

Review

Hydrophobization of Natural Polymers by Enzymatic Grafting of Hydrophobic Polysaccharides, Partially 2-Deoxygenated Amyloses

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Abstract

This review overviews the efficient hydrophobization method of hydrophilic natural polymers, which has been developed by means of glucan phosphorylase (GP)-induced enzymatic grafting of unnatural heteropolysaccharides, that is, partially 2-deoxygenated (P2D)-amyloses. The enzymatic polymerization technique is well known as a useful approach to prepare polysaccharides with well-defined structures. The authors have found that the hydrophobicity of P2D-amylose, synthesized by the thermostable GP (from *Aquifex aeolicus* VF5)-induced enzymatic copolymerization of α -D-glucose 1-phosphate (Glc-1-P)/D-glucal as comonomers, started from maltooligosaccharide primers. Based on this finding, glycogen, a hydrophilic spherical natural polysaccharide, was hydrophobized by means of the thermostable GP-induced enzymatic functionalization of the P2D-amylose chains because glycogen acted as the polymeric primer for the GP catalysis. After introducing the maltooligosaccharide primers onto hydrophilic natural polymers with carboxylate groups—such as poly(γ -glutamic acid), carboxymethyl cellulose, and alginic acid—via chemical reactions, the thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal was carried out using the resulting polymeric primers, enabling their hydrophobization through the grafting of P2D-amylose chains (the chemoenzymatic approach). Moreover, the chemoenzymatic method has extensively been employed for hydrophobization of the surfaces on natural polysaccharide nanofibers, such as cellulose and chitin nanofibers.

Keywords: chemoenzymatic synthesis; enzymatic grafting; glucan phosphorylase; hydrophilic natural polymer; hydrophobization; nanofiber; partially 2-deoxygenated amylose



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1. Introduction

Natural polymers, such as polysaccharides and proteins, comprising saccharide and amino acid residues linked through glycosidic and peptide bonds, respectively (Figure 1), are widely distributed and exhibit specific biological functions in nature [1,2]. Therefore, natural polymer-related substrates, e.g., their derivatives, have been employed as functional polymeric ingredients, such as biocompatible, biodegradable, tissue engineering, and eco-friendly materials [3]. Because natural polymers often contain numerous high polar substituents including hydroxy, amino, and various acidic groups (Figure 1), they generally exhibit a hydrophilic nature with water-solubility and -swellability, as seen in food polysaccharides, food hydrocolloids, and food proteins [4–6]. Although structural

polysaccharides and proteins, such as cellulose and collagen, are insoluble in water owing to their extended fibrous crystalline fashions and highly stiff polymeric chain packing, they are poorly resistant to water, restricting their practical applications [7].

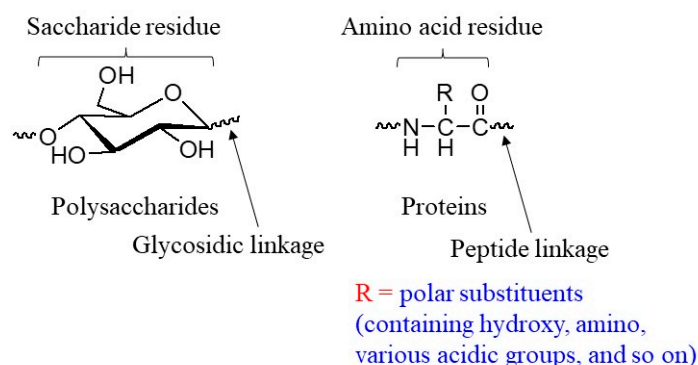


Figure 1. Representative repeating unit structures of polysaccharides and proteins.

Hydrophobization of hydrophilic natural polymers is identified as one of the necessary approaches for their application as practical bio-based alternatives to conventional petroleum-based resins and plastics, mostly with hydrophobicity [7,8]. Their efficient hydrophobization has widely been investigated by means of chemical functionalization of their polar reactive substituents by hydrophobic moieties, such as alkyl and acyl groups (Figure 2) [9,10]. For example, various acylation (ester derivatization) reactions of hydroxy groups in natural polysaccharides, e.g., starch and cellulose, have successfully been carried out to produce bio-based hydrophobic materials with useful functions, like thermoplasticity and processability [11–14]. However, the above modification methods via the introduction of synthetic hydrophobic groups into natural substrates mostly lead to the loss of useful intrinsic biological functions, e.g., biocompatibility, biodegradability, and so on, as above-mentioned. As alternatives to such artificial hydrophobic groups, hydrophobic analogs of naturally occurring substituents can be identified as candidates to alter hydrophilicity of natural polymers to hydrophobicity, while preserving the intrinsic biological functions.

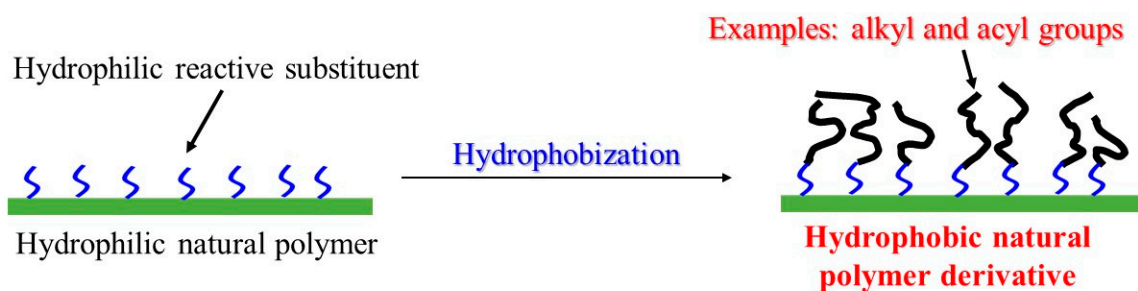


Figure 2. Hydrophobization of hydrophilic natural polymers by chemical functionalization by hydrophobic groups.

