Oxygen Permeability and Biodegradability of Polyuronic Acids Prepared from Polysaccharides by TEMPO-Mediated Oxidation

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Polyuronic acids, i.e., amylouronic acid, cellouronic acid and chitouronic acid, were prepared from starch, cellulose and chitin, respectively, by the 2,2,6,6-tetramethylpiperidine-1-oxy radical (TEMPO)-mediated oxidation, and their gas-barrier properties and biodegradability were studied in consideration to use the polyuronic acids as flexible packaging films or coating materials. Cellouronic acid and amylouronic acid had excellent oxygen-barrier properties similar to that of poly(vinyl alcohol) (PVA), while chitouronic acid did not. The regular chemical structures of the former two polyuronic acids with no bulky substituents or adducts may have brought about such high oxygen-barrier levels. An oxidized product prepared form fine microcrystalline cellulose by the TEMPO-mediated oxidation was not completely dissolved in water, but became a paste. However, this paste also formed sufficiently smooth films by coating, and had good gas-barrier property. All polyuronic acids prepared were biodegradable; cellouronic acid and chitouronic acid had high degrees of biodegradability, while amylouronic acid had quite low value. These various characteristics are significant for end use of these new polyuronic acids as gas-barrier materials for biodegradable packaging.

KEY WORDS: TEMPO; Polyuronic acid; biodegradation; gas-barrier; cellulose; amylose; chitin.

INTRODUCTION

Gas-barrier property is one of the most indispensable characteristics for flexible packaging films. Aluminum foil, metal- or metal oxide-coated films, poly(vinylidene chloride), poly(vinyl alcohol) (PVA) and others have been used as conventional gas-barrier films. It is well known that syndiotactic PVA has better oxygen-barrier properties than atactic PVA, probably because the former has hydroxyl groups with high stereo-regularity. However, these metals and synthetic polymers are facing some environmental problems, when their wastes are incinerated or buried in the ground. On the other hand, because polysaccharides including polyuronic acids have numerous hydroxyl groups to form intra- and inter-molecular hydrogen bonds, these films are expected to have superior gas-barrier properties with biodegradability for food protection and preservation.

Several papers have already been reported in terms of oxygen-permeability of polysaccharides for edible films [1, 2]. Effects of amylose/amylopectin ratios in starch films on oxygen-barrier properties were also studied, and some amylose samples had superior gas-barrier properties [3, 4]. However, more excellent and functional gas-barrier materials

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are still required as environmentally friendly packaging films for various end uses.

Nooy et al. [5] first reported that some water-soluble polysaccharides were convertible to the corresponding polyuronic acids having homogeneous chemical structures by selective oxidation of the C6 primary hydroxyl groups with NaClO and catalytic amounts of 2,2,6,6-tetramethylpiperidine-1-oxy radical (TEMPO) and NaBr in aqueous media. We also prepared β-1,4-linked polyglucuronic acid (cellouronic acid) and β-1,4-linked poly-N-acetylglucosaminuronic acid (chitouronic acid) from regenerated cellulose and chitin, respectively, by the TEMPO-mediated oxidation [6]. In the case of oxidation of chitin, we found that regenerated chitin prepared from its aqueous NaOH solutions or N-acetylated chitosan with 81–93% degrees of N-acetylation gave chitouronic acids with sufficiently high degrees of polymerization around 300 by the TEMPO-mediated oxidation [7], while regenerated chitin prepared from chitin/LiCl/N,N-dimethylacetamide solutions gave chitouronic acid with degree of polymerization of only 50 [8]. Because amylouronic acid, cellouronic acid and chitouronic acid have mostly homogeneous chemical structures with numerous hydroxyl groups, their films have potentials to be used as high level gas-barrier materials.

In this paper, oxygen-barrier properties of poly(ethylene terephthalate) (PET) films coated with some polysaccharides, cellulose derivatives, microcrystalline cellulose and polyuronic acids prepared from starch, cellulose, chitin and microcrystalline cellulose by the TEMPO-mediated oxidation were investigated to use the polyuronic acids as new packaging materials. Moreover, biodegradabilities of polyuronic acids and other materials were compared under the same conditions by the compost test method.

