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Thermodynamic considerations of starch functionality in foods

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Abstract

Functionality of starch macromolecules is considered from the viewpoint of their thermodynamic properties. The thermodynamic incompatibility, self-association and inclusion complexing of starch macromolecules are important for food formulation and digestion. Helical conformations of starch macromolecules increase their co-solubility with other biopolymers (molecular mimicry) and influence the thermodynamic activity of other macromolecules (molecular symbiosis). Molecular mimicry and molecular symbiosis are the basis of many biological functions, phase behaviour and rheology of biopolymer mixtures. Rotation of starch granules in shear flow (producing a ball-bearing effect) contributes to the fat-like texture of foods. Branched exopolysaccharides could be an evolutional predecessor of starch granules. Amylopectin-like exopolysaccharides form a biopolymer solution layer around the cell, which is not accessible to foreign macromolecules due to biopolymer incompatibility underlying a non-specific defence of the cell. However, this biopolymer solution around cells remains perfectly accessible to nutrients of low molecular weight allowing for necessary cell nutrition. This barrier solution layer of exopolysaccharides could then be regarded as a protective and alimentary capsule surrounding the cell. The principle of incompatibility of exopolysaccharide fractions could be extended to provide a mechanism by which exopolysaccharides leaving the cell are responsible for binding and evacuating metabolites. This binding of undesirable metabolites is analogous to the binding of self-antigens and in a molecular sense precedes the evolutionary development of a similar result: specific immunity. A colony of cells is formed as the assembly of individual cells with their surrounding exopolysaccharide solutions separated from the medium by the interfacial layer. The overlap of the interfacial layers produces a three-dimensional network of canals that would assist the diffusional transportation of nutrients from a larger surrounding area. At the level of individual cells, the thermodynamic properties of amylopectin-like exopolysaccharides predict the binding of nutrients within their helical structure by inclusion complexing. Carrying them to the surface of the cell would then produce an octopus-like effect of facilitated transport. The capacity of starch macromolecules to bind lipids and other hydrophobic ligands leads to a decrease in the rate of enzymatic hydrolyses of inclusion complexes, which would extend the contribution of the exopolysaccharide layer to the nutrition of the cell. An increase in concentration of cells and their competition for the exopolysaccharides as a source of energy could result in the accumulation of dietary energy reserve inside the cells in the form of starch-like granules. Like globular proteins, the primary structure of amylopectin (branch point distribution and side-chain lengths) appears to be responsible for the secondary and tertiary structure of macromolecules and the structure–function relationship in starch granules.

Keywords: Biopolymer incompatibility; Inclusion complexing; Excluded volume; Molecular mimicry; Molecular symbiosis; Ball-bearing effect; Resistant starch; Nutrition; Origin of non-specific immunity; Specific immunity; Exopolysaccharides; Prebiotics; Octopus effect; Cell nutrition; Colony of cells; Evolutionary origin of starch; Gelatinisation

1. Introduction

This paper is devoted to a paradox of food formulation. Why are starch pastes unstable? And, on the contrary, why are starchy foods of reproducible quality? Why are synergy and antagonism so typical of polysaccharide mixtures? The main reason is that a similarity of non-specific interactions of food macromolecules between each other and other food components determines the thermodynamic similarity of foods. In other words, the properties of a food system reflect more the interactions between its components than the properties of those individual components (Tolstoguzov, 2001, 2002a,b). For instance, in spite of the detailed information available on starch and gluten, dough functionality is still empirically controlled (Tolstoguzov, 1997). Consequently, the objective of the paper is to review typical interactions of starch in food and chyme. The article will focus on three topics: (i) the general properties of food macromolecular mixtures, (ii) starch interactions and

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functionality in foods and (iii) biological systems. We will start with the interactions between macromolecules.

2. Thermodynamic incompatibility of biopolymers

The results summarised in Fig. 1 illustrate that mixing biopolymer solutions can result in two different types of phase separation. They correspond to concentration of the biopolymers either in the two different phases or in one of them. These phenomena that reflect either repulsion or attraction between either dissimilar or similar macromolecules, are biopolymer incompatibility, interbiopolymer complexing, self-association and crystallisation of macromolecules. Phase separations caused by thermodynamic incompatibility, crystallisation and inclusion complexing with low molecular weight compounds are more typical of starches than interbiopolymer complexing. The latter are formed with phenolic polymers, some polysaccharides and proteins. For instance, interbiopolymer complexes are formed in many beverages (Tolstoguzov, 2000c, 2002a).

Starch forms inclusion complexes with many aliphatic and aromatic organic compounds. Inclusion complexing increases the stability of helical structures of starch macromolecules and provides a highly pronounced absorption ability of starches to food flavours. The starch-associated lipids (free fatty acids and lysolecithin) also form inclusion complexes that are usually insoluble, resistant to oxidation and can presumably delay starch hydrolysis (Morrison, 1995; Warner et al., 2001).

Biopolymer incompatibility was discovered more than 100 years ago, when Beijerinck found it impossible to prepare a homogeneous mixed solution of potato starch with gelatin (Beijerinck, 1896). He described the first water-in-water emulsion, where droplets of gelatin solution were dispersed in a starch solution (Beijerinck, 1910). Later, Ostwald and Hertel showed a difference in the phase separation threshold for mixed solutions of gelatin with cereal and potato starches (Ostwald & Hertel, 1929). Normally, the phase separation threshold, i.e. the minimal bulk concentration of biopolymers at which phase separation occurs, is dependent on the excluded volume of the macromolecules.

Fig. 2 illustrates the principle of excluded volume for rigid globular and rod-like macromolecules (Tanford, 1961; Tolstoguzov, 1991, 1992, 1999a). The adjacent spheres represent two protein molecules of the same radius, R. Illustrated in the figure are the excluded volumes that surround each molecule and are not accessible to the centres of other similar molecules. Since the radius of the excluded volume equals the diameter of molecules, the excluded volume is 8-fold larger than the molecule itself. It is significantly larger for linear rigid macromolecules, which are relatively independent in solution, when the distance between them exceeds their length. The excluded volume effects determine solution space occupancy by the macromolecules and their co-solubility. Table 1 shows that phase separation occurs at biopolymer concentrations of about 1–4% for mixtures of polysaccharides, exceeds 4% for globular protein–polysaccharide mixtures and more than 12% for mixtures of globular proteins (Annable, Fitton, Harris, Phillips, & Williams, 1994; Antonov, Grinberg, & Tolstoguzov, 1977; Antonov, Pletenko, & Tolstoguzov, 1987; Grinberg & Tolstoguzov, 1997; Kalichevsky, Orford, & Ring, 1986; Kalichevsky & Ring, 1987; Kasapis, Morris, Norton, & Gidley, 1993b; Polyakov, Grinberg, & Tolstoguzov, 1997; Tolstoguzov, Grinberg, & Gurov, 1985). Normally, synthetic polymers exhibit a phase separation threshold concentration at least 10-fold lower than globular proteins and starchy macromolecules. Actually, Table 1 shows that the co-solubility of biopolymers, especially proteins and starch macromolecules, is surprisingly high (Tolstoguzov, 1999a,b, 2000c).

