Chapter 12
Enzymatic Modification of Polymers

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Abstract In polymer applications and development, it is often necessary to modify an existing polymer structure in order to impart special end-use properties. Whereas chemical modification methods are most commonly practiced, sometimes enzyme-catalyzed modifications may be desirable because of the specificity of the reactions, reduction in the by-products produced, milder reaction conditions, and more benign environmental impact. A number of enzyme-catalyzed reactions are reviewed in this paper, covering primarily biobased materials like polysaccharides, proteins, triglycerides, and lignin. The enzymes used include mostly hydrolases, oxidoreductases, and transferases, with occasional involvement of lyases and isomerases. The types of reactions are diverse and include polymer hydrolysis and degradation, polymerization, oxidation, glycosylation, cross-linking, and transformation of functional groups. Because biopolymers are agro-based and occur abundantly in nature, they are often available in large quantities and amenable to enzymatic reactions. As such, the combination of biopolymers and enzymes represents a good product development opportunity and a useful tool for postharvest agricultural technology and green polymer chemistry.

Keywords Biopolymers · Enzymes · Functionalization · Hydrolysis · Lignin · Modification · Polymers · Polysaccharide · Protein · Triglyceride

12.1 Overview

In view of current interest in green polymer chemistry, reduction in the dependence of petroleum-based raw materials, and environmental stewardship, agro-based materials and enzyme-related technologies have become increasingly emphasized in polymer research and product development. Indeed, the use of enzymes for polymer modification reactions is now fairly well established and often encountered.

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A huge body of literature has been accumulated on numerous reactions and processes, including many books [1–7] and reviews [8–14]. A detailed literature search of Chemical Abstracts for "enzymatic modification" showed for the 2008–2018 time period 301 papers on polysaccharides, 6350 papers on proteins, 472 papers on lignin, 377 papers on triglycerides, and 7455 on synthetic polymers. In order to keep this chapter within a reasonable size, only a selected coverage is being made for the period 2012–2018, with the emphases on enzyme-catalyzed reactions that can be used for research and development (R&D) and potential industrial applications. Such reactions include polymer hydrolysis; addition, removal, or modification of functional groups; cross-linking; and (to a lesser degree) polymer synthesis.

12.1.1 Enzyme Categories

Enzymes are commonly classified into six categories, known as Enzyme Commission (EC) numbers: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Table 12.1) [15]. For polymer hydrolysis, modifications, and syntheses, three enzyme categories (hydrolases, oxidoreductases, and transferases) are most often utilized. The other three categories are helpful but less often observed for the topics covered in this review.

12.1.2 Hydrolases (EC 3)

Thus far in the literature, hydrolases have appeared more frequently in polymer studies than any other enzyme types. In addition to its natural function of hydrolyzing the substrates, hydrolases have been used extensively to carry out polymerizations and polymer modification reactions. Many hydrolases are non-specific and can accommodate a range of substrates. It is also helpful that many hydrolases are commercially available at reasonable prices.

Polymerization Reactions One successful application of hydrolases is to catalyze the synthesis of oligosaccharides and polysaccharides. This approach has been

Table 12.1 Uses of enzymes in synthesis, modification, and degradation of conventional polymers

Enzyme category	EC number	Polymer hydrolyses	Polymer modifications	Polymer syntheses
Oxidoreductase	1	√	√	√
Transferase	2	√	√	
Hydrolase	3	√	√	√
Lyase	4			√
Isomerase	5	√	√	
Ligase	6			

pioneered by Kobayashi et al. and summarized in several excellent reviews [8, 10, 16–18]. A somewhat different approach is to use glycosidases to prepare oligosaccharides [19–22].

Another very active approach is the synthesis of polyesters, polylactones, and polycarbonates. Lipases have been the most commonly used enzymes for this purpose, and a large number of polymers have been made. Particularly outstanding is *Candida antarctica* lipase B immobilized on acrylic resin (also known as CALB or Novozym® 435). This large body of literature has been reported and reviewed by a number of workers [23–34].

In addition, lipases and proteases have been utilized to make polyamides. Polyamides over 10 KDa have been enzymatically synthesized for water-soluble polyamides [35–37] or made through ring-opening polymerization of lactams [38–40]. Proteases have been used to prepare oligopeptides with controlled sequences and the effects of reaction parameters studied [41, 42].

An interesting development is silicon bioscience [43–46], where some proteins (called "silicateins") can act both as catalysts and as macromolecular templates in vitro, directing the condensation of silica and polysiloxanes from silicon alkoxides at neutral pH. The use of a lipid-coated lipase to catalyze the oligomerization of diethoxydimethylsilane (DEDMS) has been reported by Nishino et al. [47].

Polymer Modification Reactions This is a large area of endeavor where various hydrolases have been employed to insert or remove functional groups on substrate polymers or to convert one functionality to another. A good overview with selected illustrations has been given by Puskas et al. [48]. Some examples from the literature include CALB-catalyzed end-functionalization of many synthetic polymers [48, 49], lipase-catalyzed syntheses of fatty acid diester of poly(ethylene glycol) [50], lipase-catalyzed derivatization of silicone-containing polymers [51], and preparation of glycosilicone conjugates [52].