The authors have found the natural polymer analog as a good substituent candidate for hydrophobization of natural hydrophilic polymers, that is, an unnatural heteropolysaccharide, i.e., a partially 2-deoxygenated amylose (P2D-amylose), randomly comprising D-glucose (Glc)/2-deoxy-D-glucose (2dGlc) units [15]. A water contact angle measurement of cast films, formed from the P2D-amylose, resulted in values larger than 90°, supporting its hydrophobicity (Figure 3). The P2D-amylose is precisely synthesized via thermostable glucan phosphorylase (GP, isolated from the thermophilic bacteria, *Aquifex aeolicus* VF5)-induced enzymatic copolymerization of two comonomers— α -D-glucose 1-phosphate (Glc-1-P) and 1,2-dideoxy-D-glucose, called D-glucal—used as native and non-native substrates

for the GP catalysis, respectively, starting from maltooligosaccharide primers, such as maltotriose (Glc₃, Figure 3) [16]. During the enzymatic copolymerization, an actual monomer, 2-deoxy- α -D-glucose 1-phosphate (2dGlc-1-P), is produced from D-glucal in situ (the detailed mechanism is discussed in Section 2). The hydrophobicity of the P2D-amylose has been supported by the molecular dynamic simulations of the homopolysaccharide, 2-deoxyamylose, where a larger number of hydrophobic planes, which is constructed due to the absence of some hydroxy groups at the C-2 positions of the Glc residues in 2-deoxyamylose, strongly contributes to exhibiting its hydrophobic nature [17].

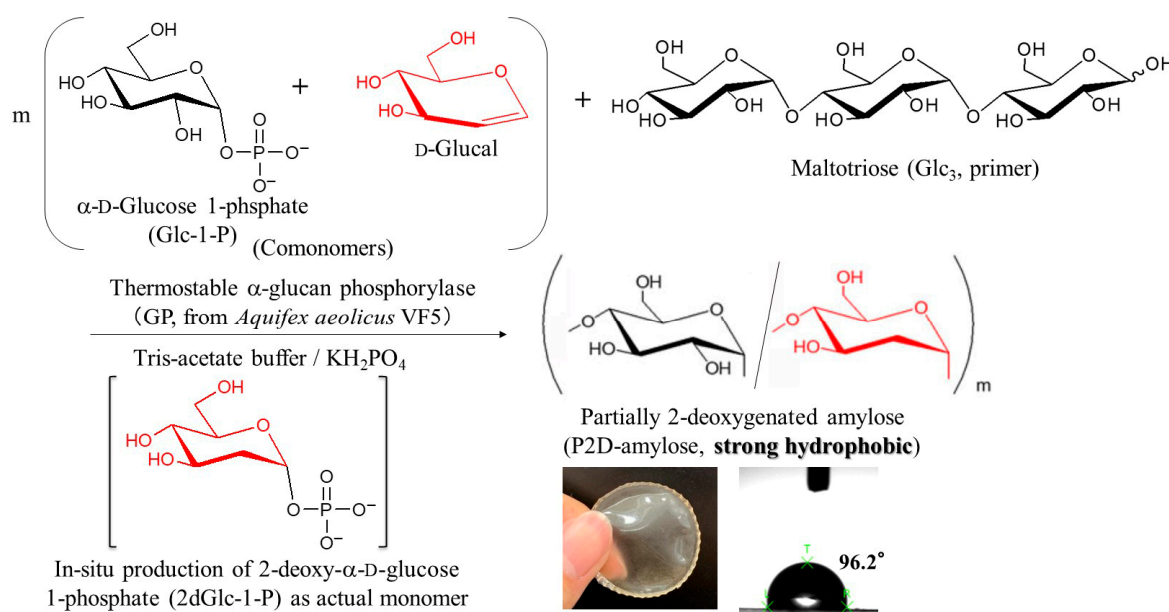


Figure 3. Thermostable glucan phosphorylase (GP, isolated from the thermophilic bacteria, *Aquifex aeolicus* VF5)-induced enzymatic copolymerization of α -D-glucose 1-phosphate (Glc-1-P)/D-glucal to construct an unnatural heteropolysaccharide, partially 2-deoxygenated amylose (P2D-amylose), and water contact angle measurement of its cast film.

Taking the above findings into account, the authors have developed the efficient hydrophobization method of hydrophilic natural polymers by means of the thermostable GP-induced enzymatic grafting approach of the P2D-amylose chains [18,19]. The method has extensively been employed for hydrophobization of the hydrophilic surfaces on natural polysaccharide nanofibers, such as cellulose and chitin nanofibers (CNFs and ChNFs, respectively). The present review article first introduces characteristic features of the GP-induced enzymatic polymerization, briefly. The hydrophobization method of several hydrophilic natural polymers and nanofibers are then discussed based on the thermostable GP-induced enzymatic grafting approach.

2. Characteristic Features of GP-Induced Enzymatic Polymerization

The enzymatic polymerization technique has been identified as one of the versatile methods to accurately construct polysaccharide chains with well-defined structures, because the highly controlled reactions in regio- and stereo-arrangements occur by enzymatic catalysis [20–23]. The enzymatic polymerization, catalyzed by GP, which belongs to the family of glycosyl transferases, GT35 (Enzyme Commission number, EC 2.4.1.1), is known to be practically employed for the precision synthesis of $\alpha(1\rightarrow4)$ -glucan, that is, amylose, using Glc-1-P as the monomer (Figure 4) [24–30]. To activate the polymerization, maltooligosaccharides (short $\alpha(1\rightarrow4)$ -glucans) are required as primers, where an initiation selectively takes place at the non-reducing end of the primer. The propagation then pro-

gresses from the elongating non-reducing end site according to consecutive glycosylations with liberating inorganic phosphate (Pi) from Glc-1-P. The enzymatically produced amylose is gradually precipitated from the reaction media because the amylose chains construct left-handed parallel double helices, mainly arising from intermolecular O(2)–O(6) and intramolecular O(2)–O(3) hydrogen bonding, leading to the formation of a water-insoluble assembly [31–34]. As the other primer chain end, called the reducing end, does not participate in the initiation, maltooligosaccharides, immobilized on various polymeric substrates at the reducing ends by appropriate chemical reactions, can also act as the primers for the GP-induced enzymatic polymerization (polymeric primers) to fabricate polymeric materials containing amylose graft chains (the chemoenzymatic method, Figure 5) [35–37].

