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De- and rehydration behavior of α,α-trehalose dihydrate under humidity-controlled atmospheres

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Abstract—Effects of humidity were investigated on de- and rehydration behavior of α,α-trehalose dihydrate (T_h) throughout simultaneous measurements of differential scanning calorimetry and X-ray diffractometry (DSC–XRD) and simultaneous thermogravimetry and differential thermal analysis (TG–DTA). When T_h was heated from room temperature under dry nitrogen atmosphere, a metastable anhydrous crystal (T_α) was formed at 105 °C after dehydration of T_h. The resulting T_α melted at 125 °C and became amorphous, followed by cold crystallization from 150 °C giving rise to a stable anhydrous crystal T_β. Under a highly humid atmosphere, on the other hand, T_β was formed at 90 °C directly as a result of T_h dehydration. T_α was readily rehydrated and turned back to T_h when nitrogen gas with low water vapor pressure of 2.1 kPa was admitted, whereas high water vapor pressure up to 7.4 kPa was required for rehydration of T_β into T_h. This study provided a picture of pathways that link various solid forms of trehalose, taking into account the effects of a humid environment.

Keywords: Trehalose; Phase transition; Crystallization; Dehydration; Rehydration

1. Introduction

α,α-Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) is a nonreducing disaccharide, naturally synthesized in various organisms such as mushrooms, yeasts, fungi, and insects.[1] One of the current interesting topics is that the larvae of *Polypedilum vanderplanki*, which breed in temporal rock pools in Northern Nigeria and Uganda, synthesize and accumulate in their bodies trehalose in amounts as high as 20% of the dry body weight when they become dormant in their completely dehydrated states. This process is called cryptobiosis.[2] The cryptobiotic larvae are able to revive themselves on rehydration, suggesting that intracellular accumulated trehalose confers the ability of desiccation tolerance even to high- and multicellular animals. Trehalose is also useful from a practical point of view, serving as a stabilizer of proteins[3] and membranes[4] in their dried states.

There have been two hypotheses, the water-replacement hypothesis[5] and the glassy state one,[6] to explain the protective effects of several carbohydrates on biological organisms and substances against dehydration stress. The former hypothesis states that as water molecules are removed during drying, carbohydrate molecules bind to proteins or lipid head groups of membranes via hydrogen-bond interactions, thereby stabilizing the targeted biological substances. According to the second hypothesis, vitrified carbohydrates form an amorphous matrix encompassing proteins and membranes. The high effectiveness of trehalose as a biological protectant against desiccation, sometimes also against

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freezing, compared with other disaccharides is explainable by its relatively higher glass transition temperature,[7] which is related to its stereochemistry as described in our previous study.[8] Suzuki et al.[9] proposed that hydrogen-bond formation of given carbohydrates with the targeted substances and vitrification of the carbohydrates were complementary. Their studies[9,10] indicated that dried proteins were highly stabilized by the amorphous matrix formed by carbohydrates, where the extent of carbohydrate–protein hydrogen-bond formation was higher than when the corresponding carbohydrates were crystallized.

Aldous et al.[11] focused on the ability of a given carbohydrate to form crystalline hydrates from the anhydrous amorphous state. For example, trehalose crystallization as hydrous forms from the amorphous state leads to a decrease in the residual water content of the remaining amorphous mass, in which the glass transition temperature T_g becomes higher, or at least its T_g depression caused by plasticization through water uptake is more or less avoidable.

In order to elucidate the mechanism by which trehalose enables biological organisms and substances to survive dehydration stress, information of its various physical forms and their related interconversions is indispensable. Many studies have reported phase transitions occurring in the heating process of trehalose dihydrate,[12–24] using X-ray diffraction (XRD), differential scanning calorimetry (DSC), thermogravimetry–differential thermal analysis (TG–DTA), and so on. So far three different crystal forms of trehalose have been widely recognized. The dihydrate form referred to as T_h (or form I), is a rhombic form and is stable at room temperature.[25,26] One anhydrous form referred to as T_β (or form III) is a monoclinic form, less hydroscopic, and stable at room temperatures.[27–29] Another anhydrous form, referred to as T_α (or form II), is readily rehydrated back to T_h, in which the crystal structure remains ambiguous.[28,29] Sussich and co-workers[16,18,19] investigated the dehydration process of T_h at different scan rates and showed kinetically controlled transformations of T_h into anhydrous forms. For example, transformation into the mixture of T_h and T_β, referred to as T_γ, formed by the dihydrate core covered with an anhydrous shell, was observed only when T_h was heated with scan rates in the range of 12 and 40 K min^{-1}.[18] Willart et al.[20] reported that for a slow heating rate (1 K min^{-1}) T_h transformed into a polymorphic phase T_α, whereas amorphous anhydrate was produced in heating T_h at 50 K min^{-1}. According to other some studies,[17,30,31] there are different routes of anhydrate formation by dehydrating T_h, dependent on particle size of T_h, where its large particles undergo direct transformation into the anhydrate corresponding to T_β, which is namely a solid–solid conversion, whereas small particles of T_h become amorphous on dehydration. Besides, it has been known that the dehydration behavior of T_h depends on the atmospheric pressure,[12,14,16] the presence or absence of nitrogen gas flow,[23] and even the type of vessels used for thermal measurements.[13,22]

Despite accumulating knowledge of various physical forms of trehalose, little is known about humid effects on the interconversions among the phases, including crystalline states and amorphous ones, although there are a few papers describing rehydration behavior of trehalose anhydrate back to T_h under humid atmospheres.[24,28,29] Recently Sussich et al.[32] gave transformation pathways among several physical forms of trehalose, where, however, the circumstances in terms of humidity are unclear. It should be pointed out that there has been a lack of controlling partial vapor pressure of water in most thermal and XRD measurements of trehalose reported so far, which exhibited considerable puzzling and scattering with respect to the dehydration behavior of T_h depending on the experimental conditions employed, as described in the above paragraph.

