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STRUCTURE OF STARCHES EXTRACTED FROM NEAR-ISOGENIC WHEAT LINES
Part II. Molecular organization of amylopectin clusters

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High-sensitivity differential scanning calorimetry (HSDSC) and small-angle X-ray scattering (SAXS) were used to investigate the structural characteristics of starch granules with different amylose content extracted from near-isogenic wheat lines with different combinations of active granule-bound starch synthase (GBSS I) isoforms. Paracrystalline diffraction model, 'two-state' model of starch melting and other physico-chemical approaches were used to estimate the sizes of amylopectin clusters, the thicknesses of crystalline lamellae and the structure of amylopectin defects for investigated wheat starches.

The joint analysis of SAXS and DSC data has shown that the size of amylopectin cluster, the thickness of crystalline lamellae and the structure of amylopectin defects do not depend on the differences in combinations of active GBSS I isozymes. The data obtained supposed that the amylopectin cluster size and the thickness of crystalline lamellae are, generally, the universal structural parameters for wheat starches. Additionally, the data obtained suggest that increase of amylose content is accompanied by accumulation of both amylose tie-chains, located as defects in crystalline lamellae, and amylose chains oriented transversely to the lamella stack within amorphous lamellae. Disordered ends of amylopectin double helices and/or pre-existing double helices not participating in formation of crystals are considered as amylopectin defects arranged in crystalline lamellae. The relationship between structure of wheat starches extracted from near-isogenic lines and their thermodynamic properties was recognized.

Keywords: *amylose and amylopectin localization, cluster and lamellar structure, GBSS I, HSDSC, native and annealed wheat starches, SAXS*

Introduction

For semi-crystalline synthetic polymers the relationship between the structure and thermodynamic properties is well known[1]. Since starch granules belong to semi-crystalline compounds, the conclusion should be valid for starches irrespective of their polymorphous structure and amylose/amylopectin ratio. Really, estimations of the thickness of crystalline lamellae for different starches both from SAXS data, using paracrystalline diffraction theory, and DSC data, using 'two-state' model describing the melting process of starch granules, were similar to each other [2]. However, as starch is a biological object, for correct comparison of the values calculated using different model approaches, the starches with the same genetic background should be investigated, i.e. starches extracted from near-isogenic lines.

The structural periodicity of semi-crystalline starch granules is formed by alternating crystalline and amorphous layers (lamellae). Crystalline lamellae are comprised of ordered double helices formed by clustered amylopectin side chains (A-chains), while amorphous lamellae are formed by branched chains of amylopectin (B-chains) [3, 4]. Amylopectin clusters can contain amylose tie-chains, i.e. amylose molecules that pass through crystalline and amorphous layers, being in straightened conformation in crystalline lamellae and in disordered conformation in amorphous lamellae [5]. For determination of the thickness of crystalline and amorphous lamellae, as well as the amylopectin cluster size, small-angle X-ray scattering (SAXS) is usually used [2, 6–11]. SAXS patterns from native hydrated starches show broad diffraction peak related to the periodical arrangement of crystalline and amorphous layers in semi-crystalline granules [2, 6–9, 11]. The size of amylopectin cluster (thickness of one crystalline plus one amorphous lamella) and the thickness of crystalline lamella can be calculated from the position of this peak by applying the Wolf–Bragg's equation or using the paracrystalline diffraction theory. Additionally, as was mentioned above, the thickness of crystalline lamellae could also be calculated from DSC data by using the 'two-state' model [2, 12–18]. For correct application of

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the 'two-state' model it is important that DSC investigations are performed in quasi-equilibrium conditions, i.e., at low concentration of aqueous starch dispersions (0.1–0.5% d.m.b.) and low heating rate ($\leq$2 K min^{-1}) [2, 13, 17–19].

SAXS investigations of starches extracted from different origins have revealed the continuous decrease in the intensity of scattering maximum on increasing amylose content in native starches [2, 8, 11]. This fact could be accounted for by a decrease in the electron density difference between crystalline and amorphous lamellae. There are three hypotheses for the explanation of this phenomenon [2, 8, 20], namely: (*i*) co-crystallization of amylose macromolecules with amylopectin side-chains within crystalline lamella, (*ii*) accumulation of amylose chains oriented transverse to the lamellar stack within amorphous lamellae and (*iii*) accumulation of amylose tie-chains both inside crystalline and amorphous lamellae. Detailed analysis of all three hypotheses was carried out in previously published paper [2], where it was taken into account that generally for polymers and in particular for starches there is a relationship between structural and thermodynamic properties [1, 2, 13, 14, 17]. It was noted that the first hypothesis is in contrast with the data published previously [21], where it was shown that amylose and amylopectin macromolecules are incompatible in aqueous solution. Despite the fact that the hypothesis concerning localization of amylose chains within amorphous lamellae is in agreement with the previously published SAXS data [7, 8], it does not allow to explain the changes in the melting temperature of starches on increasing amylose content [2], whereas using Thomson–Gibbs' approach these explanations could be given in the framework of the third hypothesis [2]. At present, however, all these hypotheses are equiprobable.

