

## GLASS TRANSITION AND ENTHALPY RELAXATION OF POLYPHOSPHATE COMPOUNDS

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### Abstract

The glass transition behaviour of polyphosphate compounds such as di- and tri- polyphosphates used as food additives and ATP, ADP existing in biosystems, were investigated by using DSC. From the DSC heating curves of the frozen solutions, the glass transition temperatures of the maximum freeze concentrated solutions,  $T_g'$ , were determined. It was found that  $T_g'$ s for polyphosphates are relatively high. The lyophilized tripolyphosphates, ATP and ADP also showed the glass transition at a relatively high temperature, depending on the moisture content. In addition, the enthalpy relaxation behaviour of glassy ATP and ADP was examined and analyzed by using the Kohlrausch-Williams-Watts (KWW) and the Vogel-Fulcher-Tammann (VFT) equations. Judging from the parameters of the KWW and VFT equations, the amorphous states of ATP and ADP were suggested to be more fragile than trehalose and sucrose.

**Keywords:** polyphosphate, ATP, glass transition, state diagram, enthalpy relaxation, KWW

### INTRODUCTION

Recently, information on the glassy state of carbohydrates has been accumulated. It is well known that among them, sucrose and trehalose are good glass formers and effective protein stabilizers on freezing or lyophilization. Understanding their stabilizing effect has been attempted through their state diagrams, i.e. glass transition temperature ( $T_g$ )-moisture relation (1, 2). Such insight provided a new paradigm in the field of food science and pharmaceutical science (3, 4), even though some doubtful results have been reported (5, 6). Thus, the current exploratory studies for good glass formers have been conducted, of which targets covered not only new materials but also a mixture of known glass formers (7, 8).

Meanwhile, it is well known that polyphosphates have often been used as a stabilizer for frozen or dry foods. They have been believed to play the role of a buffer during the process. However, there are some questions that could not be interpreted only by the buffer effect (9). It is interesting to note that there is no report as to glass transition behaviour of polyphosphates used for foods. In the field of inorganic glass science, polyphosphates have been categorized as good glass formers as well as silica (10, 11). However, polyphosphates dealt in inorganic

chemistry are highly polymerized, whereas those used as food additives are limited to di- or tri-polyphosphates. Therefore, the present study attempted to reconsider the physical properties of di and tri- polyphosphates in terms of glass transition. ATP related compounds (ATP, ADP and AMP), which are typical polyphosphates existing in biosystems and have a similar chemical structure to di- and tri- polyphosphates, were also investigated, because such information on these compounds might provide some aid for understanding the physicochemical behaviour in biosystems at subzero temperatures. As a first step, these polyphosphates were examined by using DSC whether their frozen solutions and lyophilized samples would turn into a glassy state or not.

It became apparent that for the validity judgment of a glassy stabilizer, understanding the molecular mobility above and below  $T_g$  is necessary (12, 13, 14). Hancock *et al.* (12) elucidated the difference in molecular mobility below  $T_g$  among some excipients including indomethacin, sucrose, and poly (vinylpyrrolidone) (PVP) from the investigation of the enthalpy relaxation process, and pointed out that such investigation is important to understand the stability of the glassy state. Therefore, according to the method by Hancock *et al.*, we attempted to evaluate the stability of the glassy state for polyphosphate systems through the investigation of the enthalpy relaxation phenomena, and we compared them with carbohydrates such as trehalose and sucrose.

