating for 3 h in ampoules. These results indicated that the 7_1-helix inclusion compound was readily formed for higher water content samples and at higher heating temperature. Water molecules were considered to have an important role for the breakage and rearrangements of the hydrogen-bonding network in the amylose crystal to form the 7_1-helix inclusion compound. The heating temperature could affect the vaporization of water molecules.

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