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Note

Factors affecting the crystalline type (A–C) of native starches and model compounds: a rationalisation of observed effects in terms of polymorphic structures

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All native granular starches contain regions of long-range molecular organisation (crystallinity) caused by the ordered packing of adjacent branches of amylopectin molecules[1,2]. The two crystalline types (polymorphs) that are commonly observed by X-ray diffraction of native starches, A and B, consist of ordered arrays of double helices[3,4]. A third type of diffraction pattern (C) is occasionally observed and is due to a mixture of A and B polymorphs[2,5]. Much insight into starch crystallisation has been gained from studies of malto-oligosaccharides [(1→4)-α-D-glucan oligomers] as model compounds. Both branched and unbranched compounds can be obtained following extensive acid hydrolysis of granular starches[6–13]. Debranching of glycogen with *Cytophaga* isoamylase provides a convenient means of preparing strictly unbranched malto-oligosaccharides[14]. These model compounds can be crystallised from hot aqueous solution to give A, B, or intermediate (C) polymorphs under various experimental conditions. Numerous factors (polymorphic determinants) are known to influence the polymorphic form in crystalline model starch systems, *e.g.*, temperature, concentration, salts, organic molecules, and (1→4)-α-D-glucan chain-length. Other factors being equal, the A-type polymorph is favoured over the B-type by (*a*) shorter average chain-length[14,15], (*b*) higher temperature[14,16], (*c*) higher concentration[14,16], (*d*) the presence of salts of high lyotropic number[17], and (*e*) the presence of water-soluble alcohols and organic acids[11]. An extensive study of native starches[15] strongly suggested that the average chain-length of amylopectin is the major natural polymorphic determinant. It is now shown that all of these effects can be rationalised by considering the structural differences between A- and B-type polymorphs.

The A-type structure[4] has a nearly close-packed arrangement of double helices, whereas the B-type structure[3] consists of a more open packing of helices with a correspondingly greater amount of inter-helical water. Individual double helices in the two structures are very similar, if not identical[3,5]. These packing

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arrangements are depicted in Fig. 1. Two inferences may be drawn from the differences in polymorphic packing. First, the tightly packed A-type structure would be expected to be the more stable (*i.e.*, to have a lower free energy), and secondly, glucan chains would be expected to experience a greater entropy barrier to A-type rather than B-type crystallisation due to the closer packing of the A-type polymorph. There is some evidence from calorimetric studies[18] that the A-type polymorph has a higher melting temperature and hence is more stable than the B-type. Analogous polymorphic differences are found for saturated monoacid triglycerides[19,20], for which the closest packed polymorph (β) has a higher melting point (*i.e.*, is more stable) despite having a more negative entropy of formation than the less tightly packed α polymorph[19,20].

The effect of the chain length of the (1→4)-α-D-glucan on the polymorphic form can be rationalised from considerations of entropy, *i.e.*, with increasing chain

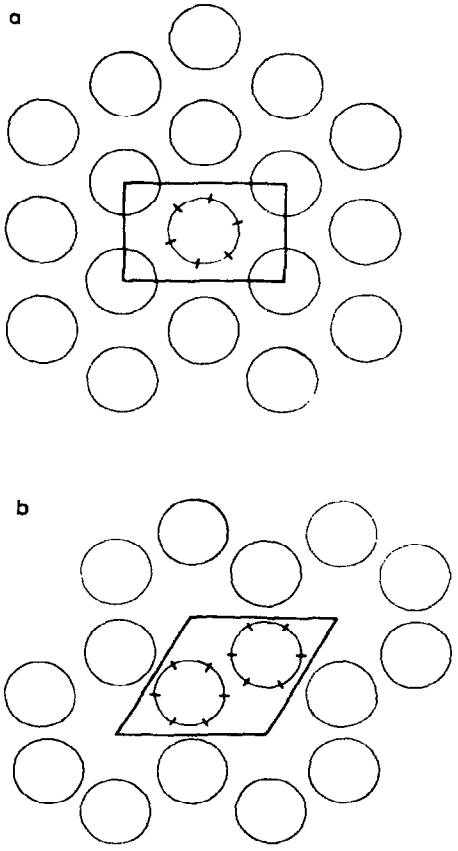

Fig. 1. Illustration of double-helix packing arrangements and unit cells in (a) A-type and (b) B-type starch polymorphs (adapted from refs. 3 and 4). Each circle represents a view down the *z*-axis of a double helix.

length, the (negative) entropy change experienced by glucan chains on crystallisation will become larger and therefore tend to favour the polymorph of highest entropy (B-type). In native starches, the result of this effect appears to be that amylopectins with average chain-lengths of <20 and >22 give rise to A- and B-crystalline types, respectively[15]. The effect of incubation temperature[14,16] is also explicable in terms of polymorphic packing arrangements. Lower temperatures would be expected to lead to more rapid crystallisation and therefore favour the polymorphic form requiring the least entropy change from solution (B-type), *i.e.*, the kinetic product; at higher temperatures, crystallisation would be slower and tend to favour the most stable polymorph (A-type), *i.e.*, the thermodynamic product. Analogous effects of temperature and (hydrocarbon) chain length are found for the saturated monoacid triglycerides[19,20]. The observation that increasing solution concentration favours A-type crystallisation[14,16] is presumably due to the greater (1→4)-α-D-glucan density in this polymorph.

In a study of the effect of inorganic salts on the crystallisation of a model starch material[17], it was found that the A-type polymorph was favoured in a concentration-dependent manner by the presence of various salts, and that those salts having the highest lyotropic numbers[21] had the greatest effect[17]. The only ion which favoured the B-type polymorph was sulphate which, of the ions studied[17], has the lowest lyotropic number[21]. Although the molecular mechanisms underlying the lyotropic series are not definitely established[21,22], it is considered that addition of salts of high lyotropic number ("structure destabilisers") makes less water available around an uncharged co-solute[22]. On decreasing the lyotropic number of the added salt, a point is reached, depending on the system, where "structure stabilising" ions "loosen" the water lattice sufficiently for there to be an increase in the water available around an uncharged co-solute[22]. Experimental support for this description is provided by recent studies of the effect of salts on protein stability[23,24]. The role of ions as polymorphic determinants in model starch crystallisations can now be rationalised by considering the water content of the two polymorphs. Thus, the A-type polymorph, which contains least water, is favoured by the presence of salts of high lyotropic number as this causes a decrease in the water available to the carbohydrate co-solute. From the results of Hizukuri *et al.*[17], it appears that only ions having a very low lyotropic number (*e.g.*, sulphate[21]) can cause an increase in the water available around starch-related materials and hence favour the B-type polymorph. Analogous effects have recently been observed in a study of the interaction of guanidinium salts with protein[24], where preferential hydration of the protein was found for the sulphate and preferential salt binding (*i.e.*, water displacement) for the acetate and hydrochloride[24].

A similar consideration of polymorphic water content provides a possible explanation for the observed effects of adding organic acids or alcohols to crystallisation media[11]. Addition of any of a range of water-soluble aliphatic acids and alcohols favours[11] A-type over B-type crystallisation. This effect increases with concentration and with the molecular size (*i.e.*, hydrocarbon chain-length) of the

additive[11]. In aqueous solutions of these molecules, their hydrophobic character would be expected to perturb the solvent structure over a long range (*i.e.*, involve significant numbers of water molecules), thus reducing the water available around a glucan co-solute and favouring A-type polymorph formation in crystallisation experiments. With increasing concentration and hydrocarbon chain-length, it would be expected that this effect would be enhanced, in line with the observations of Hizukuri *et al.*[11].

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