## MATERIALS AND METHODS

The reagents used in this study were sodium diphosphate decahydrate (WAKO Pure Chem., Inc., Japan), pentasodium tripolyphosphate anhydrous (WAKO Pure Chem., Inc., Japan), adenosine 5'-triphosphate (ATP) disodium salt (SIGMA 99%), adenosine 5'-diphosphate (ADP) sodium salt (SIGMA 95-99%), and adenosine 5'-monophosphate (AMP) sodium salt (SIGMA 99%). Approximately 10mg of 5-15(w/w)% aqueous solutions of the reagents were sealed in an aluminum pan with a crimping press, and the thermal analysis was performed on a differential scanning calorimeter (DSC) (SIMADZU DSC-50), which was calibrated for temperature and heat capacity by indium as a standard material. Samples were warmed to 293K at 2K/min after being cooled to 213K at -6K/min. To prepare low moisture samples, solutions of tripolyphosphate, ATP and ADP were lyophilized by being kept for a few days at 233K under the vacuum of  $3 \times 10^{-2}$  Torr. After that, the moisture content was adjusted by keeping in the desiccator where the relative vapor pressure was controlled. These dried samples were also sealed in an aluminum pan, and heated from 273K to 393K at 2K/min on DSC. After DSC measurements, pans were drilled on the top with a pin, and dried in the oven at 393K. Then the moisture content was determined from the weightlessness.

The lyophilized glassy samples of ATP, ADP and D (+) trehalose dihydrate (SIGMA) were sealed in an aluminum pan and scanned by DSC as the following. They were once heated to  $(T_g+30)$ K to erase their thermal history and cooled to  $(T_g-50)$ K. After that, samples were aged in a DSC for 2 to 48h at  $(T_g-10)$  to  $(T_g-45)$ K. Then, the aged samples were recooled to  $(T_g-50)$ K, and then reheated to  $(T_g+30)$ K. It is believed that the enthalpy relaxation effect during the DSC scanning can be minimized by keeping the heating and cooling rates the same (15, 16). Therefore, to eliminate such effects of enthalpy relaxation by the difference between the heating and cooling rates, every heating or cooling was performed at 5K/min. The extent of enthalpy relaxation ( $\Delta H$ ) was evaluated from the endothermic peak area around  $T_g$  obtained by the DSC heating scan. The moisture content of the samples was determined alternatively by the Karl-Fischer method.

## RESULTS AND DISCUSSION

### Glass transition of polyphosphate compounds

DSC warming curves for polyphosphate aqueous solutions indicated clear glass transition (glass-rubber) before ice-melting peaks appear as shown in Figure 1. The transition was thought to correspond to the glass transition of maximal freeze-concentrates referred to  $T_g'$  (1). The  $T_g'$ 's of di, tri-polyphosphates, ATP, ADP, and AMP were estimated to be 258, 230, 246, 244 and 249K, respectively. The only AMP aqueous solution showed an exothermic peak due to devitrification above the  $T_g'$ . It should be noted that double glass transitions, which have often been observed in low temperature DSC curves for some solution systems (17), did not appear and only one glass transition was observed.

Although, the DSC warming curves of only lyophilized ATP are shown in Figure 2, however, other lyophilized samples also indicated a clear glass transition. All the lyophilized samples were found to be in the glassy state. Moreover,  $T_g$  decreased with increasing moisture, indicating a plasticizing effect of water. The glass transition temperatures  $T_g$  versus moisture content relations for some polyphosphates are plotted in Figure 3.

