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Ester Derivatives of Microbial Synthetic Polysaccharides

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9.1 Introduction

9.1.1 Background of Bio-Based Plastics

Plastics manufactured from petroleum-based polymers are among the most conspicuous products in modern life. Light, cheap plastics that are easily processed and shaped have improved the comfort and convenience of modern life through applications ranging from automobiles to medical devices and food packaging. However, the constant increase in plastic fabrication has resulted in serious environmental problems, such as global warming, waste management and treatment, and marine microplastics pollution. With a lower environmental impact, bio-based plastics derived from sustainable and renewable natural resources, and, preferably, the biodegradable ones that degrade into carbon dioxide and water under the action of microorganisms, have attracted attention as potential alternatives to petroleum-based non-biodegradable plastics. For the definitions of bio-based plastics, we refer to the introductory chapter of this book. When it comes to bio-based plastics, these are expected to help achieving carbon neutrality. Furthermore, biodegradable plastics are considered key polymers for alleviating part of the problem of plastic waste as marine microplastics pollution.

Bio-based plastics are generally classified into three categories [1]:

1) Those with identical chemical structures to petroleum-based plastics, such as bio-polyethylene (bio-PE) derived from bio-ethanol and bio-poly(ethylene terephthalate) (bio-PET) produced from bio-based ethylene glycol.
2) Bio-based plastics that are biodegradable and have similar physical properties to petroleum-based plastics, such as poly(lactic acid) (PLA) and polyhydroxyalkanoates (PHAs).
3) Plastics with unique structures and properties derived only from biomass such as polysaccharide esters and polymers derived from aromatic plants. There are also lignin-based plastics, which fall in this category.

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Among bio-based plastics, PLA and PHAs are the most widely studied. PLA is an aliphatic polyester derived from fermented plant starch, such as corn, sugarcane, or sugar beet pulp. PLA has a glass transition temperature (T_g) of 60 °C and a melting temperature (T_m) of 175 °C [2], and films, fibers, and injection molding of PLA have been developed. PLA degrades under composting conditions (temperature >65 °C and humidity >60%) through hydrolysis but degrades slowly under lower temperature conditions. PHAs are produced in nature by numerous microorganisms from sugars or lipids [3]. More than 150 types of PHAs have been reported and can be combined to produce materials with different properties. The physical properties of poly[(*R*)-3-hydroxybutyrate] (P(3HB)) are similar to those of polypropylene (PP) with a T_g of −4 °C and a T_m of 170 °C. However, P(3HB) is relatively brittle, while P(3HB) copolymers with different second monomer units, such as 3-hydroxyvalerate, 3-hydroxyhexanoate, and 4-hydroxybutyrate, provide ductility. PHAs have high biocompatibility, which is promising for their widespread use in the medical field.

9.1.2 Polysaccharides

Polysaccharides are the most abundant natural polymer resources on Earth and are mainly composed of glucose units bonded by two types of glycosidic linkages (α and β). Figure 9.1 shows typical polysaccharides and their chemical structures, including cellulose contained in plant cell walls and plant fibers, glucomannan isolated from the tubers of *Amorphophallus konjac* plants, chitin or chitosan obtained from crustacean shells, pullulan produced by the strains of the fungus *Aureobasidium pullulans*, and paramylon derived from microalgae. Polysaccharides with different glycosidic linkages and molecular structures have varying physical properties. As the most abundant natural polymer resource, polysaccharides could potentially be used as raw materials to produce many useful bioplastics. As polysaccharides are already polymerized, they do not need to be synthesized from monomers. However, their thermal processability and solubility in organic solvents are restricted by strong intermolecular and intramolecular hydrogen bonds between the hydroxyl groups of monosaccharide units. To gain solubility and thermoplasticity, hydrogen bonds are suppressed by substituting hydroxyl groups with functional ester groups through esterification. Usually, esterification of polysaccharides is performed using carboxylic acids and trifluoroacetic anhydride (TFAA) as a catalyst at 50 °C for one hour. Then, the reaction solution is poured into methanol or water to obtain polysaccharide esters as powder samples.

Cellulose found in plant cell walls and plant fibers is the most abundant and commonly used polysaccharide with a molecular structure comprising β-1,4-glucan without branches. Cellulose triacetate (CTA) is an important polymer that is mainly used in optical films, cigarette filters, and fiber materials [4]. However, despite its excellent properties, a large amount of plasticizer is required for heat molding owing to poor thermal fluidity [5]. In addition to cellulose, many types of natural polysaccharides with various characteristic structures can be found in nature, including hemicellulose containing xylan, chitin, and chitosan, which are

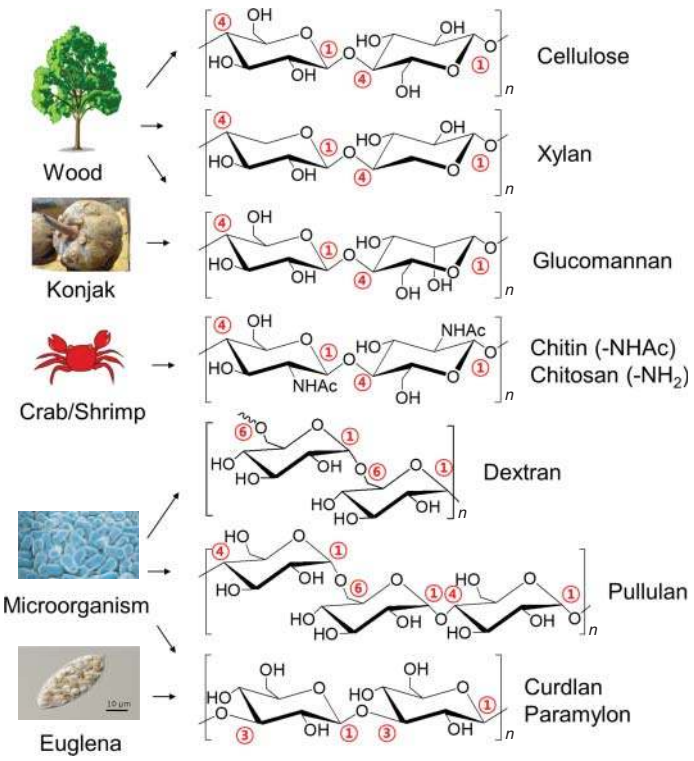


Figure 9.1 Representative polysaccharides existed in nature. Source: Tim/Adobe Stock Photos.

obtained from crabs and prawns, and curdlan and pullulan, which are synthesized by microorganisms. Although most of these materials are used as food additives and paint thickeners, there are few reports on their use as plastics.

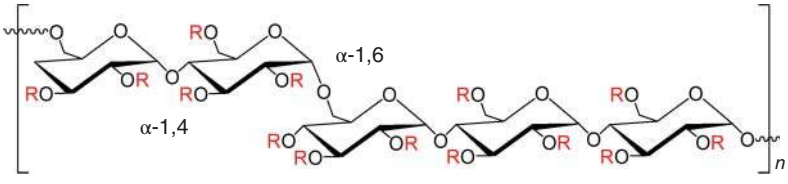
In recent years, we have developed polysaccharide esters fabricated from various polysaccharides. As a result, some unique properties have been developed using techniques of esterification processes. In particular, pullulan esters consisting of α-1,3- and α-1,4-linkages show outstanding transparency that is insensitive to light polarization (zero birefringence) and, thereby, deliver sharp transparency [6, 7], dextran esters consisting of α-1,6-glucan show excellent adhesive property [8], and β-1,3-glucan derived from paramylon or curdlan shows high thermal fluidity after esterification [9–11]. An organic solvent is generally needed to extract polysaccharides from natural resources, resulting in a decrease in molecular weight. To solve the problem, we have synthesized a non-natural polysaccharide with a new α-1,3-glucan structure *in vitro* using enzymatic polymerization, followed by esterification [12, 13]. Furthermore, to improve the physical properties, ester groups with branches were adopted in cellulose, curdlan, and α-1,3-glucan derivatives [14–16]. In the case of enzymatic synthesis, regioselective esterification of dextrin was achieved [17].

This chapter introduces the excellent thermal and mechanical properties obtained from various polysaccharide linear and branched esters and discusses the enzymatic polymerization and esterification of polysaccharides.