In this study we have investigated de- and rehydration behavior of trehalose dihydrate T_h under humidity-controlled atmospheres throughout simultaneous measurements of XRD and DSC, and of TG and DTA. It will be shown that anhydrous forms resulting from T_h dehydration strongly depend on the surrounding humid atmospheres, and that the partial vapor pressure of water required for rehydration also varies according to the type of dehydrated forms. The transformation routes among various solid forms of trehalose will also be illustrated by taking account of the effects of humidity.

2. Experimental

α,α-Trehalose dihydrate was purchased from Sigma Chemical Co., as a high purity grade (>99.9%). The dihydrate was used after mechanical grinding its crystals and their subsequent passing through a 45 μm sieve.

For the purposes of the present study described above, three physical observations were targeted: (1) the X-ray diffraction (XRD) pattern, (2) heat flow, and (3) gravimetric change. In order to measure them as simultaneously as possible under humidity-controlled atmospheres, we employed instruments for simultaneous XRD–DSC and simultaneous TG–DTA measurements. The former was carried out with a RIGAKU/XRD-DSC II instrument, which is an X-ray diffractometer, RIGAKU/RINT-ULTIMA II, combined with a heat-flux type DSC based upon a RIGAKU/ThermoPlus DSC 8230 modules. The XRD–DSC instrument was used in combination with a humidity generator RIGAKU/HUM-1. TG–DTA measurements were done using a RIGAKU/ThermoPlus 8120D system equipped with a humidity generator, HUM-1. Details of these

instruments have been described elsewhere.[33,34] A line-shape X-ray source was operated at 50 kV and 40 mA, and the data were collected over 5–38° of 2θ at a scan speed of 20°/min. In the XRD–DSC and TG–DTA measurements the temperature was scanned at 2 °C/min for heating and cooling, respectively.

Seven different experiments were performed with respect to the pattern of temperature and humidity controls, of which four consisted of only the dehydration process, and the other three did hydration and subsequent rehydration processes. The purpose of the dehydration experiments No 1–4 was to investigate the humid effects on both dehydration behavior of T_h and the subsequent crystal interconversions leading to T_β. Rehydration experiments No 1–3 aimed to investigate relative hygroscopic abilities of three different anhydrates T_α, T_β, and T_ε, each of which forms directly through dehydration of T_h according to the given humid atmosphere. Each protocol is described below, where flow rates of dry and wet nitrogen gases are 200 mL min^{-1}, respectively:

- Dehydration experiment No 1.
  Heating from room temperature to 220 °C under dry nitrogen gas.
- Dehydration experiment No 2.
  Heating from room temperature to 220 °C under wet nitrogen gas with a partial pressure of vapor water, P_{H_2O} = 3 kPa.
- Dehydration experiment No 3.
  Heating from room temperature to 220 °C under wet nitrogen gas with P_{H_2O} = 5 kPa.
- Dehydration experiment No 4.
  Heating from room temperature to 220 °C under wet nitrogen gas with P_{H_2O} = 12 kPa.
- De- and subsequent rehydration experiment No 1.
  Step 1: Heating from room temperature to 105 °C under dry nitrogen gas.
  Step 2: Cooling from 105 to 35 °C under dry nitrogen gas.
  Step 3: At 35 °C the wet nitrogen gas was admitted with increasing P_{H_2O} = 1.5 kPa → 2.1 kPa → 2.4 kPa.
- De- and subsequent rehydration experiment No 2.
  Step 1: Heating from room temperature to 105 °C under wet nitrogen gas with P_{H_2O} = 3.1 kPa.
  Step 2: Cooling from 105 to 38 °C under dry nitrogen gas.
  Step 3: At 38 °C the wet nitrogen gas was admitted with increasing P_{H_2O} = 2.5 kPa → 3.1 kPa → 4.1 kPa.
- De- and subsequent rehydration experiment No 3.
  Step 1: Heating from room temperature to 105 °C under wet nitrogen gas with P_{H_2O} = 12.2 kPa.
  Step 2: Cooling from 105 to 46 °C under dry nitrogen gas.
  Step 3: At 46 °C wet nitrogen gas was admitted with increasing P_{H_2O} = 5.3 kPa → 7.4 kPa.