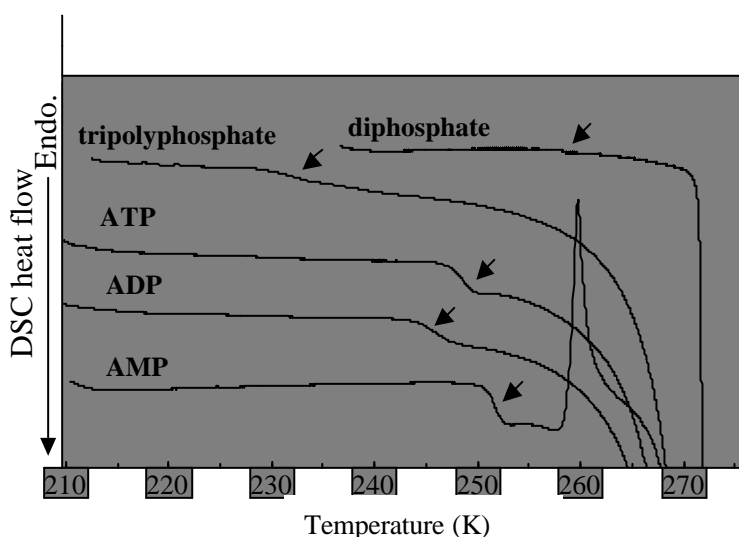


Figure 1. DSC warming curves of approximately 10% polyphosphate solutions. Every scanning was performed at 2K/min, except for diphosphate. Diphosphate sample was scanned at 0.5K/min. Arrows indicate  $T_g'$ .

In this study, every  $T_g'$  or  $T_g$  was decided by the onset point of the base line shift. In Figure 3, the fitting curves by the following Couchman equation (18, 19) Eq. 1 are also shown as solid lines,

$$T_g = \frac{X_s \cdot \Delta C_{p_s} \cdot T_{g_s} + X_w \cdot \Delta C_{p_w} \cdot T_{g_w}}{X_s \cdot \Delta C_{p_s} + X_w \cdot \Delta C_{p_w}} \quad \text{-(Eq. 1)}$$

where  $X_s$  is a mole fraction of solute, and  $X_w$  is that of pure water.  $T_{g_w}$  and  $\Delta C_{p_w}$  are the glass transition temperature and heat capacity increment for pure water, respectively, and were given as 135K and 35J/(mol·K) from a literature (20).  $T_{g_s}$  and  $\Delta C_{p_s}$  for pure solute are adjustable parameters in the fitting calculation.

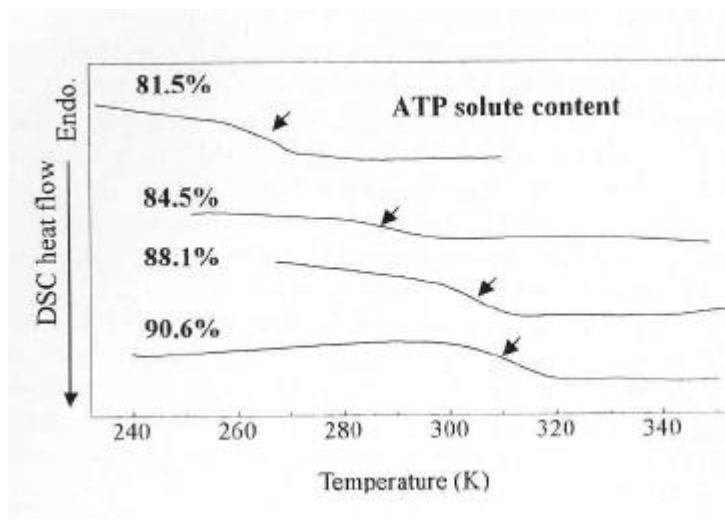


Figure 2. The typical DSC heating curves of lyophilized ATP adjusted to each solute content. Every scanning was performed at 2K/min and arrows show  $T_g$ .

For comparison, the glass transition temperature curves for trehalose and sucrose (21) are also shown in Figure 3. It was found that tripolyphosphate, ATP and ADP have relatively high  $T_g'$  and  $T_g$  values compared with carbohydrates such as trehalose and sucrose. On the other hand,  $C_g'$ , i.e., solute concentration at  $T_g'$  (2), was found to be lower than that of trehalose and sucrose systems. This result suggests that the polyphosphates have higher water holding capacity. These results are summarized in Table 1.

