

Manifestation of the Size Effect during Crystallization and Melting of Dispersed Water in Native and Amorphous Starches with Various Degrees of Hydration

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Received May 10, 2016; in final form, July 19, 2016

Abstract—Differential scanning calorimetry (DSC) was used to study the melting and crystallization of frozen water dispersed in humid potato starch. Melting and crystallization temperatures and heats as functions of the degree of hydration of the starch were obtained for native and amorphous starch states. Manifestations of the size effect were observed in the dependences of heat for the processes in both starch states. Crystallization and melting heats of frozen water were found to change nonlinearly with the increasing degree of hydration in all cases. In contrast, a size effect in the dependences of melting and crystallization temperatures of frozen water was detected only for native starch. Reasons responsible for the absence of a size effect in the amorphous state were considered. Hysteresis, which is characteristic of small particles, was observed upon melting and crystallization of frozen water and its manifestation strongly differed in the native and amorphous states of potato starch.

Keywords: calorimetry, starch, water clusters, crystallization, melting, size effect

DOI: 10.1134/S0006350917010213

INTRODUCTION

Substantial progress has been made in the past decades in studying the structure of starch, which is among the most widespread polysaccharides. Starch is a unique biopolymer; its native structure (granules) includes nanoscale crystalline elements, which are relatively easy to detect by standard X-ray diffraction [1–4]. However, only limited information is available for the detailed architecture of polymer chains in granules. Reliable data on the arrangement of water molecules in the complex hierarchic structure of starch granules is even scarcer. Studies of the hydrate shell of starch molecules generally proceed from the assumption that the properties of polymer-bound water in all biopolymeric systems substantially differ from those of bulk water. The specific organization of water in the vicinity of biomolecules has been studied for a long time using various methods [5–9]. Detection of new effects [10, 11] gives impetus to further studies of the problem [12].

In terms of calorimetry, attention is continuously given to the phase transition of water frozen at temperatures below 0°C, that is, supercooled water. This frozen water (FW) melts as the biopolymer–water sys-

tem is heated. The unfrozen water (UFW), which is an intrinsic part of the native polymer structure, does not freeze and melt. The water–biopolymer interaction energy of the unfrozen water is thought to be higher than the water–water interaction energy. Calorimetric experiments that directly support these views have been performed with DNA in particular [13, 14]. It should be understood that the water classification as bound (unfrozen) and free (frozen) in the biopolymer–water system is still used [15–19], but this is certainly a simplification [20]. The quantitative boundary between the two water states is known to depend on the detection technique, the temporal parameters of experiments, and the model used to describe the structure of the biopolymer.

Our calorimetric studies and many others have shown that small water structures (clusters) can form in biopolymers with a low frozen water content (~10%) and that they melt at temperatures appreciably lower than 0°C in contrast to bulk water. These structures have been observed in humid proteins [21–23], DNA [7], and high-concentration solutions of carbohydrates of both natural and artificial origins [6]. The melting temperature of water clusters has been found to decrease with the decreasing water concentration in the system under study [6, 7, 15–19, 23]; i.e., a mani-

Abbreviations: FW, frozen water; DSC, differential scanning calorimetry.

festation of a relatively prominent size effect can be assumed.

An increasing effect of surface energy on energy balance of small particles has been discussed over the past decades [3, 19, 24–29]. Cluster size limitations have been considered for both all three dimensions [19, 24–26] and one of them. The latter has been the case, in particular, in studies of lamellar crystals of synthetic polymers [27–29] and lamellae of starch crystallites [3]. Changes in the surface-to-volume ratio of the clusters have been identified as a major cause of the size effect.

Water clusters that substantially differ in properties from bulk water have been shown to form in starch samples with minor contents of frozen water [15–18, 23, 30–33]. As with other biopolymers, the changes observed in melting parameters of FW as a function of the frozen-water content in starch can be explained by the effect exerted by the size of clusters that form in the hydration shell of starch molecules.

However, it should be noted that appreciably different estimates have been reported in different studies for the absolute melting temperature (T_m) and absolute melting heat (Q_m) of frozen water in starch and their relationships with the corresponding parameters for the bulk water. We recently addressed the problem in detail when studying the heat properties of native and amorphous starch samples as a function of their water content [33]. In particular, we have found that absolute T_m estimates that exceed (sometimes substantially) the well-known value of bulk water as reported in many calorimetric studies [15–19, 34, 35] are most likely explained by the use of continuous heating in the cases where bound and free waters occur in comparable amounts in biopolymers. We proposed a procedure that allows taking into account a temperature delay in differential scanning calorimetry (DSC) and yields negative T_m values for water clusters [33]. Note that frozen water T_m estimates lower than 0°C have been obtained in classical studies of humid biopolymers by steady-state adiabatic calorimetry [7].