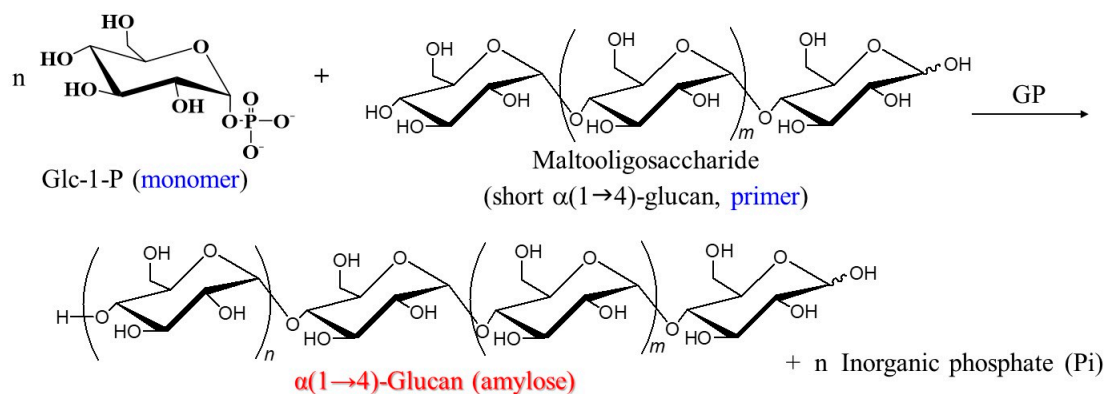


Figure 4. GP-induced enzymatic polymerization to obtain amylose.

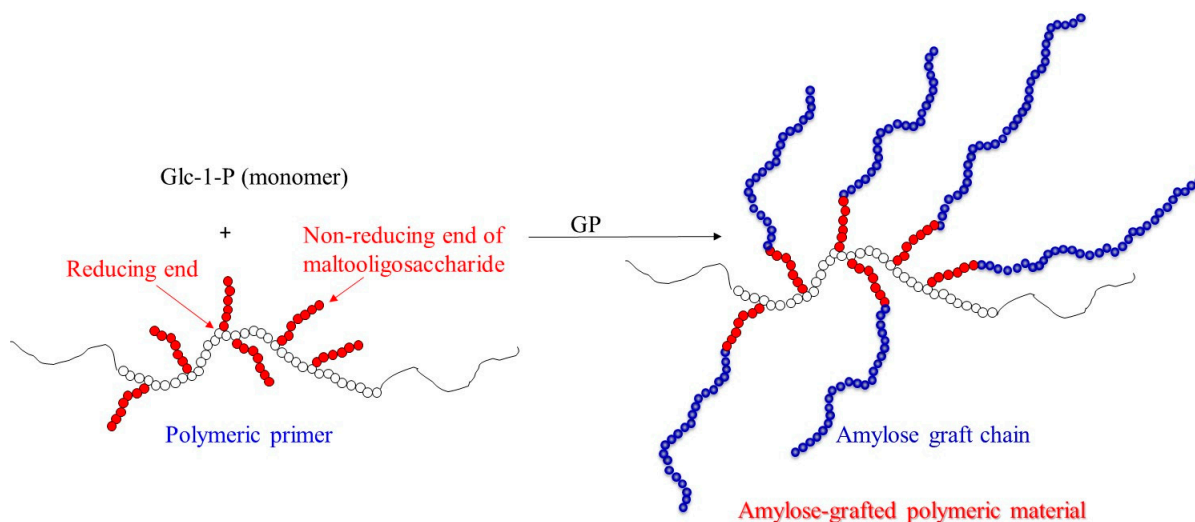


Figure 5. Chemoenzymatic synthesis of polymeric materials containing amylose graft chains.

For example, the GP-induced enzymatic polymerization of Glc-1-P using glycogen as such a polymeric primer occurs to obtain amylose-functionalized glycogens (Figure 6a) [38]. Glycogen is a hydrophilic natural polysaccharide with highly branched and spherical structures, composed of $\alpha(1\rightarrow4)$ -glucan chains additionally interlinked by $\alpha(1\rightarrow6)$ -glycosidic branching. Owing to the presence of the numerous non-reducing $\alpha(1\rightarrow4)$ -glucan chain ends on the glycogen sphere, it potentially acts as the polymeric primer, where the amylose chains are elongated from the surface of glycogen by the GP-induced enzymatic polymerization of Glc-1-P. When the enzymatic polymerization solution was left standing, it totally converted into the hydrogel form (Figure 6a). The hydrogel formation is caused by constructing polymeric networks with the double helical cross-linking points, formed from the elongated amylose chains functionalized on glycogens.