Table 1. The glass transition temperature of polyphosphate compounds

Compound	$T_g'$ (K)	$T_g$ (K)	$\Delta C_p$ (J/(mol·K))	$C_g'$ (%)	molecular weight
Diphosphate	258	-	-	-	266.1
Tripolyphosphate	230	377	267	62.4	367.9
ATP	246	390	248	76.3	551.1
ADP	244	395	226	72	427.2
AMP	249	-	-	-	347.2
Sucrose Ref. 21)	227	335	195	81.7	342.3
Trehalose Ref. 21)	233	373	187	81.6	342.3

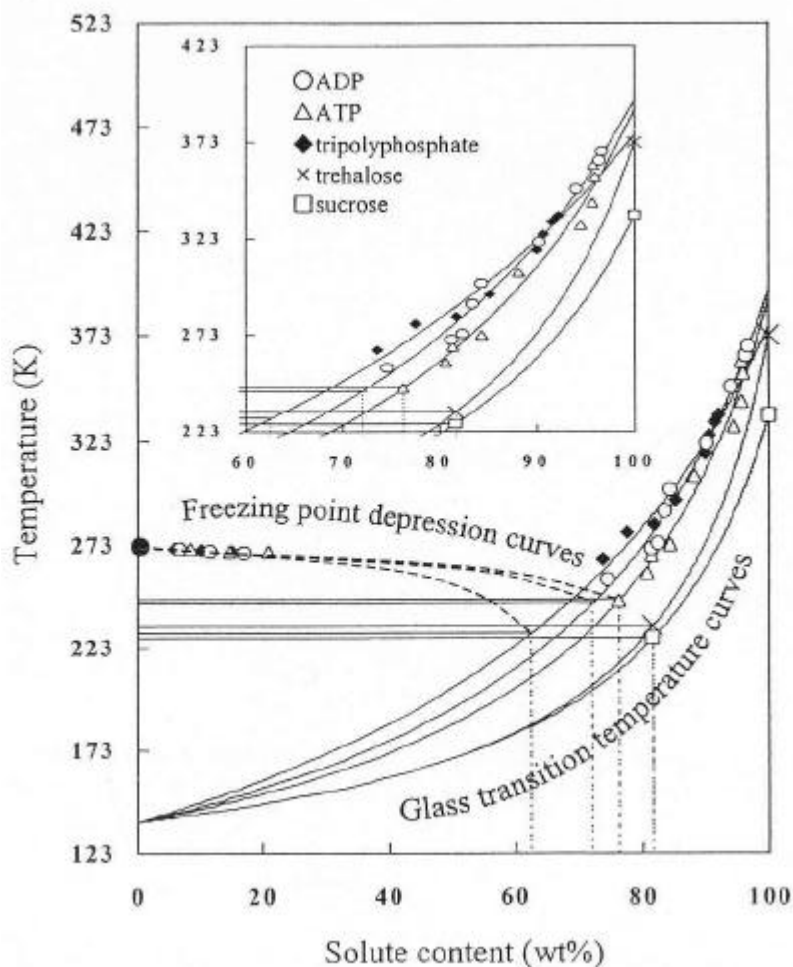


Figure 3. State diagrams of solutes - water binary systems. The data for sucrose and trehalose in the diagram were taken from ref. 21

### ENTHALPY RELAXATION OF POLYPHOSPHATES

Figure 4 shows the DSC heating curves of fresh and aged glassy ATP, ADP and trehalose as a typical result. Whereas the fresh samples showed normal glass transition feature, the aged samples indicated an endothermic peak around  $T_g$ , which represents the enthalpy relaxation of the glassy samples. Though DSC thermograms for other aging conditions are not shown here, they gave similar results. The relaxed enthalpy ( $\Delta H$ ) for the samples aged under different conditions was estimated from the peak area as shown in Figure 4. Then, the relation of the relaxed enthalpy and aging time is plotted in Figure 5. The relaxed enthalpy for glassy ATP and ADP was found to be considerably less than that of trehalose. At  $(T_g-30)K$ , though it is not shown here, the enthalpy relaxation for ATP and ADP could not be recognized within 48h aging, whereas that of trehalose was confirmed even at  $(T_g-45)K$ .