The situation is even more complex when the melting heat of FW is studied as a function of the degree of hydration in starch. It is natural to believe that the frozen water Q_m should approximate bulk water Q_m as the frozen water content in the system increases. However, when the Q_m of FW is estimated with due regard to weight of the frozen water from published data, the estimates obtained for starch samples with low frozen-water contents are lower than bulk water Q_m in some studies [15, 16] and higher than bulk water Q_m in some others [17, 18]. Our data on FW melting heats in various starches indicate that frozen water Q_m is substantially lower than bulk water Q_m at frozen-water contents below 35% [33]. In other words, Q_m also displays a size effect, which is overt in amorphous starch and less distinct in native starch according to our earlier

findings [33]. The factors underlying this situation require further investigation.

Native starch consists of granules, which include both crystalline and amorphous phases [36]. The amount of water incorporated in the crystal lattice remains constant during swelling [37], while the total water content in a native starch granule may increase to 50% as the water content in the amorphous phase of the granule increases [36, 38]. A starch granule can principally be considered as a transformable biopolymeric matrix whose pores accommodate water clusters of different sizes depending on the degree of hydration of starch, in contrast to classical solid matrices with constant pore sizes. The matrix walls are flexible in starch and the pore size is determined by how wide the pores open with changes in the degree of hydration. The pores are therefore always completely filled in such a system. It is also important to note that water clusters occur in an absolutely hydrophilic system because pore walls are formed by starch molecules surrounded by unfrozen water, whose content is 23–25% of the total starch weight [37, 39]. Thus, systems with water clusters of different sizes can be obtained with the same original matrix by changing the degree of hydration of starch granules. This offers an opportunity to study various properties of dispersed supercooled water.

To continue the previous studies, the main objective of this work was to investigate, in one DSC experiment, the crystallization and melting of dispersed water in a biopolymeric matrix over a broad range of degrees of hydration. Potato starch in native and amorphous states was used as a matrix.

We performed DSC measurements not only in a heating mode, as is usual, but also in a cooling mode. Use of the two complementing modes yielded principally new data on the processes. Experiments with amorphous starch associated thermal behavior of water clusters incorporated in a matrix with matrix properties and, on the other hand, provided further insight on how the concentration equilibrium is established in amorphous starch–water systems in various hydration regions.

In general, data on the thermodynamic parameters of melting and crystallization of water clusters that varied in size were obtained by studying the properties of supercooled water in various matrices; these data are of both theoretical and applied importance.

METHODS

The thermal properties of water in starch were studied using a differential scanning microcalorimeter DSC-111 (Setaram, France) with a sensitivity of $3 \cdot 10^{-5}$ J/s. Power calibration of the instrument was performed with a standard cell by the Joule effect and checked against the known melting heat values of reference substances (In, H₂O, and Hg) [28, 29]. The ref-

erence values mapped to the instrument sensitivity vs. temperature plot obtained with a standard cell with an accuracy better than 1%. Measurement errors were $\pm 5\%$ for the frozen water melting heat Q_m measured in starch in the heating mode and $\pm 10\%$ for the FW crystallization heat Q_c measured in the cooling mode at a rate of 5 degrees/min, which was most commonly used in experiments. The difference in accuracy is explained by the fact that heating of the calorimetric cell after its preliminary cooling to a necessary temperature was carried out without blowing the cell with liquid nitrogen vapor in our experiments. However, continuous blowing with liquid nitrogen vapor was necessary for recording the thermal effects in the cooling mode and substantially increased the noise level in heat-effect recordings.

Calibration of the temperature scale was performed using the same reference substances. The errors of temperature measurements were $\pm 0.2^\circ\text{C}$ in the heating mode and $\pm 1^\circ\text{C}$ in the cooling mode.

As is well known, melting of a crystalline substance is reflected in DSC in a heat absorption curve with the certain half-peak width and peak amplitude. The initial temperature measured from the curve is independent of the sample weight and corresponds to the T_m of the sample, while $T_{m\max}$ is a function of the heating conditions. To study how the degree of hydration affects the melting of FW presented in starch we used corrected peak temperatures determined from the melting curves of FW in this work. As has been demonstrated convincingly, the $T_{m\max}$ of the DSC curve is higher at higher values of sample weight, transition heat, and heating rate [28, 29]. In other words, experimental temperature values (T_m^{exp}) that correspond to maximums of FW melting curves recorded in a continuous heating mode are not thermodynamic parameters of the process under study.

