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STUDIES ON THE ISOTHERMAL CRYSTALLIZATION OF D-GLUCOSE AND CELLULOSE OLIGOSACCHARIDES BY DIFFERENTIAL SCANNING CALORIMETRY

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ABSTRACT

Isothermal crystallization from the glassy state of D-glucose and cellulose oligosaccharides (*e.g.*, cellobiose, cellotriose, and cellotetraose) has been studied by differential scanning calorimetry. The crystallization of amorphous D-glucose and oligosaccharides was very difficult in the absence of traces of water. Amorphous cellobiose and cellotetraose crystallized far more rapidly than amorphous D-glucose and cellotriose. The activation energy for the crystallization of cellobiose and cellotetraose was $\sim$10–12 kJ.mol^{-1}, while that for D-glucose and cellotriose was $\sim$1–2 kJ.mol^{-1}. An odd–even effect seemed to be associated with the crystallization process of these saccharides.

INTRODUCTION

The crystal structure of D-glucose and cellulose oligosaccharides has been studied by several groups of workers[1–4]. Alfthan *et al.*[5] estimated the temperatures of the glass transition of oligosaccharides from cellulose and xylan by the use of a torsional braid analyzer. Shafizadeh and Lai[6] investigated the thermal transformations and rearrangements of β-cellobiose and trehalose by dynamic thermal analysis and chemical methods, and reported that β-cellobiose showed concurrent melting and thermal anomerization, followed by condensation and ultimate decomposition at about and above the melting temperature. However, little attention has been paid to the physical properties of cellulose oligosaccharides in connection with the crystallization process from the amorphous state at lower temperatures.

The purpose of the work reported here was to clarify the crystallization process of amorphous D-glucose and cellulose oligosaccharides by using differential scanning calorimetry (d.s.c.). The crystallization process of a polymer is ordinarily observed by decreasing the temperature from that of the molten state to the crystallization temperature. It was possible to obtain amorphous D-glucose by the use of the above

procedure. However, it was difficult to maintain cellulose oligosaccharides in a molten state, as the compounds decompose immediately above the melting temperature. Therefore, the crystallization process was observed by using amorphous oligosaccharides obtained by freeze-drying their aqueous solutions[7].

EXPERIMENTAL

Sample preparation. — Cellulose oligosaccharides up to cellotetraose were prepared according to the procedure reported by Miller *et al.*[8]. Their purities were checked by thin-layer chromatography[9]. It was confirmed that each oligosaccharide gave only one spot after development. The degree of polymerization of oligosaccharides was estimated from the values of molecular weight measured from the lowering of vapor pressure.

Amorphous D-glucose was prepared by rapid cooling from the melt to the solid state. D-Glucose and cellulose oligosaccharides from cellobiose to cellotetraose were also obtained in the amorphous state by freeze-drying their aqueous solutions. First, small amounts of the samples were dissolved in water. The solution was then frozen quickly (liquid nitrogen), and dried *in vacuo* so as to evaporate off the solvent. Amorphous materials obtained were then dried thoroughly over phosphorus pentaoxide *in vacuo* for more than 2 days. The X-ray diffractograms of the products are all very similar, showing typically amorphous patterns as seen from Fig. 1.

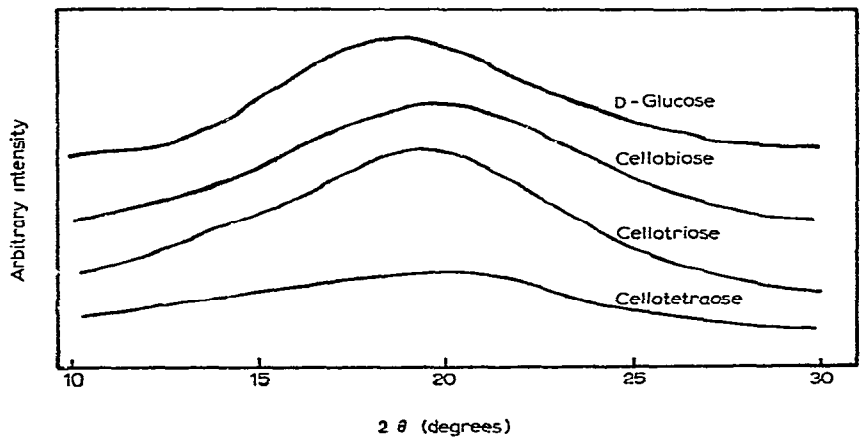


Fig. 1. X-Ray diffractograms of amorphous D-glucose and cellulose oligosaccharides.