EXPERIMENTAL

Materials

Water-soluble starch (Acros Co. Ltd., USA), chitosan with 25% degree of N-acetylation (Dainichi Seika Co. Ltd., Japan), pectin, alginic acid sodium and ammonium salts (Wako Pure Chemicals Co. Ltd., Japan), hydroxyethyl cellulose (Kanto Chemical Co. Ltd., Japan), carboxymethyl cellulose with 0.7 degree of substitution (CMC, Aldrich Co., Ltd., U.S.A.), poly(vinyl alcohol) (PVA: P124, Kurare Co., Ltd., Japan) and fine microcrystalline cellulose with average particle size of 3 μm (Ceolus® FP-03; Asahi Kasei Co., Ltd., Japan) were used without further purification treatments. Chitosan was converted to chitin or N-acetylated chitosan with 93% degree of N-acetylation according to the method reported by Hirano et al. (1976) [9]. The water-soluble starch, commercial regenerated cellulose fiber (Bemliese®; Asahi Kasei), N-acetylated chitosan and microcrystalline cellulose were used as starting materials for the TEMPO-mediated oxidation as described in the next section. Commercial PET film (Lumirror® P-60 12 μm in thickness; Toray Co., Ltd., Japan), whose surface was treated by corona discharge for increasing hydrophilicity, was used as a base film for the oxygen permeability test. TEMPO, sodium bromide, about 11% sodium hypochlorite solution, and other chemicals and solvents were of laboratory grade (Wako Pure Chemicals), and used without further purification.

TEMPO-Mediated Oxidation of Polysaccharides

Cellouronic acid (β-1,4-linked polyglucuronic acid Na salt), amylouronic acid (α-1,4-linked polyglucuronic acid Na salt) and chitouronic acid (β-1,4-linked poly-N-acetylglucosaminuronic acid Na salt) (Fig. 1) were prepared from regenerated cellulose,
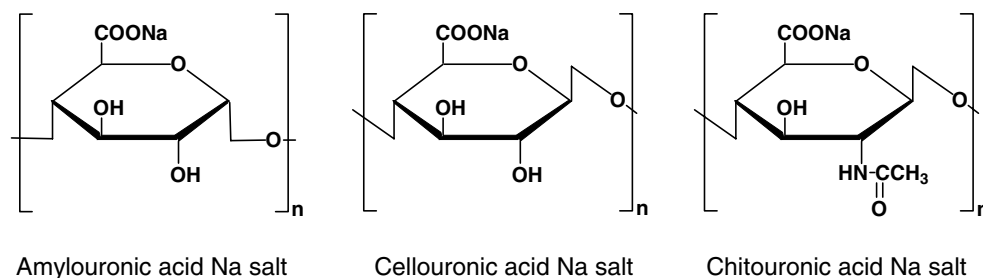

Fig. 1. Chemical structures of amylouronic acid, cellouronic acid and chitouronic acid prepared from water-soluble starch, regenerated cellulose and N-acetylated chitosan with 93% degree of N-acetylation by the TEMPO-mediated oxidation.

water-soluble starch and N-acetylated chitosan, respectively, by the reported methods [6, 7, 10]. Complete oxidation of the C6 primary hydroxyl groups to sodium carboxylates for these three polysaccharides was confirmed by NMR. Polyuronic acids having free carboxyl form were prepared from their sodium salts by acidifying their aqueous solutions with diluted HCl and isolation as precipitates by adding aqueous ethanol to the acid solutions. Complete conversion from sodium salts to free carboxyls for amylouronic acid and chitouronic acid was confirmed by ^{13}C-NMR, because these two polyuronic acids with free carboxyl form were soluble in D_2O as well as water. Microcrystalline cellulose was oxidized and isolated by the same procedure as that for preparing cellouronic acid [6], although complete dissolution of microcrystalline cellulose in the aqueous oxidation medium was not achieved.

Oxygen Permeability

Each polymer was dissolved in water to prepare a 5 wt% solution. Starch and PVA were first suspended in cold water, and heated until clear solutions were obtained. Aqueous acetic acid (0.1 M) was used to dissolve chitosan. These polymer solutions were cast and coated on the PET film using a bar-coater #10. Thin polymer layer about 1 μm in thickness on the PET film was obtained by heating the coated film at 120°C for 30 min. Microcrystalline cellulose and its oxidized product were suspended in water to prepare pastes with 5 wt% consistency, and these pastes were coated on the PET film by the same procedure as those for the polymer solutions described above.

Oxygen-permeability rates through the polymer-coated PET films were determined at 30°C and either 70 or 100% relative humidity by means of a MOCON OX-TRAN 5/50 (Modern Controls Inc., USA) under the standard conditions (ASTM 3985). Each measurement was continued until the O_2-permeability rate reached a stable value. The permeability was expressed as volumes of oxygen (cm^3) permeated through the 1 m^2 film at 1 Pa for 1 day.

