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The effect of chain length and solvent interactions on the dissolution of the B-type crystalline polymorph of amylose in water

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Abstract

To characterize the effectiveness of water as a diluent in depressing the melting temperature of amylose crystals, maltooligomer/water interactions were probed, as a function of composition, by calorimetry and vapour sorption measurements. These studies, in part analysed using the Flory–Huggins theory of polymer solutions, showed that although there is a strong energetic interaction between water and the oligomeric chain, this is balanced by an opposing, first neighbour entropic contribution. At room temperature, water was characterized as a poor solvent for the amylose chain. The dissolution in water of the B-type crystalline polymorph of amylose, crystallized from fractions of limited polydispersity, ranging in chain length from 12 to 55 residues, was examined by scanning calorimetry. With increasing chain length in this range, the dissolution temperature, at a volume fraction of water of 0.8, increased from 57 to 119 °C. The extrapolated dissolution temperature for the high molecular weight polymer at this water content was 147 °C.

Keywords: Starch; Amylose; Maltooligosaccharides; Heats of mixing; Water sorption; Crystal melting

1. Introduction

Starch consists of two main polysaccharides, amylose and amylopectin, both of which are based on chains of (1 → 4)-linked α-D-glucose [1]. Whereas amylose is essentially linear, amylopectin is highly branched [1]. The starch chain can form a number of different crystalline polymorphs. In the native starch granule A and B forms can be distinguished which are based on the packing of double helices into monoclinic [2] and hexagonal [3] arrays, respectively. During industrial usage the crystalline structure of the starch granule is usually 'melted' in the presence of a diluent, most commonly water. Although there have been studies on the effect of water content on 'gelatinization' of semi-crystalline starch in its native granular form [4], relatively little is known about the melting and dissolution behaviour of the isolated crystalline material.

In contrast to low molecular weight species, poly-

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mers do not generally form molecular crystals, that is, crystals of single molecular species, even narrow fractions may contain several chain lengths, and as a result the ordered crystalline structure can be disrupted by the chain ends [5–7]. Theoretically the melting temperature, T_{me}, of an extended chain crystal of a polymer containing n repeating units can be related to the melting temperature, $T_{m\infty}$, of the infinite molecular weight chain through the equation [5–7]

$$1/T_{me} - 1/T_{m\infty} = (R/\Delta H_u)(1 + b)(1/n) \quad (1)$$

where ΔH_u is the enthalpy of fusion per repeating unit and

$$b = \left[1 - (\zeta_e/n) + (1/n)\right]^{-1} \quad (2)$$

where ζ_e is the number of repeating units in an ordered length.

The classical description [8] of the effect of a diluent on the dissolution of a crystalline polymer represents the system on a lattice, the polymer chain is considered to be composed of segments of a defined volume — usually the molecular volume of the diluent obtained from its molar volume. The equilibrium melting temperature, T_m, of a crystalline polymer-diluent mixture is given by

$$1/T_m = 1/T_m^0 + (R/\Delta H_u) \cdot (V_u/V_1) \cdot \left[-\ln v_2/x + (1 - 1/x)v_1 - \chi v_1^2\right] \quad (3)$$

At the limit of high molecular weight, this expression reduces to

$$1/T_m = 1/T_m^0 + (R/\Delta H_u) \cdot (V_u/V_1) \cdot \left[v_1 - \chi v_1^2\right] \quad (4)$$

where T_m^0 is the melting temperature of the pure polymer, V_u and V_1 are the molar volumes of polymer repeating unit and diluent, respectively, v_1 and v_2 are the diluent and polymer volume fractions, respectively, x is the number of segments per molecule, and χ is the Flory–Huggins interaction parameter characterizing the interaction energy per solvent molecule [8]. In the original treatment, this parameter was obtainable from the enthalpy change ΔH_m on mixing solvent and polymer, although a potential entropic contribution to the interaction energy from first neighbour interactions was also recognised. This is particularly significant in aqueous polymer solutions when hydrogen bonding between water and the polymer repeating unit leads to an orientation-dependent first neighbour interaction.