9.2 Zero Birefringence Property of Pullulan Esters

Various α-glucans are found in nature depending on the glycosidic linkage position. The most representative α-glucan is a starch composed of α-1,4-glycosidic linkages. In this section, other α-glucan esters are introduced, namely, pullulan, dextran, and α-1,3-glucan.

Pullulan is a natural linear polysaccharide produced by strains of fungus *Aureobasidium pullulans*. This polysaccharide consists of α-1,6-linked maltotriose units, which comprise three α-1,4-linked glucose units [18]. The commercial production of pullulan at Hayashibara is at approximately 300 metric tons per year [18]. It is applied as a food additive and a biomedical material owing to its valuable properties, such as biodegradability, water solubility, non-toxicity, and film-forming abilities [19, 20]. However, this valuable glucan has not been previously applied as a plastic material. To date, some studies have reported the derivatization of pullulan hydroxyl groups with substituents including sulfate, cholesterol, and carboxylate groups [21]. Among these various derivatization studies, much research has focused on the esterification of pullulan (Figure 9.2) [6, 7, 22, 23]. Solution-cast and melt-pressed films were successfully prepared from pullulan esters with different carbon numbers (n), from acetic acid ($n = 2$) to myristic acid ($n = 14$) [6]. The characteristics of pullulan ester films are shown in Table 9.1. The tensile strength of these solvent-cast films is in the range of 1.4–24 MPa, while the elongation at break is in the range of 8–1120%. The higher the carbon number of the acyl groups, the lower the strength, but the higher the elasticity. Pullulan esters have no melting point, suggesting that they are amorphous polymers [6]. However, the T_g of pullulan acetate is 174 °C, which is higher than that of polycarbonate (145 °C), poly(methyl methacrylate) (115 °C), and polystyrene (100 °C). This result indicates that pullulan acetate has higher thermal stability than oil-based amorphous polymers. Transparent melt-pressed films of pullulan esters can be easily prepared above the T_g.



Pullulan: R = H
Pullulan ester: R = $CO(CH_2)_xCH_3$

Figure 9.2 Chemical structure of pullulan esters.

Table 9.1 Characteristics of α-glucan.

Name	Substituent	M_w ($\times 10^5$)	M_n ($\times 10^5$)	Polydispersity index (PDI)	T_g (°C)	T_m (°C)	$T_{d5\%}$ (°C)	Tensile strength (MPa)	Elongation strain (%)	Young's modulus (MPa)
Pullulan	Acetate ($n = 2$)	2.1	0.92	2.3	174	—	332	23.4	8	772
	Propionate ($n = 3$)	1.5	0.67	2.2	127	—	331	23.5	14	650
	Butyrate ($n = 4$)	4.1	1.9	2.1	99	—	332	15.6	9	571
	Valerate ($n = 5$)	5.9	2.3	2.4	67	—	334	9.5	37	274
	Hexanoate ($n = 6$)	5.9	2.5	2.4	46	—	349	2.1	410	23
	Octanoate ($n = 8$)	7.1	3.4	2.3	42	—	354	2.4	1120	8
	Decanoate ($n = 10$)	8.2	3.1	2.4	35	—	351	1.4	830	9
	Laurate ($n = 12$)	9.0	3.6	2.5	38	—	348	1.8	1000	16
	Myristate ($n = 14$)	8.1	3.1	2.6	38	—	365	1.4	1090	7
Dextran	Acetate ($n = 2$)	—	—	—	—	—	346	—	—	—
	Propionate ($n = 3$)	2.1	0.74	2.8	—	—	364	—	—	—
	Butyrate ($n = 4$)	2.2	0.55	4.1	75	—	360	7.9	14	71
	Valerate ($n = 5$)	1.5	0.61	2.4	18	—	285	1.4	84	10
	Hexanoate ($n = 6$)	1.6	0.63	2.5	−1.6	—	302	0.76	35	10
	Octanoate ($n = 8$)	2.3	0.60	3.8	2.4	—	330	0.44	20	5.5
	Decanoate ($n = 10$)	3.0	0.72	4.2	9.4	—	273	0.48	33	4.7
	Laurate ($n = 12$)	3.2	0.79	4.0	—	—	269	2.2	27	24
α(1 → 3) Glucan	Acetate ($n = 2$)	1.6	0.71	2.3	177	339	350	41	7	840
	Propionate ($n = 3$)	2.1	0.89	2.3	150	299	355	37	8	710
	Butyrate ($n = 4$)	1.8	0.84	2.2	110	242	354	15	12	360
	Valerate ($n = 5$)	1.6	0.77	2.1	97	210	353	8.2	32	220
	Hexanoate ($n = 6$)	1.7	0.84	2.0	91	142	350	7.7	90	150
	Octanoate ($n = 8$)	2.2	0.90	2.4	28	—	351	4.8	212	70

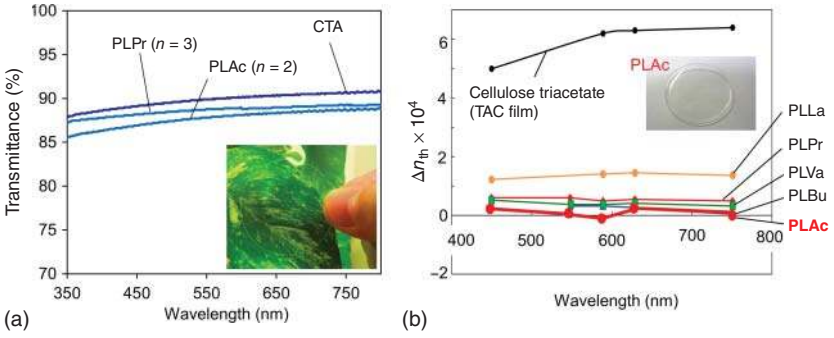


Figure 9.3 (a) Optical transmittance of pullulan acetate (PLAc), propionate (PLPr), and cellulose triacetate (CTA) and (b) out-of-plane birefringence of pullulan ester films. Source: Danjo et al. [7]/Springer Nature/CC BY 4.0.

As shown in Figure 9.3a, the optical transmittance percentage of these films was within the range of 83–88% in the visible-light wavelength range (380–750 nm) [7]. These values are similar to those of cellulose triacetate (CTA, 87–90%), which is applied as an liquid crystal display (LCD) protective film. In particular, Figure 9.3b shows a lower out-of-plane birefringence for pullulan ester films than for CTA films, regardless of the acyl carbon number [7]. The birefringence of the CTA film is known to decrease with the introduction of some additives. However, surprisingly, the out-of-plane birefringence of pullulan acetate was almost "zero" throughout the visible-light wavelength region without additives. This result suggests that pullulan acetate has an excellent zero birefringence property with high thermal stability without any additive, which has great potential for applications in optical films.

9.3 Bio-Based Adhesives from Dextran (α-1,6-Glucan)

Similar to pullulan, dextran is a water-soluble polysaccharide. However, it shows unique properties different from those of pullulan. Dextran is a natural polysaccharide comprising glucose linked by α-1,6-glycocidic linkages (Figure 9.1) [24]. This α-1,6-glucan is biologically synthesized from sucrose by lactic acid bacteria, such as *Leuconostoc mesenteroides*. The commercial production of dextran is estimated to be c. 2000 t/yr [25]. It has been widely applied as a medical material and a cosmetic binding agent owing to its water solubility and biocompatibility [24, 26–28]. However, dextran derivatives can also be used as adhesives, including hot-melt adhesives and safety solution adhesives, by esterifying the hydroxyl groups of dextran with linear carboxylic acids [8]. As shown in Tables 9.1 and 9.2, these dextran esters have different physical properties and solubilities depending on their acyl carbon number (n) and degree of substitution (DS), which is the number of hydroxyl groups substituted among the three hydroxyl groups in glucose units [8, 29]. For example, the solubility of dextran esters can reportedly be controlled by changing the DS. Table 9.2 shows the solubility of dextran esters in common solvents. Dextran butyrate (DexBu,

Table 9.2 Solubility of dextran butyrate with different DS in common solvents.

Name	DS	Water	Methanol	Ethanol	Acetone	Chloroform
Dextran-neat	0.0	○	×	×	×	×
DexBu-0.7	0.7	○	○	×	×	×
DexBu-1.1	1.1	×	○	○	○	×
DexBu-1.7	1.7	×	○	○	○	○
DexBu-2.2	2.2	×	○	○	○	○
DexBu-2.5	2.5	×	○	○	○	○
DexBu-3.0	3.0	×	×	×	○	○

○; soluble, ×; insoluble.