2.1. Molecular mimicry

We have proposed previously that the high co-solubility of biopolymers results from a principle we have termed molecular mimicry (Tolstoguzov, 1999a,b). Molecular mimicry refers to the tendency to maximise the chemical resemblance of hydrophilic surfaces of globular proteins, with most of the unique chemical information hidden within the hydrophobic interior. Molecular mimicry is first apparent in submolecular helical structures and would explain the dense packing of polypeptide and polysaccharide helices. Just as the principle of mimicry of globular proteins derives from a spatial organisation of hydrophobic and hydrophilic regions of polypeptides, the formation of polysaccharide helices results in a hydrophobic molecular interior and a common resemblance of the hydrophilic cylindrical surfaces.

The most surprising macroscopic example of an increase in surface hydrophilicity of helical macromolecules and in hydrophobicity of their interior is perhaps the very high hydrophobicity of gelatin gel surface formed in contact with air or lipids and a high hydrophilicity of the surfaces formed by cutting the same gel (Tolstoguzov, 1999b). Since association of triple helices within the bulk of the gelatin gel forms its network, the helical conformation could be responsible for the high hydrophilicity of new surfaces obtained by cutting the gel. This means that conformational change (presumably unfolding the helix) within a nano-scale surface layer during formation of the gel provides its extraordinary high hydrophobicity. Molecular mimicry predicts the nature of water–biopolymer interactions at the submolecular, molecular and supermolecular levels, as well as at the level of thermodynamic compatibility since apart from the excluded volume of macromolecules, the interactions of the biopolymers with each other and with the solvent water are responsible for their thermodynamic compatibility.

Molecular mimicry of biopolymers is inherent to their

Table 1
Critical point co-ordinates and phase separation thresholds of some biopolymer mixtures

Biopolymer pair	Conditions: temperature (°C), pH and salt concentration	Phase separation threshold (%)
Maltodextrin [potato] + Arabic gum	45 °C, 0.1 M NaCl	16
Ovalbumin + soybean globulins	pH 6.6, 20 °C	15.4
Caseinate [sodium] + amylopectin [MW = 38,000 kDa]	20 °C, 0.15 M NaOH; 25 °C, 0.15 M NaCl; pH 6.5	8.0
Soybean globulins + Arabic gum [MW = 497 kDa]	20 °C, pH 9.0	7.2
Soybean globulins + sodium alginate [MW = 150 kDa]	20 °C, pH 9.0	4.9
Soybean globulins + pectin [MW = 40 kDa]	20 °C, pH 9.0	4.3
Pectin [MW = 69 kDa; esterification degree 62.7%] + methyl cellulose [MW = 70 kDa]	20 °C, pH 5.0, 0.5 M NaCl	1.20
Pectin [MW = 69 kDa; esterification degree 62.7%] + locust bean gum	20 °C, pH 5.0, 0.5 M NaCl	0.86
Maltodextrin [potato] + locust bean gum	45 °C, 0.1 M NaCl	6.3
Amylose [MW = 700 kDa] + dextran [MW = 472 kDa]	75, 70 and 90 °C	5.0

Fig. 1. Schematic representation of phase separation in mixed starch solutions. Interbiopolymer complexes can be formed by starches and modified starches with, e.g. with phenolic polymers and with proteins. Phase separation caused by incompatibility is most typical of starch macromolecules. Both self-association of starchy macromolecules, especially of amylose, and formation of lipid–starch inclusion complexes decrease solubility and co-solubility, i.e. the phase separation threshold of starch macromolecules.

biological functions, e.g. high mixed biopolymer concentrations are necessary within the cell cytoplasm, blood, protein bodies, starch granules and for enzymatic functions for which thermodynamic incompatibility would preclude these concentrations and activities if they were not able to counteract the basic thermodynamic properties of dissimilar biopolymers by adopting effective surface mimicry.

Another type of molecular mimicry of biopolymers can be seen in the hybrid macromolecules, such as mucopolysaccharides, glycoproteins, natural and synthetic protein–polysaccharide conjugates (Tolstoguzov, 1999b, 2000d, 2002a). These hybrid, mainly branched, macromolecules illustrate that macromolecules of mixed chemical nature are an important tool for controlling compatibility of dissimilar biopolymers (Tolstoguzov, 1993a,b). The molecular construction of protein–polysaccharide conjugates is typical of vaccines imitating microorganism surfaces, particularly. Supermolecular mimicry is also widely used, e.g. by viruses and bacteriophages.

However, the similarity of macromolecules caused by mimicry reduces the range of properties of biopolymer mixtures, which become difficult to control just by changing their composition.

Fig. 2. Schematic illustration of the excluded volume for: (A) globular (protein) and (B) rod-like (rigid polysaccharide) macromolecules and submolecular helices. Minimal excluded volume of globular molecules is 8-fold higher than that of the molecule.

2.2. Molecular symbiosis

It has been proposed that an additional thermodynamic principle acting in the direction opposite to molecular mimicry is 'molecular symbiosis' (Tolstoguzov, 2000b). This term means a mutual influence of dissimilar biopolymers, whose macromolecules differ in conformation and/or composition, and which favours the biological efficiency of at least one of them. Because of excluded volume effects, the macromolecules mutually concentrate each other and as a result, each behaves as if they were, for example, in a more concentrated mixed solution (Tolstoguzov, 1985, 1991, 1995, 1996, 1998a). Changes in the excluded volume of macromolecules can arise from their conformational (e.g. helix–coil) transitions, association–dissociation of macromolecules or through incompatibility-complexing (association–segregation) transitions of the hybrid macromolecular components, e.g. protein–polysaccharide conjugates (Tolstoguzov, 1993a, 2000c,d). For instance, Fig. 3 illustrates a decrease in the excluded volume of macromolecules caused by their association. This means that the effective concentration of an enzyme can be increased without additional synthesis due to the dissociation (Fig. 3) of a macromolecular co-solute of high excluded volume.

