Enzymatic Degradation of Thermoresponsive Poly(*N*-Isopropylacrylamide) Grafted to Carboxymethylcellulose Copolymers

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ABSTRACT: The enzymatic degradation of poly(*N*-isopropyl acrylamide) (PNIPAM) grafted to carboxymethylcellulose (CMC) copolymers with a cellulasic preparation (*Trichoderma viride*) was studied. The enzymatic activity of the cellulasic preparation against CMC and the grafted copolymers was determined by the Petterson–Porath method, while their reduced viscosity variation in the presence of the same preparation was also followed. It has been shown that the enzymatic degradation behavior depends on the copolymer composition and the reaction temperature. Reducing sugars analysis showed that the experimental values for the grafted copolymers were higher than the calculated ones. At 50°C, the enzymatic reaction is completed in about 20 min for the copolymers, whereas for CMC it takes more than 40 min. It can be concluded that their enzymatic degradation is facilitated by the presence of the PNIPAM grafts. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 87: 1383–1386, 2003

Key words: degradation; biodegradable; graft copolymer; polysaccharides

INTRODUCTION

Cellulose is a most abundant natural polymer and an important raw material for many industries. Cellulose derivatives have special applications because of their properties and higher solubility in water. Carboxymethyl cellulose (CMC) in acid or salt form finds various applications in many domains such as foods, cosmetics, pharmaceuticals, suspension agents, and viscosity increasing agents, and as a formulation agent in controlled release of drugs and pesticides, paper products, adhesives, ceramics, etc.

Graft copolymers synthesized either by grafting of a macromolecular chain onto a polysaccharide backbone[1,2] or by polymerization of the appropriate monomers in the presence of a polysaccharide[3,4] have received considerable attention during the past few years due to their wide applications in various fields such as papermaking, textiles, petroleum washing, environmental protection, or biomedical applications.[5,6] Moreover, the synthesis of materials that continue to have functionality while in service but degrade after use is a rather novel concept in the development of new materials.[7,8]

Graft copolymers of CMC with poly(*N*-isopropylacrylamide) (PNIPAM) (CMC-*g*-PNIPAM) are stimuli responsive, as their conformation varies with temperature[2] due to the inverse solubility behavior of PNIPAM, precipitating out at 33°C.[9] These copolymers could be proposed as the precursors for the development of temperature-responsive switching devices, able to take over control functions in microsystems in which enzymatic reactions could take place.[5] In this work, an enzymatic degradation study of CMC-*g*-PNIPAM copolymers has been carried out.

EXPERIMENTAL

Materials

CMC (Polysciences, Inc.) was purified by dialysis and finally obtained by freeze drying. It was of a nominal molecular weight, M_w = 82,000, in accordance with the value determined viscosimetrically in a 0.2*M* NaCl solution at 25°C, by the relation: $[\eta] = 43 \times 10^{-3} [M_w]^{0.74}$.[10] The content of the carboxyl groups determined by an acid–base titration was found to be 0.78 carboxyl groups per anhydroglycose unit.

The synthesis of the CMC-*g*-PNIPAM27 and CMC-*g*-PNIPAM47 copolymers has been described elsewhere,[2] the numbers 27 and 47 indicating the weight percentage composition of the graft copolymers in *N*-isopropylacrylamide units.

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Cellulase from *Trichoderma viride* (Merck Co., Darmstadt, Germany), with an activity of 3 U/mg (CMC, pH 4.5 at 37°C), was purchased from Merck (Merck 2324).

Methods

Cellulase activity

The reaction temperature of 50°C is optimum for the cellulase activity, as was demonstrated by many authors in studies on various types of cellulose.[11,12] It has been also established that the pH optima of most cellulases tend to be acidic (pH 2.5–7), whereas the optimum temperatures are quite broad, ranging from 30 to 75°C, with the majority of the enzymes being most active at temperatures equal to or greater than 50°C. [11,13–15] Therefore, the enzymatic degradation was performed in the presence of *T. viride* at 50°C for 10 min in a buffer solution with a pH of 6.4 obtained with a sodium citrate/citric acid/sodium hydroxide mixture. Polymer concentration was constant at 1 g/100 mL.

The Petterson–Porath method[16] used in this study is based on the determination of the reducing groups of the cello-oligosaccharide fragments, resulting from the reaction of the cellulosic substrate with the enzyme, through their reaction with dinitrosalicylic acid, giving a yellow color. Specific absorption of UV–vis spectra at 640 nm was used to determine the concentration of the reducing cello-oligosaccharide fragments after calibration with freshly prepared glucose solutions of a concentration range varying from 0.1 to 1 g/100 mL.