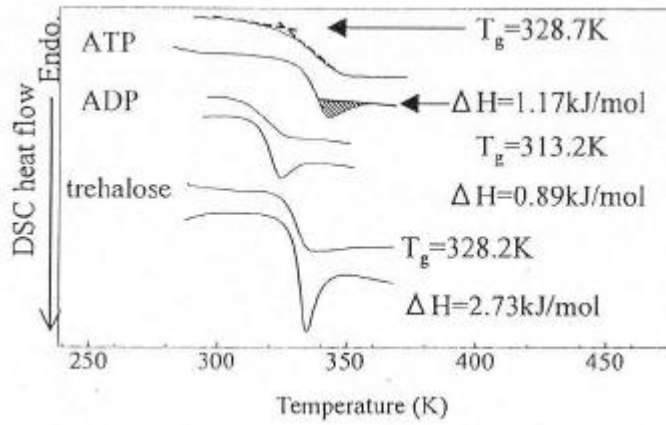


Figure 4. Typical DSC heating curves of fresh (above) and aged (below) glassy ATP, ADP and trehalose sample. The aging for these samples was performed for 48h at  $(T_g-15)K$ .

To obtain a mean relaxation time ( $t$ ), the relaxed enthalpy–aging time data were analyzed by Kohlrausch –Williams -Watts (KWW) equation (Eq. 2) (3, 12, 22),

$$F_t = \exp\left(-\left(\frac{t}{\tau}\right)^\beta\right) \text{-(Eq. 2)}$$

where  $t$  is aging time,  $\tau$  and  $\beta$  are the mean relaxation time and a relaxation time distribution parameter, respectively. And  $F_t$  is a fraction of enthalpy remained unrelaxed as defined by Eq. 3,

$$F_t = 1 - \left(\frac{H_t}{H_\infty}\right) \text{-(Eq. 3)}$$

where  $H_t$  is the relaxed enthalpy after  $t$  hour, and  $H_\infty$  is the maximum relaxed enthalpy at the aging temperature,  $T$ .  $H_\infty$  calculated from Eq. 4,

$$H_\infty = (T_g - T) \cdot C_p \text{-(Eq. 4)}$$

where  $T_g$  and  $C_p$  is the glass transition temperature and the heat capacity change at  $T_g$  measured, respectively. The above KWW equation, which has been often used to analyze a relaxation process of amorphous polymers (22), was used for small molecules by Hancock *et al.* (12). The best fitting to the KWW equation was found to be applicable to the results obtained for each sample as shown in Figure5. This means that all the relaxation processes of ATP, ADP and trehalose obey the KWW equation. So,  $\tau$  and  $\beta$  for the samples at each aging temperature were determined.

Furthermore, the relation between the  $\tau$  for each sample versus aging temperature was analyzed by using the Vogel– Fulcher- Tamman (VFT) equation (Eq. 5) (23, 24),

$$\tau = \tau_0 \cdot \exp\left(\frac{T_0 \cdot D}{(T - T_0)}\right) \text{-(Eq. 5)}$$

where  $\tau_0$  and  $T_0$  are hypothetical relaxation time constant for an unrestricted molecule and an ideal temperature at which molecular mobility is frozen perfectly, respectively (12, 23).  $D$  is a parameter that controls how closely the system obeys the Arrhenius law (25). This VFT

equation, which is known to be applicable to a non-Arrhenius type dynamic behaviour, has been used to characterize glassy materials above  $T_g$  by Angell *et al.* (23, 24, 25).

