Journal of Thermal Analysis and Calorimetry, Vol. 93 (2008) 2, 561–567

EFFECT OF ATMOSPHERIC PRESSURE ON THE PHASE TRANSITIONS OF α,α-TREHALOSE DIHYDRATE

DTA study of the dehydration behavior in open systems

T. Furuki[1]*, R. Abe*[2]*, H. Kawaji*[2]*, T. Atake*[2] *and M. Sakurai*[1*]

[1]Center for Biological Resources and Informatics, Tokyo Institute of Technology, B-62 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan
[2]Materials and Structures Laboratory, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku
Yokohama 226-8503, Japan

The phase transitions of α,α-trehalose dihydrate (T_h) were investigated by either differential thermal analysis (DTA) with an in-house apparatus of variable-pressure type equipped with an open sample holder or commercially available TG (thermal gravimetry)-DTA apparatus for comparison under the same experimental conditions as to the heating rate (2°C min^{-1}), the type of pan (open), and the particle size of T_h (63 μm). The former DTA measurements were carried out under five different total pressures, 101, 75, 61, 48 and 35 kPa, which provided quite helpful information necessary for confirmative assignments of the endothermic peaks due to either melting or dehydration of T_h. The usage of largely different amount of T_h, 126 and 14 mg for the DTA and TG-DTA measurements respectively, led to their different DTA traces, showing that there were largely different extents of the influence by the measured sample surface exposed to the surrounding atmosphere on its dehydration behavior. In addition the high thermal sensitivity achieved with such mass of T_h gave rise to an interesting discovery of an unidentified thermal event at 92°C prior to either melting or dehydration of T_h.

Keywords: *differential thermal analysis of variable pressure type, open sample holder, phase transition, surface effects, trehalose*

Introduction

α,α-trehalose (α-D-glucopyranosyl-α-D-glucopyranoside, hereafter abbreviated as trehalose) is a non-reducing disaccharide composed of two glucose molecules linked by α,α-1,1 glycosidic bond. The disaccharide occurs widely in nature, being produced and accumulated in some cases up to 20% of the dry mass of cold- or desiccation-resistant organisms such as yeast, mushrooms, fungi, soil-dwelling insects, and even polar biota [1, 2]. It has been so far recognized, in particular, that the intracellular accumulated trehalose vitrifies under dry conditions and then contributes to allowing so-called anhydrobiosis organisms to survive almost complete dehydration [3]. The glass-forming property of trehalose is also useful in food and pharmaceutical points of view [4–6], making this sugar applicable as a protectant and excipient of proteins and enzymes during their formulating process and subsequent storage involving drying treatment.

Trehalose has several different physical forms such as amorphous state, dihydrate crystal (hereafter denoted conventionally by T_h), and anhydrates, some of which are widely recognized as T_α and T_β phases, respectively [7–9]. These phases have pathways for their interconversions, which depend on the temperature and humidity, as described in our previous paper [10]. One of the earliest studies of phase transitions of trehalose was provided by Reisener *et al.* [11], who demonstrated that crystal water of T_h was lost at 100°C with a microscopic melting block exhibiting only sintering of T_h and loss of birefringence at 100°C. Crystal water release from T_h can be monitored also by the mass loss, although the observed dehydration behavior depends on the experimental conditions such as the heating rate and the particle size of T_h fractions [12–16]. A kinetic study was reported [17] on the dehydration behavior of T_h throughout thermogravimetric analysis with isothermal conditions ; when the constant temperature was set between 40 and 60°C, the dehydration kinetics was of the Arrhenius type, while in the range from 70 to 90°C, the isothermal dehydration exhibited non-Arrhenius behavior. Similar results on the dehydration kinetics of T_h were also described in [18]. Such kinetic change according to the heating temperature suggests that the dehydration mechanism varies in the temperature range from 60 to 70°C, the origin of which is unclear at the present stage.

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[*] Author for correspondence: msakurai@bio.titech.ac.jp

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

Shafizadeh and Suscott [19] reported, with DSC measurements, that T_h melted at 100°C in a closed system (in hermetically sealed pan). A similar finding was given later in [20], where the onset and peak temperatures assigned toT_h fusion was 91 and 97°C, respectively, on the DSC curve. In addition, recently, there appeared a finding that the melting peak of T_h was observed only when sealed pans were used in DSC measurements, whereas the use of any other types of pans, such as open cells or closed pans with different orifice radius (0.1–1.2 mm), exhibited no corresponding melting events on DSC curves [21]. According to an X-ray crystallographic study [12], there was no evidence of T_h melting at least around 100°C, which would be related to open cells used for X-ray irradiation allowing diffusion of water vapor from the cells.