Viscosimetry

It is well known that cellulases react against soluble cellulosic derivatives both by the breakdown of reducing sugars at the nonreducing chain ends and by the scission of glucosidic bonds of the entire chain. Enzymatic degradation takes place by CMC backbone scission, decreasing its molecular weight and viscosity.[13] Cellulase activity at different temperatures was estimated by following the reduced viscosity of CMC and of the grafted copolymers in dilute solutions at various moments of the enzymatic reaction at pH 6.4, in the presence of *T. viride* at 40 and 50°C, using an Ubbelohde-type viscometer. The beginning of the enzymatic reaction was considered the moment of addition of the cellulase preparation in the reaction medium, whereas the end of the reaction was considered stable in viscosity for three consecutive determinations.

RESULTS AND DISCUSSION

The base monosaccharide of CMC is glucose and its structural unit is cellobiose bounded by β-1,4-glycosidic bonds. The main fungi-degrading celluloses are *T. viride*, *Trichoderma reesei*, and *Humicola insolens*, etc.[15,17] It is well established that cellulases, capable of degrading cellulosic materials, are multicomponent enzyme systems acting synergistically.[18,19] The enzymatic cellulolitic complex is able to hydrolyze cellulose and its derivatives.[19–21] It contains the following different enzymes: cellulase, cellobiohydrolase, and β-glucosidase.[14] Cellulase or endo-β-1,4-glucanase or [1,4-(1,3; 1,4)-β-D-glucan-4-glucanohydrolase, EC 3.2.1.4] hydrolytically breaks down the β-1,4-glycosidic bonds of the polyglucidic chains. Cellulases randomly attack and hydrolyze the β-1,4 bonds of cellulose to produce cello-oligosaccharides. Endoglucanases, often named carboxymethyl cellulases, are able to hydrolyze the bonds in the middle of the cellulose chains and substituted substrates such as CMC or hydroxyethyl cellulose, while exoglucanases, with narrow tunneling active fragments, mainly hydrolyze the chain ends of cellulose. The substituents of cellulose derivatives are not able to pass through these tunnels and are not hydrolyzed by exoglucanases. The enzymatic scission of a glycosidic bond is a stereospecific process.[14,15]

TABLE I
Results of the Enzymatic Degradation of CMC and CMC-*g*-PNIPAM Copolymers Followed by the Peterson/Porath Method, at 50°C

Sample	Cellulase activity (U/ml min)	
	Experimental	Calculated
CMC	16.8	—
CMC-*g*-PNIPAM27	15.2	12.3
CMC-*g*-PNIPAM47	12.2	8.9

The experimental results of the enzymatic degradation of CMC and CMC-*g*-PNIPAM copolymers, followed by the Peterson–Porath method, are presented in Table I. The values calculated from the weight composition of the grafted copolymers in CMC are also presented. The considerable difference between the experimental and the calculated values indicates that the presence of the PNIPAM grafts in the copolymer increases its susceptibility to the enzymatic degradation.

Moreover, cellulases provoke a rapid decrease in the viscosity of the cellulosic solutions, thus providing a relatively specific means for assaying their activity. The size of the limited cello-oligosaccharides varies depending mainly on the source of the enzyme, but only a few cellulases are able to efficiently hydrolyze substrates with a degree of polymerization < 4.[14] The variation of the reduced viscosity during the enzymatic degradation of CMC and CMC-*g*-PNIPAM copolymers at 50°C is shown in Figure 1. We observe that the reduced viscosity of the fragments resulting at the end of the reaction from the copolymers is lower

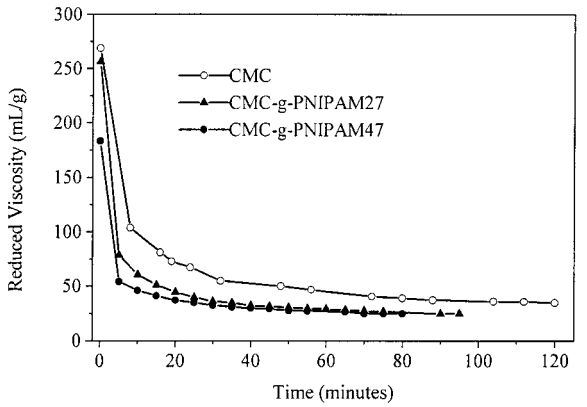

Figure 1 Variation of the reduced viscosity of CMC, CMC-*g*-PNIPAM27, and CMC-*g*-PNIPAM47 during enzymatic degradation at 50°C.