Methods. — The molecular weight of oligosaccharides was measured with a Hitachi Model-115 molecular-weight apparatus.

The X-ray diffractograms were obtained with a Rigaku Denki Model 3D-F X-ray diffractometer equipped with a proportional counter.

The thermal behavior of the samples was observed with a Perkin–Elmer differential scanning calorimeter, DSC-II. The sample weights were between 1.0 and

4.0 mg. D.s.c. curves were obtained at the heating rate of 10°/min. Isothermal crystallization was carried out in an isothermal state maintained at each predetermined temperature between glass-transition and crystallization temperatures. The area of a transition peak was noted as J/g, using the calibration factor determined from the area of the melt of a known weight of indium.

RESULTS AND DISCUSSION

As shown in Fig. 2, the d.s.c. curve (*A*) of amorphous D-glucose obtained by cooling from the molten state shows only the glass transition. Amorphous D-glucose obtained by freeze-drying shows a d.s.c. curve (*B*) which is very similar to that of amorphous D-glucose obtained from the molten state. However, the d.s.c. curve (*C*) of amorphous D-glucose exposed to an atmosphere of 40% relative humidity shows broad crystallization and successive melting peaks, which means that amorphous D-glucose crystallizes in the presence of a trace of water, whereas completely anhydrous D-glucose is very difficult to crystallize.

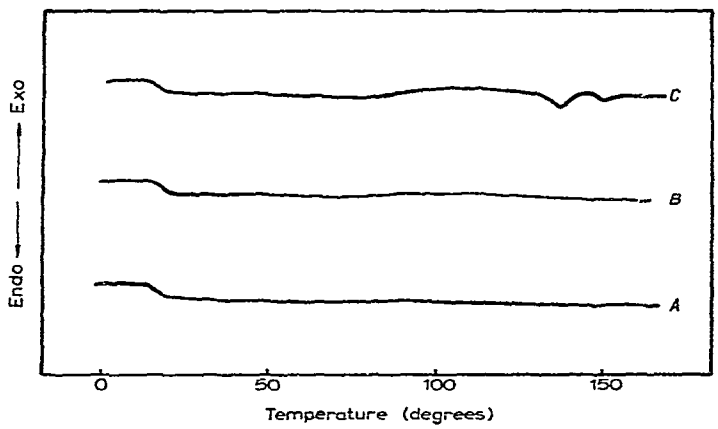
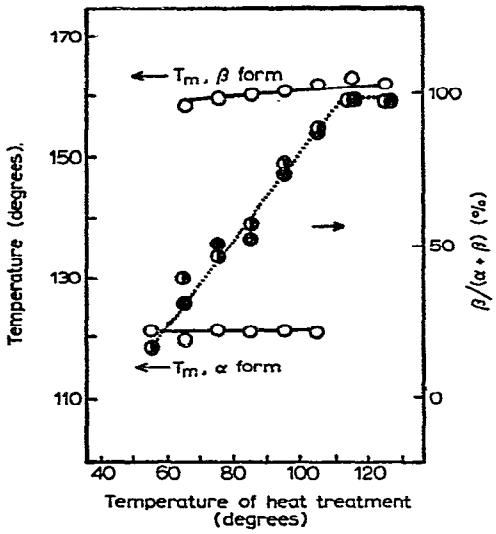


Fig. 2. D.s.c. curves of amorphous glucose. Heating rate, 10°/min. (*A*) Amorphous D-glucose obtained by cooling rapidly from molten state; (*B*) amorphous D-glucose obtained by freeze-drying its aqueous solution; (*C*) amorphous D-glucose exposed to an atmosphere of 40% relative humidity for 24 h before scanning.