In the polysaccharide area, some examples include protease-catalyzed acylation of polysaccharides [53], papain-catalyzed amidation of pectin [53], hydrolase-catalyzed amidation of carboxymethyl cellulose [50], enzymatic syntheses of fatty acid esters of cationic guar [50], and modification of starch [54]. More detailed information is given in Sect. 12.2.

Hydrolysis and Degradation In nature, hydrolases are usually designed for hydrolysis and degradation of substrate molecules. Hydrolases can be used for molecular weight reduction, cleavage of branch chains, breakdown of polymeric matrices, and other applications. Some examples in the polysaccharide area include cellulolytic enzymes for biomass conversion [55], proteases for the degradation of guar gum [53], cellulase for viscosity reduction of xanthan gum [53], and beta-D-galactosidase for pectin hydrolysis [53]. More examples can be found in Sect. 12.2.1.

Examples in the polyester area include the use of specific depolymerases for the degradation of poly(lactic acid-co-hydroxybutyrate) [56], recombinant cutinases for

polyester degradation [57], cutinases for poly(ethylene terephthalate) hydrolysis [58], protease- and lipase-type depolymerases for poly(lactic acid) [59], and lipases for the degradation and recycling of poly(butyl adipate) and poly(butylene succinate) [60]. Other hydrolases for polymer degradation and hydrolysis include Alcalase® protease for the hydrolysis of end-terminated esters in polyamide [61] and nitrilase (and nitrile hydratase) for the bioconversion of nitriles to carboxylic acids [62–65]. Two reviews mostly on the enzymatic hydrolysis of synthetic polymers have appeared in 2008 and 2003 [66, 67].

12.1.3 Oxidoreductases (EC 1)

Oxidoreductases tend to be more specific with respect to their substrates. Some oxidoreductases require the concurrent action of cofactors and some do not. Because cofactors require extra cost and more skill in handling them, the reactions obviating the use of cofactors are preferred. However, in appropriate cases oxidoreductases requiring cofactors can still be useful.

Polymer Syntheses This is potentially a very fruitful area for enzymes in polymer science. A lot of papers have been published using oxidoreductase for the polymerization of phenols and anilines. Thus, polyphenols [68–73], poly(phenylene oxide) [74, 75], and electrically conducting polymers [76–78] have been made in this way. A different and very productive approach is to engage oxidoreductases in the free-radical polymerization of vinyl monomers [79–86].

Polymer Modifications Some oxidoreductases have been used in specific ways to convert one functionality to another in a biopolymer. A well-known example is galactose oxidase that oxidizes only the C6 alcohol on galactose to an aldehyde [87–89]. Thus, this reaction has been used on galactomannans (such as guar and locust bean gum) [87–89]. Another example is tyrosinase, which catalyzes the oxidation of phenolic compounds into quinones. It has been used to functionalize chitosan [90, 91] or to graft proteins onto chitosan [92].

An interesting oxidation reaction involves lipase (formally a hydrolase), which catalyzes the conversion of a carboxylic acid to a peracid in the presence of H_2O_2 [93, 94]. The resulting peracid can then carry out polymer modifications, such as the epoxidation of polybutadiene [95] and oxidation of hydroxyethylcellulose [96].

Polymer Hydrolysis and Degradation For pulp and paper industry, a significant application of oxidoreductase is "biobleaching," which refers to the enzyme-catalyzed removal of lignin from wood pulp [97–99]. Four oxidoreductases have been studied: laccase (with concurrent use of a mediator and oxygen), lignin peroxidase, manganese peroxidase, and versatile peroxidase (in combination with H_2O_2).

Presumably the enzymatic system oxidizes the lignin, such that it is more easily removed later in the pulping process. More information is given in Sect. 12.5.

12.1.4 Transferases (EC 2)

A transferase is an enzyme that catalyzes the transfer of a specific functional group from one molecule to another. Cofactors are sometimes needed for the processes. Thus far, quite a few reactions involving transferases have been used for polymer syntheses and modifications.

Polymer Syntheses Many articles describe the application of transferases for polymer syntheses. One example is the use of glycosyltransferase for the synthesis of oligosaccharides and polysaccharides, where the cofactors are recycled [100–104]. Wang et al. [100] have reviewed several approaches relating to glycosyltransferases. DeAngelis [105] described two methods using recombinant *Pasteurella multocida* synthase to generate glycosaminoglycans (GAG). These have been utilized to make GAG via immobilized mutant enzyme reactors [106]. Another approach is to take advantage of biopathway engineering to design different carbohydrate polymers [107].

Other transferases of interest include dextransucrase, which catalyzes the formation of dextran and some oligosaccharides [108–111]. Yet another transferase reaction entails the use of potato starch phosphorylase in the synthesis of low-molecular-weight amylose [112, 113]. Glycogen phosphorylases have been employed in a two-enzyme tandem reaction to produce artificial starches with different levels of branches [114].