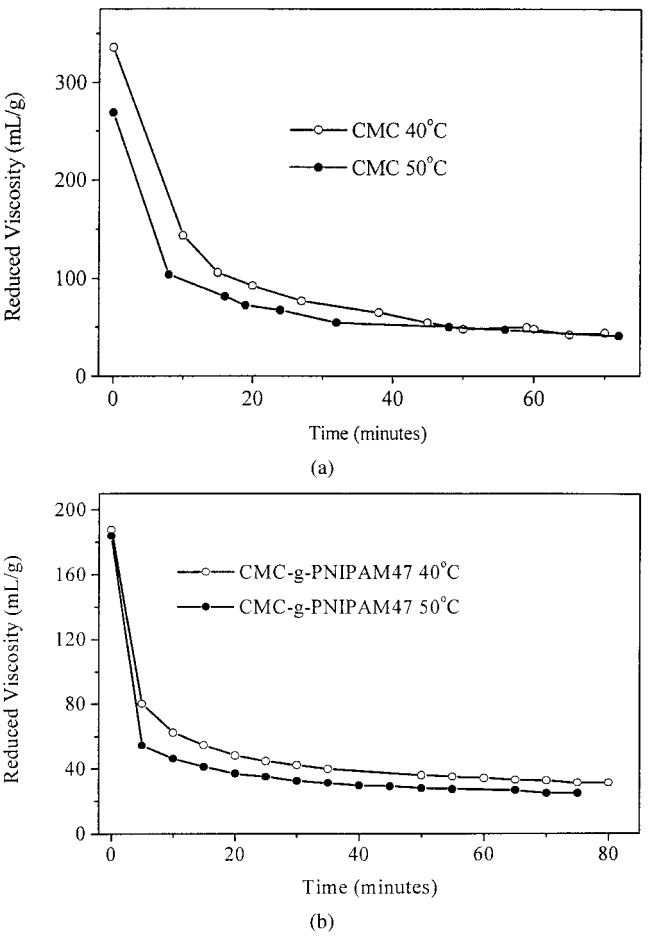

Figure 2 (a) Variation of the reduced viscosity of CMC during its enzymatic degradation at 40 and 50°C. (b) Variation of the reduced viscosity of CMC-*g*-PNIPAM47 during its enzymatic degradation at 40 and 50°C.

than that of the fragments resulting from CMC. Besides, the reaction is faster for the copolymers, being completed in 25 min in respect to 50–70 min needed for CMC. The reason for this behavior could be an influence of grafting on the enzymatic degradation, promoting the chain scission.[14]

In Figure 2 the reduced viscosity versus time for CMC and CMC-*g*-PNIPAM47, during enzymatic degradation at 40 and 50°C, is presented. Moreover, on the basis of these data, kinetic parameters of the enzymatic degradation have been evaluated, according to a well-known procedure[15,22] and the results are given in Table II. The influence of the temperature on the reaction pathway seems to be very important in the beginning of the reaction, where the reduced viscosity of CMC, Figure 2(a), and CMC-*g*-PNIPAM47, Figure 2(b), decreases much faster at 50°C than at 40°C. The same tendency is obvious from the variation of the rate constant (k), Table II, being about two times higher at 50°C than at 40°C for both polymers. It can also be easily observed that k is much higher for the grafted copolymer. The apparent activation energy (E_{app}) is two times higher for the copolymer, indicating a change of the reaction mechanism of the enzymatic degradation. E_{app} corresponding to the enzymatic degradation of CMC is comparable with the values for enzymatic degradation of other polysaccharides. For crosslinked dextran, as an example, a value of $\sim$ 55 kJ/mol was found.[22] The activation energy for enzymatic degradation generally lies between 42 and $\sim$ 200 kJ/mol.[22–24]

CONCLUSION

A preliminary study on the enzymatic degradability of CMC-*g*-PNIPAM copolymers showed that they are more susceptible to biodegradation with cellulases than the polysaccharide backbone, CMC. Grafting of CMC with PNIPAM chains favors its enzymatic degradability as the amount of the cello-oligosaccharide fragments is increased in respect to that expected. The decrease of the reduced viscosity of the copolymers is considerably faster compared to that of CMC; the rate constant of the reaction of the enzymatic degradation is also increased, as well as the activation energy.

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TABLE II
Kinetic Parameters of the Enzymatic Degradation of CMC and CMC-*g*-PNIPAM47 in the Presence of Cellulolitic Complex of *Trichoderma viride*

Sample	Rate constant $K \times 10^2$ (s^{-1})		Eapp (kJ/mol)
Temperature	40°C	50°C	—
CMC	1.4	2.5	68
CMC-*g*-PNIPAM	6.4	15.0	134

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