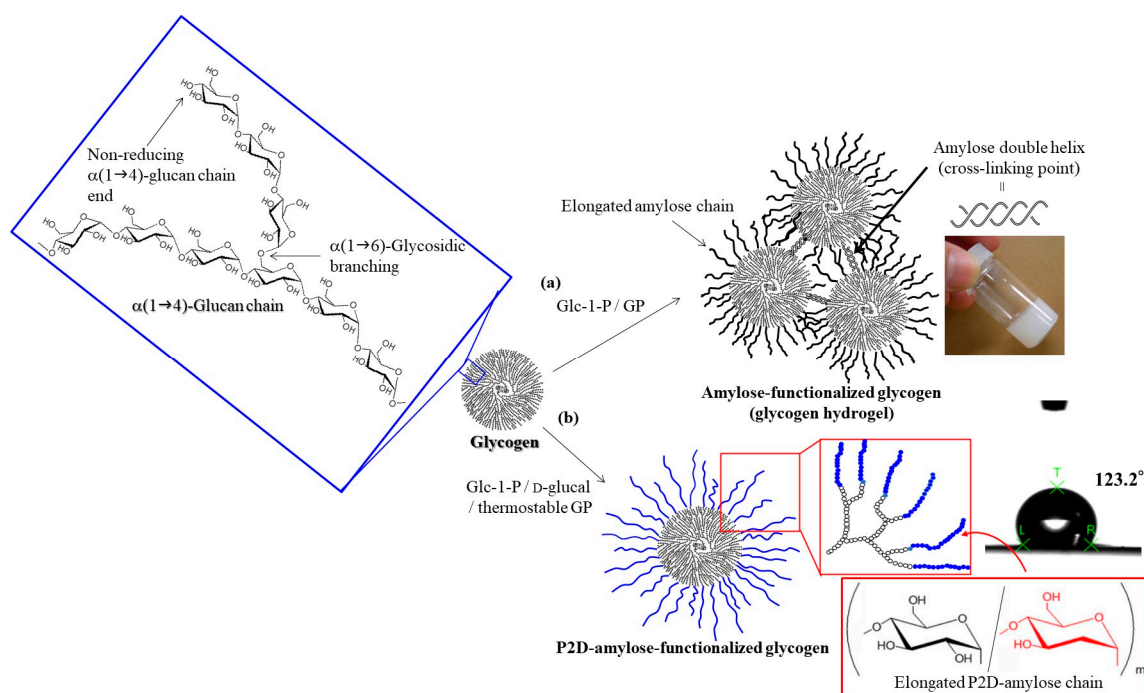


Figure 6. GP-induced enzymatic (co)polymerization from glycogen (a) using Glc-1-P to fabricate amylose-functionalized glycogen and its hydrogel and (b) using Glc-1-P/D-glucal to fabricate P2D-amylose-functionalized glycogen and water contact angle measurement of its cast film.

GP shows weak specificity for substrate recognition, and accordingly, is known to catalyze the enzymatic polymerization using some analogous substrates of Glc-1-P; that is, 1-phosphates of different monosaccharide residues from Glc [18,39–42]. For example, GP, isolated from potato—the most extensively studied source—catalyzes the enzymatic polymerization of D-glucal in Tris-acetate buffer with KH_2PO_4 (Pi source) to yield the unnatural polysaccharide, 2-deoxyamylose. In this reaction, potato GP recognizes the in situ produced 2dGlc-1-P as the actual non-native monomer, as described above [43]. In this polymerization, the GP-catalyzed enzymatic 1,2-addition from a hydroxy group at C-4 position of the non-reducing end in the maltooligosaccharide primer to a 1,2-double bond in D-glucal occurs, assisted by Pi, to form the $\alpha(1\rightarrow4)$ -linked 2dGlc non-reducing end structure (Figure 7a). The 2dGlc unit is subsequently released by the GP-catalyzed phosphorolysis (a backward reaction of the chain-elongation) in the presence of Pi to produce a 2dGlc-1-P in situ (Figure 7b). This participates in enzymatic polymerization as the monomer via consecutive glycosylation to yield 2-deoxyamylose (Figure 7c) [44]. 2-Deoxyamylose is readily precipitated from the reaction media, owing to the formation of a water-insoluble assembly. The authors have found that the crystalline structure of 2-deoxyamylose assembly is completely different from that of amylose, suggested by the powder X-ray diffraction (XRD) measurement. Molecular dynamic simulations of 2-deoxyamylose indicated that it forms an intrinsic antiparallel double helix, arising from hydrophobic building blocks, constructed via stacking of 2-deoxyglucose residues [17]. The absence of hydroxy groups at the C-2 position in 2-deoxyamylose gives rise to no possibility for intermolecular O(2)–O(6) and intramolecular O(2)–O(3) hydrogen bonding unlike amylose, which probably contributes to the formation of its intrinsic double helix.

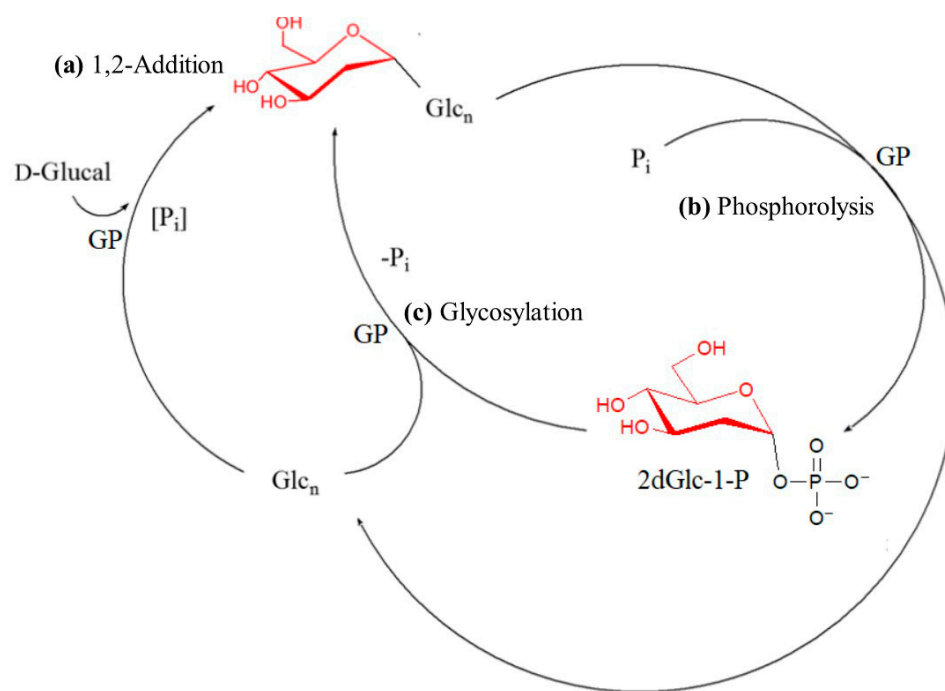


Figure 7. Mechanism for production of 2-deoxyamylose in GP-induced enzymatic polymerization of D-glucal via (a) 1,2-addition, (b) phosphorolysis, and (c) glycosylation.

The authors have found the more tolerance of thermostable GP, isolated from *Aquifex aeolicus* VF5 (thermophilic bacteria), for recognition of the substrate than that of potato GP. For example, the thermostable GP catalyzes consecutive glycosylation of α -D-glucosamine 1-phosphate (GlcN-1-P) toward the maltooligosaccharide primer, e.g., Glc₃, whereas one-step glycosylation only happens in the same reaction system by the potato GP catalysis [45,46]. In particular, the thermostable GP-catalyzed consecutive glycosylation using GlcN-1-P have been accelerated in NH₃ buffer (0.5 M, pH 8.6) containing MgCl₂ because P_i, released from GlcN-1-P via the glycosylation, is removed from the reaction media as the ammonium magnesium P_i precipitate to prevent phosphorolysis (a backward reaction of the glycosylation). Therefore, the enzymatic polymerization of GlcN-1-P, using the Glc₃ primer in such a buffer, progresses to produce an $\alpha(1\rightarrow4)$ -linked unnatural aminopolysaccharide, named as ‘amylosamine’. The thermostable GP has also been found to catalyze enzymatic copolymerization in the comonomer combinations of the non-native GlcN-1-P, α -D-mannose 1-phosphate (Man-1-P), or D-glucal with the native Glc-1-P, to construct the unnatural glucosaminoglycan composed of GlcN/Glc units, mannoglycan composed of Man/Glc units, and P2D-amylose composed of 2dGlc/Glc units (abovementioned) connected by $\alpha(1\rightarrow4)$ -glycosidic linkages [16,47,48]. Additionally, the thermostable GP-induced enzymatic copolymerization of the two non-native monomers, that is, GlcN-1-P and D-glucal, took place to obtain an unnatural $\alpha(1\rightarrow4)$ -linked glucosamino-2-deoxyglucan [49].