Polymer Modifications Glycosyltransferases have also been utilized for glycan chain modifications, especially at outer or terminal positions [22]. Transglutaminases are acyl transfer enzymes that catalyze the condensation of glutamine and lysine residues of proteins [115, 116]. A calcium-independent microbial transglutaminase has been reported by Payne et al. [92] to cross-link the protein in gelatin-chitosan blends.

12.1.5 Lyases (EC 4)

A lyase is an enzyme that catalyzes the breaking of various chemical bonds by means other than hydrolysis and oxidation. It often forms a new double bond or a new ring structure. In practical terms, lyases are alternatives to hydrolases in degrading polymers. In the polysaccharide area, a number of lyases are known and sometimes used, e.g., pectin lyase, pectate lyase, xanthan lyase, alginate lyase, hyaluronate lyase, and heparin lyase.

12.1.6 Isomerases (EC 5)

An isomerase converts a molecule from one isomer to another, often involving bond breakage and reformation. For example, alanine racemase catalyzes the conversion of L-alanine to D-alanine, and glucose isomerase converts glucose to fructose. In polymer science, isomerases are less often used. Some examples in polysaccharides include the use of epimerases to convert mannuronate to guluronate in alginates [117–120] and to carry out C5 epimerization of oxidized konjac glucomannan [121] and oxidized galactomannan [122].

12.1.7 Ligases (EC 6)

DNA ligase is involved in DNA synthesis during replication. Other ligases are also useful in various biochemical processes. For conventional polymer synthesis or modification, the use of this category of enzymes is rather rare.

12.2 Enzymatic Modification of Polysaccharides

Polysaccharides are polymeric carbohydrates found in nature that consist of monosaccharides attached together by glycosidic bonds. Some of them, like starch and glycogen, are important for food and nutrition. Many of them (e.g., cellulose, hemicellulose, starch, chitin, guar, xanthan, carrageenan, and alginate) are industrially relevant materials, used as thickeners, gelling agents, stabilizers, interfacial agents, flocculants, and encapsulants in a variety of applications [123–126]. Yet, the polysaccharides found in nature often do not have the optimal properties needed for specific applications, and chemical modifications are needed in order to produce desirable and competitive commercial products [127–129]. Particularly successful are chemically modified cellulose and starch, with a range of derivative products available. For example, commercially available cellulosic derivatives include cellulose acetate, carboxymethyl cellulose (CMC), hydroxyethyl cellulose (HEC), and methyl cellulose (MC). Modified starches include cationic starch, hydroxyethyl starch, starch acetate, and starch phosphate.

It is well known that enzymatic reactions can complement the chemical modification reactions in suitable cases [130–133]. Examples of useful enzyme-catalyzed reactions of polysaccharides include (1) molecular weight reduction, (2) addition of charge, (3) addition of polar group, (4) hydrophobic modification, and (5) formation of reactive oligomers. For illustration, some enzyme-catalyzed modification reactions are shown below.

12.2.1 Molecular Weight Reduction

Through the use of an appropriate hydrolase, the molecular weight of a polysaccharide can be reduced [133]. An example of an advantageous reduction of molecular weight is shown for "biostable" cellulosic derivatives [134, 135], where CMC or HEC can be deliberately treated with a cellulase enzyme to hydrolyze substrate sites that are susceptible to enzymatic action. The resulting cellulosic products are then resistant toward microbial degradation.

In the recent literature, there has been some interest in degrading the molecular weight of glycosaminoglycans, particularly hyaluronic acid in order to yield new bioactive compounds. Thus, in one study, many enzymes and microbes were screened for their ability to degrade a marine exopolysaccharide comprising mostly hyaluronic acid, and one candidate was found to be particularly suitable [136]. Moreover, the degradation of hyaluronic acid by hyaluronidase was studied with a protein nanopore [137].

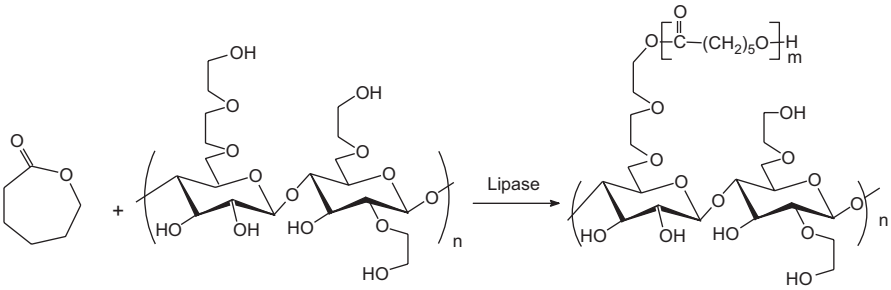
Another example of a functional product is low-molecular-weight guar, which can be used as a dietary fiber and bioactive substance [138, 139]. The molecular weight reduction can be done chemically or enzymatically [140, 141]. In an earlier study [142], the molecular weight reduction of guar was examined combinatorially with four enzymes (lipase, hemicellulase, pectinase, and protease). The hemicellulose/protease combination gave the greatest molecular weight reduction. That study pointed out the importance of protein (up to 7% in guar) in contributing toward the viscosity of guar.