$n = 4$) with a low DS dissolves in hydrophilic solvents, such as alcohol or water, while DexBu with a high DS does not dissolve in hydrophilic solvents such as water [29]. Specifically, the water resistance of dextran was expected to be improved by esterification. Notably, DexBu with a DS of 1.1–2.5 dissolves in ethanol. Figure 9.4 shows some typical examples of adhesion with dextran ester adhesives using ethanol as a

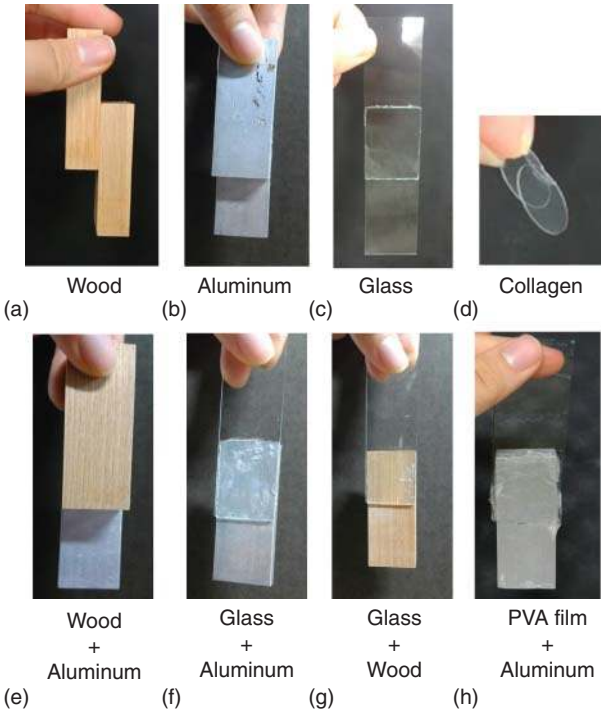


Figure 9.4 Adhesive property of dextran esters with different materials. (a) wood + wood, (b) aluminum + aluminum, (c) glass + glass, (d) collagen + collagen, (e) wood + aluminum, (f) glass + aluminum, (g) glass + wood, (h) PVA film + aluminum.

solvent. Ethanol solutions of dextran esters have shown adhesive performance with a wide range of materials, including wood, glass, glass coated by a collagen film, and gladius of squid [8, 29]. The tensile strength with wood is comparable to that of commercial polyvinyl alcohol (PVA)–water adhesives [29]. Both the water resistance and drying speed have been reported to be significantly improved by using ethanol as a solvent compared with water-based adhesives, such as PVA–water and dextran–water adhesives, although the latter leads to increased emission of volatile organic compounds. For example, in a water resistance test, neat dextran–water adhesives and commercial PVA–water adhesives completely lost adhesive strength after immersion in water for 24 hours, while DexBu ($n = 4$, DS = 1.1) maintained 51% of the adhesive strength (1.0 MPa) measured before immersion in water [29]. Another advantage of dextran ester adhesives is applicability to medical adhesives. As shown in Figure 9.4d, ethanol-born dextran esters show adhesive properties with medical materials, such as collagen films and gladius of squid.

Finally, some dextran esters, such as dextran valerate, have also shown adhesive performance as hot-melt adhesives by heating above the T_g [29]. This is a meaningful property because hot-melt adhesives are more eco-friendly because no solvent is required. Therefore, dextran ester adhesives can be applied using two eco-friendly approaches: hot-melt and ethanol as a solvent. These good adhesive properties with various materials suggest that dextran esters have high functionality as an adhesive. This valuable property is unique among polysaccharides.

9.4 Films and Fibers from Paramylon and Curdlan (β-1,3-Glucan) Esters

β-1,3-Glucan is a crystalline polysaccharide mainly derived from curdlan and paramylon. Curdlan is an extracellular microbial polysaccharide first discovered and studied by Harada et al. in 1968 [30], while paramylon is a storage polysaccharide photosynthesized by the microalga *Euglena* [31]. Both curdlan and paramylon are linear β-1,3-glucans without branches. Normally, the weight-average molecular weight of paramylon (200×10^3 g/mol) is about one-fifth that of curdlan (1000×10^3 g/mol). Both curdlan and paramylon have characteristic sixfold helical main chain structures [32–35], which differ from the twofold helical structure of cellulose.

Fully substituted β-1,3-glucan esters with different side-chain lengths can be prepared by a simple carboxylic acid/TFAA system, as shown in Figure 9.5 [9, 10]. The weight-average molecular weights of paramylon esters and curdlan esters are in the range of $(3.0–9.6) \times 10^5$ g/mol and $(6.0–16.0) \times 10^5$ g/mol, respectively, which are adequate values for manufacturing plastic materials. β-1,3-Glucan esters with C2–C6 alkyl chains are crystalline polymers, while those with C8–C12 chains are amorphous polymers, as summarized in Table 9.3. Furthermore, despite the molecular weight differences between curdlan and paramylon, their esters with the same substituted ester groups show the same thermal properties.

Table 9.3 Characteristics of β-1,3-glucan.

Name	Substituent	M_w ($\times 10^5$)	M_n ($\times 10^5$)	PDI	T_g (°C)	T_m (°C)	$T_{d5\%}$ (°C)	Tensile strength (MPa)	Elongation strain (%)	Young's modulus (MPa)
Curdlan	Acetate ($n = 2$)				171	287	318	21.2 ± 4.0	12 ± 3	448 ± 33
	Propionate ($n = 3$)	6.0	3.3	1.8	110	213	364	23.7 ± 4.2	46 ± 20	260 ± 99
	Butyrate ($n = 4$)	8.9	4.6	1.9	74	190	351	15.4 ± 1.9	26 ± 5	204 ± 12
	Valerate ($n = 5$)	13.0	6.4	2.0	51	168	296	12.4 ± 2.3	79 ± 9	149 ± 35
	Hexanoate ($n = 6$)	14.0	7.1	2.0	69	167	274	14.7 ± 1.0	150 ± 28	92 ± 10
	Octanoate ($n = 8$)	14.0	6.7	2.1	50		281	6.0 ± 2.0	112 ± 43	46 ± 3
	Decanoate ($n = 10$)	16.0	7.4	2.1	55		292	4.6 ± 0.4	144 ± 11	19 ± 1
	Laurate ($n = 12$)	16.0	7.0	2.3	47		293	1.7 ± 0.3	172 ± 35	12 ± 1
Paramylon	Acetate ($n = 2$)				168	281	327			
	Propionate ($n = 3$)	3.4	1.9	1.8	112	221	340	31.4 ± 3.4	62 ± 6	597 ± 52
	Butyrate ($n = 4$)	5.8	3.2	1.8	76	207	337	20.6 ± 1.2	87 ± 25	352 ± 54
	Valerate ($n = 5$)	8.1	3.9	2.1		196	330	7.9 ± 0.7	61 ± 28	133 ± 19
	Hexanoate ($n = 6$)	8.5	4.0	2.1		114	328	7.5 ± 0.6	446 ± 18	47 ± 3
	Octanoate ($n = 8$)	8.9	4.5	1.9			330	2.7 ± 0.3	415 ± 66	3 ± 1
	Decanoate ($n = 10$)	9.2	4.8	1.9			324	2.8 ± 0.6	328 ± 48	12 ± 1
	Laurate ($n = 12$)	9.6	5.4	1.8			333	2.7 ± 0.5	529 ± 108	5 ± 1

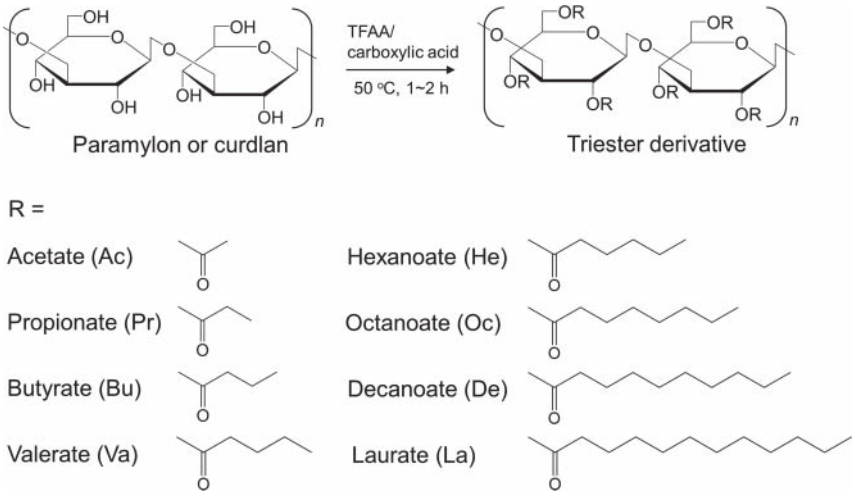


Figure 9.5 Synthetic scheme of β-1,3-glucan esters.