Fig. 3 shows the effect of heat treatment on amorphous D-glucose exposed to an atmosphere of 40% relative humidity for 24 h. Samples were heat-treated at different temperatures for 1.5 h. The temperature of a melting peak of anhydrous α-D-glucose is not much changed by the temperature of heat-treatment, whereas that of anhydrous β-D-glucose shifts to higher temperature on elevating the heat-treatment temperature. The dotted line in Fig. 3 shows the ratio of α and β forms of anhydrous D-glucose obtained at different temperatures of heat-treatment. Only the α form of anhydrous D-glucose was obtained by the heat-treatment below 50°, whereas only the β form was obtained above 110°.



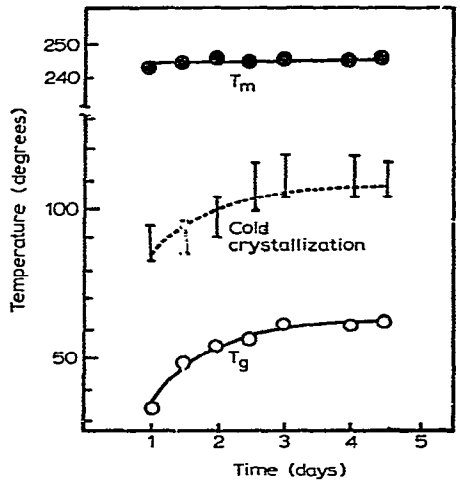


Fig. 3. Melting temperatures (T_m) and the ratio between the α and β forms of D-glucose obtained by isothermal crystallization of amorphous D-glucose: the ratio $\beta/(\alpha+\beta)$ calculated from the peak area of the d.s.c. curves (●); the ratio $\beta/(\alpha+\beta)$ calculated from X-ray diffractograms (◑).

Fig. 4. Effect of drying time on glass-transition (T_g), cold-crystallization, and melting temperatures (T_m) of cellobiose.

As seen from Figs. 2 and 3, two melting peaks corresponding to the α and β forms of D-glucose were observed in the d.s.c. curves of amorphous D-glucose treated under appropriate conditions. It is well-known that the heat of fusion of a crystalline material is proportional to the amount of crystalline structure[10]. Therefore, it is possible to calculate the amounts of α and β forms of D-glucose from the areas of the two melting peaks observed in the d.s.c. curve of a heat-treated sample. The ratio $\beta/(\alpha+\beta)$ thus calculated increases almost linearly with elevation of the temperature of heat-treatment below 110°, as shown in Fig. 3.

The main peaks of α and β forms of anhydrous D-glucose were observed at values of 2θ of $\sim$20 and $\sim$16°, respectively, in the X-ray diffractograms[1,11]. The ratio $\beta/(\alpha+\beta)$ was calculated from the peak intensity of each main peak corresponding to the α and β forms. The ratio calculated from the area of melting peaks in the d.s.c. curves agreed well with the data obtained from X-ray diffraction.

Fig. 4 shows the effect of drying time on glass-transition, cold-crystallization, and melting temperatures of amorphous cellobiose. Glass-transition and cold-crystallization temperatures shift to higher values with increase in drying time. However, the melting temperature was not affected by drying. The above phenomena suggest that water affects the molecular motion in the amorphous state more significantly than in the crystalline state.

