 DOI: 10.1002/masy.200750808

Amylose and Amylopectin Hybrid Materials via Enzymatic Pathways

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Summary: Oligo- and polysaccharides are important macromolecules in living systems, showing their multifunctional characteristics in the construction of cell walls, energy storage, cell recognition and their immune response.

Saccharides as organic raw materials can open new perspectives on the way to new biocompatible and biodegradable products which could help to overcome the problems resulting from the upcoming restrictions of petrochemical resources. Construction of well-defined carbohydrate polymer backbones is very challenging as it is difficult to realize complete regio and stereo-control of the glycosylating process. Most synthetic approaches are therefore based on the modification or degradation of naturally occurring polysaccharides resulting in less then perfect products. Enzymes have several remarkable catalytic properties compared with other types of catalysts in terms of their selectivity, high catalytic activity, lack of undesirable side reactions and operation under mild conditions. A biocatalytic pathway to synthesize saccharides is therefore very attractive as it results in well-defined polysaccharides avoiding the above drawbacks.

When biogenic polysaccharides are combined with synthetic macromolecules, surfaces etc. materials with new interesting properties arise and the processability of the designed hybrid materials is facilitated. Amylose and amylopectin hybrid materials can be synthesized via enzymatic polymerization routes utilizing transferases. This approach opens access to well-defined hybrid structures bearing amylase or amylopectin moieties that cannot be synthesized by any other means.

Keywords: amylopectin; amylose; enzymatic polymerization; polysaccharides

Introduction

Starch is the most abundant storage reserve carbohydrate in plants. Carbohydrates such as starch function as a reservoir of energy for later metabolic use. It is found in many different plant organs, including seeds, fruits, tubers and roots, where it is used as a source of energy during periods of dormancy and regrowth. Starch granules are composed of two types of α-glucan, amylose and amylopectin, which represent approximately 98–99% of the dry weight. The ratio of the two polysaccharides varies according to the botanical origin of the starch.

Amylose is a linear molecule in which the glucose units are joined via α-$(1 \rightarrow 4)$ glucosyl linkages. Amylopectin is a branched molecule in which about 5% of the glucose units are joined by α-$(1 \rightarrow 6)$ glucosyl linkages (see Figure 1).

In animals, a constant supply of glucose is essential for tissues such as the brain and red blood cells, which depend almost entirely on glucose as an energy source.

The mobilization of glucose from carbohydrate storage provides a constant supply of glucose to all tissues. For this purpose glucose units are mobilized by their sequential removal from the non-reducing

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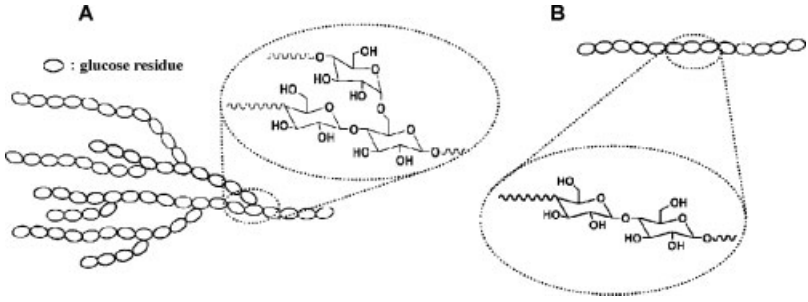

Figure 1.
Structure of A) amylopectin and B) amylose.

ends of starch utilizing three enzymes in the *in vivo* process:

- Glycogen phosphorylase catalyzes glycogen phosphorolysis (bond cleavage of the α-(1 → 4) bonds by the substitution of a phosphate group) to yield glucose-1-phosphate.
- Glycogen debranching enzyme removes α-(1 → 6) glycogen branches, thereby making additional glucose residues accessible to glycogen phosphorylase.
- Phosphoglucomutase converts glucose-1-phosphate into glucose-6-phosphate which has several metabolic fates.

The glycogen phosphorolysis of phosphorylase can be reverted, which makes it possible to enzymatically polymerize amylose as well as hybrid structures with amylose as outlined in the following.