3. Results

For dehydration experiment No 1 the XRD pattern and DSC thermogram obtained are shown in Figure 1, where the XRD pattern corresponding to each point of the DSC curve within the same temperature range is given on the left side. For example, the bottom most bottom X-ray pattern in Figure 1 corresponds to the temperature range from 54.5 to 59.0 °C for the DSC curve. The thermogram indicated a broad endotherm with a peak of 82 °C corresponding to the dehydration of trehalose dihydrate T_h, after which the XRD pattern obtained lost both an intense peak at 2θ of ca. 23.9° and a small one at 2θ of ca. 8.8°, both of which are characteristic of the XRD pattern of T_h.[17,21,25,26] When the temperature reached 110 °C where the dehydration process was almost completed as confirmed by TG–DTA measurements (data not shown), the measured XRD pattern indicated formation of the anhydrous polymorphic T_α, which is determined by agreement of the XRD pattern with corresponding data in the literature,[20,29] where one of XRD peaks exists at 16° of 2θ, which does not appear for T_h or T_β. T_α Melted around 125 °C with a small endothermic peak on the DSC curve. The observed melting point is in agreement with the literature data.[16] The resulting amorphous phase from T_α melting underwent cold crystallization, starting from about 150 °C, which is exhibited by a large exothermic peak on the DSC curve. The XRD patterns at temperatures above 164 °C confirmed transformation into the anhydrous crystal T_β throughout the cold crystallization, showing the characteristic pattern of T_β, that is, the existence of peaks at 2θ of ca. 6.7°, 20.5°, and 22.5°, respectively.[17,21,29] Such XRD patterns started to disappear over 200 °C with an endothermic peak on the DSC curve, corresponding to melting of T_β. Several previous studies provided similar series of thermal events to the present ones on nonisothermal slow heating of T_h from room temperature up to 220 or 230 °C.[16,17,23] For example, phase transitions, $T_h \rightarrow T_\alpha \rightarrow$ amorphous $\rightarrow T_\beta$, were reported by Nagase et al.[23,†] who also made a simultaneous measurement of XRD and DSC for the dehydration process of T_h at the same scan rate as ours, 2.0 °C min^{-1}, under dry nitrogen gas with a slightly lower flow rate of 150 mL min^{-1} relative to the current case of 200 mL min^{-1}. The present temperature range where T_α formed and subsequently melted to result in the amorphous state is similar to the one in the literature.[23] On the other hand, however, in the current DSC thermogram the exothermic peak corresponding to cold crystallization

[†] To avoid confusion and possible misunderstanding, the following should be noted. Nagase et al. referred to the hygroscopic anhydrate of trehalose as T_κ. However, T_κ is considered to be identical with T_α, as they themselves mentioned in their literatures.[23,29]

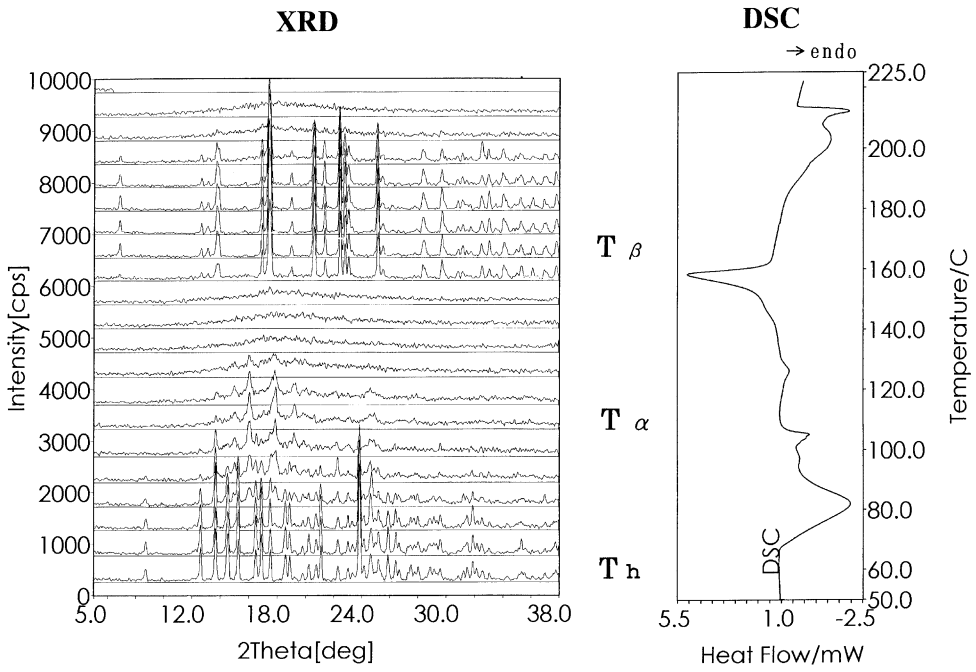

Figure 1. XRD patterns and DSC curve in thermal process of dehydration experiment No 1.

toward T_β appeared at lower temperatures and was sharper in its shape than that of Ref. 23, where it was also shown that the flow rate of nitrogen gas had little or no effects on the DSC thermogram obtained for T_h dehydration. The different behavior observed between the present study and that reported in the literature[23] measurements could be due to different particle sizes of T_h. The current sizes were less than 45 μm throughout sieving, whereas such treatment had not been carried out in the study reported in the literature.[23]

Figures 2 and 3 show the XRD patterns and DSC curves for the dehydration experiments Nos 2 and 3, respectively. One of the different findings from the experiment No 1 is that no anhydrous form T_α was observed to form after dehydration of T_h. An unknown XRD pattern, which has not been reported so far at