12.2.2 Addition of a Polar Substituent

The nature of a substituent on a polysaccharide can significantly change the properties of the polysaccharide. This is why so many cellulose and starch derivatives have been made. Whereas most of the derivatizing reactions have been conducted through chemical means, there have been some enzymatic reactions. A simple example is the reaction involving lactose and β-galactosidase to insert a galactose moiety either at the chain ends of an oligosaccharide [143] or at the end of ethylene oxide units in HEC [144]. Another fruitful approach is to enzymatically graft a polymer onto a polysaccharide, as shown in the two examples below.

In the first example, HEC, when deposited as a film, could be grafted by caprolactone in bulk, using lipases derived from porcine pancreas (Scheme 12.1) [145]. The product had a degree of substitution between 0.10 and 0.32 on the anhydroglucose basis. The reaction demonstrates that lipase-catalyzed ring-opening polymerization can be employed to graft hydrophobic polyesters onto hydrophilic cellulose-based polymers.

The second example is the chemoenzymatic synthesis of amylose-grafted cellulose [146]. A maltoheptaose was chemically introduced to the amine-functionalized



Scheme 12.1 Modification of hydroxyethyl cellulose (HEC) via graft copolymerization of ε-caprolactone by a lipase catalyst

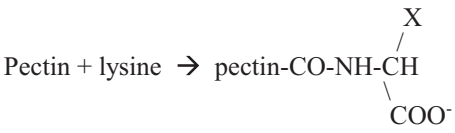
cellulose by reductive amination to produce the maltoheptaose-grafted cellulose. Then, the phosphorylase-catalyzed enzymatic polymerization of glucose 1-phosphate from the graft-chain ends on the cellulose derivative was performed, giving the amylose-grafted cellulose. The obtained material was shown to form gels and films [146].

$$\text{cellulose} + \text{maltoheptaose} \xrightarrow{\text{chemical}} \text{cellulose}\ (\text{glc})_7 \xrightarrow[\text{phosphorylase}]{\text{glucose 1 phosphate}} \text{amylose grafted cellulose}$$

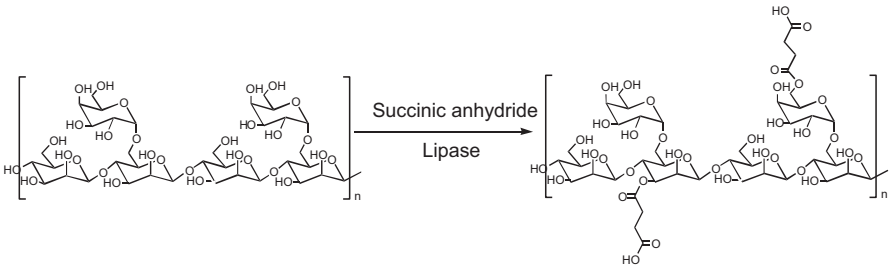
12.2.3 Addition of Charge

Sometimes it is useful to introduce an electrical charge to a polysaccharide to impart special properties. An example of the addition of an anionic group is shown by the lipase-catalyzed reaction of an anhydride (e.g., succinic and maleic) [147, 148]. Thus, lipase AK (*Pseudomonas* sp., from Amano) was found to have excellent activity for this reaction on guar, giving succinated guar (Scheme 12.2). The same reaction was also reported for HEC [147].

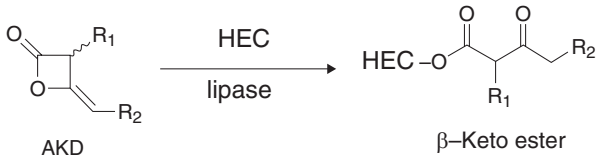
The enzyme-catalyzed addition of a cationic functionality to a polysaccharide is less common. An example is the papain-catalyzed amidation of high methoxy pectin with lysine and other diamines as shown below [149].



where X is $-(CH_2)_4-NH_2$ for lysine but can also be $-CH_2-(CH_2)_5-NH_2$, $-CH_2-(CH_2)_2$-imidazole, or $-CH_2-(CH_2)_2$-guanidine [149]. The active enzyme appeared to



Scheme 12.2 Modification of guar by succinic anhydride catalyzed by lipase



Scheme 12.3 Lipased-catalyzed modification of hydroxyethyl cellulose (HEC) with alkyl ketene dimer (AKD)

be a minor component in papain. The products were reported to show enhanced viscosity and gel-forming properties.

Another example of the addition of a cationic functionality was shown for the protease-catalyzed reaction of CMC with 1,6-hexamethylenediamine to form an amide with a free amine end group [148].

12.2.4 Hydrophobic Modification

A more commonly encountered reaction is hydrophobic modification. Because polysaccharides are usually water-soluble, a hydrophobic moiety on the polysaccharide tends to associate with each other to increase the viscosity of the polysaccharide solution at low shear rates [150]. These hydrophobically modified polysaccharides can often be used as surfactants or rheology modifiers. Whereas this reaction is mostly done chemically, an enzyme has been used in selected cases to facilitate the reaction or to provide additional hydrophobic structures. An example of the lipase-catalyzed reaction of alkyl ketene dimer with HEC is shown in Scheme 12.3 [151], where $R_1 = R_2 = C_{14}H_{29}$, for a ketene dimer derived from palmitic acid. Although the reaction can be done without an enzyme at higher temperature, the use of the enzyme increases the yield and decreases the reaction temperature. This enzyme-catalyzed reaction has also been carried out on starch [152].