Hybrid Structures with Amylose Blocks

The strict primer dependence of the glycogen phosphorylases makes them ideal candidates for the synthesis of hybrid structures of amylose with non natural materials (e.g. inorganic particles and surfaces, synthetic polymers). For this, a primer functionality (maltooligosaccharide) can be coupled to a synthetic structure and subsequently elongated by enzymatic polymerization resulting in amylose blocks.

Various examples on these types of hybrid materials are reported which are outlined in the following.

Amylose Hybrids with Short Alkyl Chains

Pfannemüller et al. showed that it is possible to obtain carbohydrate containing amphiphiles with various alkyl chains via amide bond formation. For this maltooligosaccharides were oxidized to the according aldonic acid lactones which could subsequently be coupled to alkylamines.[1–9] Such sugar based surfactants are important industrial products finding their applications in cosmetics, medical applications etc.[10–12] The authors were also able to extend the attached maltooligosaccharides with enzymatic polymerization with potato phosphorylase which resulted in products with very interesting solution properties.[13,14]

Amylose Brushes on Inorganic Surfaces

Amylose brushes (a layer consisting of polymer chains dangling in a solvent with one end attached to a surface is frequently referred to as a polymer brush) on spherical and planar surfaces can have several advantages, such as detoxification of surfaces etc. The modification of surfaces with thin polymer films is widely used to tailor surface properties such as wettability, biocompatibility, corrosion resistance and friction.[15] The advantage of polymer brushes over other surface modification methods like self-assembled monolayers is their mechanical and chemical robustness,

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coupled with a high degree of synthetic flexibility towards the introduction of a variety of functional groups.

Commonly, brushes are prepared by grafting polymers to surfaces by for instance chemical bonding of reactive groups on the surface and reactive end groups of the attached polymers. This 'grafting to' approach has several disadvantages as it is very difficult to achieve high grafting densities and/or thicker films due to steric crowding of reactive surface sites by already adsorbed polymers. The so-called 'grafting from' approach (polymers are grown from initiators bound to surfaces) is a superior alternative as the functionality, density and thickness of the polymer brushes can be controlled with almost molecular precision.

The first surface initiated enzymatic polymerization reported was the synthesis of amylose brushes on planar and spherical surfaces.[16] For this silica or silicone surfaces were modified with self assembled monolayers of (3-aminopropyl)trimethoxysilane or chlorodimethylsilane respectively. To these functionalities oligosaccharides were added via (a) reductive amidation of the oligosaccharides to surface bound amines, (b) conversion of the oligosaccharide to the according aldonic acid lactone and reaction with surface bound amines and (c) incorporation of a double bond to the oligosaccharide and subsequent hydrosilylation to surface bound Si-H functions. The surface bound oligosaccharides could be enzymatically elongated with potato phosphorylase and glucose-1-phosphate as monomer to amylose chains of any desired length. The degree of polymerization could be determined by spectrometric measurement of the liberated amount of inorganic phosphate[17] which was confirmed by cleavage of the amylose brushes (either enzymatically or by prior incorporation of light sensitive spacers) and subsequent characterization of the free amylose chains. The obtained amylose modified surfaces showed good chiral discrimination when employed as column materials in chiral affinity chromatography. Modification of the OH-Groups of the amylose brushes even enhanced the separation strength of the developed column materials.[18] The results were recently confirmed by Breitinger who attached maltooligosaccharides to surfaces via acid labile hydrazide linkers and enzymatically extended the chains with potato phosphorylase.[19]

Copolymers with Amylose

The combination of oligo- or polysaccharides with non natural polymeric structures opens up a novel class of materials. By varying the chain topology of the individual blocks as well as of the whole copolymer, the type of blocks, the composition etc. a complete set with tailor made properties can be designed.