In the polymer literature, the utility of this approach has been established, and there are tabulations of interaction parameters for a range of synthetic polymers and diluents [9]. Although the value of applying the concepts of polymer science to biopolymers, such as the starch polymers [10], has long been recognised, the experimental study of these materials is difficult. The study of the equilibrium melting of polymers requires the preparation of extended-chain single crystals [6,11]. Under appropriate conditions, short chains of the starch polysaccharide amylose, ranging in degree of polymerization, dp, from 15–30, can be crystallized as spherulites, consisting of assemblies of single crystals [12,13]. By changing the crystallization conditions it is possible to prepare both the A- and the B-type crystalline polymorphs of linear dextrins [12,14]. For the same chain length it was found that the dissolution of the A polymorph in water occurred at temperatures approximately 20 °C higher than the B polymorph. For both polymorphs, the dissolution behaviour could be fitted to the equation 4 with a predicted T_m for the dry crystals of approximately 260 °C [15]. It is not possible to confirm this value experimentally as thermal degradation precedes melting. It must also be recognised that as well as being an important diluent, water is also involved in the crystal structure [3,16] with the B polymorph containing 26% w/w. Removal of this water disrupts the crystal lattice and leads to the disappearance of crystal structure as assessed by X-ray diffraction.

In this paper we extend these observations by examination of the dependence of dissolution temperature on chain length for amylose chains of limited polydispersity. To characterize water as a diluent we have determined the Flory–Huggins interaction parameter, χ, from water sorption studies and measured the heats of solution of glassy glucose and maltooligomers.

2. Experimental

Materials.—Linear dextrins of degree of polymerization 12–25 were obtained by heterogeneous acid hydrolysis of potato starch granules in 2 M HCl at 35 °C for 40 days. The product was washed extensively to remove acid, and dissolved in water at 120 °C to make a 10% (w/w) solution. The solution was kept at 60 °C for several hours to permit the aggregation of high molecular weight material, which was removed by centrifugation. Ethanol was added to the solution to a final concentration of 50% (w/w) and the linear dextrin isolated as a crystalline precipi-

tate after storage at 2 °C. The linearity of the dextrins was assessed by comparison of chromatographic elution profiles before and after debranching with isoamylase. Synthetic linear dextrins were obtained by the action of phosphorylase. Typically a mixture of 200 mg of α-D-glucose 1-phosphate and 20 mg of maltotetraose in 5 mL MES buffer, pH 6.6, was treated with 10 units rabbit muscle phosphorylase a (Sigma–Aldrich, Dorset, UK) at 37 °C for 24 h. The dp of the product was dependent on the ratio of substrate to primer. The crude dextrin preparations were separated by preparative size-exclusion chromatography (Shodex SB2003, 30 cm × 0.2 cm) and further fractionated by reverse phase chromatography using a Polymer Labs PLRPS column (30 cm × 0.75 cm) at 65 °C with water as the eluent. The efficiency of the fractionation was monitored by analytical ion-exchange chromatography on a Dionex PA-100 column equipped with a Dionex pulsed amperometric detector. The column was eluted with 0.1 M NaOH (A) and 0.1 M NaOH containing 0.6 M NaOAc (B) using a gradient of 25 to 60% B from 5 to 48 min. The column was calibrated using commercially available maltooligomers (maltose to maltoheptaose) of > 95% purity (Sigma–Aldrich, Dorset, UK), and isoamylase-debranched wheat amylopectin. Degrees of polymerisation were assigned assuming each discrete peak to represent a single dp.

Calorimetry.—Differential scanning calorimetry (dsc) to probe crystallite dissolution over the temperature range 0 to 160 °C was carried out at 10 °C/min using a Perkin–Elmer DSC 7 instrument as described [15]. The instrument was calibrated from the melting of indium, and the peak dissolution temperature and the enthalpy of dissolution obtained using the standard software. Crystallization of the dextrin fractions from 20% (w/w) aqueous suspensions was carried out in the sample pan, using alternate cycles of heating (at 10 °C/min) followed by cooling and storage at 1 °C for 5 days, to obtain endotherms of dissolution which were superimposable. Crystallization under these conditions leads to the formation of the B-crystalline polymorph of starch [12].

