Enzymatic Degradation of Guar and Substituted Guar Galactomannans

Yu Cheng* and Robert K. Prud'homme

Department of Chemical Engineering, Princeton University, Princeton, New Jersey 08544

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Enzymatic degradation of guar galactomannan is studied using gel permeation chromatography (GPC) and steady shear viscometry. In very dilute polymer solutions, reaction rate increases with first-order kinetics with substrate concentration. In the intermediate concentration regime, the enzyme/polymer binding saturates, and the degradation kinetics is zero-order. The observations are in accord with a Michaelis−Menton kinetics model. The Michaelis−Menton parameter, K_m and V_{max}, were determined to be 0.6 mM and 7.8×10^{-10} mol/(mL s) for guar at pH = 7, where the maximal velocity of the reaction, V_{max}, was measured in terms of the molar concentration of glycosidic bonds broken per unit time. However, as the solution increases in concentration, the reaction rate decreases and the enzyme diffusion through the concentrated polymer gel becomes a limiting factor. A reaction−diffusion model is presented to express the competition between enzyme reaction and diffusion. The scaling theory and kinetic data are used to define the boundaries of the polymer concentration regimes between substrate (i.e., polymer strand) limited reactions, enzyme limited reactions, and hindered diffusion limited reactions. The influence of polymer derivatization on the degradation kinetics was also explored. The degradation rate was shown to be greatly affected by the type of substituent groups as well as the degree of substitution. The triggering mechanism and controlled degradation were found for the enzymatic hydrolysis of cationically derivatized guar solutions.

Introduction

Guar galactomannan is a naturally occurring polysaccharide consisting of a linear backbone of β-1,4-linked mannose units with α-1,6-lined galactose units randomly attached as side chains (Figure 1). The ratio of mannose to galactose units is 1.6:1 to 1.8:1.[1] Guar is widely used as a rheology modifier due to its excellent thickening ability.[2] One of the major applications is in the hydraulic fracturing process in oil and gas recovery. During the process, a guar-based fracturing fluid is blended with sand (proppant) and pumped down the wellhole to the oil formation under high pressure up to 20 000 psi. As a result, fractures are created radially from the wellbore and the proppants keep the fracture from closing once the pressure is released. After the fracture is formed, a breaker is applied to degrade the guar to a low viscosity fluid so that it can be pumped back out of the formation. Finally pressure is released from the well, and the fractures provide a network of channels for the flow of oil out of the rock with decreased flow resistance, thereby enhancing the rate of recovery.[3]

Enzymatic breakers have proven to be an efficient and environmentally benign method for the degradation of fracturing fluids.[4,5] The enzyme breakers are preferable to oxidative breakers, because (a) they are true "catalysts", and therefore can be used at low concentrations; (b) they are relatively high in molecular weight and they remain trapped with the guar polymer rather than being filtered off into the rock formation as is the case with low molecular weight oxidizers; (c) they are environmental benign; and (d) they are less susceptible to dramatic changes in activity by trace contaminants. Three kinds of bonds in guar are susceptible to enzymatic hydrolysis: the endo- and exo-β-1,4 linkages on the D-mannose backbone and the α-1,6 linkage between the mannose unit and the galactose side-chain. The enzymes that cleave these bonds are respectively endo- and exo-β-mannanase and α-galactosidase. To improve the efficiency of oil recovery and design enzymes with better properties, it is important to understand the enzymatic degradation kinetics in guar solutions as well as the correlation between the kinetics and the rheological properties of the fracturing fluids during the degradation.

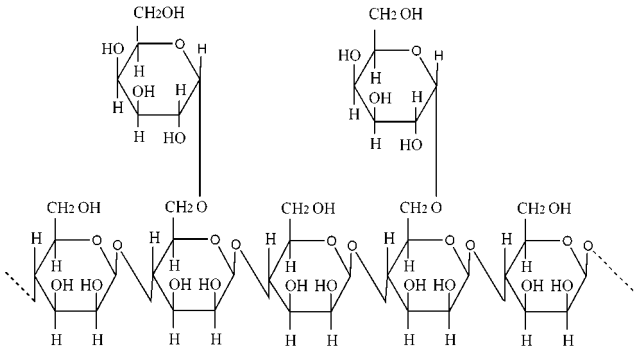

Figure 1. Structure of guar. Guar has a linear backbone of β-1,4-linked mannose units with α-1,6-lined galactose units randomly attached as side chains. The ratio of mannose to galactose units is 1.6:1 to 1.8:1.