Molecular symbiosis could underlie the predominance of the two extreme conformations and structures typical of biopolymers: globular and rod-like, linear and highly branched.

3. Thermodynamic incompatibility of starch macromolecules

Incompatibility is typical of biopolymers because of the large size of macromolecules and the very low entropy that accompanies the mixing of biopolymers. Therefore, even when the corresponding monomer sugars are co-soluble in aqueous media in all proportions polysaccharides assembled from these sugars are usually incompatible when their polymer chains differ in structure and/or composition. Only one monomer, glucose, is involved in the formation of amylose and amylopectin chains. Normally, such polymer homologues tend to self-associate, crystallise, are thermodynamically compatible and can form transparent single-phase mixed solutions. This is, presumably, the case of highly concentrated amylose and amylopectin mixtures in native starch granules under conditions typical of seeds. This is not the case of starches gelatinised in an excess of water. What is the reason, however, for the immiscibility of starch macromolecules with one another as found experimentally by Kalichevsky and Ring (1987)?

There are several differences between these two polymers that would underlie their thermodynamic incompatibility. First is the difference in length and flexibility between amylose chains and amylopectin side-chains. This difference could prevent their mutual 'recognition' as being of the same chemical nature. The second factor is the difference in the regularity of chains and their tendency to self-association. Normally, conditions promoting self-association favour the incompatibility of biopolymers. A third difference is the interactions with starch lipids. Such an inclusion complexing would increase the effective difference between the polymer chains in both their solubility and rigidity. As a net result of these multiple factors, both the phase separation thresholds and phase diagram asymmetry depend on the botanical origin of various starches.

Fig. 4 shows that unlike protein (gelatin, globular protein) solutions, gelation of starch suspensions is accompanied by a continuous increase in viscosity (Tolstoguzov, 1990, 1995). This increase in viscosity is due to the continuous leaching, entanglement and association of amylose. Fig. 5 illustrates the gelation of starch pastes. The high local concentration and high excluded volume of amylopectin molecules inside the swollen granule would increase the rate of leaching of amylose out of the granule. The rate of amylose leaching decreases with increasing concentration of starch dispersion. Since the rate of leaching can be either greater or lesser than the rate of amylose retrogradation, amylose can retrograde either between or within the starch granules. The material of semi-permeable coverage of granules is structurally similar to and compatible with retrograded amylose. Thin layers of the leached and retrograded amylose could therefore encapsulate swollen granules. Coalescence of these retrograding amylose capsules appears to form a three-dimensional 'honeycomb-like' amylose construction, a network of the gel, where the swollen granules act as an active filler.

According to Le Chatelier's principle, an increase in the concentration of macromolecules favours a reduction in their excluded volume, i.e. in the case of starch concentration favours helical conformations, self-association (Fig. 3), gelation and crystallisation of starch macromolecules (Tolstoguzov, 1997, 2001, 2002a). For instance, the rate of starch retrogradation increases with the starch paste concentration. It was shown that crystallisation of both amylopectin and maltodextrin is greatly enhanced in the presence of gelatin (Doi, 1965; Kasapis, Morris, Norton, & Brown, 1993a; Kasapis et al., 1993b). The presence of polysaccharide (dextran) also results in an increase in the rate of coil–helix transition, self-association and gelation of the gelatin (Tolstoguzov, 1985, 1992, 1995, 1998a). The variation of amylopectin crystallisation, thus, predicts bread staling. Thus, an increase in concentration of dough favours bread staling. Another example of excluded volume effects on recrystallisation of amylopectin can be seen in the higher content of resistant starch in pasta products other than in bread, and especially in pasta products after high temperature drying (Pagani, Gallant, Bouchet, & Resmini, 1986; Tolstoguzov, 2002a).

For the same reason of incompatibility, the addition of guar gum increases the amount of resistant starch in drinks

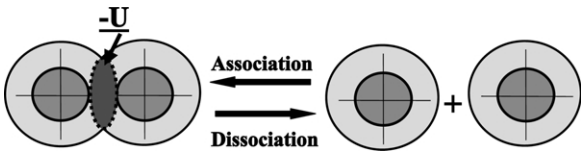

Fig. 3. Association of macromolecules decreases their excluded volume. On the contrary, dissociation increases their excluded volume and concentration of macromolecular co-solutes. According to Le Chatelier's principle, the self-association of macromolecules and submolecular helices reducing their excluded volume can be intensified by an increase in concentration of the macromolecular system.

or meals (Alam et al., 1998; Brennan, Blake, Ellis, & Schofield, 1996; Ellis, 1999; Ellis, Dawoud, & Morris, 1991). It was shown (Closs, Conde-Petit, Roberts, Tolstoguzov, & Esher, 1999; Closs, Tolstoguzov, Conde-Petit, & Esher, 1998) that amylopectin and pre-gelatinised waxy maize starch are incompatible with guar gum, so that the addition of guar gum can result in phase separation, encapsulation of the starchy phase by the guar gum-enriched phase and stopped leaching of the amylose. Fig. 6 shows a phase diagram for galactomannan-pre-gelatinised waxy maize starch mixtures at 60 °C. Phase separation could also increase the concentration of macromolecules inside the granules, the rate and degree of starch retrogradation, and hence the formation of resistant starch (Figs. 3 and 5).

Fig. 7 shows that the interfacial layer between two aqueous polymer phases can adsorb hydrophobic particles, including cells and cell organelles. Partitioning of cells usually occurs between the interfacial layer and one bulk phase. Systematic application of this phenomenon by Albertsson, Walter and Zaslavsky showed that the principle can be used for large-scale fractionation and isolation of cells (Albertsson, 1972; Walter, 2000; Walter, Johansson, & Brooks, 1991; Zaslavsky, 1995).

Fig. 8 shows that the coalescence of lipid droplets adsorbed within the interfacial layer between the phases of a water-in-water emulsion can result in a lipid capsule around the dispersed particles (Tolstoguzov, 1996, 1998b, 2000a,c, d, 2002a).

Coalescence of these lipid capsules leads to a three-dimensional honeycomb-like lipid construction. This honeycomb-like lipid phase can encapsulate up to 80%, aqueous phases and has been used in butter replacers

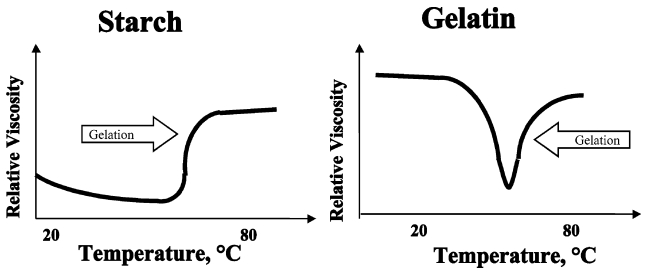

Fig. 4. Schematic representation of viscosity changes due to association of macromolecules of a gelforming agent. In the process of gelation and just before the formation of three-dimensional network, a decrease in viscosity typical of proteins does not occur in starch pastes.