The heat changes on dissolution of maltooligomer glasses in water at 25 °C were obtained using a heat-flux Setaram Micro-DSC (Lyon, France), calibrated from heat capacity measurements of sapphire. The dry carbohydrate glass (~ 10 mg) was accurately weighed into a Setaram C276 circulation vessel. Water, pre-equilibrated to 25 °C was added to reference and sample vessels at a rate of 0.1 mL/min using a Gilson Minipuls peristaltic pump. The enthalpy change on dissolution of the glassy oligosaccharide was obtained through comparison of the heat fluxes to sample and reference.

Water sorption isotherms.—Sorption isotherms were obtained by measuring the change in weight of dry maltooligomers after storage in atmospheres of known water activity [17] at 20 °C. Samples were conditioned until constant weight was achieved which typically took between 4 and 7 days.

3. Results and discussion

Maltooligomer / water interactions.—To predict the effect of water content on the dissolution of amylose crystallites using eqs (3) and (4) it is necessary to have an estimate of the polymer–solvent interaction parameter, χ, and the enthalpy of fusion per repeating unit of the pure crystal, ΔH_u. The interaction parameter describes the interaction between a solvent and polymer segment and is related to the enthalpy change on mixing of the two components, ΔH_m, and also includes entropic effects due to specific first neighbour interactions [8]. In this study we have examined the heats of solution of amorphous glassy glucose, maltotriose, and maltohexaose (Table 1). For amorphous glassy glucose we obtained a heat of solution of −4.4 kJ/mol on dissolution and dilution to 0.05 molal. This is comparable to a value of −4.7 kJ/mol obtained in an earlier experimental study [18] (the precise value depending on the thermal history of the glass). For maltotriose and maltohexaose the corresponding values were −31 and −60 kJ/mol. While recognising that the thermal history of the glass will affect these figures, we conclude that just as for D-glucose, the heats of solution of the maltooligomers indicate a strong energetic interaction between water and the carbohydrate.

Table 1
Physical data characterizing solute–water interactions of glucose and maltooligomers

Sample	Heat of solution (kJ/mol)	Water content for a T_g of 20 °C [20] (% w/w)	χ
D-Glucose	−4.37	1.5	–
Maltotriose	−31.0	10.8	0.7
Maltotetraose	–	–	0.75
Maltohexaose	−60.0	15.6	0.78
Maltoheptaose	–	–	0.78

Vapour pressure measurements of the solvent in equilibrium with the high molecular weight polymer provide a direct method of determination of χ [19,20], from the relationship

$$\chi = \left\{\ln[p_1/(1-v_2)p_1^0] - v_2(1-1/x)\right\}/v_2^2 \quad (5)$$

where p_1/p_1^0 is the solvent activity, and x is the number of segments per molecule obtained from the ratio of the molar volume of the polymer to that of the solvent, water. In Fig. 1(a–d) is shown the water sorption behaviour, at 20 °C, of maltotriose, tetraose, hexaose, and heptaose where the amount of water sorbed per 100 g of carbohydrate is plotted as a function of p_1/p_1^0. The observed behaviour for the oligomers has the same general form and is compared with the behaviour predicted from eq (5). At low water activities the observed dependence of sorption on water activity has a different functional form to that of eq (5), which is more marked for the higher oligomers. The T_g's of the maltooligomers [21] and the compositions at which T_g equals 20 °C is shown in Table 1. Convergence between Flory–Huggins and observed behaviour occurs for compositions which have a $T_g > 20$ °C, with estimates of χ being obtained in this composition range. Comparable behaviour is observed for the sorption of organic solvents by amorphous synthetic polymers [22]. χ for the maltooligomers is in the range 0.7 to 0.78, and can be compared with estimates obtained from water sorption studies on starch. For example, van den Berg [23] found a value of 0.8 at a water content of 25% at 25 °C for various starch samples including native granules. More recently [24], the variation of χ for starch water/mixtures with composition and temperature was examined. At low water contents (when the starch/water mixture was glassy) there was a marked dependence of the calculated χ on composition, as a result of non-equilibrium factors. For compositions for which T_g was exceeded, the composition dependence was much less marked with values of ~ 0.85 being obtained at 57 °C for water contents > 17.6% (w/w). For these macromolecular materials the amount of water sorbed will be influenced by the crystallinity of the sample or the extent to which it crystallizes during the course of the experiment. This will impact on the calculated value of χ. An advantage of the maltooligomers used in the present study is that they are very difficult to crystallize with no