Various studies have investigated the enzymatic depolymerization of polysaccharides due to the great utilization

* To whom correspondence should be addressed. Fax: 609-258-0211. E-mail: yucheng@princeton.edu.

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of new polysaccharide materials for the production of foods, fuels and specialty chemicals.[6] However, most of these studies concentrated on the degradation of cellulose, starch, and dextran.[7−9] β-D-Mannanases are produced by plants, bacteria and fungi. They catalyze the hydrolysis of 1,4-β-D-mannosidic linkages in galactomannan polymers. Their ability to degrade these substrates depends both on the degree of polymerization and galactose substitution levels.[10] Bulpin et al. used a α-galactosidase to modify the side chain content of natural guar. The modified guar has applications in food industry by forming synergistic gels with xanthan and κ-carageenan.[11] Tayal et al. studied the enzymatic degradation kinetics of high molecular weight native guar.[5] Guar solution viscosity was found to be very sensitive to the enzymatic hydrolysis and decreased by several orders of magnitude during the course of degradation. An empirical zero-order kinetics is recently reported by using Gammanase, a commercial mixed enzymatic system, to cleave the guar backbone.[12] However, they only studied the degradation over a certain range of polymer concentrations. There is no report on the degradation kinetics at very low and high polymer concentration ranges. In addition, they studied only native guar and did not consider guar substitution effects.

In standard laboratory practice guar/enzyme reactions are monitored by studying viscosity reductions of $^1/_2$−1 wt % polymer solutions.[13] However, no data appears in the literature on the degradation rate in high concentration guar fluids such as occur in the actual field operation where the guar filter cake has 3−7 wt % polymer.[14] Also, a range of substituted guars are used in hydraulic fracturing operations. But there has been no published data on the effect of substitution on reactivity. In this study, we followed the enzymatic degradation process over a wide range of substrate concentrations by using gel permeation chromatography (GPC) and steady shear viscometry. A Michaelis−Menton model is used to explain the results based on previous studies of Suga et al.[8] We also proposed a reaction−diffusion model to further discuss the competition between enzyme reaction and diffusion in all concentration ranges. In addition, guar can be modified by grafting side-groups (−R) onto the hydroxyl sites. Three commercially available derivatized guars were used in this study: hydroxypropyl guar (HPG), carboxymethyl guar (CMG), and hydroxypropyl trimethylammonium guar (HPTMAG) (Figure 2). HPG is a neutral polymer, while both CMG and HPTMAG are ionized in solution. The β-mannanase enzyme we used in the experiment has an isoelectric point (pI) of 3.5,[15] so in a polymer solution at pH 7 the enzyme is negative charged. Therefore, by adding either neutral or charged groups on guar, we studied both the steric and columbic interactions between the enzyme and the substrate for the three derivatized guars, and explored the effect of side groups on the degradation kinetics.

Experimental Section

Materials. The polymers we used in this study are guar (Rhone-Poulenc, Inc., Cranbury, NJ, SCN13835), HPG (Rhone-Poulenc, Inc., Cranbury, NJ, SCN13833), CMG (Rhone-Poulenc, Inc., Cranbury, NJ, SCN13834) and HPTMAG (Rhone-Poulenc, Inc., Cranbury, NJ). The guar solutions are prepared by the following procedure: a 1000 mL wide mouth jar containing 150 mL of deionized water is stirred using a mixing impeller with speed adjusted to 1000 rpm to form a deep vortex. Then 1 g of guar powder is sprinkled slowly onto the liquid-free surface over a 3-min interval to produce a uniform dispersion and stirring continues for 5 min. Another 49 mL of deionized water is added to wash all the residual powder on the beaker walls into the solution. The mixing speed is then reduced to 500 rpm for an additional 60 min. After the speed is reduced, 100 ppm of sodium azide (FisherChemical) is added as a preservative. The solution pH is adjusted to 7.0 using HCl (EM Science). Finally, the polymer solution is transferred to a container and placed on a low shear roller for approximately 20−24 h to complete hydration. To clarify solutions and remove cell debris, polymer solutions were centrifuged at 16 000*g* for 4 h. The final polymer concentration is measured by dry weight analysis. The endo-β-mannanase extracted from *Aspergillus niger* was obtained from Megazyme Inc. The enzyme was supplied as an ammonim sulfate suspension in 0.02% sodium azide. A 1.35 μL aliquot of this suspension was diluted in 2 mL of 0.1 M sodium acetate (EM Science)/acetic acid (Glacial, FisherChemical) buffer solution with pH adjusted to 6.