A special procedure was used for T_m^{exp} correction as described in detail previously [33]. Measurements with DSC-111 were carried out so that all sample heating conditions with the exception of the water content in the sample remained constant. Thus, to estimate T_m from the experimental T_m^{exp} value for frozen water, it is necessary only to allow for a temperature delay that occurs in the heating mode and is associated with the dependence on the water weight of the sample. To obtain the necessary correction (T_m^{corr}), a weight dependence of the peak temperature of the melting curve was preliminarily constructed for distilled water ($T_m^{\text{exp}}(\text{H}_2\text{O})$) in the same heating conditions. The temperature of the transition under study (T_m) was obtained for each sample as T_m^{exp} (frozen water) minus the correction temperature T_m^{corr} , which was $T_m^{\text{exp}}(\text{H}_2\text{O})$ of a pure water sample with a weight equal to the fro-

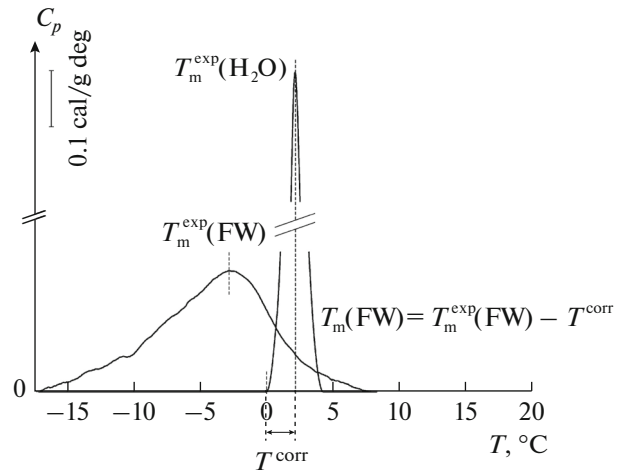


Fig. 1. Correction for a temperature delay of DSC. The frozen water weight is equal to the control H_2O sample weight; $V_{\text{heating}} = 5 \text{ deg/min}$.

zen water weight of the starch sample. The weight of the FW was obtained as a difference between the total water weight of a sample and the weight of water that is bound to the polymer and remains unfrozen upon cooling to low temperatures. Figure 1 illustrates the procedure for correcting the T_m^{exp} of FW. It should be noted that original dry samples of the same weight, 30 mg, were used to obtain starch samples that varied in their degree of hydration. We have previously observed that a similar correction of T_m^{exp} (frozen water) in two series of samples with original weights of 5 and 30 mg yielded $T_m(\text{FW})$ vs. degree of starch hydration dependences that coincided within $\pm 1^\circ\text{C}$.

Unlike with T_m , experimental temperatures at maximums of respective exothermal curves were used as temperatures of the crystallization of FW in starch.

Thus, the procedure for correcting T_c^{exp} was not carried out in the reverse scanning mode because experiments showed that the difference between the starting and maximum temperatures of a crystallization curve was no more than 2°C with both pure water and water contained in starch. That high initial rate of water crystallization compared with melting is possibly associated with the fact that the total surface area of crystallization centers as a source of heat upon cooling is several orders of magnitude greater than that of ampules as a source of heat upon heating.

It should be noted that supercooling was not detected in experiments on cooling bulk water drops weighing 1–30 mg, which were not dispersed water. The result fully agrees with the views of water supercooling that have been developed in classical works on the problem [40] and suggest that only dispersed water is capable of supercooling. Water drops with sizes of approximately $3 \mu\text{m}$ can be supercooled to a tempera-

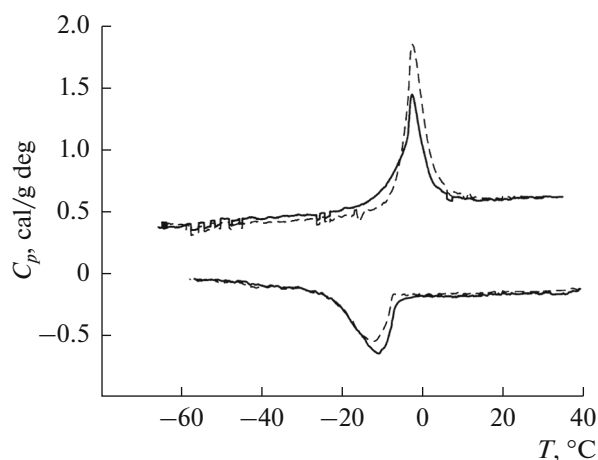


Fig. 2. The temperature dependences of the heat capacity in the region of the crystallization and melting of frozen water in native (solid lines) and amorphous (dashed lines) potato starch with a 35% degree of hydration. $V_{\text{heating}} = V_{\text{cooling}} = 5 \text{ deg/min}$.

ture of $\sim -42^\circ\text{C}$ (231 K) [40]. This temperature is thought to be the minimum temperature to which dispersed water can be supercooled at a cooling rate of $\sim 10 \text{ deg/s}$, while a cooling rate of $\sim 10^5 \text{ deg/s}$ is required for generating of so called amorphous ice or glassy water, which is observed at temperatures below 77 K.