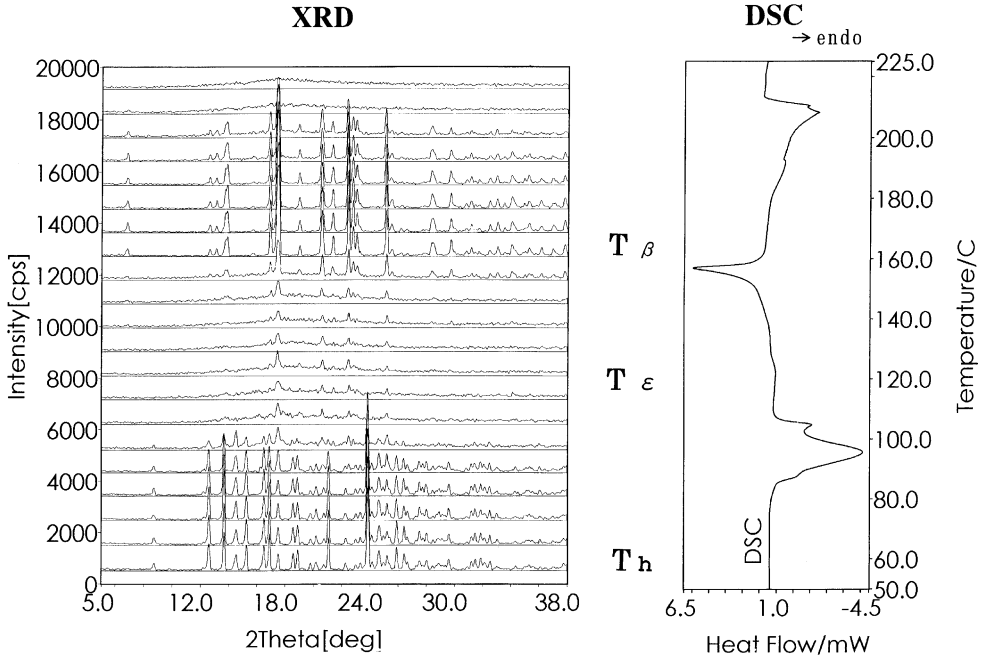

Figure 2. XRD patterns and DSC curve in thermal process of dehydration experiment No 2.

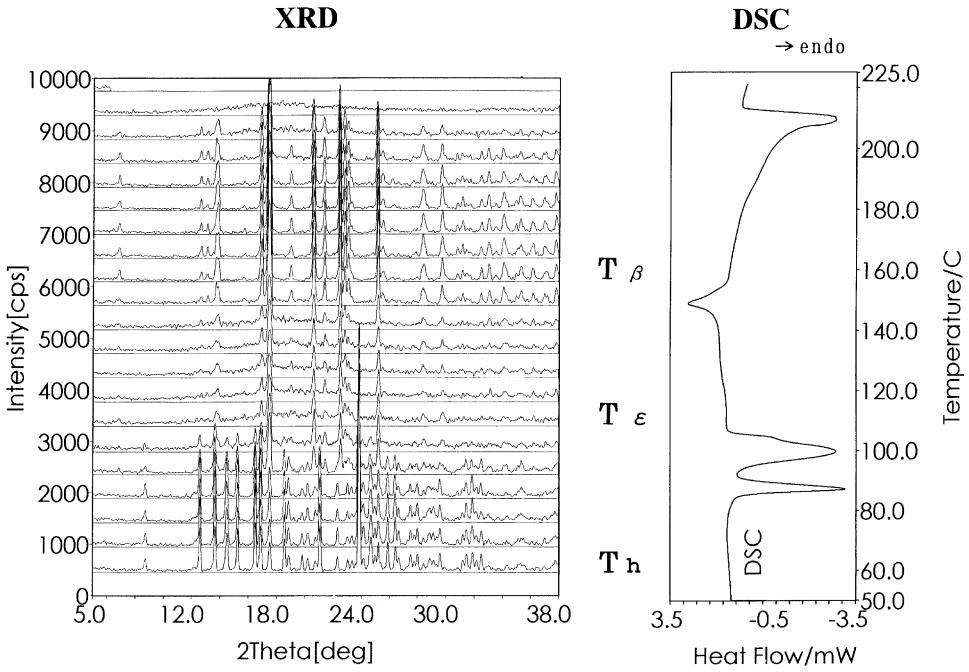

Figure 3. XRD patterns and DSC curve in thermal process of dehydration experiment No 3.

least to our knowledge, appeared at about 110 °C. The corresponding dehydrated state will be called T_ε, hereafter to be distinguished from T_h, T_α, and T_β. The dehydrated state T_ε shown in Figure 2 has a low crystallinity, as indicated by the XRD pattern that is somewhat haloed. Relatively intense XRD peaks of T_ε appeared for dehydration experiment No 3 where the controlled atmosphere had higher humidity as compared with that of experiment No 2. This indicates that the humid conditions have an effect on the crystallinity of the dehydrated state T_ε.