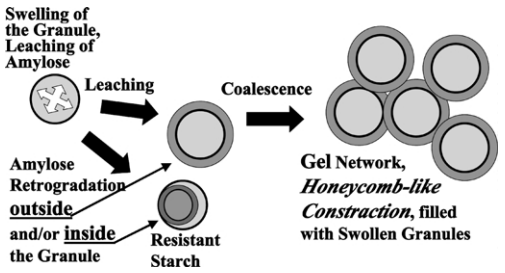

Fig. 5. Schematic representation of leaching and retrogradation of amylose out of starch granules in the processes of gelation and formation of resistant starch.

(Cain, Jones, & Norton, 1987). In such low-fat spreads, mixed solutions of maltodextrin and gelatin are usually used to prepare small gel granules separated by lipid layers. During spreading, the small gel granules rotate and behave ostensibly like ball bearings. This results in a lubricant effect and could be responsible for fat mimetic properties (i.e. fat-like texture) of many foods and cosmetics, such as cosmetic rice starch powder (Tolstoguzov, 1996, 1997).

Fig. 9 shows that the revolving of starch granules in flowing pasta dough can decrease the friction between adjacent gluten layers flowing at different rates (Tolstoguzov, 1997, 2000b). The ball-bearing effect here results in unusually high dough fluidity in spite of the fact that wheat flour dough is one of the most concentrated food systems. In flowing dough, the granules rotating between adjacent gluten layers could roll out the gluten strips. Starch granule size, much as a 'rolling-pin', exceeds the gluten strip thickness. This rolling-pin effect could greatly contribute to dough structure. For example, it could be responsible for the homogeneous three-dimensional structure of bakery goods. One more result of the revolving of starch granules is their migration towards the central layers in flowing dough. According to Bernoulli's principal, pressure is least where flow velocity is greatest. An 'upward force' moves a granule towards faster flowing dough layers during extrusion. Migration of starch granules would result in the formation of a 'starch-filled' central layer and a 'starch-reduced' surface layer (Tolstoguzov, 1997, 2001).

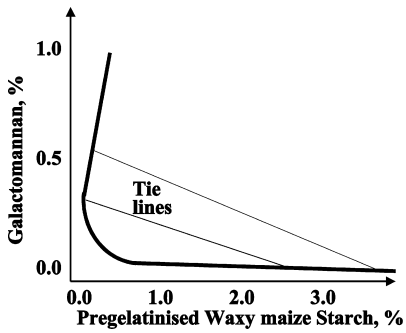

Fig. 6. Phase diagram for galactomannan-pre-gelatinised waxy maize starch mixtures at 60 °C (Closs et al., 1999). An apparent increase in co-solubility of the biopolymers with the galactomannan concentration results from gelatinisation of the amylopectin due to a greatly increased concentration of the amylopectin-rich phase and a non-complete separation of the phases.

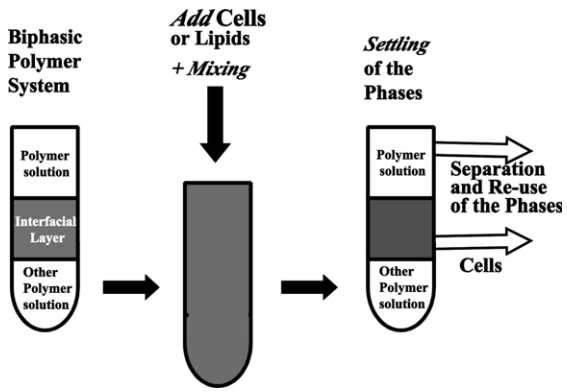

Fig. 7. Schematic representation of the interfacial layer between two aqueous polymer phases. The interfacial layer can adsorb hydrophobic particles, including cells, cells organelles and lipids.

4. Molecular evolution of starch

We now turn to the molecular evolution of starch (Tolstoguzov, 2000c, 2001, 2002a,b). Because soluble food can be absorbed into the cell, the best source of energy would be simple sugars. However, they must be converted into macromolecules to be stored. Consequently, the molecular evolution of starch could have begun with the synthesis of amylopectin-like exopolysaccharides by the cell. This deposition of sugar polymers outside the cell could have conferred selective advantage as a means of accumulating a nutritional reserve during times of surplus. Amylopectin-like macromolecules appear to correspond well to this function because of their slow diffusion, high viscosity and ease of re-conversion into simple sugars again to be used by the cell when required.

4.1. Nutrition and non-specific immunity as the same process

Fig. 10 illustrates other possible functions of exopoly-

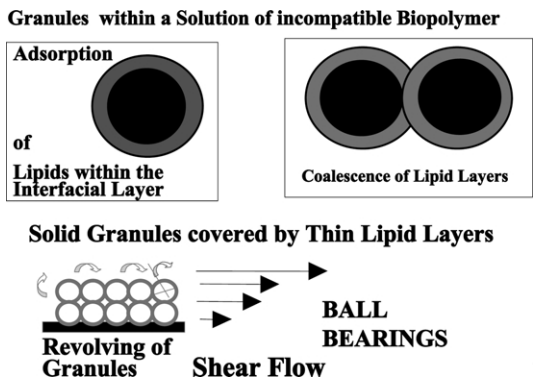

Fig. 8. Schematic representation of adsorption of lipid droplets, which are mainly within the interfacial layer and one bulk phase. Coalescence of the lipid particles adsorbed between aqueous phases results in encapsulation of aqueous dispersed particles and in honey comb-like structures. On shearing, e.g. on spreading, revolving of granules separated by lipid layers (i.e. rotation of solid spherical particles of a honeycomb-like structure) results in ball-bearing, lubricant effect.