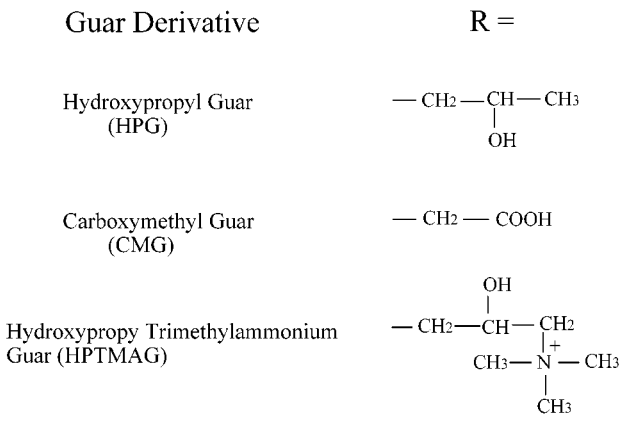

Figure 2. Side groups of derivatized guars. The hydrogen atom of hydroxyl group of guar can be substituted by a side group (−R) to form different derivatized guars: hydroxypropyl guar (HPG), carboxymethyl guar (CMG), and hydroxypropyl trimethylammonium guar (HPTMAG).

Enzymatic Degradation. The enzymatic degradation reaction was run in a sealed jar at room temperature. The pH of the guar solution was adjusted 7.0. The 0.2 mL of enzyme buffer solution was injected into 200 mL of guar solution using a microsyringe. The mixture was magnetically stirred during the reaction. After the reaction began, aliquots of the guar and enzyme mixture were taken out at various times. Each aliquot was immediately heated to 100 °C for 20 min to denature the enzyme and stop the reaction. Biochemical analyses reveal that the half-life of endo β-mannanase is 6 min at 85 °C.[12] The viscosity of the solution does not change after the enzyme is denatured. Experiments were also done to show that this denaturing protocol (i.e., heat treatment) did not produce a viscosity reduction for polymer solutions in the absence of enzymes (data not shown). To compare results from different experiments, equal endo β-mannanase concentration was used as a basis.

At high guar concentrations (>1.5 wt %), the high viscosity of the polymer solution makes (rapid) homogenization of the guar and enzyme difficult. As a result, high substrate concentration samples were made by concentrating the 1 wt % guar solution and enzyme mixture by dialysis at 0 °C where enzyme activity is low. A control experiment was run to show there is a negligible molecular weight reduction at this low temperature. The reaction rate is seven times faster at 25 °C than at 0 °C. For example, to study the degradation experiment at 3 wt % substrate concentration, 5 μL enzyme buffer solution was added to 15 g of 1 wt % guar solution and mixed well. The mixture was immediately transferred into a Spectra/Por dialysis bag with a molecular weight cutoff of 3500. Then the dialysis bag was concentrated by placing it in a bed of absorbent silica gel (Fisher Scientific). After around 2 h, the guar solution was concentrated to 3 wt % with a total mass of 5 g. Finally, the substrate−enzyme mixture was heated in a microwave for 3 s. The temperature increased from 0 to 25 °C, and the reaction began.

GPC Measurement. Gel permeation chromatography (GPC) was carried out with two columns in series (TSK G3000PW$_{XL}$ and TSK G6000PW$_{XL}$). For protection, a guard column (TSK GDNA-PW) was used. All columns were thermostated at 40 °C to decrease solvent viscosity and peak broadening. The HPLC system consisted of a pump (Waters M510), a differential refractometer (Waters 410) and an injector (Valco SSA C12P). The mobile phase was 55 mM Na_2SO_4 and 0.02% NaN_3 aqueous solution. Flow rate was 0.6 mL/min. Degraded guar samples were diluted to 0.05 wt % and filtered through a 0.45 μm filter (Whatman Autovial) before injection. Both pullulan and guar standards were used to calibrate the columns. Nine fractions of pullulan standards with average molecular weight (MW) ranging from 5900 to 1.6 million were used (Shodex Corp., Japan). Two fractionated guar standards, Meyprogat 7 (M_p = 58 000) and CSAA 200 (M_p = 2 000 000), were used as secondary standards.[16] The Mark−Houwink−Sakurada relationship $[\eta] = K(M_w)^a$ of guar and pullulan are as follows: $K = 3.8 \times 10^{-4}$ dL/g, $a = 0.723$ (guar); $K = 1.9 \times 10^{-4}$ dL/g, $a = 0.67$ (pullulan).[17,18] A universal calibration curve was plotted for guar and pullulan and all points fall on a single straight line.[19] The molecular weight (MW) and molecular weight distribution (MWD) of degraded guar samples were determined based on the universal calibration curve.