Recently it has been shown that localization of amylose macromolecules within amylopectin clusters of wheat starches extracted from near-isogenic lines is influenced by the number of active GBSS I isoenzymes synthesizing amylose [12]. Particularly, it was shown that when amylose biosynthesis is catalyzed only by one GBSS I isoform (double null line starches), amylose is predominantly arranged within amorphous background and/or amorphous lamellae with insignificant amounts of amylose macromolecules located most likely within crystalline lamellae as amylose tie-chains and amylose–lipid complexes. When biosynthesis of amylose is controlled by a combination of two GBSS I isoenzymes (single null line starches), more amylose is located in crystalline lamellae compared to triple (no active GBSS I isoforms) and double null line starches. If the conclusions are valid and the decrease in electron density difference between crystalline and amorphous lamellae on increasing amylose content is determined only by accumulation of amylose tie-chains, we could expect only insignificant changes in the difference of electron density between crystalline and amorphous lamellae at passing from triple to single null double line starches. Whereas at passing from triple to null line starches these differences should be significant. Moreover, since changes in melting temperature of wheat starches (T_m) are determined by content of defects (amylose tie-chains, particularly) [12], the values for the intensity of scattering maximum (reflecting changes in the electron density difference between crystalline and amorphous lamellae) and the T_m values for starches with different amylose content should display positive correlation.

Besides amylose tie-chains, single ends of amylopectin A-chains uncoiled into double helices, the ends of amylopectin double helices unpacked into crystallites and molecular ordered structures could be considered as defects arranged within crystalline lamellae and thus could affect the changes in electron density difference between crystalline and amorphous lamellae at increasing amylose content in starches [22, 23]. According to Genkina *et al*. and Kiseleva *et al*., for determination of the starch defects type, the comparison of thermodynamic melting parameters for native and annealed starches should be carried out [22, 23]. Recently, using this approach, an attempt was made to determine the structural features of defects in amylopectin, normal and amylose-rich wheat starches [23]. It was shown that: (*i*) an increase of amylose content in wheat starches is accompanied by the rise in the content of defects, (*ii*) amylopectin wheat starches contain free ends of amylopectin A-chains that belong to longer crystallites and are located in amorphous lamellae, (*iii*) amylose-rich starches can apparently contain disordered ends of amylopectin double helices unpacked into crystallites and/or double helices not participating in formation of crystals but arranged within crystalline lamellae (molecular ordered structures), (*iv*) normal wheat starches can contain defects typical both for amylopectin and amylose-rich wheat starches. Unfortunately, investigated starches were not extracted from near-isogenic wheat lines. It could be supposed that mechanical extending of the presentations about structural features of defects on wheat starches with other genetic background could be incorrect. In this work, investigations concerning both localization of amylose and amylopectin chains within amylopectin clusters and estimation of effects from different GBSS I combinations on structural organization of amylopectin cluster were performed for the starches extracted from near-isogenic wheat lines.

The goal of this investigation is determination of the effects from different GBSS I combinations on localization of amylose and amylopectin chains within amylopectin cluster of starches extracted from near-isogenic wheat lines. The investigation was performed by joint analysis of DSC and SAXS data using different physical approaches.

Experimental

Materials and methods

Plant materials

A spring wheat cultivar Chinese Spring (CS) and its near-isogenic partially waxy and waxy types were used in this work. The details of near-isogenic lines development are described in the work of Miura *et al*. [24]. The Wx protein profiles of wheat types were studied elsewhere [24] and are shown in Table 1.

Starch preparation

Starches from near-isogenic wheat types were extracted as described in the paper of Mangalika *et al*. [25]. Some characteristics of extracted wheat starches and the methods for their determination were published previously [12, 25] and are presented in Table 1.

Annealing of starches

The starch annealing procedure has been described in the earlier published paper [26]. The annealing temperatures (incubation temperatures) where chosen as a function of the melting temperature of native starches: the samples were annealed at a temperature slightly below (2–3 K) the onset temperature (T_o). Taking into consideration that after 10 h the thermodynamic melting parameters of annealed wheat starches remain constant irrespective of amylose content in starches, the time of annealing (incubation time) was chosen 10 h for all investigated samples.

High-sensitivity differential scanning microcalorimetry (HSDSC)

Calorimetric investigations of starch dispersions in water (0.3% dry matter, sample volume 0.5 cm^3 in sealed cells) were performed using a high-sensitivity differential scanning microcalorimeter DASM-4 (Puschino, Russia) over the temperature range of 10–120°C with a heating rate of 2 K min^{-1} and excess pressure of 2.5 bar. Deionized water was used as a reference material. The heat capacity scale was calibrated using the Joule–Lenz effect for each run. It was shown previously, that under experimental conditions used, corrections for dynamic lag and residence of the samples in calorimetric cell were not necessary, moreover, gelatinization of starch-water dispersions could be considered as a quasi-equilibrium process [17]. Additionally, for starches with symmetric DSC curves, the 'two-state' model is applicable for the description of the melting process of crystalline lamellae [17, 27]. This model implies that there is a reversible transition between native and molten states. Accordingly, parameter of cooperativity, which corresponds to the minimal number of monomers undergoing the transition, could be determined [5, 17, 27].