Another example is the hydrophobic modification of HEC, using lipase and vinyl stearate [153]. The stearoyl functionality forms an ester with the –OH of HEC with the loss of acetaldehyde, thereby providing a hydrophobic substituent on HEC.

12.2.5 Formation of Reactive Functionalities

Enzymatic reactions can be used to convert the functionality of one substituent on a polysaccharide to another. A good example is the conversion of C6 alcohol on a galactose to an aldehyde through galactose oxidase [87–89, 154–156]. Another reaction is the addition of an acrylate functionality onto HEC with vinyl acrylate and a lipase. The acrylate functionality on HEC can then be reacted further to form additional derivatives [153].

12.3 Enzymatic Modification of Proteins

Enzymes are proteins themselves, and thus one may expect the use of enzymes in protein modification to be very productive. Indeed in living systems, a lot of proteins are modified in physiological processes. However, from the point of view of industrial product development, the largest applications of enzymatic modification of proteins happen in the food arena. As in other polymers, sometimes a given protein may not have the optimal properties needed for an application, and protein modifications are needed. In the food area, chemical modifications have to be done with care to ensure that the reagents used are nontoxic (or else fully reacted or removed after the reactions), the reaction products are nonhazardous, and all by-products involved in the reactions are compatible with FDA regulations for food use. In contrast, the enzymes are more specific in their reactions (thereby generating less by-products) and usually involve milder experimental conditions (such as lower temperature and aqueous solvents), and many enzymes are nontoxic themselves, such that the FDA requirements can be more easily met.

An excellent early review on this topic was provided by Whitaker [157]. More recent reviews included Filice et al. [158], Kumar et al. [159], Panyam and Kilara [160], and Chobert et al. [161]. For the enzymatic reactions on food proteins, the most common reactions are hydrolysis and cross-linking. Other modifications have been used with less frequency, such as phosphorylation/dephosphorylation, glycosylation, and oxidation/reduction. The following review was based on a Chemical Abstracts search of enzymatic modifications of food proteins covering the years 2012–2018.

12.3.1 Hydrolysis

For food applications, hydrolysis is the most common way of modifying proteins. Since 2012 at least 27 papers and reviews have appeared on enzymatic hydrolysis of food proteins. These papers can be grouped into four categories. In the first category, 21 papers deal with enzymatic hydrolysates of proteins in order to improve

their properties, to enhance their use in normal or clinical nutrition, and to produce bioactive substances or functional food ingredients. A wide range of food proteins were enzymatically hydrolyzed, including soy proteins [162, 163], sesame proteins [164–166], milk proteins [167–171], fish proteins [172, 173], egg proteins [174], canola meal protein [175], sunflower protein [176], flaxseed protein [177], rice bran protein [178], wheat protein [179], and pea protein [180, 181]. The properties being studied included emulsification, foaming, antioxidant, and bioactivity. Canola meal protein [175], milk protein [167], and sunflower protein [176] were specifically subjected to enzymatic hydrolysis in order to improve their poor water solubility. The enzymes used included mostly proteases, e.g., Alcalase®, papain, pepsin, chymotrypsin, trypsin, neutrase®, bromelain, and Flavourzyme®.

The second category of papers in the enzyme hydrolysis area deals with encapsulation. Thus, soy protein was degraded with Alcalase® in order to make oil-in-water emulsions that encapsulated tocopherol [182]. Sunflower protein was also enzymatically hydrolyzed to form microparticles and emulsions that encapsulated tocopherol [183]. Hydrolysis was combined with enzymatic cross-linking for soy and sunflower protein [184]. In all three cases, oil retention decreased with hydrolysis but increased with fatty acid acylation.

The third category consists of publications that use enzyme hydrolysis to reduce allergenicity of the protein. Thus, whey protein was treated with trypsin [185] and chymotrypsin and bromelain in combination with high hydrostatic pressure [186] in order to develop specialty products like infant formulas, geriatric products, diet foods, and high-energy food supplements.

There are also some papers that carried out enzymatic hydrolysis of proteins to gather fundamental information, e.g., use of Alcalase® and Flavourzyme® to degrade potato protein to get amino acid composition [187] and degradation of casein to determine its chemical structure and molecular weight distribution [188].

12.3.2 Cross-Linking

In the past 5–6 years, there have been at least 23 papers on enzyme-modified food proteins through cross-linking. Most of them used transglutaminase (TG) although tyrosinase and laccase were sometimes involved as well. In an excellent paper [189], the reaction conditions for TG, *T. hirsuta* laccase, and two types of tyrosinase (*A. bisporus* and *T. reesei*) were investigated. In raw milk, *T. reesei* tyrosinase was the only enzyme that induced intermolecular protein cross-linking. After heat treatment of milk, both TG and *T. reesei* tyrosinase were able to form covalently linked oligomers. β-Casein was the most readily cross-linked protein. Susceptibility of the whey proteins to enzymatic modification was restricted due to their compact globular structure. After heat treatment, which partially unfolded the whey protein molecules, both TG and *T. reesei* tyrosinase were capable of cross-linking whey proteins, whereas *A. bisporus* tyrosinase and laccase were inefficient [189].