Amylose is a rod-like helical polymer consisting of α-(1 $\rightarrow$ 4) glycosidic units. A measurement of the stiffness of a polymer is afforded by the so-called persistence length, which gives an estimate of the length scale over which the tangent vectors along the contour of the chains backbone are correlated. Typical values for persistence lengths in synthetic and biological systems can be several orders of magnitude larger than for flexible, coil-like polymers. Rod-like polymers have been found to exhibit lyotropic liquid crystalline ordered phases such as nematic and/or layered smectic structures with the molecules arranged with their long axes nearly parallel to each other. Supramolecular assemblies of rod-like molecules are also capable of forming liquid crystalline phases. The main factor governing the geometry of supramolecular structures in the liquid crystalline phase is the anisotropic aggregation of the molecules.

Copolymeric systems with amylose are therefore systems in which at least one component is based on a conformationally rigid segment, which are generally referred to as rod-coil systems.[20–23] By combining rod-like and coil-like polymers a novel class of self-assembling materials can be produced since the molecules share certain general characteristics typical of diblock molecules and thermotropic calamitic molecules. The difference in chain rigidity

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of rod-like and coil-like blocks is expected to greatly affect the details of molecular packing in the condensed phases and thus the nature of thermodynamically stable morphologies in these materials. The thermodynamic stable morphology probably originates as the result of the interdependence of microsegregation and liquid cristallinity. From this point of view it is very fascinating to compare the microstructures originating in solution and in the bulk for such materials.

Comb type and linear block copolymer systems with enzymatically synthesized amylose are reported, which are outlined in the following.

Comb-Type Copolymers with Amylose

The first comb like structures synthesized by enzymatic grafting *from* polymerization from a polymeric backbone were reported by Husemann et al.[24,25] Acetobromo oligosaccharides were covalently bound to 6-trityl-2,3-dicarbanilyl-amylose chains and subsequently elongated by enzymatic polymerization with potato phosphorylase, the result being amylopectin-like structures with various degrees of branching. Pfannemüller et al. extended this work by grafting amylose chains onto starch molecules. The modified starches where studied by the uptake of iodine and by light scattering measurements of carbanilate derivates[26] and appeared to be star like in electron microscopy studies.[27]

A full series of star-, network- and comb-like hybrid structures with oligosaccharides were synthesized by Pfannemüller et al. (see Figure 2) and it was shown that the attached oligosaccharides can be extended via the enzymatic polymerization with potato phosphorylase.[1,2,9,28,29]

Another type of comb like amylose hybrids synthesized via enzymatic grafting with phosphorylase is based on polysiloxane backbones. To achieve these structures double bonds were incorporated to the reducing end of oligosaccharides which were then attached to poly(dimethylsiloxane-co-methylsiloxane) copolymers via hydrosilylation[30,31] or to silane monomers which were subsequently polymerized to polysiloxanes.[32] Various mono-, di-, tri and oligosaccharides were attached to siloxane backbones and their solution properties were studied with viscosimetry and static and dynamic light scattering.[33] The pendant oligosaccharide moieties could be extended with enzymatic grafting from polymerization[34,35]

Kobayashi et al. succeeded in attaching maltopentaose to the *para* position of styrene and performed free radical polymerizations towards the homopolymers[36,37] as well as copolymers with acrylamide.[36] Kobayashi et al. also reported on the

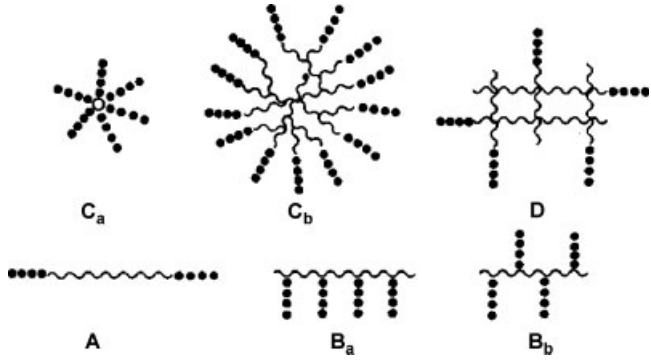

Figure 2.
Maltotetraose hybrids with various carriers resulting in different chain architecture. A: poly(ethylene oxide); B_a and B_b: poly(acrylic acid), amylose, cellulose and other polysaccharides; C_a: cyclodextrin and multifunctional acids; C_b: amylopectin; D: crosslinked poly(acryl amide).[28]

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successful attachment of maltopentaose to poly(L-glutamic acid).[38] Kakuchi et al. showed that the saccharide modified styrene monomers could also be polymerized with TEMPO-mediated controlled radical polymerization.[39] In all cases the authors could successfully elongate the attached oligosaccharide structures with enzymatic polymerization, the product being comb type block copolymers with amylose.