3. Enzymatic Synthesis and Specific Characters of P2D-Amylose

As abovementioned, the thermostable GP-induced enzymatic copolymerization of the Glc-1-P/D-glucal comonomers involves the in situ production of 2dGlc-1-P in a Tris-acetate buffer containing KH₂PO₄ to obtain P2D-amyloses [16]. The powder XRD analysis of the produced polysaccharides with different Glc/2dGlc unit ratios indicated that the certain higher unit ratios of one residue to the other formed respective crystalline structures, similar to those of homopolysaccharides, i.e., amylose and 2-deoxyamylose. Conversely, the powder XRD results supported amorphous natures of the P2D-amyloses with the moderate unit ratios (Glc/2dGlc = approximately 7–6/3–4), owing to the random Glc/2dGlc sequence.

Therefore, cast films were formed by drying their DMSO solutions. Water contact angle values of the films, which were greater than 90° , indicated hydrophobic natures of the amorphous P2D-amyloses (Figure 3) [15]. The stress–strain curve of the cast film (Glc/2dGlc = 6.3:3.7) under tensile mode showed the slightly lower values of both tensile strength and elongation at break than those of the commercial cellophane film; however, it indicated good mechanical properties (Figure 8).

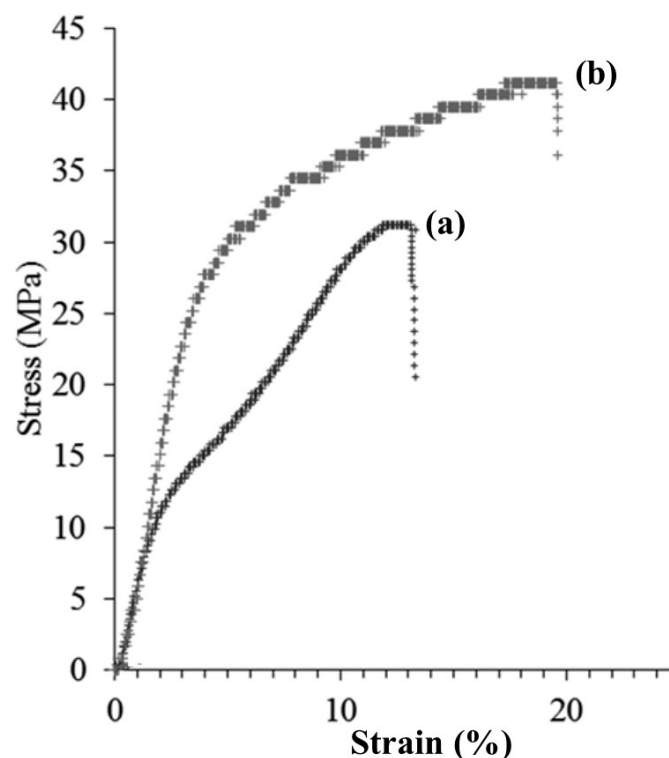


Figure 8. Stress–strain curves of films of (a) P2D-amylose (Glc/2dGlc = 6.3:3.7) and (b) commercial cellophane under tensile mode.

By means of the hydrophobicity of the P2D-amylose chains, hydrophobization of hydrophilic natural polymers was first attempted by the thermostable GP-induced enzymatic functionalization on glycogen, because of its role as the polymeric primer for the GP-induced enzymatic polymerization [15]. Therefore, the functionalization of the P2D-amylose chains on glycogen was conducted by the thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal from the non-reducing $\alpha(1\rightarrow4)$ -glucan chain ends, present on glycogen, in Tris-acetate buffer with KH_2PO_4 (Figure 6b). The powder XRD measurement of the obtained P2D-amylose-functionalized glycogens indicated an amorphous nature of the elongated P2D-amylose chains. Using the products and amylose-functionalized glycogen as a reference sample, which was provided by the GP-induced enzymatic polymerization of Glc-1-P from glycogen, cast films were formed by the drying of DMSO solutions. The water contact angle value of the amylose-functionalized glycogen film was 63.1° owing to its hydrophilicity, while the values of the P2D-amylose-functionalized glycogen films were larger than 100° , regardless of the Glc/2dGlc unit ratios (Figure 6b). The results have supported that the thermostable GP-induced enzymatic modification of the P2D-amylose chains is a powerful method for hydrophobization of hydrophilic polymers.

4. Chemoenzymatic Approach for Hydrophobization of Hydrophilic Natural Polymers

Hydrophobization of several hydrophilic natural polymers, such as poly(γ -glutamic acid) (PGA), carboxymethyl cellulose (CMC), and alginic acid (alginate), has been achieved by modification of the P2D-amylose chains, based on the abovementioned chemoenzymatic approach [19]. Because such polymers contain carboxylic acid (carboxylate) groups, the maltooligosaccharide primers have been introduced on the hydrophilic polymeric chains by their condensation with amino groups present at the primer reducing ends (amine-functionalized maltooligosaccharide) in the presence of condensing agents (Figure 9a) [50–52]. The synthesis of the amine-functionalized maltooligosaccharide had already been developed by the authors' previous study, which was carried out by the nucleophilic ring-opening reaction of maltoheptaose lactone with 2-azidoethylamine and subsequent reduction in the azido group by sodium borohydride [50].