Fig. 5 shows the d.s.c. curves of amorphous cellobiose, cellotriose, and cellotetraose. Amorphous cellotriose did not crystallize if it was completely dried. There-

fore, amorphous cellotriose was exposed to an atmosphere of 40% relative humidity for 24 h before scanning by d.s.c. The d.s.c. curves of amorphous cellotriose show a broad, cold crystallization which is similar to that of amorphous D-glucose. On the other hand, amorphous cellobiose and cellotetraose crystallized in the dry state more easily than cellotriose. The d.s.c. curves of amorphous cellobiose and cellotetraose show clear exothermic peaks of crystallization (Fig. 5). Cellobiose shows a melting peak at ~245°. However, d.s.c. curves of cellotriose and cellotetraose did not show melting peaks, as these compounds decomposed above the melting temperatures.

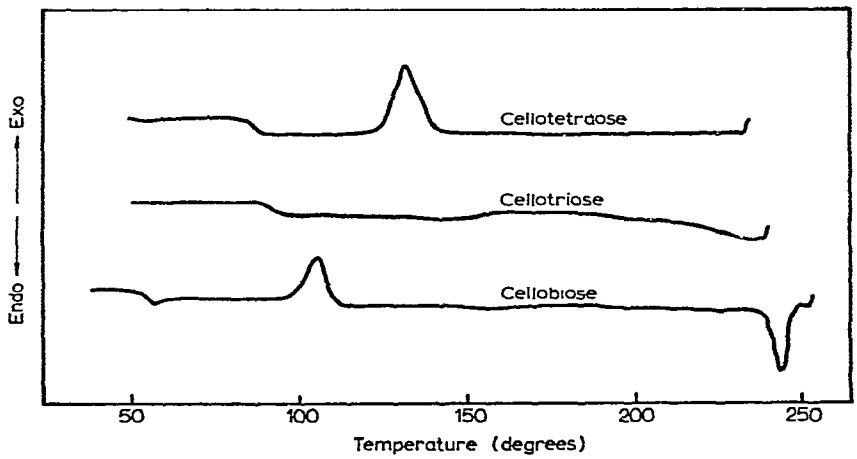


Fig. 5. D.s.c. curves of amorphous cellulose oligosaccharides. Heating rate, 10°/min.

Figs. 6–9 show isothermal crystallization curves for D-glucose, cellobiose, cellotriose, and cellotetraose, respectively. The crystallinity of each saccharide was estimated by designating the equilibrium state of crystallization at each pre-determined heat-treatment temperature as 100%. Crystallization of amorphous D-glucose and cellotriose proceeds slowly, as shown in Figs. 6 and 8. The crystallinity of D-glucose and cellotriose reached ~20% after heat treatment for 30–40 min, judging from the X-ray diffractograms obtained by the use of Wakelin's method[12]. On the other hand, crystallization of amorphous cellobiose and cellotetraose proceeds rapidly. The crystallinity of both saccharides reached ~90% after heat treatment for 1–2 min.

The data in Figs. 6–9 indicate that the observable molecular rearrangements from the amorphous to the crystalline state of oligosaccharides occur by a first-order mechanism. It is reasonable to assume that there is sufficient thermal motion of the molecules to cause some concurrent alignment of the molecules to form primary nucleation sites. Alternatively, some nucleation sites could form during the initial period, until a temperature is reached where nucleation and subsequent crystallization can occur. The dependence on time of this initial process should be influenced by the crystal structure growing from these nuclei. Trace amounts of water probably play an important role in some of the above processes. It may be appropriate to consider

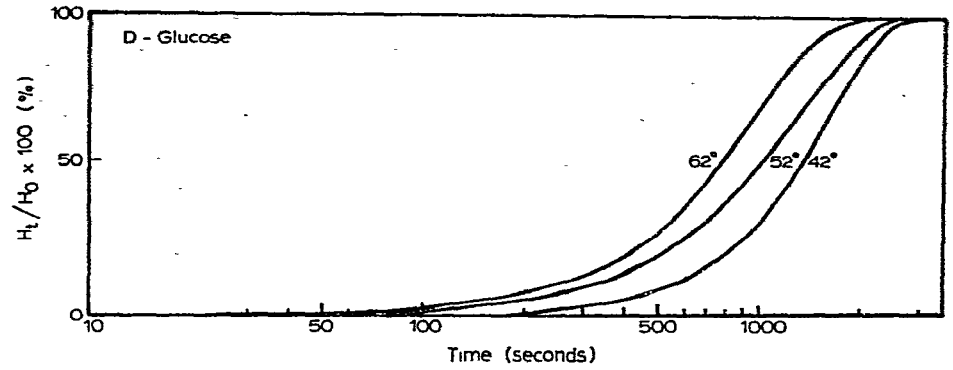


Fig. 6. Isothermal crystallization curves of amorphous D-glucose; H_0 is the equilibrium heat of transition, and H_t is the heat of transition at time t.