For dehydration experiment No 4, neither T_α or T_ε was observed to form as indicated by the XRD pattern shown in Figure 4. The anhydrous form T_β was produced at 90 °C, following a large endothermic peak on the DSC curve corresponding to the dehydra-

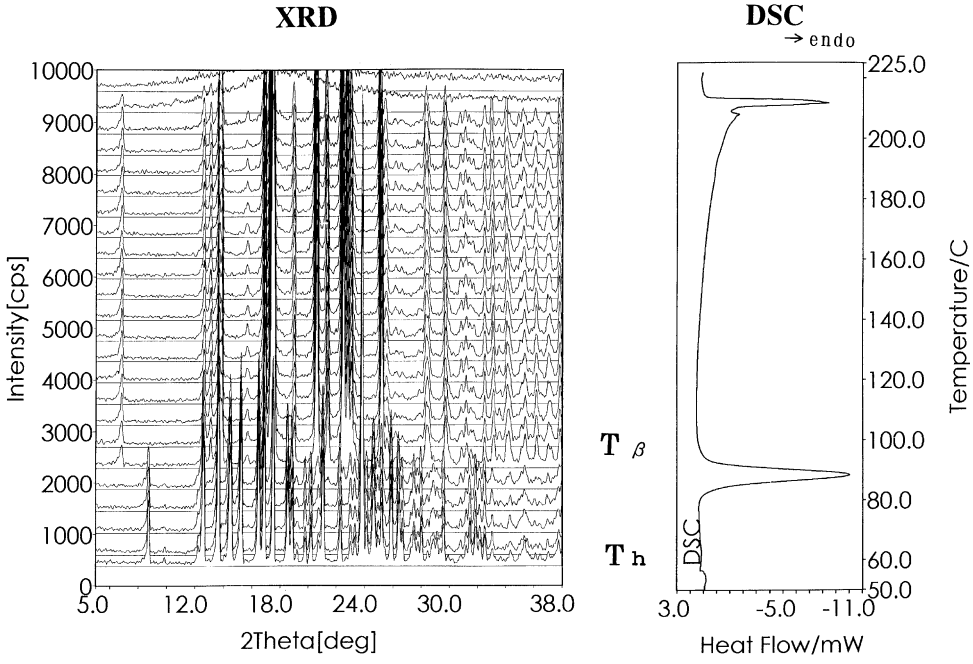

Figure 4. XRD patterns and DSC curve in thermal process of dehydration experiment No 4.

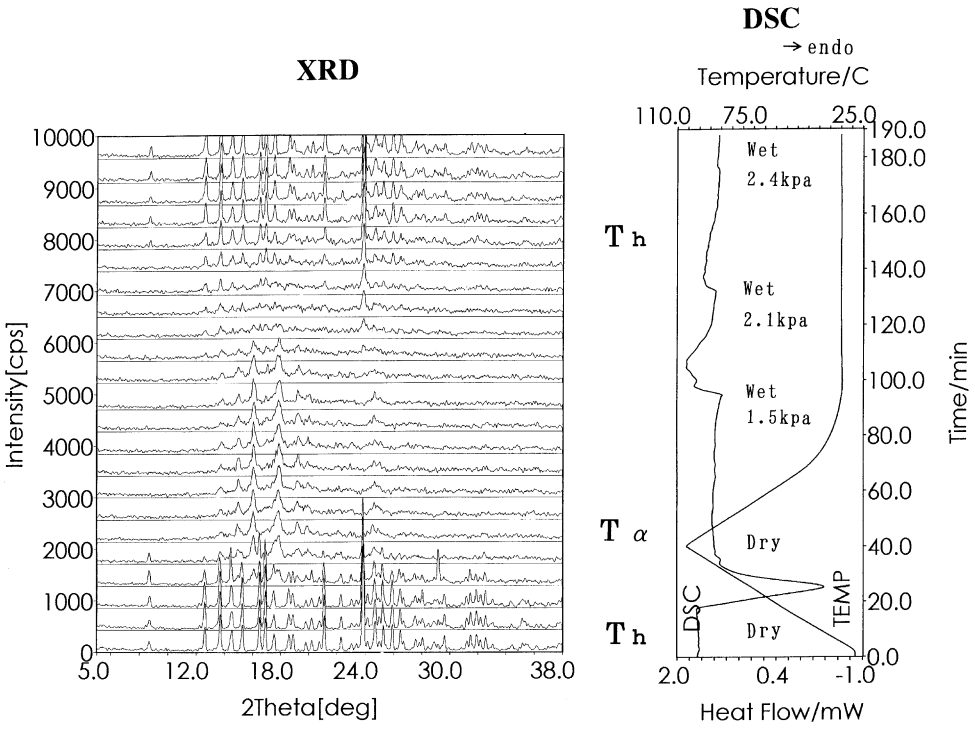

Figure 5. XRD patterns and DSC curve in thermal process of de- and subsequent rehydration experiment No 1.

tion of T_h. The phase transition from T_h to T_β is clearly exhibited by the XRD pattern change, particularly in the range of 2θ from 5° to 10°.

The findings obtained from the dehydration experiments Nos 1–4 indicate that the dehydrated states of T_h and the subsequent phase transitions on heating strongly depend on the moist conditions. It is of particular interest that the nonhygroscopic anhydrous form T_β was produced under highly humid atmospheres.