The melting temperature (T_m) was attributed to the temperature of the maximum on the DSC curve. The heat capacity jump (ΔC_p^{exp}) during the melting process was determined by linearly extrapolating the partial heat capacity change of the native C_p^n and molten C_p^m states to the melting temperature T_m and was calculated as follows:

$$\Delta C_p^{exp} = C_p^m - C_p^n \quad (1)$$

Calorimetric enthalpy (ΔH_m) was determined as the area under the peak above the extrapolation lines. The average values of the thermodynamic parameters

Table 1 Wx protein profile of wheat types, amylose content in extracted starches and T_m of crystalline lamellae

Wheat type	Wx protein profile*			Amylose content*/%	T_m**/K
	Wx-A1	Wx-B1	Wx-D1		
wxABD	–	–	–	2.1	334.5
wxBD	+	–	–	17.1	334.1
wxAB	–	–	+	18.6	333.1
wxAD	–	+	–	19.5	332.6
wxD	+	+	–	21.1	331.5
wxB	+	–	+	22.0	328.9
wxA	–	+	+	22.4	331.0

*Wx protein profiles and amylose content in starches were determined in the previous work [24] (presence (+) and absence (–) of Wx proteins). Amylose content in each wheat starch type was determined in the work [24] using the Concavalin A method.
**The T_m values of crystalline lamellae for investigated starches were determined previously [12].

were determined using five measurements at 95% significance level and normalized per mole of anhydroglucose units (162 g mol^{-1}). The error in determination of T_m is 0.1 K, values for ΔH_m and ΔC_p^{exp} were determined with the error of not more than 10%.

Values for van't Hoff enthalpy (ΔH^{vH}) were calculated according to previously published papers [5, 13, 27] as:

$$\Delta H^{vH} = 2T_m R^{1/2}(C_p - 0.5\Delta C_p^{exp})^{1/2} \quad (2)$$

where R is gas constant, T_m is the melting temperature of starch crystalline lamellae, C_p is the difference between the maximum ordinate on the DSC curve and the value of C_p^n, linearly extrapolated to the melting temperature T_m. Values for the parameter of cooperativity (ν) and the thickness of crystalline lamellae (L_{crl}) were calculated according to the following equations:

$$\nu=(\Delta H^{vH})/(\Delta H_m) \quad (3)$$

$$L_{crl}=0.35\nu \quad (4)$$

where ΔH_m is the experimental melting enthalpy of crystalline lamellae and ΔH^{vH} is the van't Hoff enthalpy; the pitch height per anhydroglucose residue in the double helix is 0.35 nm [28].

To analyze the changes in thermodynamic parameters of melting, the Thomson–Gibbs equation was used [1]:

$$T_m = T_m^0[1 - 2\gamma_i / (\Delta H_m^0 \rho_{crl} L_{crl})] \quad (5)$$

where T_m^0 and ΔH_m^0 are the melting temperature and the melting enthalpy of a hypothetical crystal with unlimited size (a perfect crystal), γ_i is the free surface energy of the face sides of crystalline lamellae, while ρ_{crl} and L_{crl} are, respectively, the density and the thickness of the crystals. Since the values of the melting temperature (T_m^0) and the melting enthalpy (ΔH_m^0) for perfect starch crystals are not available, calculations were performed assuming the following values: T_m^0=366.5 K, ΔH_m^0= 35.5 J g^{-1} for A-type spherulitic crystals [29]. Additionally, density of A-type structures (ρ_{crl}=1.48 g cm^{-3}) and the L_{crl}, ΔH_m and T_m values for investigated starches were used. Parameters characterising the surface of crystalline lamellae (free surface energy (γ_i), enthalpy (q_i) and entropy (s_i) of crystalline lamellae face sides) were determined using Thomson–Gibbs equation as described previously [2, 12, 15, 16, 23, 26].

Calorimetric data for native starches from near-isogenic wheat lines were determined in the recently published paper [12].

Small-angle X-ray scattering (SAXS)

For SAXS measurements powders of native starches were dispersed in excess of distilled water to form slurries with ~50 mass% fraction of native starches according to previous works [2, 7, 8]. The measurements were carried out in transmission geometry using the X-ray diffractometer designed in the Institute of Biochemical Physics. During the X-ray exposure the starch slurries were kept in sealed cells to prevent dehydration. The X-ray beam emitted from the fine-focus Cu X-ray tube (30 kV/30 mA) was line-focused with a glass mirror. The SAXS patterns were recorded with a one-dimensional position-sensitive detector. Experimental SAXS curves were corrected for the background scattering, corrected (desmeared) for collimation distortions and plotted as a function of s=(2sinθ)/λ, with λ and θ being the Cu K_α-wavelength (0.1542 nm) and half of the scattering angle, respectively. Collimation correction was performed with the SAXS data processing program 'PRO', developed in the Institute of Crystallography RAS (Moscow, Russia). Parameters of the SAXS peak (intensity I_{max}, position S_{max} and full width at half maximum ΔS) were determined by the simple graphical method as described in the earlier published paper [2]. The Bragg spacing D was calculated according to the Wolf–Bragg equation as $D=(S_{max})^{-1}$. Desmeared SAXS curves were also processed according to the paracrystalline diffraction theory [30–32]. The average thickness of amylopectin cluster α and the crystalline lamellar thickness ($L_{crlSAXS}$) were estimated as described previously [2].