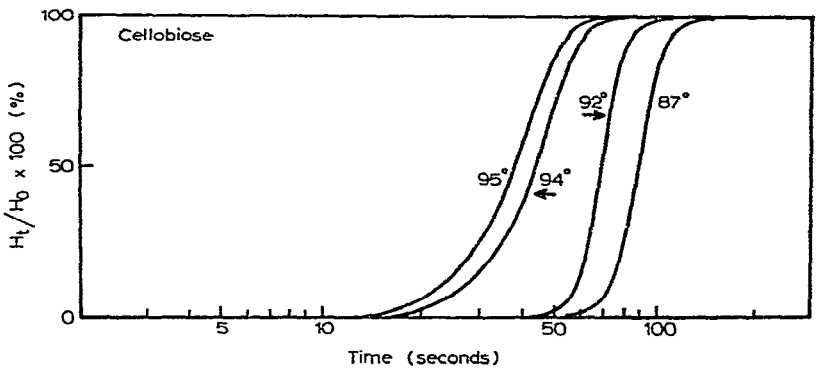


Fig. 7. Isothermal crystallization curves of amorphous cellobiose; H_0 is the equilibrium heat of transition, and H_t is the heat of transition at time t.

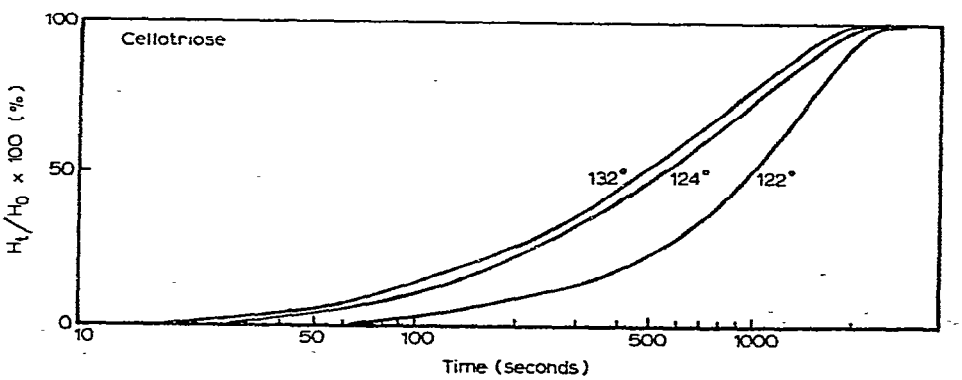


Fig. 8. Isothermal crystallization curves of amorphous cellotriose; H_0 is the equilibrium heat of transition, and H_t is the heat of transition at time t.

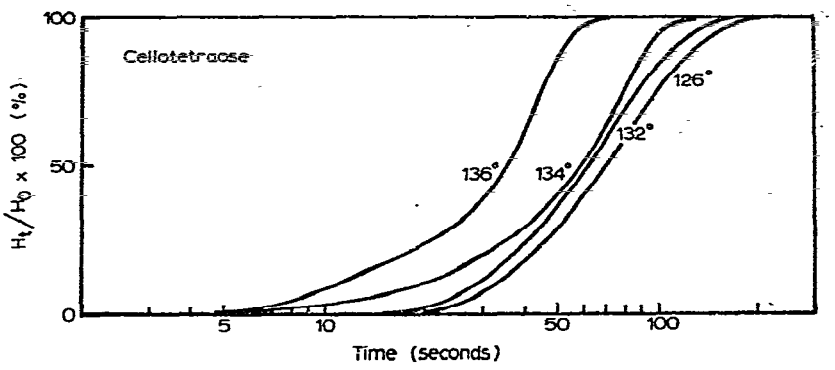


Fig. 9. Isothermal crystallization curves of amorphous cellotetraose; H_0 is the equilibrium heat of transition, and H_t is the heat of transition at time t.