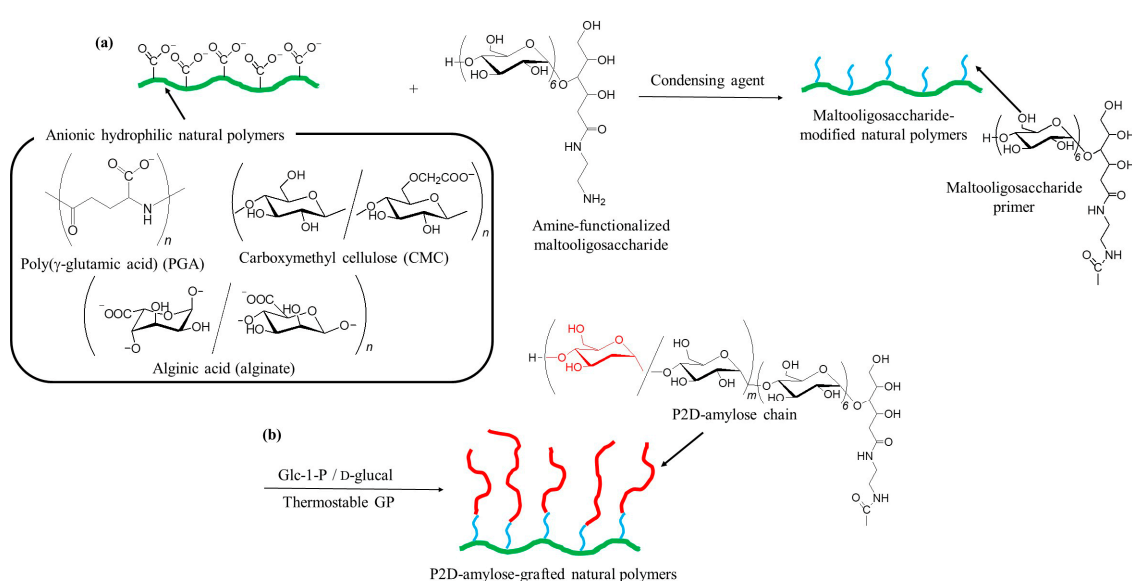


Figure 9. Hydrophobization of anionic hydrophilic natural polymers via (a) introduction of primers by condensation with amine-functionalized maltooligosaccharide and (b) subsequent thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal.

Grafting of the P2D-amylose chains on PGA was conducted by the thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal using the prepared maltooligosaccharide-modified PGA (Figure 9b) [53]. The powder XRD results of the products with different Glc/2dGlc unit ratios did not observe obvious diffraction peaks, supporting their amorphous structures, attributed to random sequence of the Glc/2dGlc units. Because the P2D-amylose-grafted PGAs were soluble in DMSO, the DMSO solutions were dried to form cast films. The strong hydrophobic nature of the P2D-amylose-grafted PGA was suggested by the water contact angel values of the films, larger than 100° , regardless of the Glc/2dGlc unit ratios of the graft chains.

Similarly, hydrophobization of CMC and alginate has been achieved by the thermostable GP-induced enzymatic grafting of the P2D-amylose chains using the maltooligosaccharide-modified CMC and alginate, respectively [54]. When carboxylate groups in the resulting P2D-amylose-grafted CMCs were converted into free carboxylic acid groups by the treatment with cation exchange resin in water, the water-insoluble products were obtained, which formed films by drying. The water contact angel measurement of the films indicated the hydrophobic nature of the P2D-amylose-grafted CMCs.

Figure 10 shows the ^1H NMR spectrum of the P2D-amylose-grafted alginate in 1.0 mol/L NaOD/D₂O, obtained by the thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal using the maltooligosaccharide-modified alginate (degree of substitution = 18.7%) in Tris-acetate buffer with KH₂PO₄ (feed ratios of primer/Glc-1-P/D-glucal = 1:25:75, run 3 in Table 1). The two anomeric signals at δ 5.29 and 5.49, assignable to α (1 $\rightarrow$ 4)-linked Glc and 2dGlc units, are detected in addition to the anomeric signals at δ 4.5–5.2, derived from alginate. Furthermore, the characteristic C-2 methylene signals derived from the 2dGlc units are detected at around δ 1.7–1.9 and 2.1–2.3 (axial and equatorial protons, respectively). The NMR result fully supported the structure of the P2D-amylose-grafted alginate. The Glc/2dGlc unit ratios in the products, which were estimated by integrated ratios of the corresponding anomeric signals, were changed according to the feed ratios of Glc-1-P/D-glucal, as listed in Table 1.

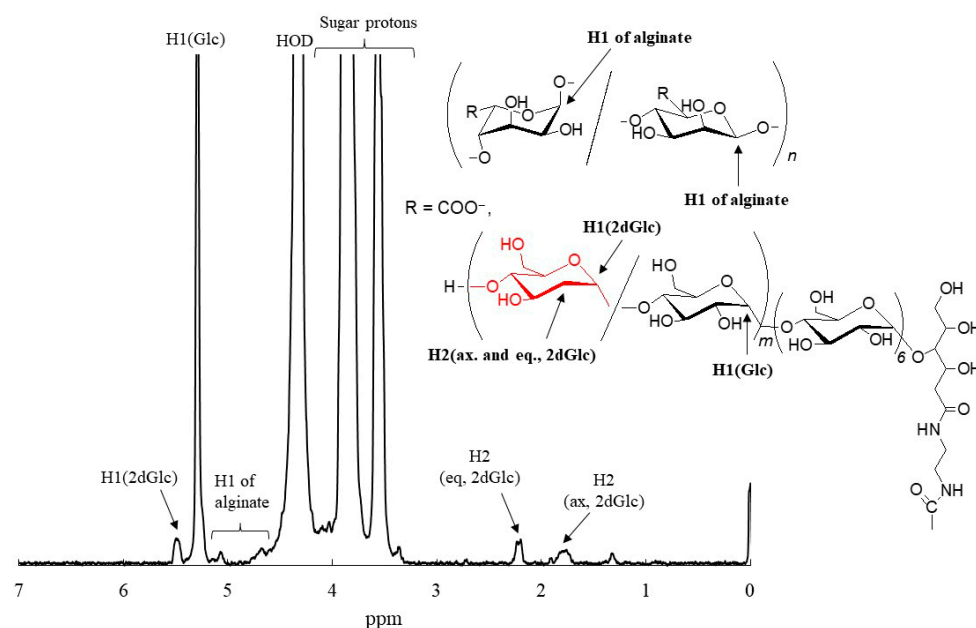


Figure 10. ^1H NMR spectrum of P2D-amylose-grafted alginate (run 3, in Table 1) in 1.0 mol/L NaOD/D₂O.