Biodegradability

Biodegradability of polysaccharides, cellulose derivatives and polyuronic acids were evaluated by determining CO_2 formed from the samples in a soil chamber containing microorganisms at 35°C with carrier gas flow rate of 40 mL/min using a Micro Oxidative Degradation Analyzer (Saita Tekkoujo, Japan). Here, the soil chamber was prepared by sufficient mixing of the polysaccharide sample (10 g), water (200 g), compost (60 g) and sea sand (320 g).

Analyses

X-ray diffraction patterns were collected using a vertical powder diffractometer (Rigaku RU-300, Japan), where Ni-filtered CuK$_\alpha$ radiation (λ, 0.154 nm) was used. Turbidity of the films was determined by using a haze meter (NDH 2000; Nippon Denshoku, Co., Ltd., Japan). Adhesion strength of microcrystalline cellulose or its oxidized product coated with the PET film was evaluated by a cellophane tape-pealing method.

RESULTS AND DISCUSSION

Oxygen Permeability of Water-Soluble Polyuronic Acids

Chemical structures of three polyuronic acids prepared from starch, cellulose and chitin by the TEMPO-mediated oxidation are shown in Fig. 1. These polyuronic acids with both sodium salt and free carboxyl forms gave smooth thin layers on the PET film by the coating method (Fig. 1). Although amylouronic acid and chitouronic acid with free carboxyl form were completely soluble in water, a slightly cloudy solution due to insoluble fractions was obtained for cellouronic acid with free carboxyl form. However, all these polyuronic acids with free carboxyl form turned to transparent and smooth thin films on the PET.

The results of permeability of these films are summarized in Table I. Although the oxygen-permeability rates clearly decreased by coating of the polysaccharides-related compounds on the PET film, the rates varied, depending on the chemical structures of the coating materials. Especially, amylouronic acid and cellouronic acid had quite high oxygen-barrier levels similar to that of PVA. Moreover, the structures of carboxyl groups, either Na salt or free carboxyl form, had little influence on the results. The presence of carboxyl groups in the polymers is not necessarily required for high oxygen-barrier levels, because the levels of CMC, pectin, alginates and chitouronic acid were clearly lower than those of amylouronic acid and cellouronic acid. Viscosities of the aqueous solutions or

Table I. Oxygen-Permeability Rate of PET Films Coated with Various Polysaccharides

Polysaccharide coated on PET film	Form of carboxyl or amine	O_2 permeability (10^{-5} cm^3/m^2 day Pa)
Water-soluble starch	–	29
Chitosan	$-NH_3^+ \cdot {}^-OCOCH_3$	110
Hydroxyethyl cellulose	–	14
Carboxymethyl cellulose	$-COONa$	98
Pectic acid	$-COONa$	15
Alginic acid	$-COONa$	41
Alginic acid	$-COONH_4$	33
Amylouronic acid	$-COONa$	1.2
Amylouronic acid	$-COOH$	1.5
Cellouronic acid	$-COONa$	2.4
Cellouronic acid	$-COOH$	1.2
Chitouronic acid	$-COONa$	94
Chitouronic acid	$-COOH$	81
PVA	–	1.0
Blank PET	–	150

degrees of polymerization of the polymers do not seem to affect the oxygen-permeability rates, because aqueous solutions of CMC and alginates had viscosities clearly higher than those of amylouronic acid and cellouronic acid.

There results indicate that chemical structures of the polymers are the most significant factors influencing the resultant oxygen-barrier properties. The lowest values for amylouronic acid, cellouronic acid and PVA support the idea that polymers having homogeneous chemical structures with no bulky substituents provide high oxygen-barrier levels as film forms. Chitosan with 25% degree of N-acetylation, CMC with 0.7 degree of substitution, alginates consisting of D-mannuronic and L-guluronic acid units, chitouronic acid with 93% degree of N-acetylation are all categorized as heteropolymers in a precise sense. Furthermore, CMC, chitosan and chitouronic acid have relatively bulky substituents or adducts, such as carboxymethyl groups, acetates as counter ions of amino groups and N-acetyl groups, respectively. These bulky side structures may also have negative effects on the oxygen-barrier properties. Water-soluble starch has the heterogeneous branch structure due to the amylopectin component. As reported in the previous paper [10], however, α-1,6-linkages present in starch as the branch structure are mostly removed during the TEMPO-mediated oxidation process, thus giving almost pure α-1,4-linked polyglucuronic acid (amylouronic acid). Therefore, both amylouronic acid and cellouronic

acid can be regarded as homopolyuronic acids. Of course, intra- and inter-molecular hydrogen bond networks formed in the polymer films must consequently govern the resultant oxygen-barrier properties. Probably, the network formation must be influenced by homogeneity of chemical structures of the polymers used for coating.