We used potato starch (Aldrich, United States), whose thermal properties were investigated in our previous works [23, 41–44]. Native potato starch granules contain B-type nanocrystallites, which differ from A-type crystallites in having a relatively high content, approximately 25%, of crystalline hydrate water [1, 2, 37], which does not freeze. The content of unfrozen and nonmelting water in native starch has experimentally been estimated at $\sim 23\%$ of the total weight by DSC [31–33]. (This is the boundary concentration, below which an endothermic peak of frozen water melting is not observed.) Note again that frozen water is in the amorphous phase and is irregularly distributed throughout a starch granule [38, 45, 46].

The crystallization and melting processes of FW were studied not only in native, but also in amorphous starch. Amorphous starch samples were obtained directly in experiments by heating native starch samples with various degree of hydration in a calorimetric cell to allow thermal destruction of biopolymer nanocrystallites.

In this work, the degree of hydration was varied from 27 to 45% for DSC measurements of crystallization and melting of dispersed water in starch. To achieve the necessary water concentration, samples were moistened (up to $\sim 30\%$) or added with water (up to 45%) at room temperature in various experiments. The initial humidity was preliminarily measured (to $\pm 1\%$) with control samples by vacuum pumping at

$T = 105^\circ\text{C}$ to a constant sample weight. The initial humidity was 10–13% depending on the ambient conditions. The water concentration (humidity, or degree of hydration; %) in a sample was obtained as $(\text{water weight/dry starch weight} + \text{water weight}) \times 100$. To achieve a uniform water distribution, native samples were placed in hermetic steel ampoules and kept at room temperature for 1–2 days. Amorphous starch samples were examined immediately after amorphization of native starch.

RESULTS AND DISCUSSION

Our DSC measurements in the cooling mode yielded new data on thermodynamic parameters of the crystallization process (transition temperatures and heats) for water contained in the potato starch–water system at concentrations varying from 27 to 45%. Both native and amorphous starch states were examined. The results obtained for frozen water melting in starch and amorphization of the biopolymer fully agreed with the data published previously by us for a broader range of degree of hydration. A self-consistent picture of the processes was obtained in the study.

It should be noted that an experiment consisted in a serial recording thermograms in both direct and reverse scanning modes for each sample. Measurements were carried out over a broad temperature range from -80 to 120°C . A minimal series of measurements included preliminary sample cooling (frozen water crystallization in native starch) \rightarrow heating to allow frozen water melting and then to disrupt the native starch structure \rightarrow cooling immediately following amorphization (frozen water crystallization in amorphous starch) and \rightarrow recording the frozen water melting curve in the amorphous polymer. As an example, Fig. 2 shows typical thermograms recorded for the crystallization and melting of FW in native and amorphous potato starch in such a series of measurements.

New data on how the crystallization temperature of FW in native and amorphous potato starch depends on its degree of hydration in the range from 27 to 45% are shown in Fig. 3. This figure shows the respective melting temperature T_m values in the concentration range examined in this work and the data obtained previously in a broader range as well [33]. To explain the relevant processes, we consecutively consider the changes observed in the parameters in question.

The melting temperature of water clusters in native and amorphous starch. A size effect was distinct in the dependence of frozen water T_m on the degree of hydration in the case of native starch; that is, the lower the degree of hydration of starch was, the lower the frozen water T_m was (Fig. 3, curve 1). Such an effect was not detected in the case of amorphous starch. Moreover, the pattern reversed after starch amorphization. The lower the degree of hydration was, the higher the T_m of

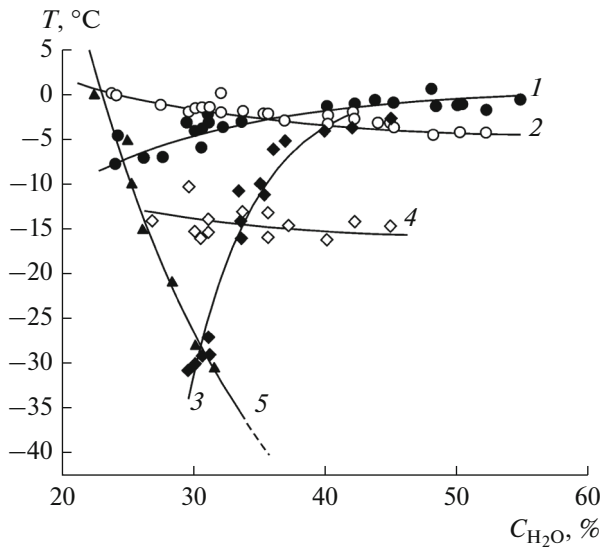


Fig. 3. The melting temperatures (curves 1 and 2) and crystallization temperatures (curves 3 and 4) of frozen water in native and amorphous potato starch as functions of the degree of hydration. Curve 5, starch glass transition temperature as a function of the degree of hydration [43].

water clusters in amorphous starch was (Fig. 3, curve 2).