Figure 9.6a shows a comparison of the thermal properties of β-1,3-glucan esters with those of commonly used polymers. By adjusting the carbon number of the ester groups, esters with T_m values of 114–281 °C can be obtained. This range covers the T_m values of commonly used plastics, such as polyethylene (PE, 120 °C), polypropylene (PP, 170 °C), and polyethylene terephthalate (PET, 280 °C). The T_g values of β-1,3-glucan esters are between 47 and 168 °C, with those of β-1,3-glucan acetate (168 °C), β-1,3-glucan propionate (112 °C), and β-1,3-glucan butyrate (76 °C) higher than that of PET (70 °C). Furthermore, the 5% mass reduction temperatures evaluated by thermogravimetric analysis (TGA) are all above 300 °C, indicating that β-1,3-glucan esters, except acetate derivatives, have a wide window of thermal process temperatures.

All β-1,3-glucan esters can form self-standing films using the solvent-cast method, while those with C2–C6 alkyl chains can also make melt-quench films. With increasing alkyl side-chain length, the tensile strength and Young's modulus decrease, while the elongation at break tends to increase. Figure 9.6b shows a comparison of the tensile properties of β-1,3-glucan esters with those of commonly used polymers. By adjusting the side-chain length, the tensile properties of β-1,3-glucan esters can be tuned to be similar to those of PP and PE. Regarding the thermal and mechanical properties, they can have similar mechanical properties, and much higher thermal stabilities, compared with those of popular petroleum-based plastics. These properties show that β-1,3-glucan esters are prospective biomass plastics with excellent thermal and mechanical properties.

In addition to films, melt-spun fibers can be manufactured from β-1,3-glucan propionate, butyrate, and valerate derivatives owing to their favorable thermal fluidity at the melt-processing temperature indicated by differential scanning calorimetry (DSC) and melt flow test results [11]. The image of β-1,3-glucan propionate fiber is

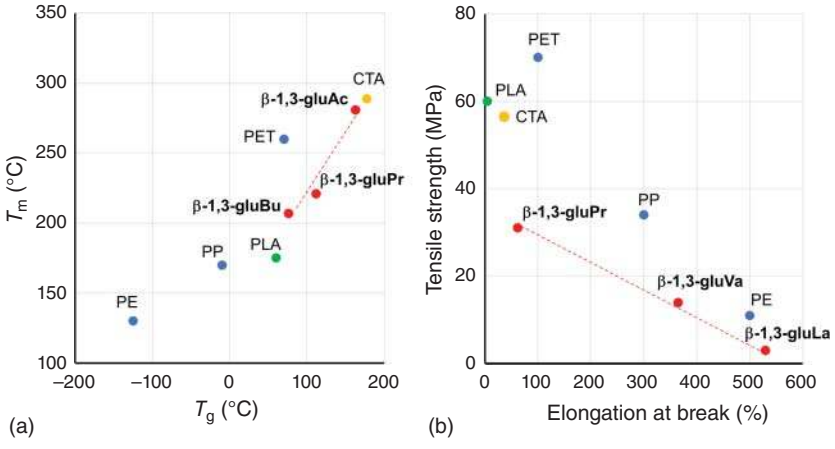


Figure 9.6 (a) Thermal and (b) mechanical properties of β-1,3-glucan esters compared with those of general petroleum- or bio-based plastics.

Figure 9.7 (a) Melt-spun fiber and (b) injection-molded specimens of β-1,3-glucan propionate.

shown in Figure 9.7a as a representative one. Each of the β-1,3-glucan esters was successively melt-spun at various take-up rates, without the need for additives, and the molecular weight did not change significantly. By adjusting the take-up rate, crystalline or amorphous melt-spun fibers could be produced. The fiber strength could be improved by increasing the take-up rate or annealing treatment, both of which promoted the crystallization of molecular chains. For β-1,3-glucan propionate, in addition to fibers, a bulk specimen could also be fabricated in different shapes by injection molding, as shown in Figure 9.7b. The specimens are yellowish because of the heating procedure during thermal processing, which might be prevented by adding an antioxidant.

In addition to homoesters, Shibakami et al. prepared paramylon esters with mixed short and long alkyl side chains, aiming to control the physical properties precisely by adjusting carboxylic acid types and ratios [36–38]. Among injection-molded specimens of the mixed esters, some showed bending strength and bending elastic modulus comparable to those of petroleum-based acrylonitrile–butadiene–styrene (ABS) resin and cellulose acetate stearate mixed ester. Meanwhile, the impact strength of mixed paramylon esters was comparable to that of PLA, indicating that

mixed paramylon esters have potential uses as thermoplastic materials in practical applications.

In summary, β-1,3-glucan esters show a wide range of controllable physical properties with changing side-chain lengths. By changing the ester group from valerate to acetate, the glucan ester shows thermal and mechanical properties that vary from PE-like to PET-like properties.

9.5 Polymerization of α-1,3-Glucan and Films of α-1,3-Glucan Esters

α-1,3-Glucan is a polysaccharide in which glucose is linked by α-1,3-glycosidic linkages. This glucan is a structural polysaccharide in the cell walls of fungi and yeast [39–42] and is also known as a major component of plaque produced by *Streptococcus* in the mouth [43, 44]. However, no pure and complete linear α-1,3-glucan is found in nature because natural α-1,3-glucans are branched by other linkages or are mixed with other polysaccharides. Recently, the synthesis of completely linear α-1,3-glucan was achieved by enzymatic polymerization [12]. As such, glucan sucrase is used to prepare α-1,3-glucan from sucrose, a disaccharide composed of glucose and fructose (Figure 9.8). Glycosyltransferase (Gtf) is among the extracellular enzymes that decompose sucrose and simultaneously polymerize only glucose to synthesize α-glucan [45–48]. In nature, the synthesized polysaccharide is an α-glucan network with complex bonds and branches because the bacteria secrete multiple types of glucan sucrase. Therefore, *in vitro* enzymatic polymerization with the GtfJ enzyme cloned from *Streptococcus salivarius* (ATCC25975) was adopted in their research. The GtfJ enzyme was produced by culturing *Escherichia coli* expressing GtfJ. After the produced enzyme was added to sucrose aqueous solution adjusted to pH 5.5, a large amount of white precipitate was observed within a few days (Figure 9.9). As shown in Figure 9.9d, this white precipitate was found to have a crystalline fiber structure with a width of 10–20 mm and length of about 1 μm. The synthesized linear α-1,3-glucan was readily collected by filtration or centrifugation because it was insoluble in water. This enzymatic polymerization is an environmentally friendly one-pot water-based reaction without organic solvent.

The yield and molecular weight of the synthesized glucan are important factors in its application in plastic materials because they directly affect production cost and the physical properties of the materials, respectively. By conducting an enzymatic reaction at 30 °C, α-1,3-glucan with a M_w of 2.0×10^5 g/mol and a polydispersity of 2.1 can be reportedly synthesized at about 50 g for the 1 l system in four days. Although the optimum temperature for this enzyme is around 37 °C, similar to the human oral temperature, the glucan molecular weight increased to over 7.0×10^5 when conducting the enzymatic reaction at 15 °C. This shows that the synthesized molecular weight can be controlled by adjusting the reaction conditions of this enzymatic polymerization.