Results and discussion

The SAXS patterns of the investigated starches are shown in Fig. 1. These patterns are at the same relative scale and therefore are directly comparable. The parameters of amylopectin clusters for investigated starches derived from the SAXS curves by graphical analysis of the SAXS peak and through the Wolf–Bragg equation (Table 2), as well as using paracrystalline diffraction theory (Table 3) are presented. As can be seen from Fig. 1, the intensity of diffraction maximum (I_{max}) for triple null line starch (wxABD, amylopectin starch) is higher compared to the values for double (wxAB, wxBD) null line starches containing 17.1 and 18.6% amylose. Moreover, the following increase of amylose content in starches from 18.6 to 22.0%., i.e. at passing from double to single (wxD, wxB) null line starches, leads to the further decrease of I_{max} values.

Analysis of the presented data shows that the changes in calculated parameters with increasing amylose content are very similar, irrespective the method of determination. The changes observed in I_{max} values, as well as in other calculated parameters are in general agreement with results published for wheat [2, 10] and other starches [6–9, 11, 33]. It is worth not-

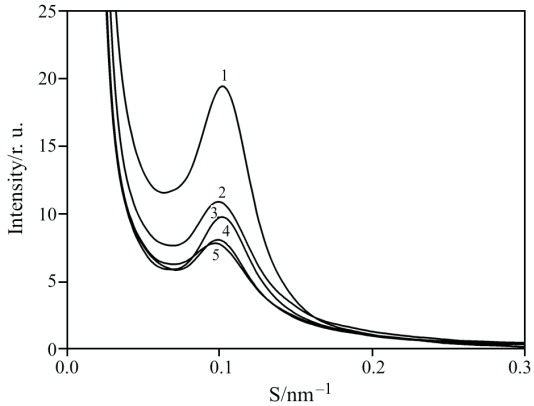

Fig. 1 Desmeared SAXS patterns of wheat starch slurries (1 – wxABD, 2.1% amylose; 2 – wxAB, 18.6% amylose; 3 – wxBD, 17.1% amylose; 4 – wxD, 21.1% amylose; 5 – wxB, 22.0% amylose)

ing, that the average repeat distance α (thickness of amylopectin cluster), determined by using the paracrystalline approach (Table 3) is somewhat smaller than the Bragg spacing *D* (Table 2). However, this result is also expected, since the same data were published earlier [2] for wheat starches with other genetic backgrounds [16]. It is important that an overall thickness of amylopectin cluster (one crystalline plus one amorphous lamellae) is constant (8.9±0.2 nm) irrespective of isozyme combinations controlling amylose biosynthesis in wheat cultivars (Table 3). Moreover, the mean value of (8.9±0.2 nm, Table 3) is equal to the corresponding value (8.75±0.17 nm) calculated for other amylopectin, normal and amylose-rich (39.5% amylose) wheat starches [2]. This means that the thickness of amylopectin cluster is universal parameter independent both on quantity of isozymes controlling amylose biosynthesis and on their combinations (Tables 1 and 3). It is supposed that such physical effect may be combined with some aspects of the biosynthesis pathway through enzymatic activity. Generally, the conclusion presented here is an additional argument to the hypothesis about universality of this parameter for different starches proposed by Donald *et al*. [7, 8, 11].

The same conclusion could be made considering influence of amylose content and isozymes combinations on the values of thickness of crystalline lamellae. Indeed, joint analysis of SAXS and DSC data evidences that the values of crystalline lamellae thickness are virtually the same for all investigated starches, despite the different approaches used for treatment of DSC data ('two-state' model) and corresponding SAXS-patterns (paracrystalline model). It follows that the investigation of wheat starches extracted from near-isogenic lines gives an example of the relationship between structure and thermodynamic properties of starches. It allows to use the values of L_{crl} estimated both from DSC [2, 12, 15, 16] and SAXS (Table 3 and [2]) data for determination of the average L_{crl} value for wheat starches. The calculation of the average L_{crl} value from DSC and SAXS data for 21 wheat starches shows that this value is 4.6±0.4 nm and it does not depend on amylose content in the range from 1.5 to 45%, quantity of isozymes controlling the amylose bio-

Table 2 Intensity (I_{max}), position (S_{max}), full width at half maximum (ΔS), Bragg spacing (D) for the SAXS peaks for wet wheat starches, and the melting temperature of crystalline lamellae (T_m) for investigated wheat starches [12]

Starch type	Amylose content/%	I_{max}/r. u.	S_{max}/nm^{-1}	ΔS/nm^{-1}	D/nm	T_m/K
wxABD	2.1	11.8	0.102	0.035	9.8	334.5
wxBD	17.1	5.3	0.102	0.041	9.8	334.1
wxAB	18.6	5.2	0.1	0.042	10.0	333.1
wxD	21.1	3.3	0.1	0.042	10.0	331.5
wxB	22.0	3.0	0.97	0.045	10.3	328.9

*The error in the measurement is ±0.001 nm^{-1} for S_{max} and ΔS and ±0.2 relative units for I_{max}.