As is seen from Fig. 3, curves 1 and 2 intersect at a ~35% degree of hydration of starch. Many studies have demonstrated that this degree of hydration separates the processes that occur in the starch–water system on heating into two groups [9]. To highlight the difference between them, destruction of the native starch structure at water concentrations higher than 30–35% is termed gelatinization, while the term heat moisture treatment is used for the processes that occur at lower concentrations. Our results (Fig. 3) agree well with this separation.

Assuming that the $T_{m_{max}}$ of the melting curve of FW reflects the average size of water clusters that form in starch and that Q_m reflects their quantity, our results indicate that both the cluster size and quantity increase because of amorphization at water concentrations below 35% (~2 cal/g at the level of 4–5 cal/g; see Fig. 4). When water concentrations are higher than 35%, the cluster size decreases, while the total cluster quantity increases by only ~1 cal/g at the level of 10–15 cal/g. The last finding does not contradict the fact that the frozen water content in starch partly decreases on amorphization, as has been observed in our previous [31–33] and other [15, 16] studies. Note that the above data were obtained via continuous measurements during heating, including starch amorphization, and cooling that were carried out the same day, as mentioned above. In contrast, the establishment of a steady-state water concentration after amorphization and a consequent gradual decrease in frozen water content in amorphous starch are slow processes and

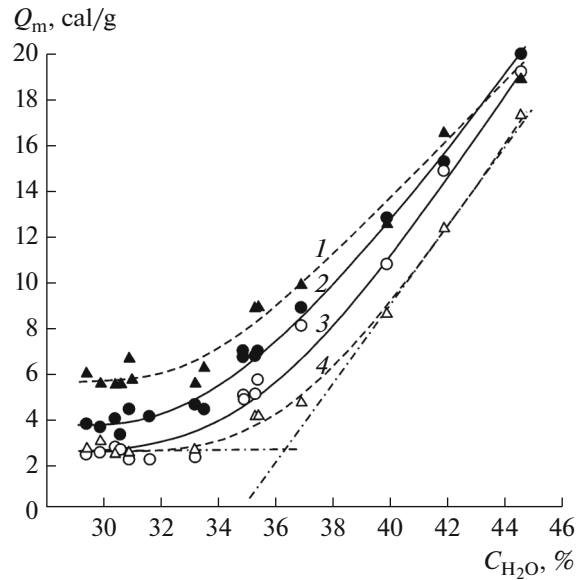


Fig. 4. The heats of the melting (curves 1 and 2) and heats of the crystallization (curves 3 and 4) of frozen water in native (solid lines) and amorphous (dashed lines) potato starch as functions of the degree of hydration in the 29–45% range. Data were normalized to the total sample weight.

take at least 10 h [32, 33]. (The problem of how the boundary between FW and UFW changes in amorphous starch over time and in native starch with a low degree of hydration upon heating within the native state range will be considered in detail separately.)

To better understand the concentration dependences of the T_m of FW in starch (Fig. 3) it is necessary to consider the specifics of heat-induced amorphization of starch. Our studies and many others have shown that the temperature of transition from the native to the amorphous state (T_{den}) strongly depends on the water content in starch, as with other biopolymers [22, 23, 41–44]. Amorphization of native starch with a low degree of hydration has been shown to occur at relatively high temperatures; i.e., a decrease in the degree of hydration from 40 to 25% increases T_{den} from 80 to 105°C [43, 44]. We assumed that high T_{den} values are responsible for an increase in the average size of water clusters in starch with a low content of frozen water [33]. This assumption is to a great extent based on the fact we have previously established that a size effect is seen in the dependence of a water cluster T_m in Sephadex samples, which consist of amorphous polysaccharides of a special design and are employed in gel filtration [47]. A special experiment was performed; its results supported the assumption.

Figure 5 shows the melting curves of water clusters for native starch and amorphous starch samples with a complex thermal history. Immediately after amorphization, both T_m and Q_m of frozen water in amorphous

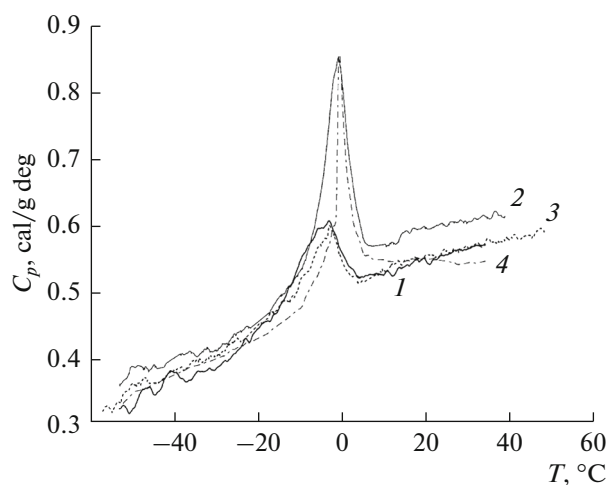


Fig. 5. The thermograms of heating a potato-starch sample with a complex thermal history in the melting region of frozen water. Curves were obtained for (1) a native starch sample, (2) the sample examined immediately after amorphization at 100°C, (3) an amorphous sample incubated at room temperature for 5 days, and (4) an amorphous sample heated again to 100°C. $C_{H_2O} = 27\%$; $V_{\text{heating}} = 5$ deg/min.