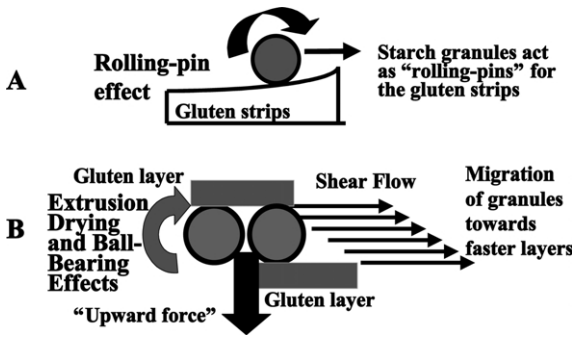

Fig. 9. Schematic representation of some structural effects of starch granules: (A) in flowing dough, revolving of starch granules (ball-bearing effect) results in rolling out of gluten strips. (B) Migration of revolving starch granules towards the central layers in flowing dough according to Bernoulli's principle.

saccharides (Tolstoguzov, 1999b, 2000c, 2001). From the earliest evolutionary stages, both nutrition and universal immune defence of the cell were presumably the same process based on biopolymer incompatibility. Polysaccharides secreted by many bacteria form an exocellular layer, that is, impenetrable to foreign proteins, other macromolecules and viruses. The exopolysaccharide layer around the cell is, however, easily penetrable by low molecular weight organic compounds, amino acids, peptides, sugars, oligosaccharides, etc. and mineral ions. This exopolysaccharide solution layer could therefore be regarded as both the 'alimentary' and 'protective' capsule of the cell.

Amylopectin-like molecules could be of special efficiency in providing these exopolysaccharide functions, i.e. the simultaneous protection and nutrition of the cell. Their advantages could reflect the self-association of amylopectin, its ability to bind different ligands (Biliaderies, 1991; Morrison, 1995; Osman-Ismail & Solms, 1973; Polaczek, Starzyk, Malenki, & Tomasik, 2000; Rutschmann, Heiniger, Pliska, & Solms, 1989; Rutschmann & Solms, 1990a–d; Schiraldi & Fessas, 2000; Tufvesson & Eliasson, 2000) and a high local concentration of side-chains due to the periodical distribution of branch points (Thompson, 2000). The incompatibility of amylopectin with both cell-wall polysaccharides and proteins of globular and unordered structures (Tables 1 and 2) (Annable et al., 1994; Antonov et al., 1977; Antonov et al., 1987; Closs et al., 1999, 1998; Grinberg & Tolstoguzov, 1997; Kalichevsky et al., 1986; Kalichevsky & Ring, 1987; Kasapis et al., 1993a,b), and also the fact that bacteriophages and microorganisms are the most important sources of enzymes hydrolysing

Fig. 10. Schematic illustration of non-specific immunity and nutrition as the same process. An alimentary and protective capsule around the cell is an exopolysaccharide solution layer impenetrable for foreign proteins, viruses and other polysaccharides.

Table 2
Phase state of mixed polysaccharide solutions (Antonov et al., 1987)

Polysaccharide	Amylopectin	Arabinogalactan–protein	Gum Arabic	Carob gum	Dextran
Pectin	II	II	II	II	II
Alginate sodium	II	II	II	II	II
Gum Arabic	II	II	–	II	I
Carob gum	II	II	N	II	II

I, single-phase; II, phase separated; N, unstudied.

exopolysaccharides (Sutherland, 1999) provide evidence of the protective functions of the exopolysaccharide capsule of the cell. Its protective functions could also underlie antivirial and antimicrobial activities (Tolstoguzov, 2000b, 2002a) and the efficiency of polysaccharides as effective barriers to spontaneous migration of foreign biopolymers would even protect a wound from the environment in multicellular organisms (Lloyd, Kennedy, Methacanon, Paterson, & Knill, 1998).

Thermodynamic incompatibility of biopolymers implies that macromolecules show a preference to be surrounded by their own type in mixed solution. Consequently, self-association, which is typical of amylopectin (due to molecular mimicry and symbiosis), is intensified in the presence of other macromolecules (molecular symbiosis). This means that the density of the protective capsule formed by amylopectin-like exopolysaccharides around the cell could increase in the presence of foreign macromolecules and supermolecular particles, e.g. phages and viruses. The self-association of amylopectin-like exopolysaccharides, which is enhanced in the presence of other macromolecules, also means that the exopolysaccharide capsule around the cell could act as a mechanical (skin-like) filter of controllable density. Its density is dependent on the nature, concentration and excluded volume of foreign macromolecular particles. An increase in the local concentration of exopolysaccharide chains and their excluded volume within the exopolysaccharide capsule could intensify enzymatic hydrolysis by: (i) an increase in an effective local concentration of exocellular hydrolytic enzymes and (ii) an enhancement of substrate–enzyme complexing, since according to Le Chatelier's principle, an increase in concentration of macromolecules favours both their self-association and complexing (Fig. 3). This would enhance the protective efficiency of the capsule against foreign macromolecules, their hydrolysis and re-use, i.e. nutrition of the cell. Consequently, it could be predicted that exocellular hydrolytic enzymes are of necessity relatively better co-soluble in exopolysaccharide solutions. This may be of importance for industrial biotechnology.

Since both the highly pronounced self-association and high viscosity of amylopectin-like exopolysaccharides are intensified by the presence of other macromolecules, the protective exopolysaccharide capsule would be valuable for mechanical protection of the cell and as a nano-shock absorber.

Fig. 11 illustrates a proposed mechanism of prebiotic activity of dietary fibre, which is thought to be responsible for the preferential colonisation of the intestinal tract by symbiotic bacteria (Tolstoguzov, 2001, 2002b). It is proposed that the exopolysaccharide capsule surrounding the cell could have developed during evolution into vegetable cell-wall polysaccharides and into the mucopolysaccharide of the brush membrane lining the gut (Tolstoguzov, 2001, 2002a,b). The relative thermodynamic affinity between these three groups of exopolysaccharides could underlie the efficiency of food fibre as prebiotics.

4.2. Evacuation of metabolites and specific protection

Fig. 12 illustrates a proposed evacuation technique of metabolites from the interior of the cell. This proposed mechanism of benefit is based on the thermodynamic incompatibility between exopolysaccharide fractions covered and leaving the cell, i.e. between amylopectin-like and modified exopolysaccharides. The fraction leaving the cell could have developed during evolution by the introduction of polar and charged side groups along the exopolysaccharide chains and by cross-linking of the chains by polypeptides. Antimicrobial peptides secreted by the cell would also logically be used for cross-linking the exopolysaccharides to be transported out of the cell and kept in the surroundings of the cell. The cross-linked large-size polysaccharide subunites could provide solubility of the antimicrobial peptide and protect it against proteases.