Table 3 Parameters of the lamellar structure: average repeat distance (thickness of amylopectin cluster) (α), its mean square deviation (σ) and crystalline lamellae thickness ($L_{crlSAXS}$) estimated using paracrystalline diffraction theory; values of the crystalline lamellae thickness calculated from DSC data (L_{crlDSC})

Starch type	Amylose content/%	α/nm	σ/nm	$L_{crlSAXS}$/nm	L_{crlDSC}/nm*
wxABD	2.1	9.1±0.1	2.45±0.05	4.7±0.1	4.3±0.3
wxBD	17.1	8.9±0.1	2.4±0.1	4.7±0.1	5.5±0.1
wxAB	18.6	8.7±0.2	2.8±0.1	4.3±0.2	5.8±0.2
wxD	21.1	9.2±0.2	2.5±0.1	4.4±0.5	4.6±0.2
wxB	22.0	8.8±0.2	3.0±0.1	4.4±0.2	5.4±0.2
mean value		8.9±0.2		4.5±0.2	5.1±0.6

*The L_{crlDSC} values were determined in the previous paper [12]. The mean L_{crlDSC} value was calculated using the values for seven types of wheat starches with different GBSS I combinations

synthesis, as well as on isozyme combinations. It could be supposed, that the thickness of crystalline lamellae for wheat starches is universal parameter as well as the thickness of amylopectin cluster.

As can be seen from Table 2 and Fig. 1, an increase of amylose content leads to continuous decrease in the intensity of the scattering maximum (I_{max}). According to X-ray diffraction theory [30–32] and practice of SAXS method application for starches with different amylose content [2, 6–9], it can be postulated that the observed changes in I_{max} reflect the difference in electron density between crystalline and amorphous lamellae. The observed differences in the I_{max} values for investigated starches could be due to two different, but possibly interconnected reasons, namely: (*i*) different localization of amylose macromolecules within amylopectin clusters, (*ii*) changes in molecular characteristics of amylopectin molecules in amorphous lamellae and their packing within crystalline lamellae.

To explain the differences in the I_{max} values for wheat starches with increasing content of amylose macromolecules, the thesis about the relationship between the structure of starches and their thermodynamic properties was used [2, 12–14, 18]. Using joint analysis of SAXS and DSC data, the following assumption was made: a decrease in the I_{max} for wheat starches with increasing content of amylose macromolecules synthesized by different GBSS I combinations is due to accumulation of amylose tie-chains, acting as defects in crystalline lamellae, thus decreasing their density and simultaneously increasing the scattering density of amorphous lamellae [2]. Since changes in the melting temperature of wheat starches (T_m) are determined by content of defects (amylose tie-chains, particularly) [12, 14, 18, 22], it would be logical enough to suppose that the observed changes in the I_{max} values (Table 2) correlate with the changes in the melting temperature of crystalline lamellae (Table 1), when passing from triple through double to single null line starches. However, joint analysis of the SAXS and DSC data shows a lack of linear correlation (Fig. 2). It could be seen, that at passing from triple null to double null line starches only insignificant changes in the T_m are observed [12], i.e. accumulation of amylose tie-chains is insignificant [2, 12], while for the same samples a sharp decrease in the I_{max} is observed. It could be supposed that the main changes in the I_{max} can be due to accumulation of amylose macromolecules that are not defects with respect to crystalline lamellae and, therefore, do not influence their melting temperature. Since this population of amylose molecules decreases the difference in electron density between crystalline and amorphous lamellae, it is believed to accumulate within the amorphous lamellae. According to Gidley [20], these chains are oriented transversely to the lamella stack. It is worth noting that an opportunity for such localization was afterwards noted by Jane *et al*. [34], Bertoft and Wiik [35], Smith *et al*. [36] and Ball *et al*. [37]. At passing from double to single null line starches, there is a reversed picture, i.e. against a background of insignificant changes in the I_{max}, a sharp decrease in the T_m is observed (Table 2). Such behaviour can be due to the fact that against a background of insignificant accumulation of amylose chains oriented transversely to the lamella stack within amorphous lamellae, a significant accumulation of amylose tie-chains is observed, to the decrease of the T_m values for crystalline lamellae.

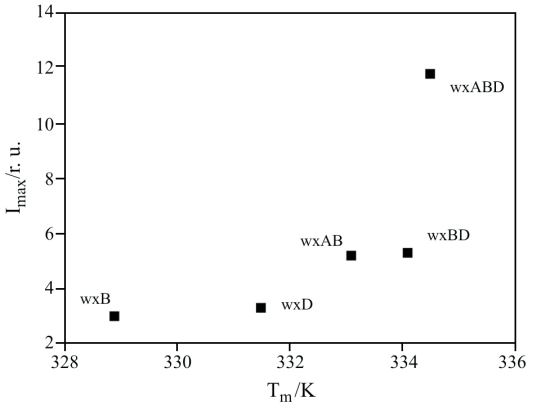

Fig. 2 Changes of intensity of the scattering maximum (I_{max}) *vs*. melting temperature of crystalline lamellae (T_m)

Summarizing the data obtained it could be supposed, that the decrease in I_{max} for wheat starches on increasing amylose content has two reasons, namely: (*i*) accumulation of amylose tie-chains acting as defects with respect to crystalline lamellae and thus decreasing their melting temperature [2, 12, 14] and (*ii*) accumulation of amylose chains oriented transversely to the lamella stack within amorphous lamellae [20]. Thus, accumulation of amylose chains of these two types in amylopectin clusters explains the observed changes in melting temperature [12] and I_{max} on increasing amylose content. It should be noted that this thesis is valid only for the starches extracted from near-isogenic wheat lines.