Linear Block Copolymers with Amylose

Various linear block copolymers of the AB, ABA and ABC type with enzymatically polymerized amylose blocks were reported. Ziegast and Pfannemüller converted the hydroxyl end groups of poly(ethylene oxide) into amino groups via tosylation and further reaction with 2-aminoalkylthiolate.[40] To the resulting mono- and di-amino functionalized poly(ethylene oxide) maltooligosaccharide lactones were attached and subsequently elongated to amylose via enzymatic polymerization.[41] Pfannemüller et al. performed a very detailed study on the solution properties of the synthesized A-B-A triblock copolymers as they can be considered model substances for ''once broken rod'' chains.[42] With static and dynamic light scattering they found that the flexible joint between the two rigid amylose blocks has no detectable effect on the common static and dynamic properties of the chain. With dielectric measurements it however became obvious that the directional properties of the electric dipoles of the broken rigid chains showed a different behavior to the non-broken rods (pure amylose). Akyoshi et al. also synthesized amylose-block-poly(ethylene glycol) block copolymers via enzymatic grafting from oligosaccharide terminated poly(ethylene oxide) and studied the solution properties of these amphiphilic block copolymers by static and dynamic light scattering.[43,44]

It was also shown that the enzymatic polymerization of amylose could be started from oligosaccharide modified polymers that are not soluble in the medium of polymerization (aqueous buffers). Amylose-block-polystyrene-copolymers could be synthesized by attaching maltooligosaccharides to anionically synthesized amino terminated polystyrene and subsequent enzymatic elongation to amylose.[45,46] Block copolymers with a wide range of molecular weights and copolymer composition were synthesized via this synthetic route. The solution properties of star type as well as crew cut micelles of these block copolymers were studied in water and THF and the according scaling laws were established.[47] In THF up to four different micellar species were detectable, some of them in the size range of vesicular structures, whereas the crew cut micelles in water were much more defined. Bosker et al. studied the interfacial behavior of amylose-block-polystyrene-copolymers at the air-water interface with the Langmuir-Blodgett technique.[48]

Recently two groups reported on controlled radical polymerizations started from maltooligosaccharides (ATRP[49] and TEMPO mediated radical polymerization[50]) which will certainly lead to new synthetic routes towards amylose containing block copolymers.

Even though the products are not block copolymer structures the work of Kadokawa et al. should be mentioned here. In a process the authors named ''vine-twining polymerization'' (after the way vine plant grow helically around a support rod) the enzymatic polymerization of amylose is performed in the presence of synthetic polymers in solution and the authors showed that the grown amylose chains incorporate the polymers into its helical cavity while polymerizing.[51–55]

Hybrid Structures with Amylopectin Like Structures

The results reviewed above clearly show that the combination of enzymatically polymerized amylose with surfaces of inorganic materials and synthetic polymers results in very interesting materials with superior properties. We are currently

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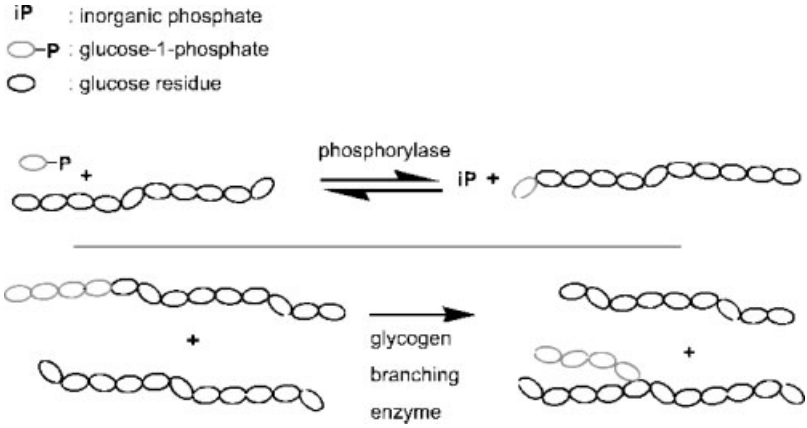