Next we will describe the de- and subsequent rehydration experiments No 1–3, and it should be noted that the ordinate of their DSC thermograms shown in Figures 5–7 indicates time, where temperature is given as a function of time. For the corresponding experiment No 1 of which observations are shown in Figure 5, the dehydration of T_h was carried out under dry nitrogen gas, followed by transformation into the anhydrous polymorphic phase T_α. This thermal event was just the same as that described for dehydration experiment No 1 above. When wet nitrogen gas with P_{H_2O} of 2.1 kPa was admitted, T_α was readily rehydrated into T_h as confirmed by the XRD pattern. In the corresponding TG–DTA measurement (data not shown) a stoichiometric loss of 9.5% of the initial weight was observed on heating T_h until the temperature reached 105 °C, which indicated that the two water molecules of the dihydrate were vaporized. No thermogravimetric change occurred on subsequent cooling from 105 to 35 °C under the same dry nitrogen atmosphere. The measured TG curve showed that at 35 °C admitting the wet nitrogen gas with P_{H_2O} = 2.1 kPa lead to a stoichiometric recovery of 9.5% of the initial weight of T_h in half an hour. This time scale required for rehydration of T_α back to T_h was in good agreement with that of the corresponding XRD–DSC measurement.

Figure 6 shows the XRD patterns and DSC curve for the de- and subsequent rehydration experiment No 2, indicating formation of the unidentified phase T_ε after T_h was almost completely dehydrated. No rehydration behavior was observed for T_ε when the wet nitrogen gas with P_{H_2O} = 2.5 kPa was admitted. In increasing P_{H_2O} = 3.1 kPa, however, rehydration began to occur and was then completed with P_{H_2O} up to 4.1 kPa.

For the de- and subsequent rehydration experiment No 3 the XRD patterns and DSC curve measured are given in Figure 7. The humid conditions in the dehydration of T_h were as highly wet as that of dehydration experiment No 4, leading to formation of the anhydrous crystal T_β without transforming into T_α or T_ε. When wet nitrogen gas with P_{H_2O} = 7.4 kPa was admitted after cooling to 46 °C, T_β was totally rehydrated back to T_h in about half an hour as confirmed by the XRD pattern and TG curve (the latter data not shown), although no rehydration phenomena were observed when P_{H_2O} of the admitted gas was 5.3 kPa. Thus highly humid conditions were required to rehydrate T_β back to T_h.

4. Discussion

For the dehydration behavior of trehalose dihydrate, T_h, there is growing information based upon thermal or

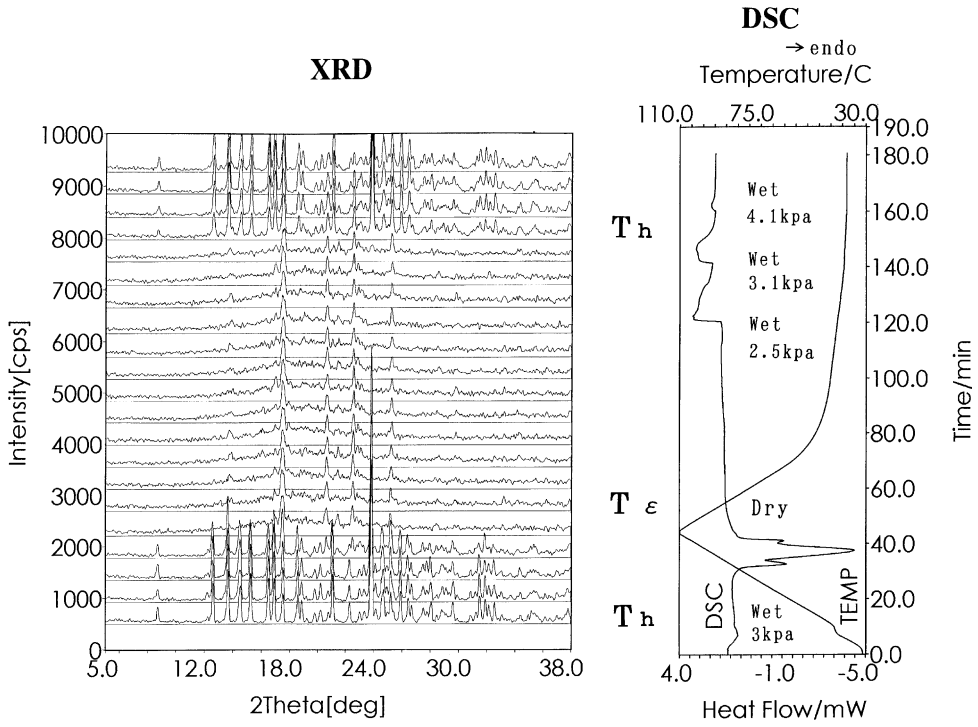

Figure 6. XRD patterns and DSC curve in thermal process of de- and subsequent rehydration experiment No 2.