As was mentioned above, the changes in molecular characteristics of amylopectin molecules in amorphous lamellae and their packing within crystalline lamellae could be the reason of observed differences in the I_{max} values for investigated starches. However, the study of amylopectin molecules of starches extracted from near-isogenic wheat lines, i.e. the same starches as studied here, showed that the first assumption is incorrect. Particularly, it was found that when amylopectin chains were classified into three groups, fa (DP 6–12), fb_1 (DP 13–24) and

fb_2 (DP 25–36), no obvious differences were revealed by chain-length distribution analysis [24].

Typical DSC traces for native and annealed wheat starches from single, double and triple null lines and their thermodynamic characteristics are presented in Fig. 3 and Table 4. It is seen from these data, that annealing of starches narrows the melting interval and increases the melting temperature of the crystalline lamellae. These results are expected and, generally, well agree with the data for potato, barley and sweet potato starches, as well as with the results for starches extracted from wheat cultivars with other genetic background [22, 23]. It is well known that such behavior for wheat starches is caused due to formation of more perfect and, respectively, more thermostable crystals [23].

According to approaches of polymer physics [1], the differences in the melting temperatures (ΔT_m) of polymer crystals and starch crystals, particularly, can be described using Thomson–Gibbs equation [2, 12, 14–16, 22, 23, 26]. In this equation three parameters determine the change in the melting temperature of crystals, namely: the polymorphous structure of crystal, the thickness of crystalline lamellae (L_{crl}) and the free surface energy of the face sides of crystalline lamellae (γ_i). It is worth noting that the γ_i value is mainly governed by the surface entropy (s_i) that is proportional to the content of defects [1].

As could be seen from Figs 4 and 5, the changes in the values of $\Delta T=T_{m\ annealed}-T_{m\ native}$ depend on the γ_i and s_i values, i.e. on the parameters proportional to amount of defects in starch crystallites. Recently it was shown, that crystallites of wheat starches from triple and double null lines contain similar insignificant amounts of defects, whilst this amount is strongly increased at passing from double to single null line starches [12]. Therefore, changes in the increments of $\Delta T=f(\gamma_i)$ and $\Delta T=f(s_i)$ functions, observed at passing from double to single null line starches, are expected. It follows that the shape of $\Delta T=f(\gamma_i)$ and $\Delta T=f(s_i)$ functions could be really attributed to changes in content of starch crystalline defects, that is determined by the differences in combinations of GBSS I isoforms participating in amylose biosynthesis [12]. Such defects could be amylose tie-chains, amylose–lipid complexes and, possibly, amylose chains located within the amorphous lamellae and oriented transversely to the lamella stack [12, 20].

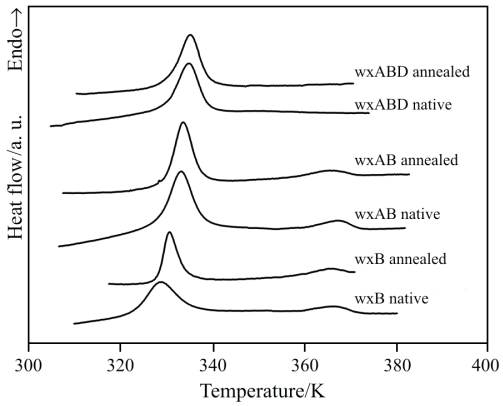

Fig. 3 Example of typical DSC traces for native and annealed wheat starches from single (wxB), double (wxAB), triple (wxABD) null lines. DSC-traces for native starches were obtained previously [12]

Besides γ_i and, respectively, s_i values, the changes in the melting temperature of crystals, according to Thomson–Gibbs' equation, depend on the type of polymorphous structure and the thickness of crystalline lamellae (L_{crl}). Since wheat starches contain extremely stable A-type polymorphous structure, it can be supposed that changes in ΔT values depend on the thickness of crystalline lamellae.

Estimation of cooperative melting unit for investigated starches and the thicknesses of their crystalline lamellae (Table 5) shows that: (*i*) the values calculated for native and annealed starches are generally in agreement with the data for starches extracted from wheat cultivars with other genetic background [2, 15, 16], (*ii*) irrespective of the differences in combinations of GBSS I isoforms, an increase in the ν and the L_{crlDSC} values is observed for all investigated starches (Table 5). Taking into account the error in determination of the L_{crlDSC}, these changes are minor for triple and double null line starches (the average value is 1.0±0.5 nm), while for single null line wheat

Table 4 The values of the melting temperature of crystalline lamellae (T_m) and the melting enthalpy (ΔH_m) for native and annealed starches extracted from near-isogenic wheat lines

Starch type	Amylose content/%	T_m/K		ΔH_m/kJ mol^{-1}	
		native*	annealed	native*	annealed
wxABD	2.1	334.5±0.1	335.2±0.1	2.8±0.1	2.6±0.1
wxBD	17.1	334.1±0.1	334.4±0.1	2.5±0.2	2.2±0.1
wxAB	18.6	333.1±0.1	333.5±0.1	2.4±0.2	2.1±0.1
wxAD	19.5	332.6±0.1	333.2±0.1	2.6±0.2	2.2±0.2
wxD	21.1	331.5±0.1	332.5±0.1	2.2±0.2	1.9±0.1
wxB	22.0	328.9±0.1	330.6±0.1	1.8±0.1	1.95±0.05
wxA	22.4	331.0±0.1	332.4±0.1	2.0±0.2	1.8±0.1

*The T_m and ΔH_m values for native starches were determined previously [12].