starch with a low degree of hydration were substantially higher than the respective values observed in native starch, indicating that larger water clusters formed in amorphous starch. When the sample was kept at room temperature for a long period of time after amorphization, the Q_m of FW decreased significantly, while T_m also decreased to a minor extent (Fig. 5, curve 3). This finding indicates that the quantity of large water clusters decreases upon long storage. The cluster size also decreased, but did not reach the average cluster size characteristic of native starch. When the amorphous sample was then heated to a high temperature equal to the amorphization temperature, Q_m of water clusters increased again (Fig. 5, curve 4). The T_m value for frozen water observed after heating the amorphous sample at a high temperature was the same as the T_m of FW detected immediately after amorphization. Therefore, high-temperature heating, which is necessary for destruction of the native structure of the biopolymer at low degrees of hydration, was responsible for the increase of water-cluster size in amorphous starch with a low content of FW compared with its native state. In turn, this indicates that the frozen-water distribution in starch became more irregular in general. The experiment additionally demonstrated that the establishment of concentration equilibrium is a long process that takes several tens of hours of keeping at room temperature in the case of amorphous water–starch systems with low degrees of hydration.

Crystallization of supercooled water in starch. Now, we consider the results obtained for crystallization of

dispersed water in starch (Fig. 3, curves 3 and 4). A significant shift towards lower temperatures was observed for the crystallization temperature T_c values for FW relative to T_m for both native and amorphous starch. In other words, water clusters crystallized when substantially supercooled relative to their T_m values.

When the thermodynamic properties are studied for nanoscale crystalline structures that form in low-molecular-weight or atomic compounds (in classical solid matrix) the term hysteresis is commonly used to describe the relationship between the melting and crystallization processes upon supercooling relative to T_m ; $\Delta T = T_m - T_c$. The same relationship between crystallization and melting temperatures has been established for synthetic polymers and oligomers [48]. In this case, the shift $\Delta T = T_m - T_c$ is thought to be due to the kinetic limitations that affect the crystallite growth rate and are associated with a low molecular mobility of macromolecules. However, the term hysteresis is not used. We consider water, which is a low-molecular-weight compound, in this work.

The data on crystallization of frozen water are discussed for native and amorphous starch separately.

Crystallization temperature of water clusters in native starch. As is seen from Fig. 3, $T_c < T_m$ at all degrees of hydration; i.e., hysteresis was observed. The water cluster T_m varied from ~ -30 to -3°C in native starch in the water concentration C_{H_2O} range of 29–45%. The hysteresis value $\Delta = T_m - T_c$ can be associated with cluster size, which has been calculated previously from the Gibbs–Thomson equation and data on water cluster melting [31, 33]. Hence, the smaller the cluster size is, the higher the hysteresis is. $\Delta T = 2\text{--}3^\circ\text{C}$ when clusters are relatively large (~ 35 nm at a water concentration of $\sim 40\%$), while $\Delta T = \sim 25^\circ\text{C}$ when clusters are small (~ 7.5 nm at a water concentration of $\sim 29\%$). The result agrees well with the common views that the smaller the cluster size is, the higher the hysteresis is [25, 26].

The fact that a heat effect was not observed for crystallization of frozen water at water concentrations lower than 29% agrees well with the following explanation of the observed hysteresis. The changes observed in hysteresis between melting and crystallization for clusters of different sizes can be associated with the relative positions that two processes occupy on the temperature scale. The processes are the formation of crystallization centers and their growth, which occur at different rates during crystallization [48]. Naturally, the lower the cluster volume is, the smaller the overlap between the two processes is; this determines the resulting crystallization rate of water clusters. Thus, it is clear that the hysteresis in question is also of a kinetic nature.

Note additionally that crystallization of frozen water is clearly detectable in amorphous starch with a water concentration of 29% or lower (e.g., at 27%; Fig.

3, curve 4), when water clusters are far larger than in native starch. This fact indicates again that a decrease in water-cluster size plays the main role in the absence of a heat effect associated with the crystallization of frozen water in native starch in the same range of degree of hydration.

The crystallization temperature of water clusters in amorphous starch. The frozen water crystallization temperature T_c was studied in amorphous starch as a function of its degree of hydration (Fig. 3, curve 4). Substantial hysteresis between melting and crystallization of water clusters was observed in amorphous starch as well. However, the hysteresis value ($\sim 12^\circ\text{C}$) varied in amorphous starch to a far lesser extent (by no more than $3\text{--}4^\circ\text{C}$) than in native starch in the water-concentration range that we examined.