In nature the so-called anhydrobiotic organisms mentioned in the first paragraph are dried in an ordinary atmosphere, i.e. under open conditions. Also when enzymes and pharmaceutical proteins are lyophilized, the drying operation is carried out in open systems so as to promote the sublimation of ice. Several years ago we reported, by Fourier transform infrared (FTIR) measurements, that the crystal water molecules of T_h are just in the same state as ones in ice, up to as high as 70°C [22], which was furthermore thermodynamically supported by our recent study on the low temperature heat capacity of T_h [23]. All of these backgrounds make it both academically and practically important to get fundamental information on the dehydration and melting of T_h in open systems as well as under systematically different experimental conditions to overcome such confused situation as described above. In the present study, high-sensitive differential thermal analysis (DTA) of variable-pressure type equipped with an open sample holder, which is the in-house DTA apparatus, was employed to detect phase transitions occurring during heating of T_h. The DTA measurements under different total pressures have given useful data to help assign convincingly a series of thermal events involved in the melting and dehydration of T_h. We will also describe effects of the sample surface exposed to the surrounding atmosphere on the dehydration behavior of T_h throughout two comparative experiments using either the above in-house DTA or commercially available TG-DTA apparatus, where T_h in largely different quantities were subjected to these thermal analyses, respectively.

Experimental

α,α-trehalose dihydrate was kindly gifted from Hayashibara Biochem. Lab. Inc. (Okayama, Japan) with purity of as high as 100%. The dihydrate was placed in a desiccator for a few hours immediately before use, which was evacuated to reduce pressure so as to promote removal of adsorbed water of the dihydrate. The particle size fraction of the crystal was almost uniform, being ca. 63 μm.

TG-DTA measurements were done with commercially available apparatus, Thermoflex TAS TG8101 manufactured by Rigaku Co. (Japan). α,α-trehalose dihydrate of 14 mg was placed in an open aluminum pan with the diameter and depth being 5 and 2.5 mm, respectively. The ratio of the surface area of the open site to the volume for the loaded α,α-trehalose dihydrate was 1.0 mm^{-1}. The heating rate was 2°C min^{-1} and no flush gas was used, both of which were the same experimental conditions as those of the in-house DTA measurements described below.

DTA measurements were carried out using an apparatus developed by one of the authors (Atake). Schematic overview of the cryostat used in the apparatus has been given elsewhere [24]. The inside of the cryostat can be evacuated and sealed airtight, which enables us to avoid frosting problems when the cryostat is cooled with liquid nitrogen. A sample vessel made of glass is inserted into the hole formed in the copper block wound with a heater coil. The vessel has a shape of tube with a spherical-shaped bottom, of which inner diameter is 4 mm. Developed theoretical backgrounds of classical DTA have been described in [25–27] and an experimental application under reduced pressures has been also published elsewhere [28].

In the present experiments, α,α-trehalose dihydrate powders of 126 mg were packed into the glass vessel and α-Al_2O_3 was used as the reference. The ratio of the top surface area to the volume of trehalose dihydrate used was 0.11 mm^{-1}. At first, DTA measurements were carried out under five different total pressures, 101, 75, 61, 48 and 35 kPa, with a heating rate of 2°C min^{-1}, in order to get information to be helpful for assignments of thermal events observed on the DTA traces. Furthermore thermal cycle experiments (Table 1) were performed to investigate the thermal properties in each state of trehalose yielded as a result of the first heating scan as well as to confirm the assignment of thermal events observed in the DTA traces.