Additionally, two other publications also studied the reaction parameters for tyrosinase [190] and laccase [191].

Most of the protein cross-linking papers deal with improved functional properties achieved, particularly with TG. In general, cross-linking affects solubility, gelation, emulsification, foaming, viscosity, and water-holding properties of the protein. Two reviews cover these effects well, one dealing with proteins in general [192] and one with dairy proteins [193]. Eight more papers deal with improvements in the properties of specific proteins with TG treatment. These include milk proteins [194], casein [195], chicken proteins [196, 197], oat and faba bean protein [198], pea protein [199], peanut protein [200], and fish protein [201]. Four papers studied the composite proteins made through cross-linking of two different proteins and their functional properties, e.g., casein/gelatin [202], and soy protein/gelatin [203–205]. Although the papers cited in this paragraph all deal with TG, the paper on oat and faba bean protein [198] also looked at the effect of tyrosinase on the colloidal and foaming properties, and one paper on soy protein/gelatin composite [204] studied the combined effect of TG and trypsin on the functional properties. A separate paper assessed the use of glucose oxidase to cross-link pork myofibrillar protein [206]. Glucose oxidase catalyzes the oxidation of glucose into gluconic acid and H_2O_2, which can degrade to the hydroxy radical. When applied to pork myofibrillar protein, firmer and more elastic gels were obtained by the enzymatic route than by the Fenton reagent at comparable H_2O_2 levels [206].

Two papers deal with a different use of cross-linking in food proteins. A review [207] indicated that gluten-free flours lack the viscoelastic network required to resist gas production and expansion during baking. Enzymatic cross-linking of gluten-free flours was proposed for creating protein aggregates that mimic gluten functionality but also for modifying proteins to improve their functionalities. Another paper was published on gluten-free amadumbe flour [208], where laccase was used; rheological data confirmed the presence of cross-linking due to protein and polysaccharide reactions.

One paper on wheat protein [209] pointed out that TG could decrease the immunoreactivity of the protein. Even greater decrease was observed for the combined TG/hydrolase treatments. Another paper on TG treatment of whey protein [210] noted that protein cross-linking and denaturation could change the rate of digestion of the resulting food and may be useful for people with special digestive needs.

One paper studied films made with soy protein and TG [211]. The mechanical properties of the films were measured. It was pointed out that such a system may be a suitable bioplastic.

12.3.3 Phosphorylation/Dephosphorylation

In nature the phosphorylation of protein is involved in many cellular processes, such as signal transduction, gene expression, cell cycle, cytoskeletal regulation, and apoptosis [212, 213]. Since the early work by Burnett and Kennedy [214],

numerous enzymes are known that carry out phosphorylation and dephosphorylation [157, 212]. For the purpose of in vitro synthesis of industrial proteins, the kinase reaction seems to be preferred. For example, two milk proteins, β-casein and α-lactalbumin, were compared as substrates for casein kinase from bovine mammary gland [215]. Soybean proteins were enzymatically phosphorylated with the catalytic subunit of cAMP-dependent protein kinase [216, 217]. Some of the functional properties of the phosphorylated soy protein were also reported [218, 219]. A 2010 review on the phosphorylation of food proteins by both chemical and enzymatic methods has more details [220].

A good example of both phosphorylation and dephosphorylation is given for genetically engineered spider silk (though not a food protein) [221]. In that publication, the protein was phosphorylated with cyclic AMP-dependent kinase and dephosphorylated with calf intestinal alkaline phosphatase.

12.3.4 Glycosylation

The Maillard reaction is well known in food chemistry, where heating of amino acids and reducing sugars brings about a coupling reaction that gives food its brown color and distinctive flavor. The same reaction can be used to glycosylate proteins [222–224]. However, this chemical reaction is non-specific, requires high temperatures, and often generates undesirable colored or other by-products. In contrast, enzymatic glycosylation reactions are usually more specific and can be carried out at lower temperatures. Indeed, in physiology, protein glycosylation is an important posttranslational modification that enhances the functional diversity of proteins and influences their biological activity. Many elaborate enzymatic glycosylation routes have been identified in a host of organisms. Several reviews have appeared on these physiological pathways [157, 225, 226] and their mechanisms [227]. It was pointed out [157] that some of these pathways (often involving glycosyltransferases) can function in vitro to glycosylate proteins. Nevertheless, in practical terms, these reactions tend to be more difficult to handle and probably less amenable to popular usage.