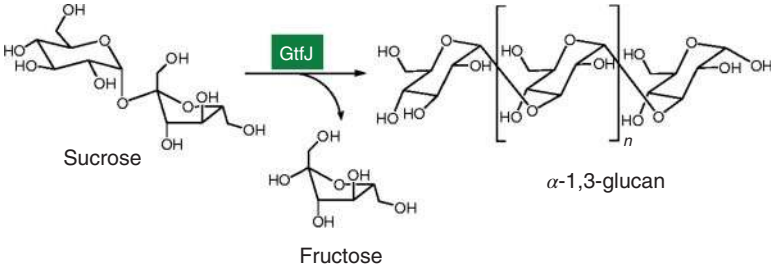


Figure 9.8 Enzymatic polymerization of α-1,3-glucan.

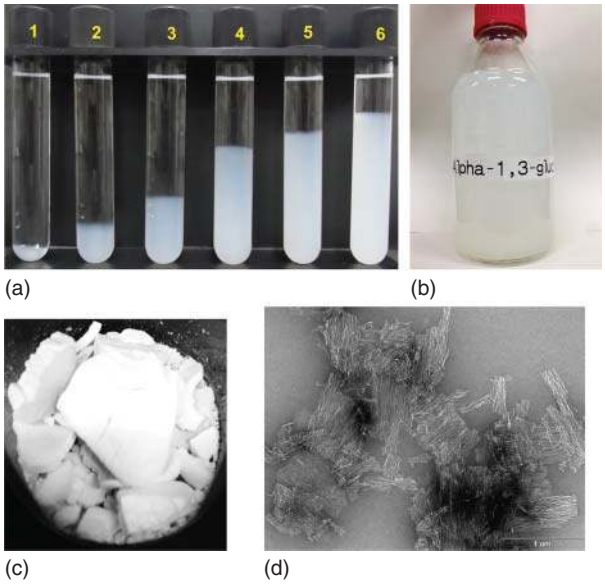


Figure 9.9 (a) *In vitro*-synthesized α-1,3-glucan; (b) synthesis of α-1,3-glucan in large scale; (c) filtered and dried α-1,3-glucan; and (d) transmission electron microscopy (TEM) image of synthesized α-1,3-glucan.

The designed synthesis of high-molecular-weight α-1,3-glucan is indispensable for improving the mechanical properties and processability into fibers. The establishment of high-yielding and high-molecular-weight enzyme reaction conditions is required. Like other polysaccharides, this synthesized α-1,3-glucan does not exhibit thermal plasticity and solubility in common organic solvents because of hydrogen bonding derived from its hydroxyl groups. However, esterification of the hydroxyl groups imparts the glucans with thermal plasticity and solubility in organic solvent [12]. α-1,3-Glucan esters have much higher T_m values than other polysaccharide esters and common petroleum-based plastics, such as CTA and PET. For example, the T_m of α-1,3-glucan acetate is 340 °C. Furthermore, the T_g of α-1,3-glucan esters is in the range of 120–180 °C, which is higher than that of nylon-6 (55 °C) and PET (70 °C). This suggests that novel bio-based plastics derived from α-1,3-glucan have

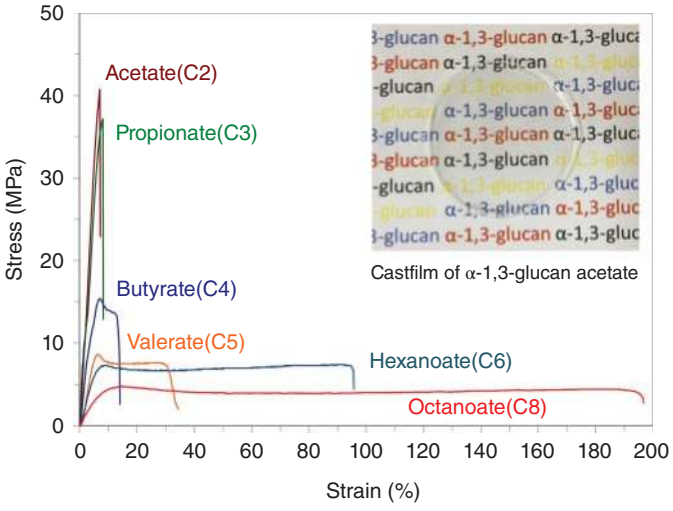


Figure 9.10 Stress–strain curves of α-1,3-glucan ester films.

new thermal properties different from petroleum-based plastics and conventional polysaccharide derivatives, such as cellulose and starch derivatives.

The films of these α-1,3-glucan esters are transparent. As shown in Figure 9.10, the mechanical properties of these films are controlled by the acyl carbon number [13]. For example, α-1,3-glucan esters with short side chains, such as acetate, show high maximum stress and low strain at break, while those with long side chains, such as octanoate, show low maximum stress and high strain at break. Based on these mechanical properties, α-1,3-glucan esters are expected to be applied as engineering plastics in the future.

9.6 High-Performance Polysaccharide-Branched Esters

In addition to the length, or carbon number, the end structure of side chain ester groups plays a prominent role in the physical properties of polysaccharide esters as thermoplastic resins. In particular, ester groups with terminally branched shapes (*iso*-branched or *tert*-branched ones such as isovalerate or pivalate, respectively, as shown in Figure 9.11) give unique thermal properties, as characterized by high melting temperatures and high glass transition temperatures that cannot be attained by linear esters. Table 9.4 shows the characteristics of polysaccharide-branched esters compared with those of cellulose, curdlan, and α-1,3-glucan.

9.6.1 Cellulose-Branched Esters [14]

Iso-branched acyl groups can be introduced into cellulose by a heterogeneous reaction with the corresponding carboxylic acids and TFAA, which is the same method used to introduce linear acyl groups. Fully substituted cellulose *iso*-branched esters

Table 9.4 Characteristics of polysaccharide-branched esters.

Name	Substituent	M_w ($\times 10^5$)	M_n ($\times 10^5$)	PDI	T_g (°C)	T_m (°C)	$T_{d5\%}$ (°C)	Tensile strength (MPa)	Elongation strain (%)	Young's modulus (MPa)
Cellulose	Isobutyrate ($n = 4$)	4.4	2.3	1.9	115	248	300	28 ± 1	25 ± 4	350 ± 20
	Isovalerate ($n = 5$)	6.3	3.1	2.1	79	141	294	25 ± 1	44 ± 20	330 ± 40
	Isohexanoate ($n = 6$)	9.3	4.7	2.0	70	106	330	19 ± 2	208 ± 41	300 ± 50
	Isoheptanoate ($n = 7$)	6.3	2.6	2.4	61	104	331	16 ± 2	403 ± 27	150 ± 20
Curdlan	Isobutyrate ($n = 4$)	8.7	4.0	2.2	110	339	360	28.6 ± 2.0	68 ± 28	470 ± 170
	Isovalerate ($n = 5$)	20.6	8.0	2.6	91	250	368	15.5 ± 1.4	52 ± 20	250 ± 60
	Isohexanoate ($n = 6$)	21.2	6.8	3.1	64	199	369	18.6 ± 2.1	180 ± 21	280 ± 50
	Isoheptanoate ($n = 7$)	17.7	6.1	2.9	41	126	368	5.0 ± 0.6	349 ± 64	50 ± 10
	Pivalate ($n = 5$)	14.3	6.8	2.1	173	337	360	35.7 ± 1.3	29 ± 5	580 ± 60
α-1,3-Glucan	Isobutyrate ($n = 4$)	1.9	0.9	2.1	206	251	385	22.9 ± 3.4	8 ± 1	500 ± 70
	Isovalerate ($n = 5$)	2.1	1.0	2.2	132	292	387	14.5 ± 1.3	9 ± 2	410 ± 50
	Isohexanoate ($n = 6$)	2.3	1.1	2.1	131	306	388	4.1 ± 0.4	18 ± 1	140 ± 50
	Isoheptanoate ($n = 7$)	2.7	1.3	2.1	102	322	386	4.6 ± 0.2	22 ± 2	220 ± 10
	Pivalate ($n = 5$)	1.8	0.9	2.1	202	307	376	22.3 ± 0.6	7 ± 2	430 ± 70
	Tertiary butyl acetate ($n = 6$)	1.9	0.9	2.1	168	348	382	—[a)]	—[a)]	—[a)]

a) A cast film is too brittle to undergo a tensile test.