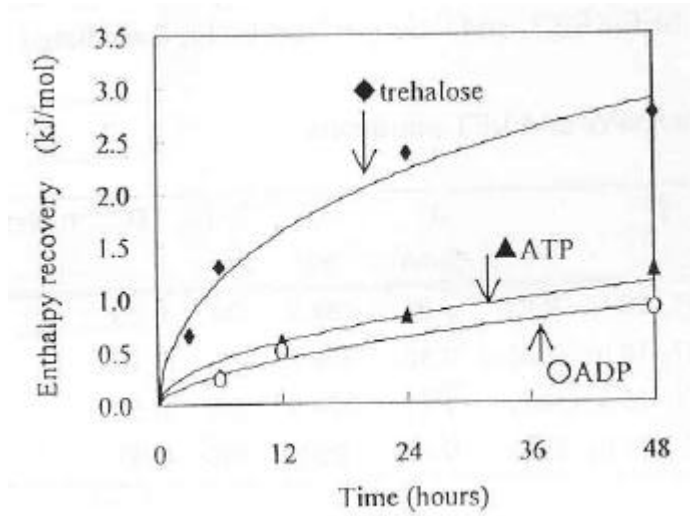


Figure 5. Enthalpy relaxation-aging time at  $(T_g-15)K$ . The solid lines were obtained by fitting the KWW equation.

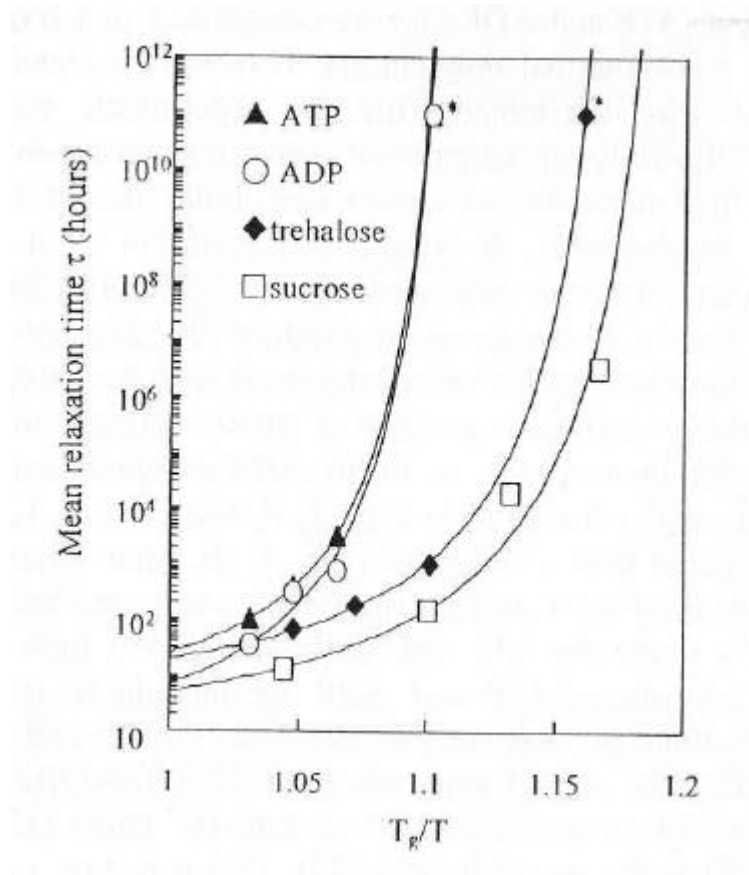


Figure 6. The relation between the mean relaxation time  $t$  and  $T_g/T$ . The solid lines were calculated from the VFT equation. The point denoted by superscript \* means that at the point no enthalpy recovery has been observed at least within 48h. The plots for sucrose were taken from ref. 12, and the fitting curve was recalculated in this study.

On the other hand, Hancock *et al.* first applied the VFT equation to the analysis of the relaxation process below  $T_g$  for glassy materials. In this study, to evaluate the relaxation process with a wide temperature range below  $T_g$ , we also attempted to use this equation. Thus, the plots of the  $\ln t$  and  $T_g/T$ , and the best fitting curves by Eq. 5 are shown in Figure 6. The values of parameters,  $t_0$ ,  $D$  and  $T_0$ , giving the best fit for Eq. 5, and  $\beta$  determined by Eq. 2 are listed in Table 2.