Two recent developments are worthy of note. In one approach [228], tyrosinase was used to convert tyrosine residues in gelatin to o-quinone moieties, which then underwent nonenzymatic reactions with nucleophilic amino groups of chitosan to furnish in vitro conjugation of gelatin to chitosan. In a variation of this approach [229], protein-oligosaccharide conjugates were produced by laccase and tyrosinase through coupling of the tyrosine side chains of α-casein and phenolic acids of hydrolyzed oat spelt xylan. The second approach used transglutaminase (TG) to conjugate an amino-containing saccharide with soy protein [230–232], caseinate [233–235], fish gelatin [236], and actomyosin [237]. Thus far, glucosamine and chitosan (up to 5 kDa in molecular weight) have been found to be suitable saccharides for this reaction. Enhanced emulsification, rheology, and solubility were reported for the glycosylated proteins [230, 234, 237]. Further development of these synthetic methodologies is expected in the future.

12.3.5 Redox Reactions

Like all polymers, proteins are susceptible to oxidative reactions. In fact, the normal aging process involves an increase in the accumulation of damaged polymers, and the buildup of oxidized proteins is regarded as a hallmark of cellular aging [238, 239]. Proteins can undergo oxidation in many different ways [240]. As regards enzyme-catalyzed oxidations, the greatest occurrence in the literature is the use of oxidative enzymes to carry out protein cross-linking reactions (as shown in Sect. 12.3.2) and conjugation of casein with oat spelt xylan (Sect. 12.3.4). Specific enzymatic oxidation reactions may be useful in selected applications, e.g., horseradish peroxidase- or lactoperoxide-catalyzed iodination of proteins at tyrosine residues [157], but these uses are relatively infrequent.

12.4 Enzymatic Modification of Fats and Oils

Fats and oils are used extensively for food and industrial applications. Although many chemical modifications are known and practiced, enzymes offer specific advantages, such as chemo-, regio-, and stereoselectivities and mild reaction conditions. A lot of work has been done, and this area has been often reviewed [241–249].

For convenience, the enzymes used for lipids can be grouped in four categories: (1) lipases, (2) enzymes for oxy-functionalization, (3) phospholipases, and (4) special enzymes obtained by protein engineering.

12.4.1 Lipases Used for Lipid Modifications

The lipases are the most commonly used enzymes for lipids. The lipase reactions can be separated into five types [247]: hydrolysis, esterification, alcoholysis, acidolysis, and transesterification. A large number of lipases from different microorganisms are commercially available. Immobilized lipases are also available, the most well known being Novozym® 435 from *Candida antarctica* B lipase. These enzymes have been used extensively to make triglycerides, diglycerides, monoglycerides, and structured lipids, as documented in the reviews [241–249].

12.4.2 Enzymes for Oxy-functionalization

These include P450 monooxygenases, hydratase, hydroxylase, lipoxygenase, and diol synthase [241, 250]. Cytochromes P450 (CYPs) are hemoproteins that are found widely in different organisms [251]. In the P450 monooxygenase reaction,

one atom of oxygen is inserted into the aliphatic position of an organic substrate (RH), while the other oxygen atom is reduced to water:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

For example, a saturated fatty acid can be converted into a ω-hydroxy fatty acid in this way [251, 252].

The fatty acid double-bond hydratase catalyzes the addition of water to the cis double bond in fatty acids to form a hydroxy fatty acid. It works with a cofactor like FAD. Thus, oleic acid is converted to 10-hydroxystearic acid and linoleic acid to 10-hydroxy-9-cis-octadecenoic acid [253, 254]. The enzyme 12-hydroxylase converts oleic acid into 12-hydroxyoleic acid (ricinoleic acid) [255]. Diol synthase converts an olefin in fatty acid to a diol [250, 256]; for example, 9-hexadecenoic acid was converted to 9,10-dihydroxyhexadecanoic acid [256].

Lipoxygenases are enzymes under the family of dioxygenases, which catalyze the synthesis of hydroperoxy fatty acids of polyunsaturated fatty acids (PUFAs) having one or more cis,cis-pentadiene units by insertion of molecular oxygen [257, 258].

12.4.3 Phospholipases

A phospholipase is an enzyme that hydrolyzes phospholipids into fatty acids and other lipophilic substances. Several types of phospholipases are known that catalyze reactions on different bonds of the glycerophospholipid [259]. The major use of these enzymes is to remove phospholipids from natural fats and oils, a process known as degumming. These enzymes have also found applications in food areas [260].

12.4.4 Special Enzymes Obtained by Protein Engineering

A large number of enzymes have been subjected to protein engineering in order to improve their attributes or properties. A major accomplishment [261] was the creation of a lipase that was highly selective for trans- and saturated fatty acids when applied to partially hydrogenated vegetable oil. Those fatty acids, identified as a major risk factor for human health, can then be removed by selective enzyme hydrolysis. Another useful modification of lipase was the chain-length selectivity; thus the medium-chain fatty acids can be selectively hydrolyzed [262]. In yet another modification, the stability of a *R. oryzae* lipase toward oxidation was improved [263, 264]. These developments have been given in more detail in a review written by Bornscheuer [243].

Another interesting development is the use of the yeast *Candida tropicalis* that was engineered with a select cytochrome P450 that converted methyl tetradecanoate

to 14-hydroxytetradecanoic acid with high yield and purity [265]. This ω-hydroxy fatty acid was then made into a polyester through chemical means (titanium-catalyzed polymerization) [266].