Steady Shear Viscometry. Steady shear rheological tests on a strain-controlled rheometer (RFS−II, Rheometrics, Piscataway, NJ) were used to characterize the samples. A Couette geometry, with inner bob and outer cup radii of 16 mm and 16.925 mm, respectively, and a bob length of 33.3 mm, was chosen. Samples without enzyme were also tested as controls to assess the initial rheology of the solutions. All viscosity measurements were made at 25 °C.

Results and Discussion

Enzymatic Degradation Kinetics. Figure 3 is a typical viscosity vs shear rate plot of six guar solutions upon exposure to β-mannanase enzyme at room temperature and a pH of 7. The β-mannanase, which cleaves the mannose backbone, is very effective in reducing the viscosity of guar solutions. Under enzymatic hydrolysis, the solution viscosity decreased by over 2 orders of magnitude after 20 h. For each sample we observed a Newtonian region at low shear rates and a shear thinning region at higher shear rates, as has been observed in previous studies.[12] Figure 4 shows the evolution of molecular weight distributions (MWD) of guar during the enzymatic hydrolysis. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were obtained by integrating the MWD curve and using the universal calibration curve. The MWD broadens considerably with time and shifts to lower molecular weight.

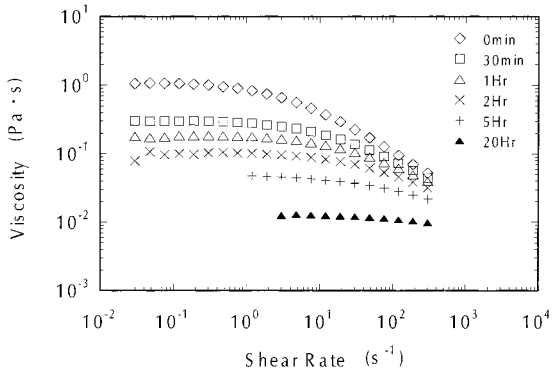

Figure 3. Viscosity of 0.5 wt % guar solution as a function of shear rate plotted at different periods during enzymatic degradation. The reaction is run at ambient temperature and pH of 7. The concentration of β-mannanase is 0.0002 units/mL polymer solution.

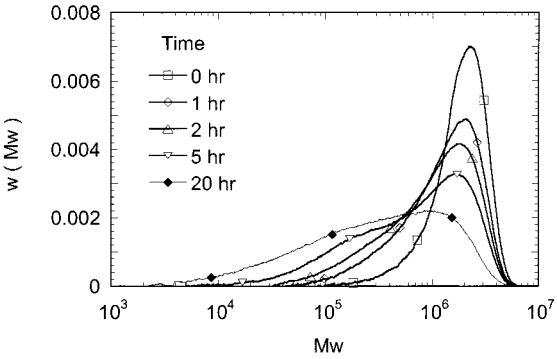

Figure 4. Evolution of molecular weight distribution (MWD) during the enzymatic degradation of guar at room temperature and a pH of 7. The weight fraction vs molecular weight is shown for five reaction times. The guar concentration is 0.5 wt %. The concentration of β-mannanase is 0.0002 units/mL polymer solution.

The reaction between enzyme and substrate is asssumed to be of the common Michaelis−Menton type:[20]

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} P + E$$

i.e., first, the enzyme (E) attaches to the polymer substrate (S) and forms an enzyme−substrate complex (ES). Then the enzyme cuts the substrate, followed by a release of the product (P) and enzyme itself. If we consider the substrate as every breakable bond in the system L and the scission of these bonds on a polymer chain to be random, the degradation kinetics are [8]

$$\frac{dL}{dt} = -\frac{k_{cat}C_e L}{K_m + L} \quad (1)$$

where k_{cat} and K_m are the rate constant and the Michaelis−Menton constant, respectively. C_e is the concentration of the enzyme. L represents the molar concentration of cleavable bonds in the system.