Table 2. The parameters in KWW and VFT equations

Compound	$\beta$	$t_0$ (hour)	$T_0$ (K)	$T_g-T_0$ (K)	$D$	moisture content (wt%)
ATP	0.53-0.55( $T_g-10$ to $-20K$ )	0.81	294.2	34	0.39	6.7
ADP	0.54-0.62( $T_g-10$ to $-20K$ )	0.12	278.2	33	0.48	11.2
Trehalose	0.56-0.87( $T_g-15$ to $-30K$ )	1.10	274.5	51	0.52	8.2
Sucrose <sup>Ref. 12)</sup>	0.40-0.80( $T_g-15$ to $-45K$ )	0.25	290	60	0.62	-

As shown in Table 2, the obtained values of parameter  $\beta$  were within the range of 0.4 to 0.8 for every compound, and showed an increasing tendency as the aging temperature decreases. According to Ediger *et al.* or Böhmer *et al.*, the  $\beta$ , representing the degree of ununiformity in the distribution of relaxation time, gives around 0.5 at  $T_g$  for most fragile glassy systems while near to 1.0 for strong glasses such as silica (26, 27). Then, judging from  $\beta$  obtained in this experiment, ATP and ADP may be categorized in a fragile glass type that has structures that do not resist thermal degradation. However, it should be noted that  $\beta$  referred by Böhmer *et al.* was determined from the experiments conducted under the temperature condition of  $T > T_g$  while our experiment was carried out below  $T_g$ . With respect to  $\beta$  below  $T_g$ , there are a limited number of reports even today though Hancock *et al.* also reported some data (12). Furthermore, theoretical interpretation as to the parameter  $\beta$ , including the temperature dependency, is on confusing and not yet established. For example, it is considered that  $\beta$  decreases with the aging temperature (28, 29), however, some studies reported that  $\beta$  does not always show such a decreasing trend with the aging temperatures (30, 31). The complexity of enthalpy relaxation process of glassy materials might have reflected such results, and more data will be necessary for the essential interpretation of glass.

It has been proposed through experiments below  $T_g$  that the less  $T_g-T_0$  value is, the more fragile and effective the material is as a stabilizer (13).  $T_g-T_0$  value obtained for ATP, ADP and trehalose, which were determined from the experiments conducted below  $T_g$ , were 34, 33 and 51K, respectively. It was found that ATP and ADP take not only high  $T_g$ ' and  $T_g$  but also small  $T_g-T_0$  value. Then, amorphous ATP and ADP are thought to be more fragile and effective as a stabilizer than trehalose and sucrose. However, both  $t_0$  and  $D$  listed in Table 2 were far from literature values for various materials determined from the experiments above  $T_g$ . For example,  $t_0$ s of almost glassy materials are less than  $10^{-9}$  hours (12), and  $D$  of fragile system such as glassy  $Ca(NO_3)_2$  is reported to be 6 (23). That is to say,  $t_0$  was too large and  $D$  was too small. The inconsistent results may be derived from the difference in the condition of measurements, i.e. above or below  $T_g$ . In other words, the result in this study indicates that the parameters obtained by the measurement above  $T_g$  do not always coincide with those obtained below  $T_g$ . There remains difficulty in the theoretical interpretation of such parameters as Hancock *et al.* have suggested (12). At this stage, it might be thought to be worthwhile that the parameters were got and could be compared as listed in Table 2.



## CONCLUSIONS

It was found that polyphosphates including ATP related compounds are good glass formers as well as carbohydrates, and they have a relatively high glass transition temperature. Among them, ATP and ADP were the best glass formers having high  $T_g'$  and  $T_g$ . Moreover, from the result of the enthalpy relaxation study below  $T_g$ , it was found that ATP and ADP form a fragile glass with small  $T_g-T_0$  value compared with trehalose or sucrose. Therefore, these polyphosphate materials might be expected as an effective protein stabilizer. However, the mechanism why ATP and ADP form such a stable glass could not be understood in this study. It should be noticed that they have many phosphate ester groups with charge. Such characteristics may affect the glass transition behaviour and stability of amorphous polyphosphates.

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