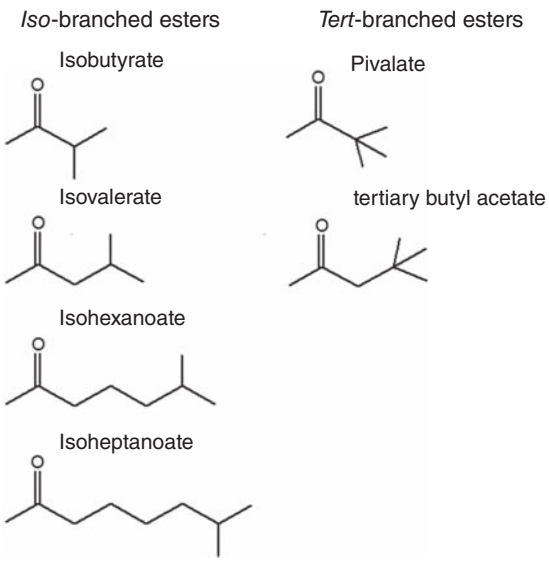


Figure 9.11 The structures of terminally branched ester groups introduced into polysaccharides.

with acyl carbon numbers of 4–7 are crystalline polymers, similar to the cellulose linear esters. Although the *iso*-branched esters with acyl carbon numbers of 5–7 have T_m and T_g values similar to those of the corresponding linear esters, the *iso*-branched esters with an acyl carbon number of 4, cellulose isobutyrate, showed a higher T_m (248 °C) and T_g (115 °C) than linear cellulose butyrate ($n = 4$, $T_m = 182$ °C, and $T_g = 87$ °C). Therefore, shorter *iso*-branched ester side chains resulted in higher thermal phase transition temperature, strength, and stiffness.

Furthermore, all cellulose *iso*-branched esters form colorless and transparent cast films. With increasing side-chain carbon number, the cast films become softer in the same manner as linear esters. Furthermore, the cast films of branched esters are harder (higher Young's modulus) and more brittle (lower elongation at break) than linear esters with the same acyl carbon number.

Regarding the crystalline structure, an explanation for the higher thermal phase transition temperatures and rigid mechanical properties of the branched esters can be proposed. Specifically, cellulose isobutyrate has threefold helix crystalline parameters similar to those of cellulose propionate. This suggests that isobutyric substituents are closely packed in the helix molecular chain conformation, resulting in unique physical properties compared with the linear esters.

9.6.2 β-1,3-Glucan (Curdlan) Branched Esters [15]

For curdlan, *tert*-branched acyl (pivalic substituent) and *iso*-branched acyl groups can be introduced using the same method as for cellulose-branched esters. Curdlan *iso*-branched esters with acyl carbon numbers of 4–7 and *tert*-branched esters with an acyl carbon number of 5 are all crystalline. The *iso*-branched esters have significantly higher T_m and T_g values than those of linear esters with the same acyl carbon number. Above all, curdlan isobutyrate has an extremely high T_m of 339 °C owing

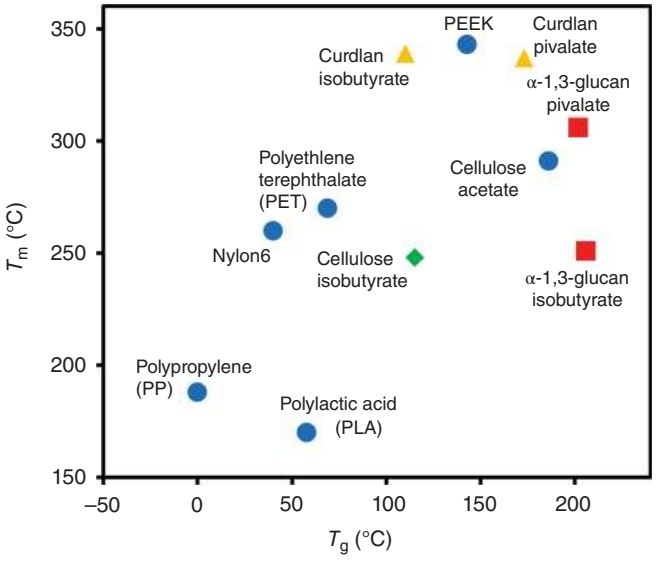


Figure 9.12 T_m and T_g values of polysaccharide-branched esters and conventional plastics.

to the closely packed crystalline structure, which cannot be realized by other curdlan esters and cellulose esters. Furthermore, *tert*-branched ester derivative, curdlan pivalate, has even higher T_m and T_g values than those of linear and *iso*-branched esters with the same carbon number. Remarkably, the T_g of curdlan pivalate (173 °C) is the highest of all the curdlan esters. As the T_m of curdlan pivalate (337 °C) is almost equal to that of curdlan isobutyrate, curdlan pivalate is the most thermally stable of all the curdlan esters, leading to the conclusion that introducing *tert*-branched ester groups is highly effective for improving thermal stability (Figure 9.12).

As observed for other polysaccharide esters, curdlan-branched esters form colorless and transparent cast films, which become softer with increasing side-chain length. Furthermore, cast films of the branched esters have higher tensile strength, higher Young's modulus, and lower elongation at break compared with linear esters with the same acyl carbon number. Regardless of the terminal shape (linear, *iso*-branched, or *tert*-branched), the tensile strength and Young's modulus of curdlan propionate, curdlan isobutyrate, and curdlan pivalate are similar. In contrast, the elongation at break values of curdlan isobutyrate and curdlan pivalate are about 5 and 10 times higher than that of curdlan propionate, respectively. Therefore, the introduction of branched ester groups also enhanced film toughness.

9.6.3 α-1,3-Glucan-Branched Esters [16]

For α-1,3-glucan, *iso*- and *tert*-branched esters can be synthesized in the same manner as those of cellulose and curdlan. These derivatives show crystallinity, although α-1,3-glucan pivalate is almost amorphous. The T_m of α-1,3-glucan-branched esters is proportional to the acyl carbon number, unlike all other linear or branched

polysaccharide esters. The change in T_m with acyl carbon number is, as usual, inversely proportional owing to the internal plasticizing effects of side chains. This usual feature led to a conspicuous T_m of 348 °C for the α-1,3-glucan *tert*-butyl acetate, which is the highest T_m among all fully substituted polysaccharide esters reported to date. Furthermore, the T_g values of α-1,3-glucan-branched esters, which decrease with increasing acyl carbon number, are slightly higher than those of the linear esters. Among them, α-1,3-glucan isobutyrate and pivalate have T_g values of over 200 °C (Figure 9.12), which are highest among all fully substituted polysaccharide esters reported to date, and comparable to, or higher than those of conventional super engineering plastics, as represented by polyether ether ketone ($T_g = 142$ °C) [49].

Furthermore, α-1,3-glucan isobutyrate and pivalate show thermoplasticity at temperatures too low for thermal degradation to occur. Specifically, α-1,3-glucan isobutyrate shows thermofluidity at around 270 °C, which is slightly higher than T_m (251 °C), while α-1,3-glucan pivalate shows thermofluidity at around 250 °C, which is between the T_g (202 °C) and T_m (307 °C), owing to its low crystallinity. The presence of both high-dimensional stability and thermoplasticity is uncommon and highly attractive for industrial applications and mass production by injection molding. Furthermore, even commercial oil-based super engineering plastics with comparable T_g values, which generally have aromatic rings in the polymer backbone, seldom realize thermoplasticity alone. Accordingly, α-1,3-glucan isobutyrate and pivalate are promising thermoplastic resins with valuable properties utilizing a bio-based polysaccharide main chain and eventually fossil-based ester side chains.

9.7 Enzymatic Esterification of Polysaccharides

Enzymes have attracted much attention as biocatalysts in chemical reactions owing to their numerous advantages, including high selectivity, ability to operate under mild conditions, biocompatibility, and catalyst recyclability. Compared with reactions using chemical catalysts, the use of enzymes in the synthesis of polysaccharide esters has several characteristic differences. The main advantages are not only an environmentally benign process under mild conditions but also having high substrate specificity, allowing for the synthesis of polysaccharide esters with well-designed structures and functionalities. As mentioned in Section 9.1.2, polysaccharide esters are commercially manufactured using strong acid catalysts under severe conditions. These chemical processes usually lead to the production of highly substituted polysaccharide esters presenting significantly increased thermoplasticity and hydrophobicity. However, this chemical route has several disadvantages in the final product, such as toxicity from the residual reagent, undesired by-products, and partial polymer degradation. Furthermore, this chemical route has limitations when producing amphiphilic polysaccharide esters with adjusted hydrophilic–hydrophobic balance.