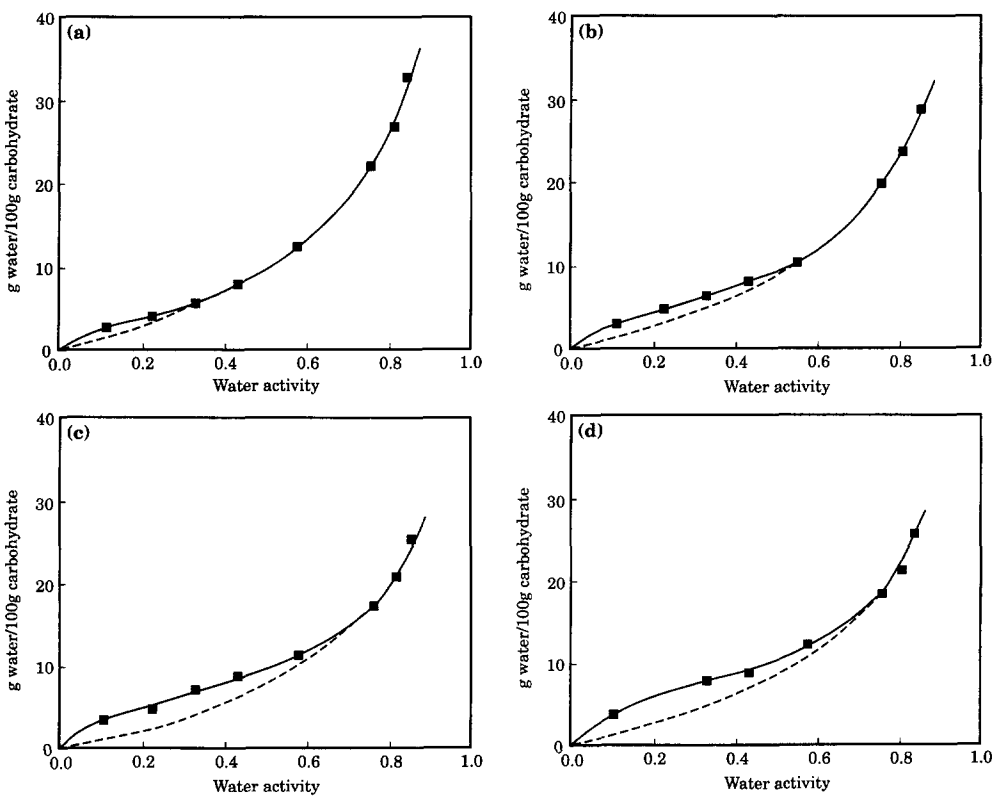

Fig. 1. Water sorption isotherms for maltooligomers measured at 20 °C (————) and Flory–Huggins fit to compositions above their glass transition temperature (– – –) for (a) maltotriose, (b) maltotetraose, (c) maltohexaose, and (d) maltoheptaose.