Results and discussion

TG-DTA measurements

When thermal events to be observed include decomposition processes like dehydration, thermogravimetric analysis (TG) is useful to complement the information necessary for interpretation of the thermal events. A lot

Table 1 Thermal cycles used for DTA measurements of α,α-trehalose dihydrate (T_h) under ordinary atmospheric pressure (P_t=101 kPa)

Experiment
No.1 30°C – (1st heating) → 93.4°C (immediately after the event A)[a] – (1st cooling) → 30°C – (2nd heating) → 140°C
No.2 30°C – (1st heating) → 102.6°C (immediately after the event B)[a] – (1st cooling) → 30°C – (2nd heating) → 140°C
No.3 30°C – (1st heating) → 140°C (immediately after the event C)[a] – (1st cooling) → 30°C – (2nd heating) → 140°C

[a] The upper limit temperatures of 1st heating scans were determined on the basis of the thermal findings in DTA measurements of T_h at P_t=101 kPa. (Cf. Fig. 3)

Table 2 Comparison of TG experimental conditions and findings on heating T_h from ambient temperature

Ref.	Heating rate/K min^{-1}	Atmosphere	Cell type	Particle size	Findings
9	1	N_2 gas of 30 mL min^{-1}	Pinholed pan	No description	9.5% loss of the initial mass by ca.100°C
	20	N_2 gas of 30 mL min^{-1}	Pinholed pan	No description	Detectable mass loss only above 130°C
12[a]	10	N_2 gas flow	No description	Less than 45 μm	9.5% loss of the initial mass by ca.110°C
14	5	N_2 gas flow	Open pan	No description	Mass loss starts at ca. 50°C and then ends at ca. 100°C
15	10	No description	Pinholed pan	50–100 μm	Mass loss is almost completed above 120°C
16	5	N_2 gas flow	Open pan	No description	Mass loss starts at ca. 60°C and then ends at ca. 120°C

[a] Many experimental conditions were studied, only one of which is exemplified here

of TG data for α,α-trehalose dihydrate (T_h) have been already reported up to now [9, 12, 14–16]. As summarized in Table 2, however, they show strong dependency on the experimental conditions employed. This situation compelled us also to carry out TG-DTA measurement of T_h to compare with findings from the in-house DTA measurements, where the experimental conditions were set in a similar way to each other as described above. Figure 1 shows the current TG-DTA trace, exhibiting minor loss of the initial sample mass commencing at 70°C, about 1 mass% decrease by 90°C. Subsequently the major mass reduction, ca. 7 mass% loss of the initial mass, occurred by 130°C. The total mass loss, around 8.7 mass% of the initial mass, by up to 150°C is a little smaller than the stoichiometric value 9.5 mass% corresponding to two crystal water molecules of T_h. This finding could imply that there was partial removal of the crystalline water when α,α-trehalose dihydrate was subjected to drying treatment under reduced pressure at room temperature immediately before use. On the other hand the above step-wise mass loss was parallel with the endothermic thermal events recorded on the concomitant DTA trace.

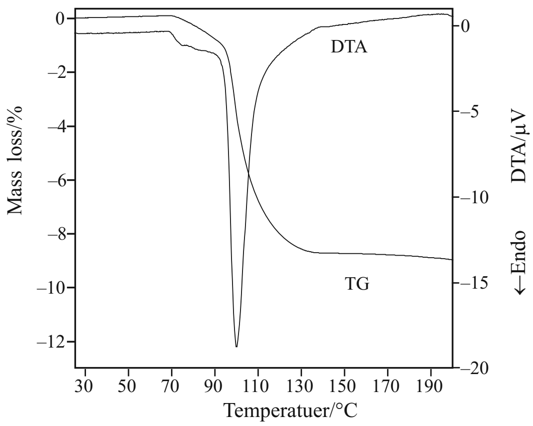

Fig. 1 TG-DTA trace of trehalose dihydrate

DTA measurements

Figure 2 shows a comparison of DTA traces obtained at P_t=101 kPa for T_h as received and as dried under reduced pressures prior to the measurement. An obvious difference between two traces is that the exothermic peak at 115°C (pointed by an arrow in Fig. 2) almost disappears in the dried sample, suggesting that the thermal event observed at 115°C could arise from the effect of water adsorbed on the crystal surface rather than from the pure component of T_h itself, although the possibility is not ruled out that the slightly defects of crystal water in T_h prevent the exothermic event at 115°C, of which details are unclear now. On the other hand other thermal events such as A, B, and C are observed in similar ways for T_h as received and as dried before use.