When $L \ll K_m$, eq 1 reduces to a first-order reaction

$$\frac{dL}{dt} = -\frac{k_{cat}C_e L}{K_m} \quad (2)$$

When $L \gg K_m$, eq 1 reduces to a zero-order reaction

$$\frac{dL}{dt} = -k_{cat}C_e \quad (3)$$

To get the reaction rate, we can let

$$k = \frac{k_{cat}C_e}{K_m + L} \quad (4)$$

and rewrite eq 1 in a first-order form as

$$\frac{dL}{dt} = -kL \quad (5)$$

in which k denotes the apparent rate constant.

Since there are $(M_n/m - 1)$ linkages per polymer molecule, where M_n is the number-average molecular weight of the chain and m is the molecular weight of a monomeric unit (for guar, $m \approx 266$), we can express L, the total number of hydrolyzable bonds, as $N(M_n/m - 1)$. N is the total number of molecules. If $M_n \gg m$ during the degradation, the following relation between molecular weight and degradation time can be derived:

$$\frac{1}{M_n(t)} = \frac{1}{M_{no}} + \frac{kt}{m} \quad (6)$$

in which M_{no} is the initial M_n of the polymer. Therefore, we followed the change of M_n with reaction time by GPC and plotted $1/M_n$ as a function of degradation time at different guar concentrations (Figure 5). The inverse M_n was shown to change linearly with time, consistent with eq 6. Accordingly, the apparent rate constant k can be obtained from the slope of the straight line. We can see that the apparent rate constant k decreases with guar concentration. On the basis of the obtained rate constant and eq 5, the enzymatic degradation rate can be calculated and plotted vs polymer concentration (Figure 6). At very low guar concentration (<0.03 wt %), the reaction rate increases with substrate concentration. This is the first-order regime predicted by the Michaelis−Menton kinetics. In this regime, polymer concentration is much lower than the overlap concentration of native guar ($C^* \sim 0.1$ wt %).[21] Guar appears as random coils in solution,[18] and its molar concentration is extremely low. At intermediate concentrations (0.05−3 wt %), the degradation rate becomes independent of substrate concentration: this is the predicted zero-order regime. This arises because at high polymer concentrations, there are sufficient substrate sites for the enzyme to bind. Therefore, the increasing of substrate concentration does not increase the number of enzyme−substrate complexes. The reaction rate is limited by the enzyme cleavage kinetics and not by the enzyme diffusion to the substrate: the reaction becomes zero-order in polymer substrate. At very high polymer concentrations (>∼5 wt %), the degradation rate begins to decrease with substrate concentration, corresponding to a diffusional resistance to the enzyme mobility in the concentrated polymer mesh. To our knowledge, this study is the first to show the three transitions from substrate limited, to enzyme limited, to diffusion-controlled. The experimental results in the first two regimes were fitted according to the Michaelis−Menton model to yield K_m and V_{max}.[22] In this study, V_{max} is measured in terms of the molar concentration of glycosidic bonds broken per unit time. K_m and V_{max} were determined to be 0.6 mM and 7.8×10^{-10} mol/(mL s).

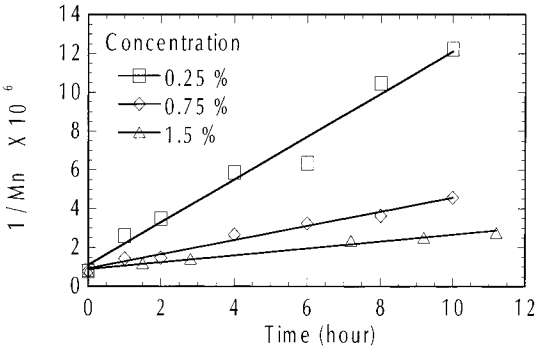

Figure 5. Inverse of number-average molecular weight, M_n, plotted vs enzymatic degradation time at different guar concentrations: 0.25, 0.75, and 1.5 wt %.