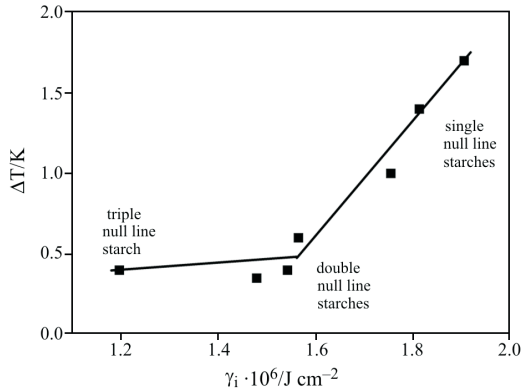

Fig. 4 Changes of ΔT *vs*. free surface energy of crystallite face side (γ_i) for annealed wheat starches extracted from near-isogenic lines. The error in determination of ΔT is ±0.2 K

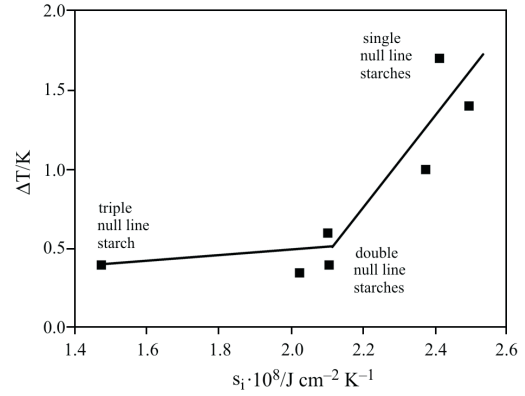

Fig. 5 Changes of ΔT *vs*. surface entropy of crystallite face side (s_i) for annealed wheat starches extracted from near-isogenic lines. The error in determination of ΔT is ±0.2 K

starches the changes are more pronounced (the average value is 2.3±0.3 nm). It is necessary to note that the changes in the L_{crlDSC} for triple and double null line starches are similar to the corresponding values for amylopectin (1.5% amylose) and normal (26.0% amylose) wheat starches from 'Leona' and 'Imeni Rapoporta' cultivars [23]. The changes in the L_{crlDSC} for single null line starches are closer to high-amylose (39.5% amylose) wheat starch from 'Bulava' cultivar [25], that is single null line starch as well [16].

As can be seen from Table 5, lengthening of double helices and, respectively, an increase in the thickness of crystalline lamellae, lead to the rise in the melting temperature of starches, while the melting enthalpy remains practically intact (Table 4). A lack of changes in the ΔH_m during annealing of starches can be observed in two cases [22, 23]. The first case can be realized for the starches containing single-chain defects such as amylose tie-chains and/or amylopectin B-chains in energy disadvantageous conformation, that are formed due to nonequilibrium conditions during starch biosynthesis and crystallization. Obviously, after anneling these defects possess an energy advantageous conformation. Such changes in starches during their annealing could be accompanied by an increase in melting temperature of starches without changes in the values of ΔH_m. In principle, this explanation is in agreemet with existing presentations about the nature of defects in investigated starches [12]. However, it can not describe the observed changes in the L_{crl}, γ_i and s_i parameters during annealing of starches (Table 5).

The second case is more complex. According to Bershtein and Egorov [1] and Kiseleva *et al*. [23], the main contribution to the ΔH_m is provided by disruption of the hydrogen bonds stabilizing the amylopectin double helices. Since the ΔH_m values do not change after annealing of starches, the lengthening of amylopectin double helices could not be explained by additional coiling of single ends of amylopectin A-chains [22]. Thus, the increase in the thickness of crystalline lamellae during annealing of starches could be caused by ordering of ends of double helices unpacked into crystallites and/or crystallization of double helices pre-existing in native starches but not participating in formation of crystals. According to the theory of semi-crystalline synthetic polymers [1] and its application to starches [23], the formation of crystals in this case is determined by the disturbance of the positional and orientational, but not conformational orders and

Table 5 The values of the cooperative melting unit (ν), the thickness of crystalline lamellae (L_{crlDSC}), the free surface energy (γ_i) and entropy (s_i) of crystallite face side for native and annealed starches

Starch type	Amylose content/%	ν/anhydroglucose residues		L_{crlDSC}/nm		$\gamma_i \cdot 10^6$/J cm^{-2}		$s_i \cdot 10^8$/J cm^{-2} K^{-1}	
		native*	annealed	native*	annealed	native*	annealed	native*	annealed
wxABD	2.1	12.3±0.3	15.2±0.3	4.3±0.3	5.4±0.3	1.15	1.20	1.30	1.48
wxBD	17.1	15.8±0.4	18.3±0.4	5.5±0.1	6.4±0.3	1.18	1.48	1.66	2.00
wxAB	18.6	16.8±0.5	18.6±0.6	5.8±0.2	6.5±0.2	1.24	1.56	1.78	2.10
wxAD	19.5	13.4±0.3	18.6±0.2	4.7±0.2	6.5±0.3	1.22	1.54	1.39	2.11
wxD	21.1	13.2±0.5	20.6±0.4	4.6±0.2	7.2±0.2	1.28	1.75	1.47	2.40
wxB	22.0	15.5±0.4	21.1±0.5	5.4±0.2	7.4±0.2	1.37	1.90	1.82	2.41
wxA	22.4	14.7±0.4	21.2±0.5	5.1±0.1	7.4±0.2	1.30	1.81	1.68	2.50