As an alternative approach to addressing these problems, the esterification of polysaccharides using enzymes has received much attention. This renewable and

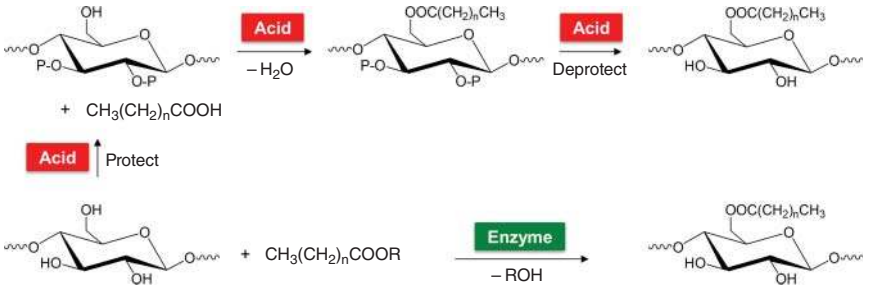


Figure 9.13 Representative routes of chemical and enzymatic esterification in the synthesis of regioselectively substituted polysaccharide ester.

eco-friendly process emphasizes environmental efficiency as an example of green chemistry. The synthesis of polysaccharide esters using enzymes is conducted under mild conditions at a relatively low temperature, which avoids polymer degradation. Enzyme esterification is also substrate specific and regioselective. These features facilitate the regioselective synthesis of polysaccharide esters with amphiphilic properties. Although regioselectivity substituted polysaccharide esters can be obtained through chemical esterification, this process usually requires sequential protection and deprotection reactions. These additional steps can cause unexpected side reactions and result in an expensive and uneconomical purification. Accordingly, as a one-step reaction for synthesizing regioselectivity substituted polysaccharide esters without the need for protection and deprotection reactions, enzymatic esterification has industrial advantages. Figure 9.13 shows the representative routes of chemical and enzymatic esterification for the synthesis of regioselectivity substituted polysaccharide esters.

9.7.1 Enzymes as Biocatalysts

As polysaccharide esterification with an enzyme is based on the original features of the enzyme, identifying the nature of enzymes is important. Enzymes are biological molecules, typically proteins comprising amino acids linked together via peptide bonds. Within its structure, the enzyme has an active site that can bind to the reactive groups (such as acetamido, amino, carboxyl, and/or hydroxyl groups) of polysaccharides. In other words, enzymes have unique interactions with specific substrates that can fit into their active site. Therefore, the selection of enzymes and polysaccharides to obtain substrate specificity is the most important factor for a successful reaction. In a prior study, many enzyme series were investigated in terms of their specificity for monosaccharides and oligosaccharides [50, 51]. Enzyme specificity related to various polysaccharides, such as cellulose, starch, chitosan, and dextran, has also been reported [52].

Among many enzymes, lipase (EC 3.1.1.3, triacylglycerol hydrolases), esterase (EC 3.1.1.1 carboxyl ester hydrolases), proteases (EC 3.4.21.x, peptidase or proteinase), and cutinases (EC 3.1.1.74, cutin hydrolases) have gained particular attention as efficient biocatalysts in the synthesis of polysaccharide esters. These enzymes are

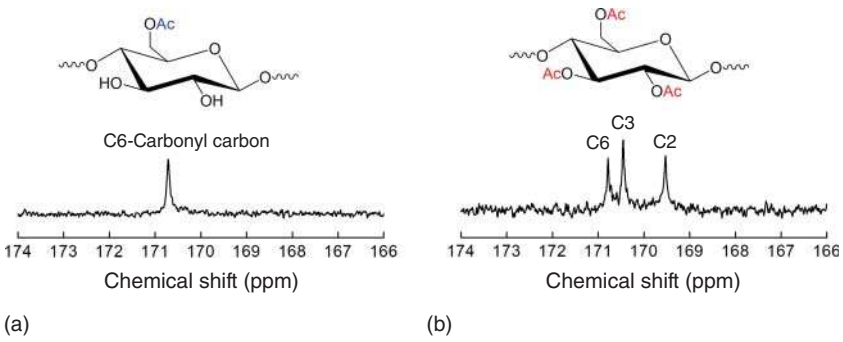


Figure 9.14 ^{13}C-NMR of dextrin acetate by enzymatic (a) and chemical esterification (b) related to the region of carbonyl carbon.

intrinsically catalysts for ester hydrolysis owing to the presence of serine in their catalytic active site but catalyze the reverse esterification reaction in the absence of water (namely, in an organic solvent). As this reaction is in the thermodynamic equilibrium state, water produced by the reaction must be controlled continuously to increase the conversion. An efficient strategy is transesterification using a vinyl ester because the alcohol by-products are easier to remove compared with water [53]. Figure 9.14 shows ^{13}C-NMR spectra of a regioselectively substituted polysaccharide acetate (from dextrin produced by partial starch hydrolysis) [17]. The spectrum of dextrin acetate catalyzed by the enzyme indicates a completely regioselective reaction, showing a single peak corresponding to a carbonyl carbon at the C6 position. To synthesize polysaccharide esters with a well-designed structure, optimum conditions in which various factors are taken into account are required.

9.7.2 Reaction Mechanism

Enzymatic esterification has an ordered mechanism including acylation and deacylation. First, the enzyme active site is adjusted to an accurate position by interacting with the substrate. The polysaccharide ester is then released from the enzyme surface, and the enzyme is regenerated for cycling of another reaction. Among the most important enzymes exhibiting high catalytic efficiency is lipase, which can esterify the primary hydroxyl group (C6-OH) of glucose units [54]. Figure 9.15 shows the catalytic mechanism of lipase for synthesizing polysaccharide ester. Lipases include a catalytic triad, comprising aspartic acid, serine nucleophile, and histidine base, as the active site. The reaction occurs at this site, with the first step proceeding to form a primary tetrahedral intermediate with acyl donor and serine. The enzyme then forms a link with the acyl group by electron transfer. The covalent acyl–enzyme intermediate finally derives a polysaccharide ester via a substitution reaction with a reactive group in the polysaccharide and is converted back to the original structure [55].

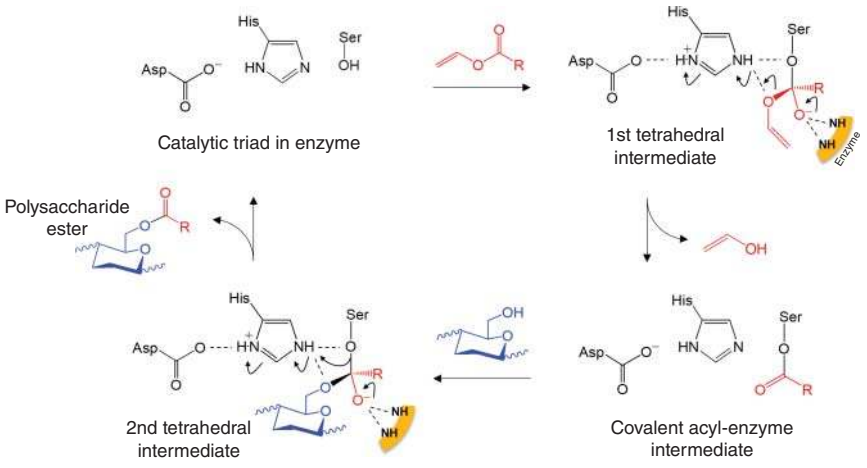


Figure 9.15 Reaction mechanism of polysaccharide ester using lipase in the organic solvent.

9.7.3 Factors Influencing Enzyme Activity

Enzymatic esterification is extremely sensitive and susceptible to various factors. Therefore, reaction under appropriate conditions is required for enzyme activation. The rate and degree of enzyme activation is basically affected by the frequency of collision with the substrate, which depends on reaction temperature, enzyme amount, and substrate concentration.

First, the temperature directly affects the structure and activity of the enzyme protein, strongly influencing the enzyme activity. At high temperatures (usually more than 50 °C), enzyme denaturation can occur, resulting in low activity. Enzymes have activity only within a limited temperature range, with many enzymes having specific temperature for catalytic activity.