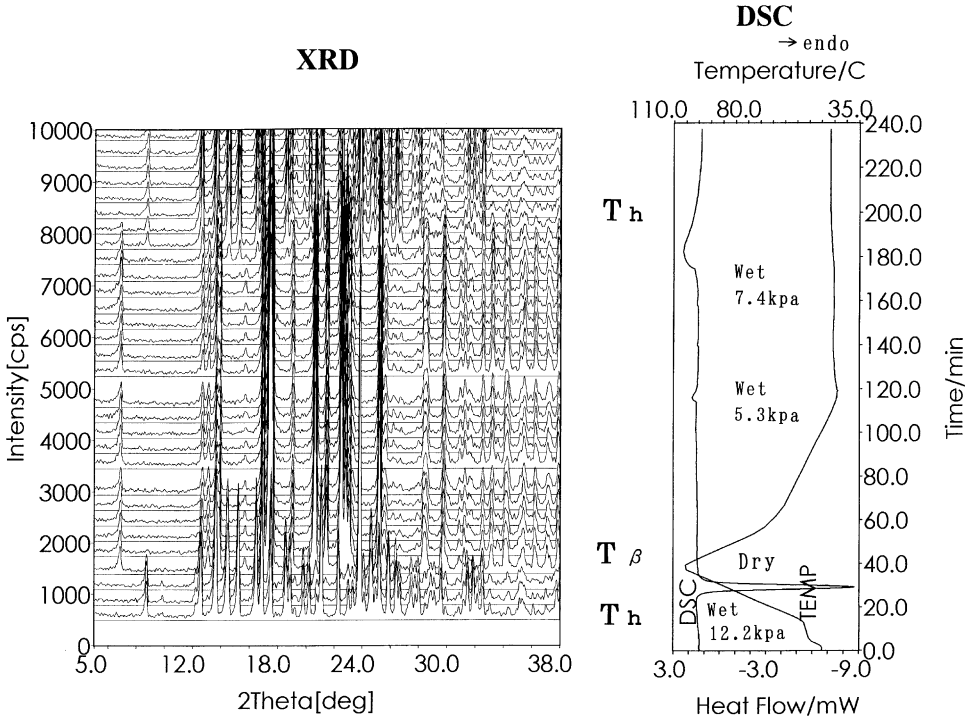

Figure 7. XRD patterns and DSC curve in thermal process of de- and subsequent rehydration experiment No 3.

XRD measurements, which have been mostly carried out under dry nitrogen or helium atmospheres, with less concern about the effects of humidity.[12–24] In the present work it was revealed that the dehydration behavior of T_h strongly depends on the humid conditions throughout simultaneous measurements of XRD and DSC under various humid atmospheres. In addition another new point is to get insight into the rehydration behavior

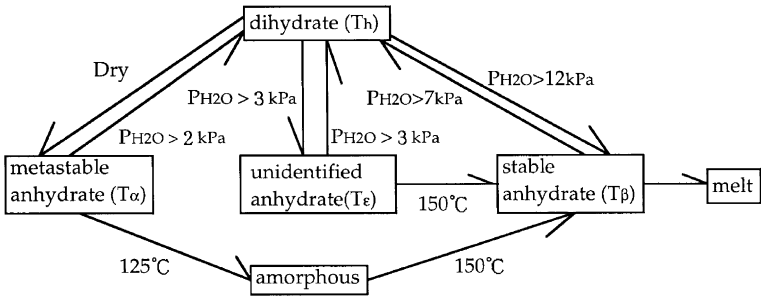

Figure 8. Phase and state transitions of trehalose.

of several different physical forms of trehalose back to T_h, although only a few literature reports have described the rehydration behavior of T_α.[28,29,†] Mainly based on above described findings from Figures 1–7, pathways are illustrated in Figure 8, that link different solid forms of trehalose.

One of the most interesting pathways newly found is between T_h and T_β. Under a given highly humid atmosphere T_h was transformed directly into T_β through dehydration, whose endothermic peak on the DSC curve was ca. 90 °C, whereas in dry or low-humidity conditions T_β was produced at as high as 170 °C via several phase transitions following dehydration. Similar dependence on humidity in relation to the dehydration behavior of hydrates has been seen for pharmaceutical compounds[33] and metal organic salts.[34] For example, when a pharmaceutical hydrate, nitrofurantoin monohydrate, was dehydrated under highly humid atmospheres, the monohydrate was transformed directly into a well-crystallized anhydrate at 140 °C, whereas in dry atmospheres the monohydrate turned into a mixture of crystalline and amorphous at temperatures, 124–180 °C, followed by crystallization to the anhydrate at ca. 185 °C.

The cause or mechanism of the above phenomena remains unclear at this stage. The current T_h dehydration experiments performed under various humid conditions, however, seem to have provided many clues necessary to interpret these phenomena. One of the factors we have to focus on first would be the so-called bridge effects given by the surrounding humid environment, which can trap, through hydrogen-bond formation, water molecules released from a given hydrate. That is, humid atmospheres act like an eluent to extract water from the hydrate. At the same time the humid atmospheres may not only prevent the crystal structure from collapsing, but also may help the molecules undergo rearrangement in the crystal. In addition, kinetic effects also should not be overlooked. When T_β, which adopts the monoclinic form,[27] is produced throughout dehydration of T_h with the rhombic form,[25,26] time is required for the trehalose molecules to rearrange in the crystal structure within an appropriate range of temperatures. In general, taken together, a careful control of both humidity and temperature is necessary to produce an anhydrous crystal via a one-step transformation by dehydrating the parent hydrate.