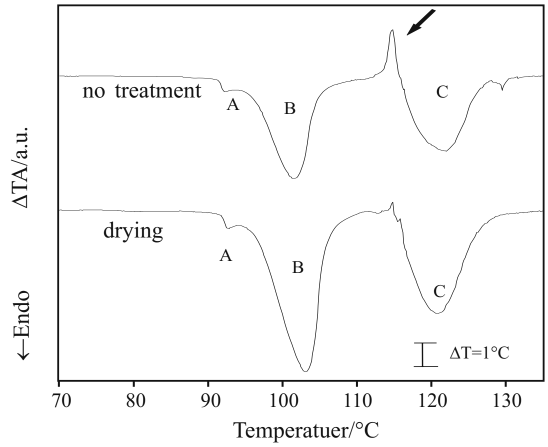

Fig. 2 DTA traces of trehalose dihydrate. ‘No treatment’ in this figure means that trehalose dihydrate was used as received for DTA measurement, whereas ‘dried’ means that trehalose dihydrate was dried in vacuo before use

Figure 3 shows the DTA traces of trehalose dihydrate T_h under various total pressures, P_t. When P_t was 101 kPa, we observed three thermal events; the endothermic baseline jump at 92°C (denoted by A in Fig. 3), two large endotherms which commenced at 96 and 120°C (denoted by B and C, respectively, in Fig. 3), and a small endothermic peak at 129°C. The large endotherm at higher temperature, denoted by C in Fig. 3, shifted to lower temperatures with decreasing P_t. In contrast, the other large endotherm (denoted by B in Fig. 3) exhibited no temperature shift. As a result, the two endothermic peaks almost merged when P_t decreased to 35 kPa. This finding indicates that the origins of these two endotherms are different from

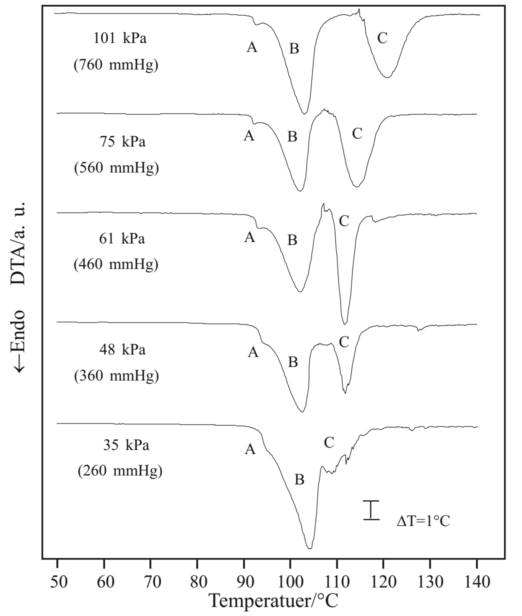

Fig. 3 DTA traces of trehalose dihydrate under various total pressures

each other, with peaks B and C mainly due to the melting and dehydration of T_h, respectively. The observed onset, peak, and end temperatures of T_h melting are 96, 103 and 106°C, respectively, which are in good agreement with the literature data of the T_h melting point measured by DSC [19, 20].

In what follows, we will show DTA traces in Figs 4–6, which were obtained from the thermal cycle experiments No. 1–3, respectively. When T_h experienced an endothermic baseline jump at 92°C on the first heating, like the experiment No. 1, the second heating scan exhibited no endothermic jump at 92°C any more, but showed two endothermic peaks at 100 and 120°C (Fig. 4). For the thermal cycle experiment No. 2, the first heating scan was terminated at 102.6°C corresponding to the stage immediately after complete T_h fusion. On the second heating, no thermal event was observed until as high as 100°C, followed by a small endotherm starting at 105°C, a small sharp exotherm at 116°C, and a large endotherm of which the peak temperature was 120°C. For the thermal cycle experiment No. 3 (Fig. 6), the first heating DTA trace had the following thermal events; a small endotherm which started at about 90°C, a sharp endotherm at 102°C, a small exotherm at 117°C, and a broad endotherm at 118°C. In contrast, no thermal event was observed on the second heating.