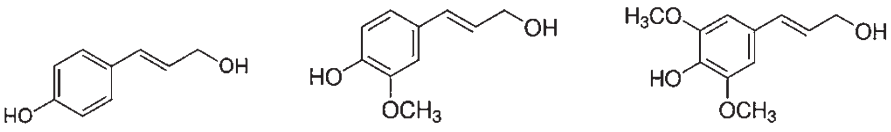
12.5 Enzymatic Degradation of Lignin

Lignin is one of the major components of woody tissue, together with cellulose and hemicellulose. It is an irregular, randomly cross-linked, heterogeneous polymer of three phenylpropanoid monomers: *p*-coumaryl alcohol (I), coniferyl alcohol (II), and sinapyl alcohol (III) (Scheme 12.4). The polymer contains both C-O-C (ether) and C-C linkages, the proportions of each varying depending on wood type. Most of the hydroxy group on the ring is methoxylated. In wood, the lignin polymer also forms covalent bonds with hemicellulose and probably cellulose.

Only a few enzymes are known to modify lignin. The ones that are most well-known are the oxidative enzymes, such as the heme-containing glycoproteins, lignin peroxidase (LiP), manganese peroxide (MnP), and versatile peroxidase (VP), and the copper-containing glycoprotein laccase. The hemoproteins (LiP, MnP, and VP) require H_2O_2 for their action, whereas laccase needs oxygen. The activity of all four enzymes (particularly laccase) can be enhanced with mediators. These enzymatic systems have been extensively studied [267–270]. They are used particularly in the pulp and paper industry in order to bleach pulp or to optimize the use of plant biomass.

Some recent development can be noted here. In an interesting paper [271], lignin was reacted with laccase and glucosamine or the tripeptide glycyl-tyrosyl-glycine in acetone/water mixture. IR and NMR data suggested that glucosamine and the tripeptide were somehow grafted onto the lignin. The detailed structures of the products and the mechanism were yet to be deciphered.

One of the active areas of research involves the mediators for laccase. In one paper [272] some phenolic compounds that seemed to be natural laccase mediators were studied; these natural phenolic compounds are eco-friendly and may facilitate the use of laccase in biorefining. In another paper [273], the mechanism of action for mediators was studied with ESR; the results suggested that electron shuttling by mediators was not a significant contributor to enhanced laccase oxidation of lignin. Yet another paper [274] addressed the question whether laccase can directly cata-



I II III

Scheme 12.4 Structures of *p*-coumaryl alcohol (I), coniferyl alcohol (II), and sinapyl alcohol (III)

lyze modification of lignin via catalytic bond cleavage. (Laccase is known to catalyze polymerization of lignin.) The authors concluded that bond cleavage indeed takes place for low-molecular-weight lignin model compounds, but for lignin itself, the laccase-mediator combination is needed.

In a review article, Gonzalo et al. [267] noted that several bacterial enzymes might be involved in lignin degradation, including bacterial laccases, glutathione-dependent β-etherases, superoxide dismutases, catalase-peroxidases, and bacterial dioxygenases. Additional delignification enzymes were discussed by Pollegioni et al. [268], such as β-etherases, demethylases, peroxidases, mycelium-associated dehydrogenase, and other oxidoreductases. The use and advantages of immobilized ligninolytic enzymes were delineated in a review article [275]. Laccase engineering, including both rational design and directed evolution, were reviewed in another publication [276].

12.6 Perspectives

It is clear from the above review that enzymatic modifications of polymers constitute an important and vibrant area of research and development. This is a multidisciplinary field, requiring the expertise in polymer chemistry (for polymer handling, polymer properties, and structure/property relationships), organic chemistry (for synthesis and workup procedures), biochemistry (for enzymology), and chemical engineering (for process development). Thus, a multidisciplinary team is often needed for an optimal research and development endeavor.

Several current trends favor the continued growth of this field. The first trend is the increasing popularity of green polymer chemistry. Indeed, the materials covered in this chapter (e.g., polysaccharides, proteins, triglycerides, and lignin) are all bio-based, degradable, renewable, and sustainable. They can be recycled if needed so that the resulting products would not produce polymer waste and cause disposal problems. The enzymes are themselves proteins, usually nontoxic, and easily disposable. The enzymatic reactions are often done in water, thereby minimizing the hazards associated with organic solvents. The enzymes are frequently specific in their reactivity and produce less by-products relative to chemical reactions. The enzymatic reactions usually entail lower temperatures, which minimize the energy usage. Thus, the use of enzymes is fully compatible with green chemistry.

The second trend is the continued development of gene technologies. Indeed, new enzymes can be (and have been) developed as needed for specific reactions. Improved enzymes can be obtained to enhance a specific functionality or to meet the demands of a given process. In the future, many advances in enzyme technologies are expected, and these should be helpful as researchers seek new or improved ways to modify the polymers or to optimize their processes.

An inherent advantage of agro-based materials like polysaccharides, proteins, oils/fats, and lignin is their availability in large quantities and their relatively low cost. As natural polymers, they are often amenable to enzymatic action. Thus, the

combination of agro-based materials and enzyme reactions should be a powerful platform for the development of green polymeric products and a useful tool for postharvest agricultural technology.

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