Thus, the high oxygen-barrier levels of amylouronic acid and cellouronic acid are characteristic for the new polyuronic acids prepared from starch and cellulose by the TEMPO-mediated oxidation. In the case of practical use of these polyuronic acids as oxygen-barrier materials for packaging, however, increases in water- and moisture-resistance of polyuronic acids in coated films are highly required.

Oxygen Permeability of Microcrystalline Cellulose and Its Oxidized Product

It has been reported that cellulose samples having cellulose I crystal form cannot be converted to water-soluble cellouronic acid by the TEMPO-mediated oxidation [6, 11]. In this study, fine microcrystalline cellulose, which is prepared by acid hydrolysis of linter cellulose followed by mechanical grinding or milling, was used as the coating material on the PET film as well as the starting material for the TEMPO-mediated oxidation. This is because the original microcrystalline cellulose was sufficiently fine to form smooth film-like layer on the PET film by the coating method. After the TEMPO-mediated oxidation of the cellulose, all polymer components both soluble and insoluble in the oxidation medium were isolated quantitatively from the mixture by adding ethanol to the oxidation medium. X-ray diffraction patterns of the original microcrystalline cellulose and its TEMPO-oxidized product, both of which were dried from their aqueous pastes at 45°C in a vacuum oven, are shown in Fig. 2. Mechanical grinding or milling of native celluloses having cellulose I crystal form brings about partial decrystallization to amorphous, accompanied by depolymerization [12]. When the amorphous cellulose region thus obtained adsorbs water or moisture, the structure turns to cellulose II by molecular rearrangements [13]. Thus, the original sample used as microcrystalline cellulose in this study had an X-ray diffraction pattern consisting of both celluloses I and II (Fig. 2).

When this microcrystalline cellulose sample was subjected to the TEMPO-mediated oxidation, the amorphous and cellulose II regions were converted
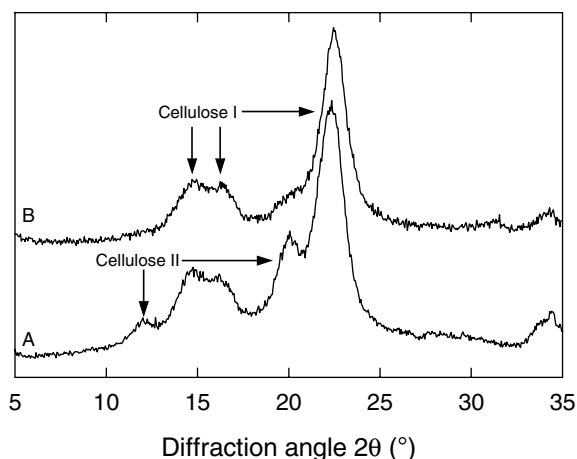
Fig. 2. X-ray diffraction patterns of the original microcrystalline cellulose (A) and its oxidized product by the TEMPO-NaBr-Na-ClO system in water at pH 10.75 (B).

to water-soluble and amorphous cellouronic acid, while the cellulose I region remained as the insoluble residue in the reaction medium [6, 11]. Thus, the cellulose II region present in the original cellulose was removed from the sample to form amorphous cellouronic acid, resulting that only cellulose I form was present in the oxidized product in Fig. 2.

Table II shows oxygen permeability of the coated films, adhesion strength with the PET film and turbidity of the film. Although the original microcrystalline cellulose gave a smooth layer on the PET film, its oxygen-barrier level and adhesion strength were quite low. Insufficient hydrogen bond networks between the cellulose particles brought about these results. In contrast, even though complete dissolution in water could not be achieved for the oxidized sample, it had a sufficient oxygen-barrier level as well as the adhesion strength. The low turbidity value of the oxidized product also supports the idea that the amorphous and cellulose II regions present in the original microcrystalline

cellulose turn to water-soluble cellouronic acid by the TEMPO-mediated oxidation.