It was shown that charged polysaccharides, mucopolysaccharides and glycopolysaccharides are usually incompatible with cell-wall polysaccharides and neutral exopolysaccharides (Tables 1 and 2). For instance, incompatibility between pectin and gum Arabic has been used to concentrate pectin solutions used to concentrate and fractionate skimmed milk proteins (Tolstoguzov, 1988, 1998b; Tolstoguzov et al., 1985; Tolstoguzov & Rivier, 1996).

It could be assumed that incompatibility of some exopolysaccharide fractions between each other could result in separation of their functions. The modified exopolysaccharides leaving the cell could be used to bind, encapsulate and evacuate some non-desirable metabolites, toxic compounds and parasitic organisms. It can also be assumed that during evolution the exomucopolysaccharides and exopolysaccharides leaving the cell could be developed into the

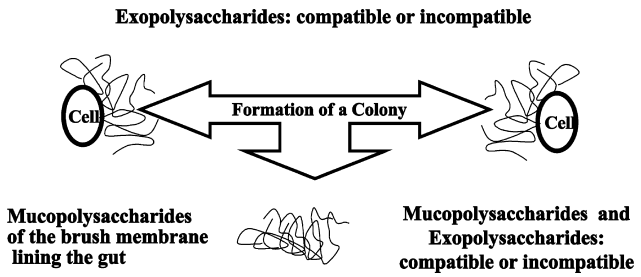

Fig. 11. Schematic representation of exopolysaccharide interactions underlying colonisation of the alimentary canal by symbiotic microflora. The colonisation could be due to a difference in relative affinity (compatibility) between exopolysaccharides of the symbiotic, other bacteria, mucopolysaccharide membrane lining the gut and dietary fibre (cell-wall polysaccharides) and causes prebiotic activity of the latter.

vegetable gums. In other words, incompatibility of the exopolysaccharides living the cell with the cell-wall-polysaccharides and the exopolysaccharides surrounding the cell could underlie their functioning as container for metabolic rubbish (i.e. as garbage-bin). Some exomucopolysaccharides leaving the cell could possibly act similarly as encapsulating agents and vehicles preventing pathogenic microorganisms from adhering to the gastrointestinal epithelia and evacuating them.

To preserve homeostasis, the evolution of cellular metabolism could only be based on the continuous identification of those metabolites to be further used by the cell and those to be evacuated from the cell. Since proteins are the main instruments of metabolism, they could be used for chemical modification of exopolysacchartides, e.g. to increase their hydrophobicity, their adhesion to hydrophobic ligands, and act as binders (antibody-like) of self-produced antigens, and also induce antimicrobial polypeptides. The exopolysaccharides leaving the cell could therefore evolve into the direction of increasing polypeptide/polysaccharide ratio to develop specific immunity tools (i.e. to evolve from gum Arabic-like particles to

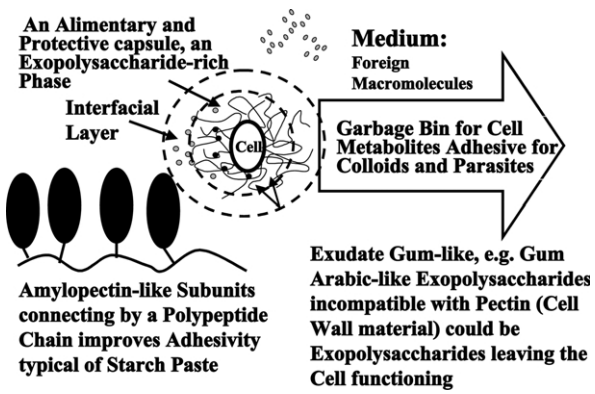

Fig. 12. Schematic representation of phase separation within the exopolysaccharide capsule (Fig. 10) caused by incompatibility of exopolysaccharides and cell-wall polysaccharide with modified exopolysaccharides (e.g. mucopolysaccharides, arabinogalactan–proteins) leaving the cell. The latter could act as a garbage bin for cell metabolites and evolute into vegetable exudate gum (e.g. gum Arabic).

lymphocyte-like particles). A long-term training in the recognition of self-produced antigens, substances to be completely bind, could lead to building up of a huge number of corresponding binders, i.e. polypeptide–polysaccharide hybrid compounds as better adhesives, for all possible metabolites to be evacuated. An evolutionary improvement of the synthesis of hybrid macromolecules as encapsulating agents more structurally corresponding to variable cellular metabolites and competitors could result in microorganisms with specific immunity. Such a perfectly protected parasitic microorganism could be able to infect an animal and form a symbiotic association for the nutritional and protective benefit of each of the partners. This infection of the host animal by parasitic microorganisms and the further development of the symbiotic co-operation could quite recently occur. The host-internal (organ) microflora collaboration underlying activity of specific immunity appears to be mainly supported by continuous vaccination by diversity of consumed food components and microflora metabolites as the most powerful sources of various antigens. In other words, like viruses that are parasites of cells, the protective specific immunity tools could be formed in cells, evolved together with cells and then changed their functions together with specialisation of cells.

If this hypothesis is reasonable, the specific recognition of antigen was formed to identify self-produced (and/or absorbed) antigens, i.e. macromolecules that may be hazardous for the cell. The means that antigens are presumably not only foreign macromolecules but both exogenous and endogenous macromolecules that from previous experience are hazardous and/or useless macromolecules for the cell. For instance, this implies that self-antigens cannot be degraded by lysosomal enzymes, be metabolised by the cell, that their chemical information and activity are dangerous for the cell.

The non-specific immune systems of microorganisms and plants widely use polysaccharides and are, presumably, more universal though more static than that of animals. Non-specific protection of animals living in changeable surrounding is based on proteins and mucopolysaccharides (e.g. skin, epithelia membranes). An other words, among two immune systems, non-specific and specific immunity, the latter, which became only typical of (from fish) vertebrates (Janeway, 2001; Paul, 1999; Travis, 1993) is significantly younger than the former. The evacuation of self- and foreign antigens from the cell, presumably, includes their transfer into the medium, which are separated from the cell by the protective capsule and an interfacial layer.