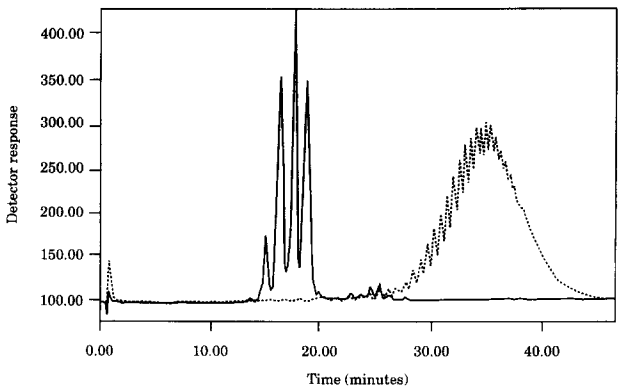

Fig. 2. Constituent chain profiles of a potato starch lintner and a fraction obtained after further preparative chromatography as characterized using high-performance anion-exchange chromatography with pulsed amperometric detection.

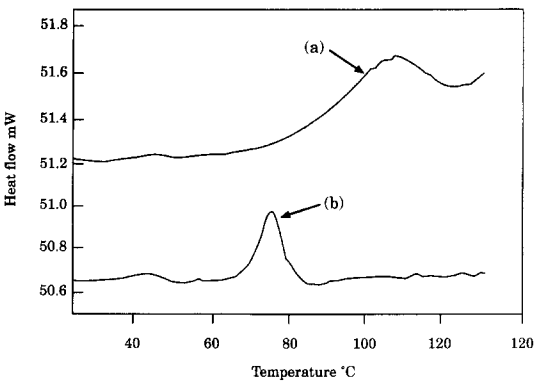

Fig. 3. Comparison of the observed endotherms of dissolution for (a) a relatively polydisperse preparation and (b) a relatively narrow fraction measured using differential scanning calorimetry.

crystal forms reported for the tetraose, hexaose, and heptaose.

The above results indicate that in concentrated solution, water is a relatively poor solvent for the (1 → 4)-α-D-glucan chain at room temperature. The interaction parameter χ is generally concentration dependent. Light scattering studies on dilute aqueous solutions of amylose characterize water as a theta solvent at room temperature [1]. At the limit of infinite molecular weight this indicates [8] that χ is 0.5 for the dilute amylose solution, rising to ~ 0.8 at a polymer concentration of 80% (w/w). These values of χ are qualitatively consistent with the aqueous solution behaviour of the amylosic chain. Although lower maltooligomers form stable aqueous solutions at room temperature, the effect of increasing chain length leads to instability, this is revealed with the observation of gelation and aggregation from concentrated aqueous solution [25].

Although water is a poor solvent for the amylosic chain, there remains a strong energetic interaction between water and the carbohydrate. In Flory's original treatment [8] it was recognised that in cases for which there are specific interactions between neighbouring components, the configurational entropy of mixing, obtained from the lattice model, may not represent the total entropy of mixing. These additional contributions are included in the interaction parameter χ. With increasing amylose chain length, the strong, orientation-dependent, hydrogen bonding between water and amylose leads to an increasing entropic contribution to χ which balances and eventually dominates the favourable energetic interaction.

Effect of chain length on dissolution.—The previous study [15] on the dissolution of spherulites of short amylose chains used a linear dextrin obtained from the lintnerization of potato starch granules. In Fig. 2 the chain profile of this product is compared with that obtained by further fractionation by size-exclusion and reverse phase chromatography. Whereas the original product shows a rather broad polydispersity with a dp_w in the region of 30, the polydispersity of the fractionated product is much more limited with a dp_n and dp_w of 13 and 12, respectively. Polydispersity also affects the width of the transition observed on crystallite dissolution (Fig. 3), with the narrow fraction giving a sharper endotherm on dissolution. Preliminary experiments on the dissolution of crystallites prepared from more polydisperse fractions ($dp_w/dp_n > 1.5$) showed a depression in T_m, relative to the narrow chain fraction, of ~ 30 °C for a dp_w of 30.