Second, the amount of enzyme used in enzymatic esterification is also a factor directly influencing the degree of reaction. If the reaction rate at which an enzyme catalyzes is proportional to the enzyme amount, the reaction system with doubling the amount of enzyme should allow twice as much substrate to be catalyzed. However, the presence of excess enzyme in the reaction system can lead to a lower degree of reactivity. The lower degree of reactivity caused by excess enzyme is attributed to enzyme movement to neighboring hydroxyl groups being interrupted by other enzymes in a sequential manner [56].

Lastly, substrate concentration plays an important role according to the collision theory of chemical reaction. As molecules must collide with each other to react, increasing the substrate concentration increases the collision frequency, resulting in higher degree of reactivity. However, a substrate concentration that is too high might increase the overall viscosity of the response system, thereby limiting enzyme movement, which causes the reaction efficiency to deteriorate.

9.7.4 Strategies for Efficient Biocatalyst Processes

Enzymes are water-soluble proteins. An enzyme is generally dissolved in solution when performing a reaction. Therefore, the enzyme activity in organic solvents is different to that in aqueous solution. Generally, most enzymes degenerate easily in organic solvents, losing their activity. To mitigate this loss of function, two representative strategies can be used, which are enzyme immobilization and the use of ionic liquids.

Immobilization is a technical process in which enzymes are fixed to a support carrier, creating a heterogeneous immobilized enzyme system. This immobilized enzyme maintains its original catalytic activity and substrate specificity with enhanced thermal and solvent stability. The use of immobilized enzymes in the enzymatic esterification of polysaccharides has advantages, including improved yield and purity of polysaccharide esters [57, 58]. Immobilized enzymes are easily separated from the reaction product and can be reused owing to their catalytic activity being maintained. There are several techniques used to immobilize enzymes, including adsorption, covalent-bonding, cross-linking, entrapment, and encapsulation. For the immobilized enzyme to exhibit its catalytic activity, the active site must be prevented from changing during immobilization. When selecting the immobilization method, the purpose of use must be considered in accordance with the original enzyme properties.

Ionic liquids are ionic compounds (salts) that exist as liquids at ambient temperatures. Recently, ionic liquids have been effectively used as green solvents owing to their unique characteristics, such as low volatility, high chemical and thermal stability, and tunable solvent properties. Ionic liquids are advantageous as alternatives to organic solvents in enzymatic reaction systems. First, the ionic liquid reaction system is more stable and eco-friendly than the organic solvent system because it avoids the high volatility, flammability, and environmentally harmful characteristics of organic solvents. Second, solvent characteristics, such as polarity, acidity, and hydrophobicity, can be adjusted according to the combination of positive and negative ions. Therefore, enzymatic reactions can be conducted over a wider range of reaction conditions without denaturation of the enzyme protein in contrast to polar organic solvents. The use of well-controlled ionic liquids in the enzymatic reaction of polysaccharides can react regioselectively with improved activity [59]. Furthermore, ionic liquids have the advantage of dissolving polysaccharides. As most polysaccharides form strong hydrogen bonds between molecules, they are generally insoluble in ordinary organic solvents. In contrast, ionic liquids are effective solvents for polysaccharides. For example, an ionic liquid consisting of 1-butyl-3-methylimidazolium (C4mim) and chloride ions shows excellent cellulose solubility [60].

9.7.5 Development Trend and Prospects

In the last few years, many studies on the enzymatic synthesis of various carbohydrate molecules (monosaccharides, oligosaccharides, and polysaccharides) have been reported for their applications with environmental potential, including

biodegradable emulsifiers, plasticizers, compatibilizers, and detergents [61, 62]. Based on the production of monosaccharide and/or oligosaccharide esters using an enzyme, some studies have been conducted on the synthesis of polysaccharide esters, such as cellulose, chitosan, starch, and dextran. Esterified polysaccharides with an appropriate acyl chain length can act as internal plasticizers, exhibiting improved thermoplasticity. Accordingly, cellulose and its derivatives have received particular research attention. Gremos et al. have reported the enzymatic esterification of cellulose with a series of vinyl esters (12–18 carbon atoms), which present a low degree of esterification of 0.9–1.3% [63]. This low enzyme activity is due to restricted enzyme penetration, which is caused by strong molecular interactions in the crystalline polymer. Comparatively, starch achieves a high conversion in the synthesis of starch esters using enzymes. Many studies have reported the synthesis of starch esters with a wide range of degrees of esterification of up to 90% [57, 58, 64, 65]. However, these reactions exhibit a lack of regioselectively owing to the specific conditions used to amplify the enzyme catalytic activity (substrate pretreatment, microwave treatment, and the addition of ionic liquids). A completely regioselective reaction has been reported using a branched polysaccharide derived from starch (namely, dextrin) [17]. Generally, branched polysaccharides show better efficiency than linear polysaccharides in enzymatic esterification, owing to good solubility in the organic solvent (a consequence of the extra degrees of freedom provided by rotation at the branched position). Dextran is another branched polysaccharide whose derivatives produced by enzyme catalysis have been studied in terms of potential for biomedical applications in particular [66, 67]. Dordick and coworkers have prepared dextran-based hydrogels using an enzymatic esterification reaction between dextran and divinyladipate [68]. This material exhibited superior mechanical properties (higher elastic modulus for a given swelling ratio) compared with chemically prepared dextran-based hydrogels.

Although much research has been conducted on the enzymatic esterification of polysaccharides, their potential has yet to be fully explored owing to the problem of low solubility resulting from the abundance of hydroxyl groups in polysaccharides. Furthermore, in terms of enzyme activation, predicting substrate specificity between enzymes and polysaccharides is difficult because polysaccharides have different complex structures and, therefore, exhibit unique properties depending on the constituents and conformations of repeating units (α- or β-glucosidically linked structures based on about 40 different monosaccharides). Therefore, no commercial process is yet available for the production of polysaccharide esters using enzymes. Nevertheless, many studies on enzymatic reactions of polysaccharides are being conducted for sustainable and environmentally friendly social development. Compared to chemical esterification of polysaccharides, enzymatic reactions do not show satisfactory results in terms of final properties, but this issue can be solved through continuous research. Among many research fields, the development of protein engineering and solvent engineering is important for improving catalytic efficiency, specificity, and stability in the enzymatic synthesis of polysaccharide esters. In the future study of this topic, the optimization of enzymatic processes should be focused on improving the process efficiency. The development of

innovative enzymatic reactions as green chemistry is expected to meet the needs of various industries and, therefore, make sustainable contributions to all of the society.

9.8 Biodegradation of Polysaccharide Ester

The conventional research in the biodegradability of polysaccharide esters is mainly for cellulose acetate [69]. The biodegradation rate depends on the DS of acetate in cellulose hydroxyl groups. On the condition that the DS of acetate is lower than 2.5, cellulose acetate biodegrades at the almost same rate as virgin cellulose. However, with the increase of DS in a range of over 2.5, the biodegradation rate decreases, and fully substituted cellulose acetate (DS = 3) hardly biodegrades. As the problem of plastic waste in the ocean garners increased attention, in 2020, Daicel Co., a Japanese chemical company, developed highly biodegradable cellulose acetate named as CAFBLO™ [70].

As for other polysaccharide esters, the biodegradability has not been investigated yet, and an intensive study is strongly needed. From 2019, our group is investigating the years-long biodegradability of various kinds of polysaccharide esters and other biodegradable and common plastics on the sea floor at a depth of over 850 m near Hatsushima island, Shizuoka prefecture, Japan, and 5000 m near Minami-Tori-Shima island in the northwestern Pacific Ocean, located 1800 km southeast of Tokyo.

9.9 Summary

As an alternative for petroleum-based plastics, polysaccharides have great potential as a bio-based backbone for high-performance plastics. In the last few years, we are committed to develop polysaccharide esters having a wide range of adjustable physical properties by choosing different kinds of polysaccharides, as well as adjusting length and branch of ester groups. Through these efforts, we have succeeded in producing a considerable variety of plastics with excellent properties such as high thermal stability, outstanding transparency, and adhesive property. There are still many methods for expanding the performance, for instance, using the mix esterification technique, changing functional groups or achieving regioselectivity of ester groups with controllable DS using enzymatic esterification. Above all, we believe that we can build a sustainable material production system and protect the global environment for future generations.

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