The anhydrous crystal of trehalose, T_β, was described to be less hygroscopic in Refs. 28 and 29, where at 25 °C and relative humidity (RH) of 43% and 40% T_β exhibited little or no moisture uptake in an hour and a week, respectively. In the current study, however, even T_β was reversibly hydrated into T_h under highly humid atmospheres in which the partial vapor pressure of water, P_{H_2O}, was up to 7.4 kPa at 46 °C, which is equivalent to a RH = ca. 85% (Fig. 7). T_β was also rehydrated back to T_h at P_{H_2O} = 6.2 kPa at 40 °C (RH = 84%, figure not shown). An interesting example to be compared with is again the rehydration behavior of raffinose hydrates. Transformation of the trihydrate to the tetrahydrate required at least 10% of RH at 30 °C.[35] An RH value of 50% was necessary to make the tetrahydrate take up moisture with transformation to the pentahydrate at 30 °C.[35] Thus there seems to exist a threshold of RH as to rehydration behavior of crystals. The above findings of trehalose suggest that for T_β the threshold of RH lies between 40% and 85%, if any.

In contrast to T_β, the other anhydrous form of trehalose, T_α, is very hygroscopic.[28,29] For example, when this anhydrate was exposed to a humid atmosphere with an RH of 43% at 25 °C, it underwent rehydration into T_h in a week.[28]Under the moist conditions of RH being 40% at 25 °C, a mixture of T_α and T_γ, the latter of which was explained in the Introduction, was totally rehydrated in half an hour, as confirmed by the steep increase in the weight of the mixture over half an hour followed by its reaching a plateau.[29] The part of T_γ in the mixture seems hardly to absorb moisture at RH = 43% since T_γ is composed of T_h and T_β.[18] If the rapid uptaking of water reported for the mixture of T_α and T_γ derives from the contribution of the T_α part, the time scale required for rehydration is in good agreement with that as seen in the present de- and subsequent rehydration experiment No 1. The rehydration behavior of T_α is insensitive to other crystals that co-exist.

The crystal structure of T_α, such as unit cell dimension, remains unclear. According to the previous study using FTIR measurements,[28] trehalose molecules in T_α

have a C_2 symmetry around the glycosidic linkage, which is similar to the case of T_β. In both crystals trehalose molecules have an open site that can trap water molecules. Judging from the unit cell dimension data of T_h and T_β,[25–27] trehalose molecules are more densely packed in T_β than in T_h, which would make it difficult for T_β to absorb water molecules. It was suggested for T_α, on the other hand, that passages for water molecules along the *c*-axis of the T_h crystal were preserved.[23,†] If T_α has such a characteristic aspect of the crystal structure, low vapor pressure of water required for its rehydration seems reasonable.

An unidentified dehydrated state, referred to as T_ε in this study, was found to be formed on dehydrating T_h under atmospheres with intermediate humidity for which the XRD–DSC results are shown in Figures 2 and 3. The XRD pattern of T_ε is somewhat haloed, showing low crystallinity of T_ε. It is found from Figures 2 and 3 that the measured XRD peaks of T_ε are more intense in the case of the higher humidity. In other words the crystallinity of T_ε formed is higher under the wetter atmosphere. The other finding to be noted is that the temperature at which cold crystallization occurs toward T_β formation is somewhat lower under the higher humid conditions. At $P_{H_2O} = 3$ and 5 kPa, for example, the corresponding exothermic peak on the DSC curve was observed at 157 and 150 °C, respectively. These findings indicate that highly humid atmospheres promote the crystal growth and molecular rearrangement in the dehydrated state of trehalose. The microscopic structure of T_ε is unclear at the current stage. Detailed studies will be required to clarify the T_ε structure, which is beyond the scope of this paper.

5. Conclusions

This study reveals that the observed dehydration behavior of trehalose dihydrate, T_h, strongly depends on humid atmospheres employed in thermal and XRD measurements, and that the resulting anhydrous forms under different conditions of humidity require different partial vapor pressures of water for their rehydration back to T_h. Under dry atmospheres a metastable anhydrous crystal T_α is formed at 105 °C on dehydrating T_h, which is highly hygroscopic and can be readily rehydrated back to T_h when exposed to even low humid atmospheres. Under high humid atmospheres, on the other hand, dehydration of T_h undergoes a direct transformation into a stable anhydrous crystal T_β at 90 °C, which is formed at as high as 170 °C in the case of dry conditions. T_β is less hygroscopic, and a high partial vapor pressure of water is necessary for its rehydration back to T_h. Under intermediate humid atmospheres the dehydration of an T_h leads to formation of an unidentified state T_ε, of which crystallinity is higher in the more humid atmospheres. Its rehydration requires environments with intermediate humidity. In addition it should be also emphasized from a series of these findings that humidity controls are indispensable for thermal and XRD measurements.

Pathways were finally clarified which describe solid–solid transformation of trehalose, which is strongly dependent on humid atmospheres. One of the most interesting findings is that an anhydrous crystalline form of trehalose, T_β, is produced at relatively low temperatures through a one-step transformation by dehydrating the dihydrate, T_h, in highly moist conditions, although this route may not be necessarily related to bioprotective usefulness of trehalose. In the fields of a pharmaceutical and material science, trehalose will be a good target to obtain insight into the general dehydration mechanism of hydrates depending on humid atmospheres.

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