This striking difference in the crystallization of frozen water in amorphous and native starches is probably related to the fact that after amorphization humid starch becomes a typical system undergoing a glass transition. This means that crystallization of FW in native and amorphous starches takes place, in situ, in different systems that differ in molecular mobility.

As studies of the glass transition of biopolymers have shown [23, 43, 44, 49, 50], amorphous starch is capable of a glass transition at certain combinations of temperature and water content, like other humid denatured biopolymers. It has been demonstrated that a transition of dehydrated starch from a glass state to a fully hydrated state with a high mobility is completed at a water concentration of 30–35%. Accordingly, the water-content dependence of the heat capacity, which characterizes the molecular mobility of the total starch–water system, also changes at a concentration of 30–35%, displaying a jump [23, 43].

Figure 3 also shows a water content dependence of the glass transition temperature (T_g) for amorphous potato starch. The dependence was obtained previously [43] and extrapolated to water contents of $\sim 40\%$. It is seen that not only the T_m values of water clusters, but their T_c values lie above T_g as well. When the water content is higher than 30%, a distinct jump in thermal capacity is not seen in heating thermograms of amorphous starch because it is masked by the ice melting curve. However, this does not mean that a glass transition does not occur, or that mobility of the polymeric matrix remains unchanged at these temperatures.

It is evident from the above that in this concentration range the amorphous biopolymeric matrix occurs in a state similar to a highly elastic state; i.e., the matrix is a far more flexible system than a native starch granule. Granules are relatively more rigid because they contain nanoscale crystalline structures associated with passing chains. This is mostly responsible for the differences observed for crystallization of water clusters in amorphous starch compared with native starch. The water-cluster size plays only a secondary role in the case of amorphous starch. This is reflected

in the experimental plot (Fig. 3, curve 4), which displays only a weak water content dependence of T_c . In contrast, the size of water clusters plays the main role in their crystallization in the case of the native starch state, where the internal structure of the granule is relatively more rigid.

In spite of this difference, hysteresis between T_c and T_m of water clusters was observed in both native and amorphous starch. The relationship between frozen water T_c observed during crystallization as well as melting of water clusters changed at $C_{\text{H}_2\text{O}}$ of approximately 35% in both native and amorphous starch (Fig. 3). This finding reflects the issue of the reason that the processes that accompany destruction of native structures in starch differ for starches with low and high degrees of hydration. The problem has been discussed for many years, but is still unclear.

Taken together, the above data indicate that studies of the thermal properties for starch itself (amorphization and a glass transition) and water contained in starch (melting and crystallization) clearly demonstrate the existence of two hydration regions that differ in the effect of hydration on these processes.

Heat of crystallization of frozen water in native and amorphous starch. Like with T_m , new results obtained for the transition heat as another parameter that characterizes the melting of water clusters in starch mapped well to the water concentration dependence of Q_m in native starch that has been obtained previously for a broader concentration range [31–33]. This is clear from Fig. 6. The dependence shows that the melting heat of FW at low degrees of hydration is lower than at high ones, which is certainly a manifestation of the size effect. Note that the Q_m values plotted in Fig. 6 were normalized to the weight of frozen water melting in the sample.

Data obtained with normalization to the total sample weight for the melting heat Q_m and crystallization heat Q_c of FW in native and amorphous starch with degrees of hydration ranging 29–45% are shown in Fig. 4. The different methods used to normalize the heat effects revealed certain specifics in these processes.

The effect of the water-cluster size is seen in all water concentration dependences of transition heat (Fig. 4), which were nonlinear in character. We have shown previously that two straight lines with different slopes approximate the water content dependence of Q_m well (with normalization to the total sample weight) for various starches [33]. The values of the Q_m of frozen water obtained for starch samples with low degrees of hydration were considerably lower than the Q_m values observed at higher (exceeding 35%) concentrations and lower than the Q_m of pure water. We note that the division into two hydration regions in accordance with their different effect on the thermal properties was initially proposed for the results of adiabatic

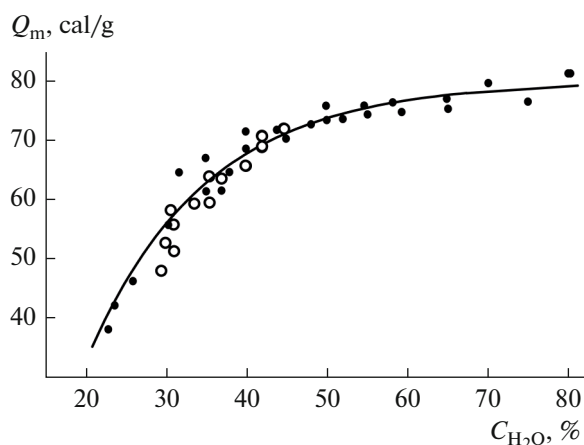


Fig. 6. The melting heat of frozen water in native potato starch as a function of degree of hydration. The results were normalized to the weight of frozen water. Data obtained in this work (open circles) were combined with previous data (filled circles) [33].