Table 1. Thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal from maltooligosaccharide-modified alginate ^(a).

Entry	Feed Ratio (Primer/Glc-1-P/D-glucal)	Unit Ratio (Glc/2dGlc) ^(b)
1	1/75/25	0.98 / 0.02
2	1/50/50	0.97 / 0.03
3	1/25/75	0.93 / 0.07

^(a) Reaction was conducted at 40 °C for 7 h. Degree of substitution of maltooligosaccharide on alginate = 18.7% per repeating unit. ^(b) Calculated from ^1H NMR analysis.

The water contact angle values of the cast films, formed by drying DMSO solutions of the P2D-amylose-grafted alginates, showed greater than 90°, regardless of the Glc/2dGlc unit ratios in the graft chains (Figure 11b–d). Conversely, the amylose-grafted alginate film, as a reference sample, observed a hydrophilic nature because of 78.4° of the water contact angle value (Figure 11a). The data suggested that the enzymatic grafting of the P2D-amylose chains is a powerful method for the hydrophobization of alginate film surfaces. Moreover, the SEM image of a spin-coated sample, prepared from an aqueous dispersion of the P2D-amylose-grafted alginate (run 3 in Table 1, 1 mg/1.0 mL), detected nanoparticle

morphology with an average diameter of 30 nm (Figure 12, the average diameter was calculated based on the lengths of the long and short axis, respectively, of the selected nanoparticles in the SEM image). This result indicates the occurrence of controlled self-assembly from the P2D-amylose-grafted alginates in water, owing to its amphiphilic nature with hydrophilic main-chain and hydrophobic graft-chains.

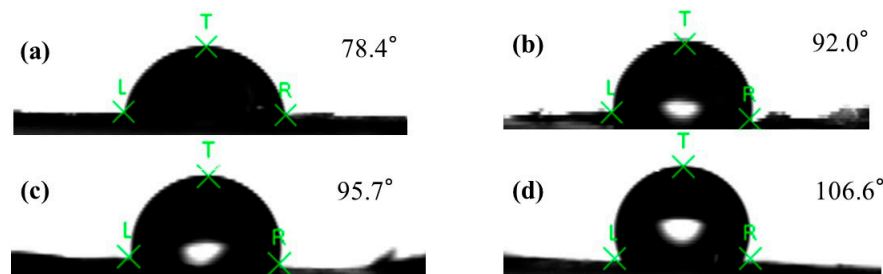


Figure 11. Water contact angle measurement of (a) amylose-grafted alginate film and (b–d) P2D-amylose-grafted alginate films of runs 1–3 in Table 1.

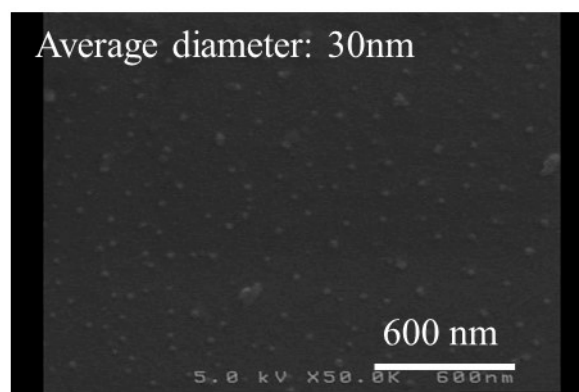


Figure 12. SEM image of spin-coated sample, prepared from aqueous dispersion of P2D-amylose-grafted alginate (run 3 in Table 1).

5. Hydrophobization of Natural Polysaccharide Nanofibers by Enzymatic Grafting of P2D-Amylose Chains

The thermostable GP-induced enzymatic grafting of the P2D-amylose chains has also been employed for hydrophobization of the surfaces on natural polysaccharide nanofibers, i.e., CNFs and ChNFs (Figure 13a), composed of $\beta(1\rightarrow4)$ -bonded Glc and *N*-acetylglucosamine units, respectively (Figure 13b). 2,2,6,6-Tetramethylpiperidine-1-oxyl-oxidized CNFs (TOCNs) are one of the representative CNF materials [55]. Because TOCNs contain carboxylate groups, the introduction of the maltooligosaccharide primers on the TOCN surface was achieved by condensation with the amine-functionalized maltooligosaccharide in the presence of a condensing agent, as the same reaction to that employed for the abovementioned anionic natural polymers. The thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal was investigated from the primer chain ends modified on the TOCN surfaces for grafting of the P2D-amylose chains [56]. The resulting P2D-amylose-grafted TOCNs were dispersed in DMSO and not dispersed in aqueous media. The DMSO dispersions were cast on glass substrates and dried to produce films. The water contact angles on the resulting films showed a tendency to increase according to 2dGlc/Glc unit ratios. Consequently, the water contact angle value of the P2D-amylose-grafted TOCN film composed of the 54% 2dGlc unit ratio was 103.9° (Figure 13c).