4.3. Interfacial layer separating the cell and the medium

Fig. 13 shows that if the exopolysaccharide capsule is incompatible with biopolymers of the medium, it must be separated from the medium by an interfacial layer. This low viscosity (depletion relative to macromolecules) layer forms

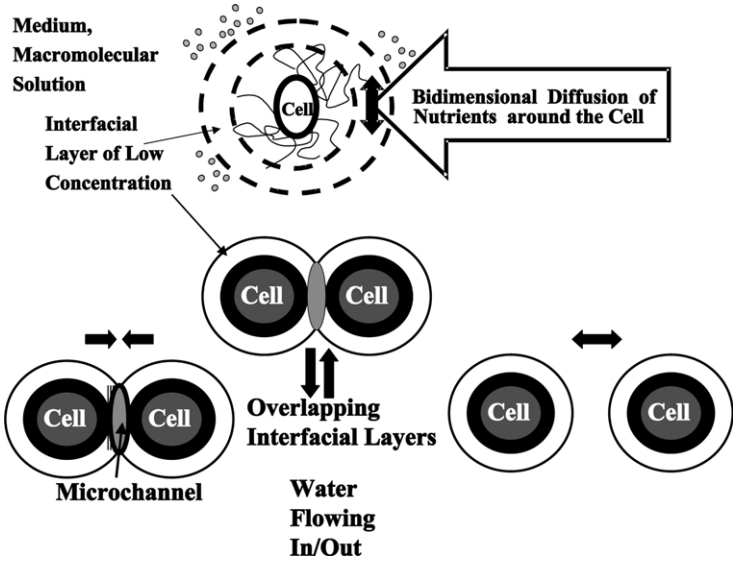

Fig. 13. Schematic representation of the spherical interfacial layer around the cell. This spherical corridor of low viscosity around the cell is formed between the exopolysaccharide capsule (Fig. 10) and the incompatible medium. Rapid diffusion of nutrients within this layer surrounding the cell could intensify its nutrition.

a spherical 'corridor' surrounding the cell. This layer could facilitate fast two-dimensional diffusion flow of nutrients, increase their redistribution rate around the cell and intensify the nutrition of the cell. Fig. 13 shows that when the moving cells come closer together, their interfacial layers become overlapping and form a microvolume with a concentration different from that of the medium. This difference in concentration corresponds to a gradient of the solvent, water, chemical potential. When the concentration (e.g. of nutrients) in the depletion microvolume exceeds that of the medium, the diffusion flow of water is oriented from the medium into the microvolume between the cells and separates the cells. This would mean that an excess of food (especially, low molecular weight nutrients) makes the nutrition of independent single cells preferential. On the contrary, when the concentration in the depletion microvolume is lower than in the medium the water is transferred from the volume between the cells to the medium and aggregation of the cells becomes favoured. It is proposed that the thermodynamic consequences of overlapping interfacial layers together with the thermodynamic incompatibility of exopolysaccharides covering different cell species could favour the formation of homogeneous cell colonies. In a colony of cells, overlapping of interfacial layers results in microvolumes forming a three-dimensional network of channels. This channelling increases the diffusion rate of nutrients from a larger area of the medium into the bulk of the colony. This means that formation of a colony is useful for nutrition of individual cells. The mechanism of channelling (channel formation) could have developed further as a selective advantage during the evolution of multicellular organisms and their organs. Another contributing factor to cell nutrition is the fact that hydrophobic nutrients are concentrated within the interfacial layer between two aqueous phases and adsorbed by the capillary channels (Figs. 7 and 8).

4.4. Transport of nutrients. An octopus-like effect

Fig. 14 shows one more contributory factor to cell nutrition. The exopolysaccharides could work like the arms of an octopus. The reversible complexing by polymers could select and transport nutrients from the interfacial layer surrounding the cell. This mechanism could be called an 'octopus' effect, based on a reversible binding of nutrients by exopolysaccharide chains, an increased hydrophobicity of the chain segments formed the complexes, their precipitation and release near the surface of the cell (Tolstoguzov, 2001, 2002b). When an inclusion complex is formed, the hydrocarbon part of the lipids (e.g. fatty acids) is only included (immobilised) within the end of the polysaccharide helical segment and the functional group of the bound lipid molecule is positioned outside of the helical cavity. Insoluble inclusion complexes can precipitate and dissociate on the surface of the cell. The precipitation of inclusion complexes can orient a bound lipid molecule by its polar or charged functional group to the surface of the cell. Since an inclusion complex contains only one lipid molecule, cell nutrition is organised at the molecular level. An octopus-like functioning of the exopolysaccharides (Fig. 14) could be regarded as mechanism of molecular sizing and adsorbing of hydrophobic nutrients forming micelles in aqueous media.

Fig. 14 shows that the amylopectin-like exopolysaccharide seems to have been specially developed as an ideal octopus. End-segments of amylopectin-like chains function like 'suckers'. Branching increases the number of 'tentacles' with suckers able to form inclusion complexes with

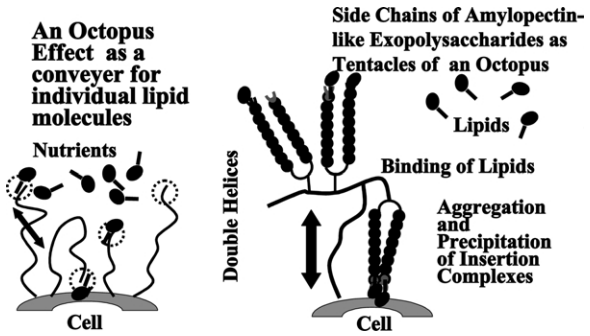

Fig. 14. Schematic representation of 'octopus effect', i.e. mechanism of binding and transportation of nutrients by linear or branched exopolysaccharide chains to the cell, e.g. by an amylopectin-like exopolysaccharide.

nutrients. In other words, the cell seems to be equipped with edible conveyor belts for the binding and transport of nutrients in the form of molecules. A more efficient fuel, lipids, when transported to the cell could preserve the amylopectin-like exopolysaccharide as a reserve for the future. The ability of lipid-inclusion complexes to decrease the digestion rate of amylopectin reduces its functions as a nutrient and, possibly, evidence for the benefits of this mechanism to the cell's nutrition.

4.5. Starch granules

The development of starch granules could have resulted from an increase in the concentration of competing cells (e.g. within cell colonies and multicellular organisms). The nutritional function of exopolysaccharides could be reduced because of competition between cells for the energy reserve concentrated outside the cells. The next step of starch molecular evolution could therefore have been the formation of starch granules inside the cell. The dense package of amylopectin macromolecules forming semi-crystalline starch granules is to provide homeostasis of the cell by ensuring controllable swelling and hydrolysis of the granules within the cell. The swelling of starch granules in cool water is limited. The reason could be stretching of macromolecular chains within amorphous layers during their swelling between the rigid crystalline lamellae acting as rigid clips. The stretching of macromolecular chains decreases their entropy and results in an elastic retractive force stopping further swelling. The swelling degree is dependent on the osmotic pressure of the surrounding solution, which includes the contribution of the hydrolysis products. Semi-crystalline starch globule covered by semi-permeable membrane could function as a microelasto-osmometer (Tolstoguzov, 1999b; Yamada, Prins, & Hermancs, 1963). Elasto-osmometry was developed by Prins and Hermans (Yamada et al., 1963) for determination of molecular weight of polymers. This method is based on measurement of the retractive force of the gel swollen in a dilute solution of a polymer, which does not penetrate into the gel. Using a strip of polydimethylsiloxane rubber immersed in toluene (as an osmometric cell), the authors showed that the retractive force of a gel at its constant deformation is inversely proportional to the number average molecular weight of the dissolved polymer. Fig. 15 illustrates a possible role of an osmotic pressure of the medium and a gel retractive force in controlling of the swelling and digestion kinetic of starch granule. In other words, semi-crystalline starch globule seems to act as the fuel and carburettor at the same time.