Thus, water-soluble cellouronic acid and cellulose I residue co-existing in the oxidized product form a dense layer having high oxygen-barrier level. For comparison, the original microcrystalline cellulose and water-soluble cellouronic acid prepared from regenerated cellulose by the TEMPO-mediated oxidation were just mixed in water, and the paste thus obtained was coated on the PET film. The oxygen-barrier level of this film was sufficiently high, while the film was clearly cloudy due to the water-insoluble microcrystalline cellulose. Water-soluble cellouronic acid has, therefore, capability to form hydrogen bond networks even with solid microcrystalline cellulose surfaces. However, introduction of carboxyl groups on cellulose microcrystal surfaces to some extent by the TEMPO-mediated oxidation is required to form composite-like layers with high homogeneity and adhesion strength with the PET film.

Biodegradability of Polyuronic Acids

Environmental impacts are significant factors for recent oxygen-barrier materials in packaging. Especially, alternatives for synthetic polymers such as PVA and poly(vinylidene chloride) have been extensively explored in terms of biodegradability or chlorine-free materials. Figure 3 shows degrees of biodegradability of polyuronic acids and the other materials subjected to the oxygen permeability test. Although biodegradability of cellouronic acid and CMC has already been reported by another method [14], the materials used in this study were subjected to the biodegradation test for comparison under the same conditions using a new evaluation apparatus.

Although all compounds tested had more or less biodegradability, their rates and patterns varied. Cellouronic acid and chitouronic acid had

Table II. Oxygen-Permeability Rate of PET Films Coated with Microcrystalline Cellulose, its Oxidized Product and Microcrystalline Cellulose/Cellouronic Acid Mixture, Turbidity of the Films, and Adhesion Strength of the Polysaccharides with the PET Film

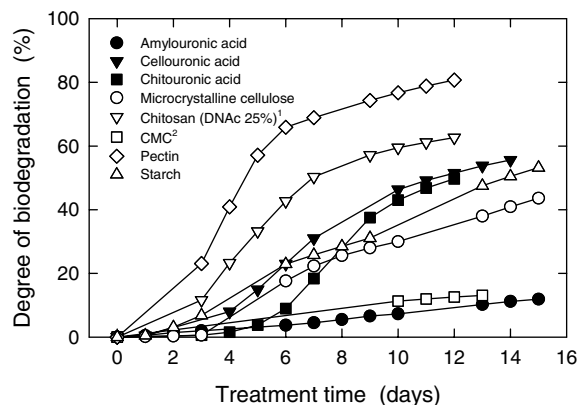| Polysaccharide coated on PET film	O_2 permeability $(10^{-5} cm^3/m^2$ day Pa)	Turbidity (Haze ratio, %)	Adhesion strength
Blank PET	150	2.9	
Microcrystalline cellulose	40	41.7	−
Oxidized product of microcrystalline cellulose by the TEMPO system	1.2	8.9	+ +
Mixture of microcrystalline cellulose and cellouronic acid (1:1 by weight)	1.8	42.6	+

Fig. 3. Biodegradability of polyuronic acids, prepared from polysaccharides by the TEMPO-mediated oxidation, and other polysaccharides. [1]DNAc: degree of N-acetylation. [2]Data are cited from the previous paper [14].

similar and sufficient biodegradability, while pectin and chitosan had much higher levels. Amylouronic acid and CMC had quite low biodegradability. In the case of CMC, artificially introduced carboxymethyl substituents may have brought about such high resistance to biodegradation. However, it is unexpected that amylouronic acid has high resistance to biodegradation similarly to that of CMC. The characteristic biodegradation behavior of cellouronic acid and chitouronic acid is significant for various end uses of these polyuronic acids.

CONCLUSION

Amylouronic acid and cellouronic acid prepared from water-soluble starch and regenerated cellulose, respectively, by the TEMPO-mediated oxidation have excellent oxygen-barrier properties irrespective of their sodium salt and free carboxyl forms. The oxygen-barrier levels of these polyuronic

acids are similar to that of PVA. However, chitouronic has an oxygen-barrier level lower than those of the former two polyuronic acids and similar to those of chitosan and CMC. The oxidized product of microcrystalline cellulose by the TEMPO system also has good oxygen-barrier property, although it is a mixture consisting of water-insoluble cellulose I region and water-soluble cellouronic acid. All the polyuronic acids prepared by the TEMPO-mediated oxidation have biodegradability. However, cellouronic acid and chitouronic acid have higher biodegradability, compared with that of amylouronic acid, whose level is as low as that of CMC.

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