In general the water release behavior from a given hydrate is investigated with thermal analysis such as DTA and DSC shows that the dehydration mechanism depends on the experimental conditions employed [10, 12–16, 28]. Thermal dehydration can be regarded as the solid-state decomposition reaction as follows:

$$\mathrm{T}n\mathrm{H_2O(s)}\rightarrow \mathrm{T}(n-k)\mathrm{H_2O(s)}+k\mathrm{H_2O(g)}$$

where n and k represent the number of crystal water molecules in the hydrate and that of water molecules released from the hydrate ($1\leq k\leq n$). The parent hydrate and its partially or completely dehydrated compound are in solid states, denoted by (s), whereas the released water is in the gaseous state, denoted by (g). The thermal dehydration can occur when the equilibrium vapor pressure of the given hydrate reaches the surrounding vapor water pressure.

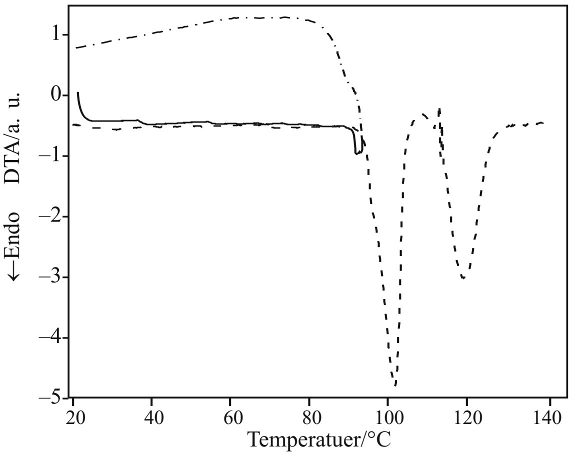

Fig. 4 DTA traces of the thermal cycle experiment No. 1. The lines correspond to the scans of the first heating (solid), cooling (dot-dashed), and the second heating (broken)

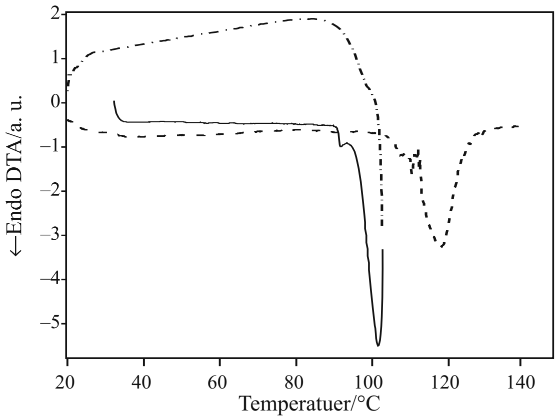

Fig. 5 DTA traces of the thermal cycle experiment No. 2. The lines correspond to the scans of the first heating (solid),

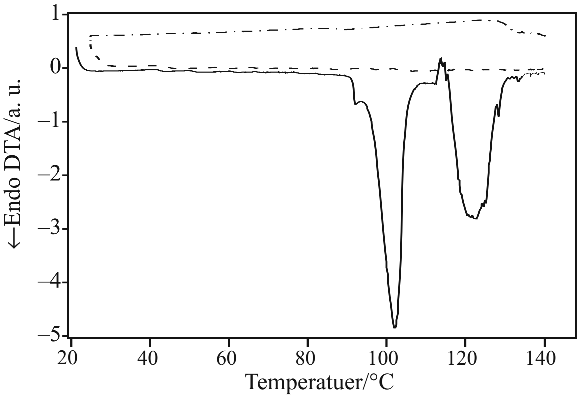

Fig. 6 DTA traces of the thermal cycle experiment No. 3. The lines correspond to the scans of the first heating (solid), cooling (dot-dashed), and the second heating (broken)

In the present study we performed at first TG-DTA measurements of T_h dehydration in the static ordinary atmosphere (Fig. 1) . The major endothermic peak around 100°C in the DTA trace corresponds well with the major mass loss, which follows continuously the minor loss of the initial mass starting at around 70°C. These thermal observations may be discussed in conjunction with our previous Fourier transform infrared (FTIR) spectroscopic study [22] on the structural property of T_h, which demonstrated that the crystal water of T_h changed from ice-like to liquid-like water a little below 70°C. Lin and Chien [29] also observed a polymorphic transition of T_h between 60 and 80°C in both FTIR and DSC measurements, although the experimental conditions in these literatures [22, 29] were quite different from the present

TG-DTA measurement. In our previous study [22], for example, the measured sample T_h was sealed between two KBr plates with heating rate of 1°C min^{-1}, whereas herein the measured sample was placed in the open pan and the heating rate was 2°C min^{-1}. For the FTIR measurements the phase transition temperature observed was independent of the heating rate at least in the range from 1 to 5°C min^{-1} (data not shown). According to [22], the state change of the crystal water in T_h was observed at 64°C when the measured sample was pressed on one KBr pellet corresponding to such an open pan as used in the current thermal measurements. Thus the combination of these findings either from TG-DTA or FTIR measurements indicate that the minor dehydration of T_h follows the phase transition of the crystal water from the ice-like to liquid-like states.