Digestion of starch starts in the mouth, before digestion of proteins, and finishes by fermentation in the colon. The macromolecular products of starch hydrolysis could facilitate swallowing and increase the hydrolysis rate of both proteins and polysaccharides. The reason is that phase separation of the chyme results in partitioning of enzymes between phases enriched in the corresponding substrates. Amylolytic enzymes would be expected in the continuous starch-rich phase, while proteolytic enzymes would be expected in the dispersed protein-rich phase. This partitioning of the proteolytic enzymes could underlie the protective mechanism of the intestinal walls against self-digestion (Tolstoguzov, 1999b, 2000c, 2001).

5. Primary, secondary, tertiary and quaternary structures of starch and gelatinisation of granules

The construction of starch granules is based on the two alternated crystalline and amorphous phases made out of the same amylopectin macromolecules modified by amylose chains. Like globular proteins there is, presumably, a strong dependence of the secondary and tertiary structure of amylopectin upon its primary structure. Disulphide intermolecular cross-links in a protein molecule presumably correspond to the branching in starch. The branching fixes relative positions of the side-chains and together with their polydispersity in length controls the secondary and ternary structures. The secondary structure of starch could cover double and single helices differing in length. These helices aligned parallel to one another organise a three-dimensional array of crystalline lamellae and quaternary structure of the globule as a rounded pile of semi-crystalline blocks (Appelqvist & Debet, 1997; Gallant, Bouchet, & Baldwin, 1997; Kweon, Haynes, Slade, & Levine, 2000; Oates, 1997; Zobel, 1988). In spite of some structural similarity, however, different terms (gelatinisation–retrogradation and denaturation–renaturation) are used to describe structural transformations of native globular proteins and starch granules.

After gelatinisation, i.e. destruction of native structure of globule, thermodynamic behaviour of starchy macromolecules comes closer to that of linear and branched polymers, e.g. exopolysaccharides. Their behaviour is dependent upon the energy and entropy factors governing non-specific interactions in macromolecular solutions. Together with excluded volume, the chain flexibility, regularity, and their

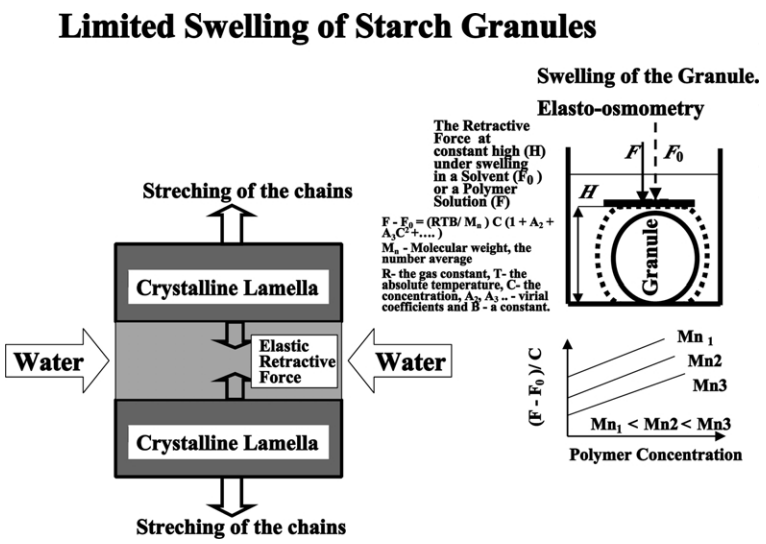

Fig. 15. Schematic representation of limited swelling of starch granules as a cell of elasto-osmometer.

interaction energy with each other and with the solvent are the main factors responsible for incompatibility of macromolecular substances in solution. For instance, since the attainable level of crystallinity depends on structural regularity of polymer chains it is significantly higher for amylose. Gelatinisation changes therefore structural functions of both starch macromolecules into the opposite directions compared to that in native granules. Amylopectin, whose laterally packed double helices form the crystalline lamellae of native granules, is greatly self-associated in gelatinised starch, slowly crystallised and molecularly immiscible with amylose. On the contrary, amylose, which is initially in the amorphous lamellae and decreases crystallinity degree of the amylopectin (i.e. presumably thermodynamically compatible with amylopectin in the absence of water), is dissolved in hot water. On cooling, amylose is crystallised, precipitated or gelled. It was shown that molecularly dispersed dilute starch solutions could be prepared only under autoclaving at temperatures of 135–160 °C (Aberle et al., 1997; Keetels, van Vliet, & Walstra, 1996).

On cooling, crystallisation of amylose chains forms the junction zones, the knots of the gel network, separated by the amorphous phase and the interfacial layers. Some amount of individual polymer chains can traverse all these three regions. When the temperature decreases thermal motion of chain segments of both types of starchy macromolecules becomes less intensive and gradually 'frozen out' (for more and more long chain segments). The frozen glassy state of associated (or co-crystallised) amylose and amylopectin side-chains act as dense physical cross-links. These physical cross-links hindering further crystallisation of long-side-chains presumably make staling the bread slower (Matveev, Grinberg, & Tolstoguzov, 2000; Nicholls, Appelqvist, Davies, Ingman, & Lillford, 1995; Tolstoguzov, 1997, 2000b).

6. Conclusion

In conclusion, it should be stressed that non-covalent, non-specific interactions of macromolecules underlie both an immense structural diversity and thermodynamic similarity of foods.

The main aim of this short consideration was to examine the functionality of starch from the viewpoint of thermodynamic properties and its assumed evolutionary origin from exopolysaccharides. More detailed discussion is difficult at the moment because of the lack of the information available on thermodynamic properties of biopolymer mixtures, e.g. about the probable compatibility of exocellular proteolytic, amylolytic enzymes and exopolysaccharides and amylopectin.

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