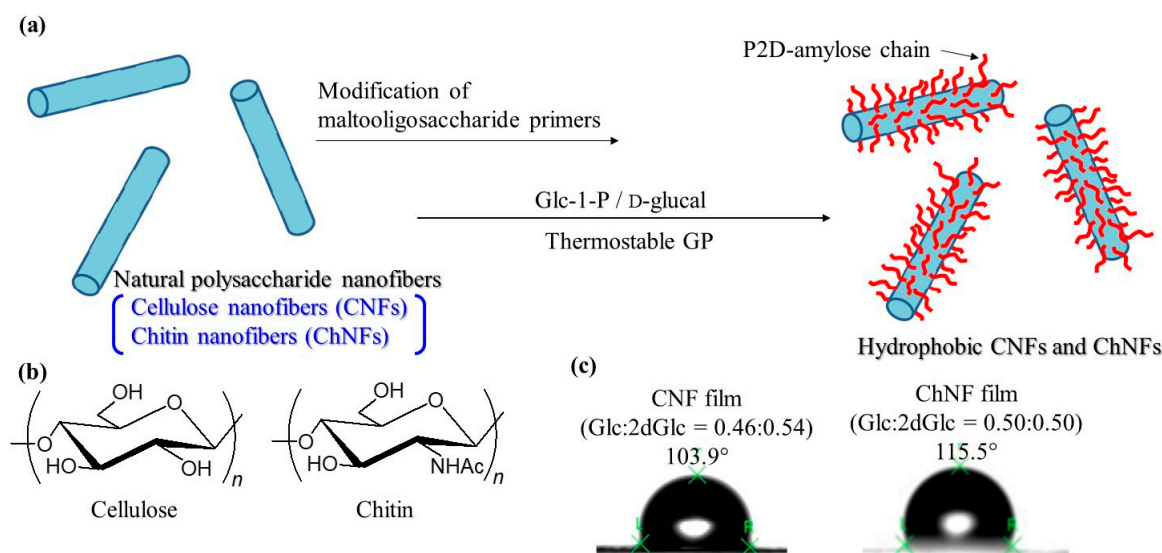


Figure 13. (a) Hydrophobization of CNFs and ChNFs by modification of maltooligosaccharide primers and subsequent thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal, (b) structures of cellulose and chitin, and (c) water contact angle measurement of obtained CNF and ChNF films.

The authors reported that regeneration of chitin from ion gels with the ionic liquid, 1-allyl-3-methylimidazolium bromide, using methanol, led to nanoscale self-assembly, followed by filtration and drying, to yield ChNF films [57,58]. Furthermore, partial deacetylation of the resulting self-assembled ChNF film using the aqueous NaOH treatment induced dispersibility with aqueous acetic acid by electrostatic repulsion among protonated amino (ammonium) groups to fabricate thinner nanofiber dispersion, called scale-down ChNFs (SD-ChNFs) [59]. The authors have also developed efficient approaches for the modification of monosaccharide and oligosaccharide substituents, such as maltoheptaose, on the surfaces of partially deacetylated and SD-ChNFs, through reductive alkylation, using NaBH_2CN as a reducing agent (Figure 14a) [60–62].

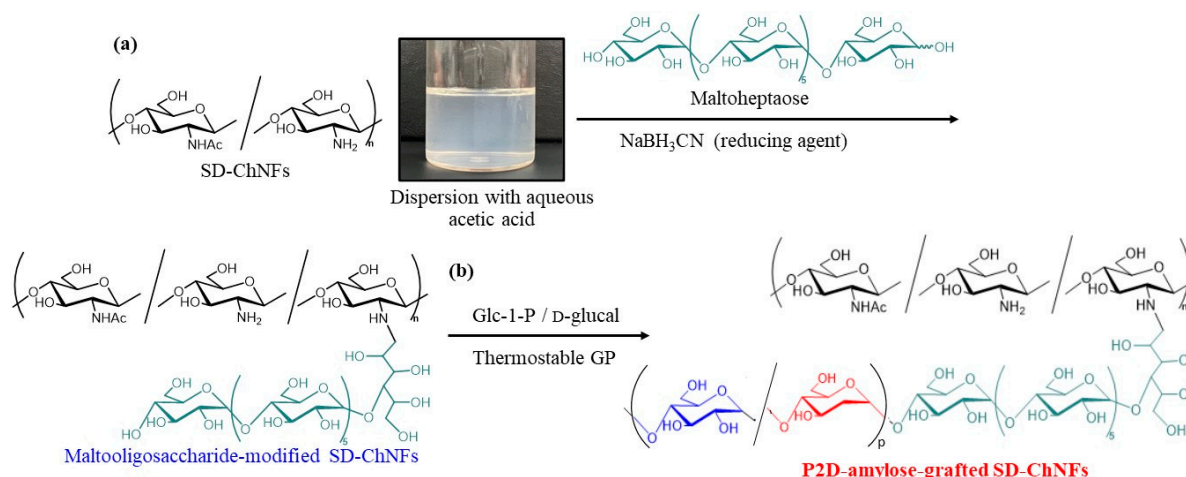


Figure 14. (a) Modification of maltoheptaose on SD-ChNFs and (b) subsequent thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal.

Accordingly, the obtained maltooligosaccharide-modified SD-ChNFs were used for the enzymatic grafting of the P2D-amylose chains to provide the hydrophobic surfaces on SD-ChNFs (Figure 14b) [63]. The powder XRD analysis of the products, produced by the thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal in different feed

ratios, concluded that unit ratios and the lengths of the graft chains affected their whole crystalline structures. The water contact angle values of the films, which were prepared by drying DMSO dispersions of the products, resulted in a tendency to increase above 100° depending on the 2dGlc/Glc unit ratios as an example of 115.5° for the film with a Glc/2dGlc unit ratio of 0.5:0.5 (Figure 13c).

6. Conclusions

In the present review article, the authors discussed the efficient hydrophobization of hydrophilic natural polymers by means of the thermostable GP-induced enzymatic functionalization of the P2D-amylose chains, based on the efficiency of the enzymatic polymerization approach to synthesize polysaccharides with well-defined structures. The authors previously have found the hydrophobic nature of the P2D-amylose, which is prepared by the thermostable GP-induced enzymatic copolymerization of Glc-1-P/D-glucal. The hydrophobic building blocks were constructed via stacking from 2dGlc residues for hydrophobicity of the P2D-amylose chains. In addition to the hydrophobic nature of the P2D-amylose-functionalized glycogen obtained by the thermostable GP-induced enzymatic method, a chemoenzymatic approach—including the introduction of the maltooligosaccharide primers and the subsequent thermostable GP-induced enzymatic copolymerization—has been achieved for hydrophobization of hydrophilic natural polymers and natural polysaccharide nanofibers. The present processes for incorporating the P2D-amylose chains can be considered an effective approach for hydrophobization of water-soluble and hydrophilic natural polymers, while retaining their biodegradability and eco-friendliness, because 2-deoxyamylose has been found to be enzymatically degraded by amylases [64]. As an example of applications, the resulting hydrophobized nanofibers can be used as reinforcing agents to form composites with conventional hydrophobic resins. For the applications, the feasibility of scaling up the present processes should be explored. Further investigations on the thermostable GP-induced enzymatic functionalization of the P2D-amylose chains will also provide new natural polymer-based hydrophobic polymeric materials with unique properties and functions in the future.

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