Figure 3.
Schematic representation of the reactions catalyzed by glycogen phosphorylase (above) and glycogen branching enzyme (below).

extending this concept by synthesizing hybrids with enzymatically synthesized amylopectin. This has several advantages including the better solubility of amylopectin versus amylose, the higher amount of functional group which will facilitate further modification etc.

The exact way of amylopectin biosynthesis in plants is still not known today. In our current research we are using a tandem reaction of two enzymes to synthesize ''artificial'' amylopectin or rather (hyper)-branched amylose *in vitro*. One enzyme is responsible for building the linear (amylose) part while the other enzyme introduces the branches, phosphorylase and glycogen branching enzyme respectively.

Phosphorylase can be easily isolated from potatoes and, after purification, used to catalyze the polymerization of glucose-1-phosphate in order to obtain linear polysaccharide chains with α-(1 $\rightarrow$ 4) glycosidic linkages, as can be seen in Figure 3.

The glycogen branching enzyme belongs to the transferase family and is able to transfer short, α-(1 $\rightarrow$ 4) linked, oligosaccharides from the non-reducing end of starch to an α-(1 $\rightarrow$ 6) position (see Figure 3).

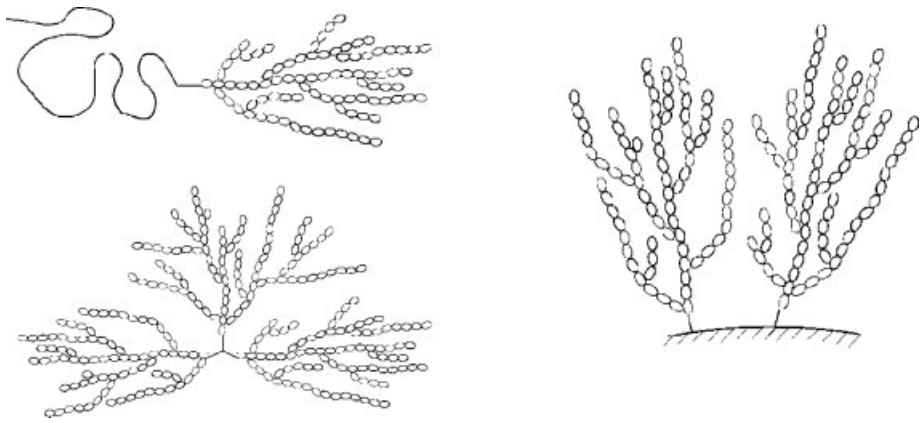

Figure 4.
Schematic representation of hybrid structures with amylopectin.

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By combining the glycogen branching enzyme with glycogen phosphorylase it becomes possible to synthesize branched structures via an one-pot synthesis as phosphorylase will polymerize linear amylose and the glycogen branching enzyme will introduce the branching points which are again extended by phosphorylase.

Both described enzymes are isolated from natural sources. Phosphorylase is isolated from potatoes whereas the glycogen branching enzyme is produced by various bacterial sources.[56,57] Depending on the source the properties of the products and the reaction conditions may differ.

As shown above, hybrid structures bearing amylose blocks can be synthesized by covalent attachment of primer recognition units for phosphorylase and subsequent enzymatic *grafting from* polymerization. Following the same route we are currently synthesizing hybrid materials bearing (hyper)branched polysaccharide structures as shown in Figure 4 with the described tandem reaction of two enzymes.

The branched structure, high amount of functional groups, biocompatibility of these structures make these architectures suitable for applications in the biomedical field and in the food industry.

Acknowledgements: We are indebted to M. Palomo Reixach, M.J.E.C. van der Maarel and L. Dijkhuizen from the Center for Carbohydrate Bioprocessing for their indispensable help with the branching enzymes.

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