Fractions of a limited polydispersity with dp's ranging from 12 to 55 were used in the subsequent experiments. In Fig. 4 is shown the dependence of T_m

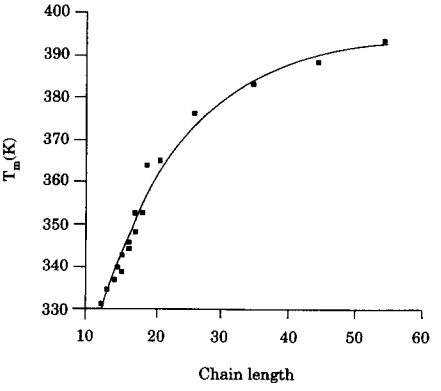

Fig. 4. Plot of dissolution temperature T_m versus chain length for the B-crystalline polymorph of starch.

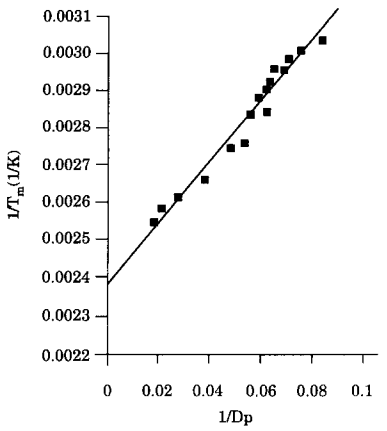

Fig. 5. Reciprocal plot of $1/T_m$ versus 1/dp for the dissolution of starch.

on chain length for the dissolution of dextrin crystallites in water at a volume fraction of water of 0.8. T_m shows a marked dependence on chain length in the range 12–20, but appears to be approaching a plateau value of ~400 K (127 °C) as the chain length approaches 60. A reciprocal plot of $1/T_m$ versus 1/dp (Fig. 5) is linear, and permits estimation, by extrapolation, of a high molecular weight limit of T_m of ~420 K (147 °C) at a volume fraction of water of 0.8. This behaviour, observed in the presence of a diluent, is comparable to the observed dependence of melting behaviour of synthetic polymers on chain length in the absence of a diluent [6]. The enthalpy of fusion for crystalline synthetic amyloses (dp 40–50) was typically 36 J/g in good agreement with that observed previously [15]. For synthetic polymers ΔH_u and T_m^0 can be generally obtained in a single calorimetric experiment, and so from a knowledge of χ it is then possible to make a prediction of the composition dependence of melting using the Flory approach (eqs (3) and (4)). For starch, thermal degradation of the crystalline polymorphs precedes melting, and it is not possible to obtain ΔH_u and T_m^0 experimentally. An estimate of ΔH_u can be obtained from examination of the melting of low molecular weight carbohydrates, for example the enthalpy of fusion of D-glucose is 30 kJ/mol, falling to 22.6 kJ/mol per anhydrohexose unit for maltose monohydrate [26]. Use of the latter value enables a good fitting to the experimentally observed composition dependence of melting [15]. To refine the prediction further it is necessary to estimate the temperature dependence of χ, in temperature and composition regimes where the non-equilibrium effects due to vitrification are absent. Present data is limited, although a recent contribution [24] suggests that, for a high molecular weight amylose chain, χ will fall to 0.5 at 160–180 °C. These values of χ and ΔH_u enable the prediction of a T_m of ~250 °C at a water content of 26% (w/w) rising to ~480 °C for T_m^0. The usefulness of this prediction can be evaluated through examination of the effect of diluents and plasticisers other than water on the observed melting and dissolution behaviour.

4. Conclusions

Although there is a strong energetic interaction between water and the starch chain at room temperature, there is an opposing entropic contribution to the free energy of interaction. Using the Flory–Huggins approach for polymer solutions this can be expressed as an interaction parameter, χ, the value of which at 20 °C of 0.78 indicates that water is a poor solvent. This observation is consistent with the observed gelation and crystallization behaviour of the starch polysaccharides. The dissolution of the B-crystalline polymorph of amylose is strongly dependent on chain length. A reciprocal plot of $1/T_m$ versus 1/dp is linear with an extrapolated high molecular weight limit of T_m of 147 °C at a volume fraction of water of 0.8.

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