*The ν, L_{crlDSC}, γ_i and s_i values for native starches were determined previously [12]

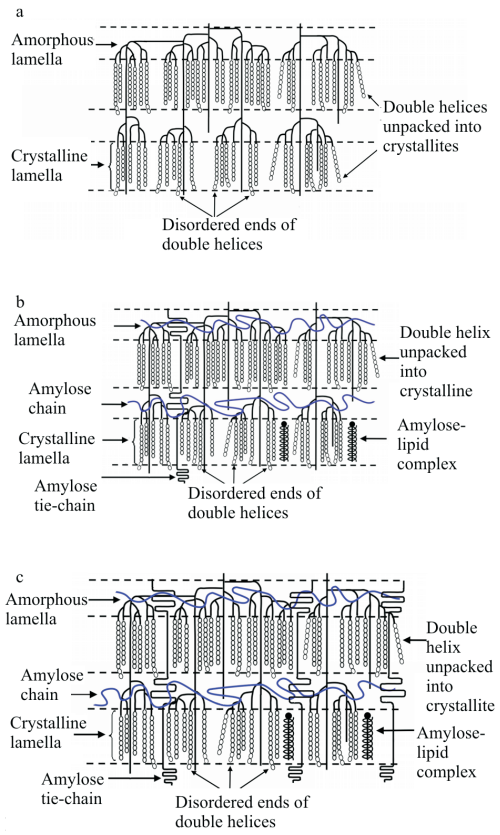

Fig. 6 Schematic presentation of amylose and amylopectin localization in amylopectin clusters for a – triple, b – double and c – single null line wheat starches

annealing is accompanied by an increase in the surface entropy of the face side of starch crystallites, as could be seen from Table 5. It follows that besides amylose-tie-chains and amylose-lipid complexes located in crystalline lamellae, the disordered ends of amylopectin double helices unpacked into crystallites and/or pre-existing double helices not participating in formation of crystals could be considered as defects located in amylopectin cluster. This conclusion is valid irrespective of the differences in active GBSS I isoforms combinations and, respectively, amylose content in starches extracted from near-isogenic wheat lines. It is worth noting that the latter point is utterly important, since the conclusions concerning the structure of defects in investigated starches and in wheat starches with different amylose content but unknown genetic prehistory are somewhat different. For example, it was shown that amylopectin starch (1.5% amylose) contain untwisted disordered single ends of amylopectin A-chains appurtenant to longer crystallites [23], while such defects were not found in investigated samples. The structure of defects in investigated wheat starches is more similar to wheat starch containing 39.5% amylose. Elimination of these discrepancies is the aim of the further investigations. However, it could be supposed that the discrepancies observed could be resulted from the differences in the genetic backgrounds of wheat cultivars.

Taking into consideration the differences in GBSS I combinations controlling amylose biosynthesis in investigated wheat starches and summarizing DSC and SAXS data described in this and earlier published papers [12], the schematic presentations of the changes in amylose and amylopectin localization within amylopectin clusters for triple, double and single null line starches are shown in Fig. 6.

Conclusions

Despite the differences in the approaches used for treatment the SAXS patterns (paracrystalline model) and DSC curves ('two-state' model), the calculated L_{crl} values are practically similar. It follows that for starches there exists a relationship between the structural and thermodynamic parameters. However, a lack of correlation between the I_{max} and T_m values on increased amylose content in starches shows that this relationship is not simple. At the same time, this result allows to define more exactly our presentations concerning the localization of amylose macromolecules in starch granules. Earlier it was shown that increased amylose content in the granules is accompanied by accumulation of these macromolecules (as amylose tie-chains and amylose–lipid complexes) within the crystalline lamellae resulting in defective crystals. The accumulation of such defects leads to decrease of the melting temperature. Taking into consideration that increase of amylose content (*i*) is accompanied by a decrease of difference in electron density between crystalline and amorphous lamellae, but (*ii*) does not provide a correlation between the I_{max} and the T_m values (i.e. accumulating amylose does not play a role of defects affecting the melting temperature of the crystalline lamellae), it is proposed that at increasing amylose content, besides accumulation of amylose tie-chains in crystalline lamellae, another population of amylose tie-chains is located within the amorphous lamellae and oriented transversely to the lamella stack according to Gidley's hypothesis [20]. Accumulation of amylose chains of both types is accompanied by the decrease in electron density difference between crystalline and amorphous phases.

The main function of amylopectin in structural organization of starch granule is the formation of crystallites built from clustered amylopectin A-chains. However, these crystallites are not ideal crystals and contain defects of different types. The type of amylopectin defects is not determined by the differences in GBSS I combinations, but depend both on differences in environmental temperature during wheat maturation and genetic background of wheat cultivars.

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