that the presence of water enables molecules to move more easily than those in the completely dry state. Then if the free energy of the crystalline phase were lower than that of the amorphous phase, crystallization would occur under proper conditions.

This initial process is defined by $-dx/dt = k(a-x)$, where k is independent of a, x is the amount of crystal formed, and a is the amount of amorphous available for crystallization. The calculated rate constants for crystallization of amorphous D-glucose, cellobiose, cellotriose, and cellotetraose, shown in Table I, indicate that amorphous cellobiose and cellotetraose crystallise far more rapidly than D-glucose and cellotriose.

TABLE I

RATE CONSTANTS FOR CRYSTALLIZATION OF AMORPHOUS D-GLUCOSE AND OLIGOSACCHARIDES

Sample	*Temperature (degrees)*	*Rate constant ($sec^{-1} \times 10^2$)*
D-Glucose	42	0.35
	52	0.48
	62	0.59
Cellobiose	87	1.34
	92	2.52
	94	2.91
	95	3.34
Cellotriose	122	0.48
	124	0.63
	132	0.75
Cellotetraose	126	0.48
	132	1.10
	134	1.34
	136	1.97

The apparent activation energy for the crystallization process of the foregoing saccharides was calculated by using the Arrhenius relationship. Fig. 10 shows Arrhenius plots employing rate constants as a function of temperature. As shown in Table II, the activation energy for the crystallization of cellobiose and cellotetraose is $\sim$10–12 kJ.mol^{-1}. This value is similar to that of the activation energy for crystallization of amorphous cellulose[13] and other polymers[14,15].

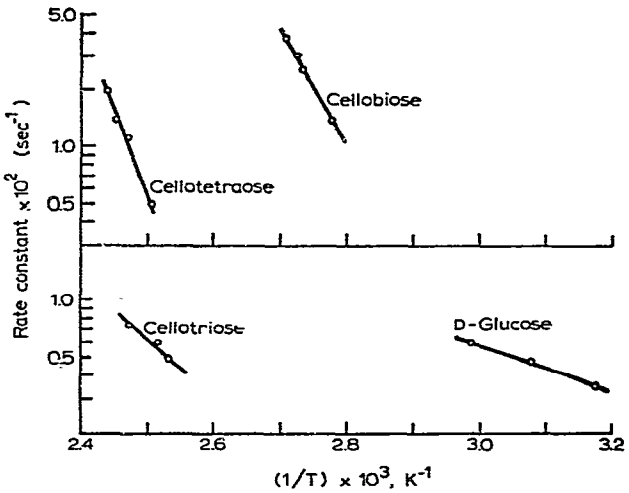


Fig. 10. Arrhenius plot, employing rate constants as a function of temperature.

TABLE II

ACTIVATION ENERGY CALCULATED FROM CRYSTALLIZATION CURVES FOR D-GLUCOSE AND OLIGOSACCHARIDES

Sample	*Activation energy* (*kJ.mol*$^{-1}$)
D-Glucose	1.2
Cellobiose	9.7
Cellotriose	1.7
Cellotetraose	12.1

The activation energy for the crystallization of D-glucose and cellotriose is 1–2 kJ.mol^{-1}. These small values seem to reflect the initial process of crystallization, as the observed crystallinity of both saccharides was very low.

The evidence thus obtained supports the view that (*1*) crystallization of amorphous saccharides from D-glucose up to cellotetraose proceeds with difficulty in the absence of traces of water, and (*2*) an odd–even effect seems to be associated with the crystallization process of these saccharides.

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