On the other hand the DTA thermogram measured with the in-house apparatus at P_t=101 kPa is quite different from the DTA trace described above, exhibiting, for instance, the endothermic peak around 120°C (denoted by C in Fig. 3), despite the similar experimental conditions as to the heating rate (2°C min^{-1}), the particle size of T_h (63 μm), the atmosphere (static air only at P_t=101 kPa), and the type of cell (open). For the in-house DTA measurements, however, one of the remarkable features is the usage of a large amount of T_h up to 126 mg, whereas only 14 mg of T_h was subjected to commercially available TG-DTA measurements. The ratios of the surface area exposed to the surrounding air to the volume of T_h used were largely different from each other, being 0.11 and 1.0 mm^{-1}, respectively, for the DTA and TG-DTA measurements. From this point of view the thermal events observed in different manners between the DTA and TG-DTA experiments reflect the different extents of the surface effects of T_h subjected to thermal analyses. The former would exhibit the properties as a mass or material of T_h rather than as a particle of T_h itself.

Another thermal event to be noted is the endothermic baseline jump, denoted by A in Fig. 3, at 92°C at P_t=101 kPa , prior to T_h fusion. To our knowledge such event has not been reported so far. This new finding also relates to the employed experimental condition with respect to thermal sensitivity. As described above, a large amount of sample, 126 mg of T_h, was used for our heat flow measurement, which is in contrast with previously reported DSC studies using a small amount of T_h, 5–15 [9], 7.5 [12], 4 [14], 2–4 mg [15]. The thermal event A was similarly observed for both of T_h as received and as dried under reduced pressures before use (Fig. 2), although this event became less obvious when the temperature ramp was decreased to 1°C min^{-1} or lower (data not shown). Thus the endothermic jump A observed at 92°C at P_t=101 kPa should be at least distinguished from the phase transition of crystal water from ice-like state to liquid-like one, of which temperature is almost insensitive to heating rate of 1 to 5°C min^{-1} [22]. At the current stage further details are unknown for the event A.

It is difficult to identify physical origins of the endothermic peaks B and C, respectively, throughout the comparison with literature data [9, 12–16]. As described in the first paragraph of this section (i.e. discussion), however, their different sensitivity to the total pressure, P_t, assists us in interpreting these thermal events. The event C shifted to lower temperatures with decreasing P_t, being attributable in major part to the dehydration of trehalose dihydrate, T_h. On the other hand, the endothermic peak B is mainly due to the fusion of T_h since it showed no dependence on P_t.

Conclusions

The thermodynamic properties of α,α-trehalose dihydrate were studied by commercially available TG-DTA in the ordinary atmosphere as well as by the in-house DTA under various pressures (P_t) using an open sample vessel. Thermal study with pressure dependence provided precious clues to distinguish the endothermic peaks due to either melting or dehydration of T_h, although at the same time we should be careful for the effects of surface at the open site of the measured sample on the way the thermal events will appear. In addition the experimental condition with high thermal sensitivity by using a large amount of sample, 126 mg of T_h, led to an interesting discovery of an unidentified state prior to melting and dehydration of T_h.

From a general viewpoint, the crystal dehydration is very important, since it relates directly to producing pharmaceutical [30] and electronic device [31] compounds. We hope that new knowledge obtained in the present study will contribute widely to further progress in the field of such crystal dehydration study as well as will lead to opening a door of new science and application of trehalose.

Acknowledgements

This work was supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN). And this work was supported in part by Grants-in-Aid for Scientific Research on Priority Areas (no.18031012) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Received: January 26, 2007
Accepted: May 29, 2007
OnlineFirst: October 14, 2007

DOI: 10.1007/s10973-007-8362-7