calorimetry for the melting of water dispersed in two biopolymers, histone H1 and poly-L-proline [51]. Our results (Fig. 4) demonstrate that approximation of the water-concentration dependence with two straight lines with different slopes is possible not only for the Q_m of frozen water, but also for the Q_c of FW in both native and amorphous starch states. In other words, the results obtained for Q_m and Q_c of FW in native and amorphous starch support the idea that small water clusters with thermal properties that differ from those of bulk water predominantly occur in starch with degrees of hydration below $\sim 35\%$, while large clusters with parameters similar to those of bulk water occur at higher degrees of hydration.

The Q_m of frozen water was higher than Q_c of FW in both native and amorphous starches at the same degree of hydration (Fig. 4), suggesting the hysteresis ΔQ , as with temperatures of the transitions under study. The maximum hysteresis $\Delta Q = Q_m - Q_c$ was approximately 2 cal/g for native starch and 4 cal/g for amorphous starch and ΔQ decreased with the increasing degree of starch hydration (accordingly, with the increasing heat values itself). As is seen the changes observed for Q_c of water clusters in starch with variation in the degree of its hydration were similar to the changes in frozen water Q_m .

Note that the data (Fig. 4) were obtained by heating or cooling samples at the same rate, 5 deg/min. When a lower cooling rate (3 deg/min) was used to study water crystallization the crystallization heat Q_c was higher for both native and amorphous starch states. This finding supports the kinetic nature of the hysteresis that is observed. In turn, the presence of hysteresis provides additional evidence that small water clusters form in the hydrate shell of starch molecules at low degrees of hydration.

CONCLUSIONS

In this work, DSC measurements were performed to study the crystallization and melting of frozen water dispersed in native and amorphous potato starch with varying degrees of hydration. The results showed the extent to which the sizes of water clusters that form in the biopolymeric matrix affect the parameters of the two processes. Experiments showed that a size effect was distinctly seen in the water concentration dependences of the melting and crystallization temperatures of frozen water in native starch. The lower the degree of starch hydration is, the lower the T_m and T_c of water clusters are. Such an effect was not detected with amorphous starch. Moreover, an inverse pattern was observed after amorphization. Water cluster T_m and T_c increased with the decreasing degree of hydration in amorphous starch. The reasons that are responsible for the lack of a size effect in amorphous starch compared with native starch differ for the dependences of T_m and T_c of frozen water.

In the case of the melting of dispersed freezing water in amorphous starch, we believe that the main factor is related to high-temperature heating, which is necessary for destruction of the native structure of the biopolymer with low degrees of hydration. An additional amount of FW occurs in the starch–water system as a result of this treatment, the average size of water clusters increases compared with the native starch state and the T_m of FW increases accordingly. In the case of the crystallization of frozen water in amorphous starch with a low degree of hydration, the process occurs, in situ, in another system, which is a typical biopolymer that undergoes a glass transition and is far more flexible than a native starch granule in this water concentration range (30% and higher concentrations). The size of water clusters plays only a secondary role in this case. In contrast, the size of water clusters plays the main role when their crystallization occurs in native starch, which has a relatively more rigid internal structure of granules owing to the presence of crystallites associated via passing chains. Thus, the performed study demonstrated at once that the thermal properties of a biopolymeric matrix are inextricably associated with those of its hydrate shell.

For the manifestation of the size effect in the dependences of the melting heat Q_m and the crystallization heat Q_c of frozen water, the results indicate that the heats of the transitions depend on the degree of hydration of starch in both its native and amorphous states. Both of the transition heats are substantially lower than the corresponding parameters of bulk water in the region of lower degrees of hydration. The water clusters that differ in thermal properties from bulk water predominantly exist in both native and amorphous starch at low degrees of hydration. Special attention was paid to the problem of a prolonged establishment of the water-concentration equilibrium after amorphization of the system.

Hysteresis between the melting and crystallization parameters of dispersed water was observed for both native and amorphous starch and was found to vary in extent and the character of changes with changing degree of hydration. Hysteresis was of a kinetic nature, which is supported by its dependence on the crystallization conditions. The finding of hysteresis between T_m and T_c and between Q_m and Q_c of frozen water in the region of lower degrees of starch hydration also reflects the small sizes of water clusters that form in the biopolymer.

In total, the results obtained for the crystallization and melting of frozen water in starch in the direct and reverse temperature scanning modes agree well with each other and complement each other to demonstrate different aspects of the manifestation of the size effect in the thermal properties of dispersed water in a biopolymeric matrix.

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