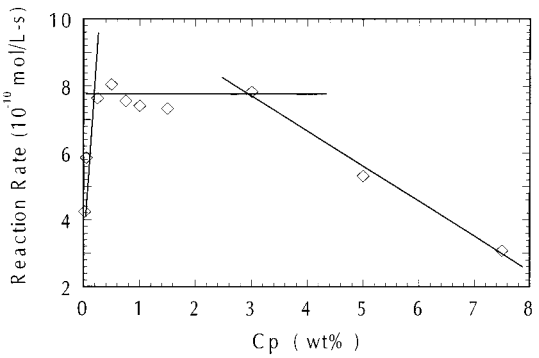

Figure 6. Enzymatic degradation rate at pH 7 is plotted vs substrate concentration. At very low concentration regime, reaction rate increases in a first-order manner with polymer concentration. In the intermediate regime, the reaction rate is independent of substrate concentration and the kinetics is zero-order. At high concentration regime, the reaction becomes diffusion limited. The concentration of β-mannanase is 0.0002 units/mL polymer solution.

It was mentioned that a low temperature mixing process was used to achieve homogeneous enzyme and polymer concentrations in the diffusion-limited regime. Figure 7 shows that the change in molecular weight (plotted as $1/M_n$) vs time at 25 and 0 °C. Clearly over the 2 h required for osmotic concentration of the polymer there is a minimal amount of degradation at 0 °C. For samples prepared in this way the initial molecular weight for the degradation experiment was taken as the value obtained from the 0 °C calibration curve in Figure 7.

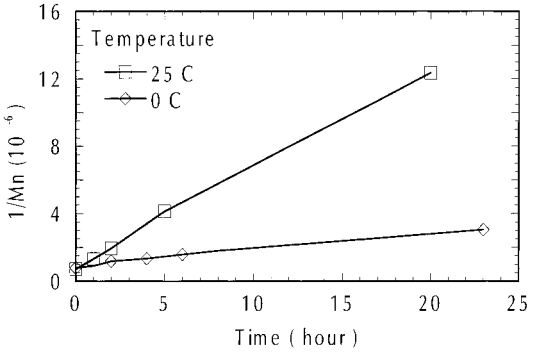

Figure 7. Influence of temperature on the degradation rate. The reaction rate is more than seven times faster at 25 °C than at 0 °C. Over the 2 h required for osmotic concentration of the polymer, there is a minimal amount of degradation at 0 °C.

Reaction−Diffusion Model. The enzymatic degradation of polymers can be divided into a two-state process, a mobile state and a bound state. The mobile state is the free diffusion of enzyme molecules through a polymer solution; the bound state consists of the binding of enzyme to polysaccharide chain, the cleavage of glycosidic bonds and the release of the enzyme from the binding site. The time of the mobile state and the bound state are denoted as t_d (diffusion) and t_r (reaction), respectively. The total time per enzyme molecule per cut of bond is $(t_d + t_r)$. Since the polymer is uncharged, we assume the interactions between enzyme and the polymer substrate are short-ranged. As a result, the diffusion of an enzyme molecule can be treated as a random diffusive process, where the time to diffuse a distance between polymer strands, ξ (i.e., the polymer correlation or screening length[23]), is $t_\xi = (\xi^2/D)$. D is the self-diffusion coefficient of the enzyme molecule. However, the diffusion time t_d between binding/reaction steps is greater than the time to diffuse the distance between polymer strands. Most collisions between the enzyme and polymer chains do not result in binding because the molecular recognition for binding requires that the collision event occurs at the enzyme active site, and has the proper spatial configuration. Therefore, the time for free diffusion, t_d, is longer than t_ξ by a "collision efficiency factor", α, such that $t_d = \alpha(\xi^2/D)$.[24] The polymer mesh size ξ defines the network through which the enzyme diffuses. In the entanglement regime and good solvents the mesh size ξ decreases with polymer concentration C_p by[23]

$$\xi = R_g\left(\frac{c}{c^*}\right)^{-3/4} \quad (c > c^*) \tag{7}$$

where R_g and c^* are the radius of gyration and the overlap concentration of guar, respectively.

Enzymes are compact globular macromolecules. The diffusion of solid spherical particles in polymer solutions has been widely studied.[25−28] In many systems, the diffusion coefficient decreases with concentration as a stretched exponential, $D/D_o = \exp(-\alpha c^\gamma)$, in which D_o is the diffusion coefficient of the probe in pure solvent, and γ is a constant which varies from 0.5 to 1 [27] (Figure 8a). de Gennes also argued that when the hydrodynamic diameter of the enzyme, d, is much smaller than the mesh size. ξ, we expect the enzymes to move rather easily, with a friction coefficient related to the viscosity of the pure solvent; when d is greater than ξ, the enzymes experience high frictions from the polymer and the friction is related the viscosity of the entangled solution.[29]

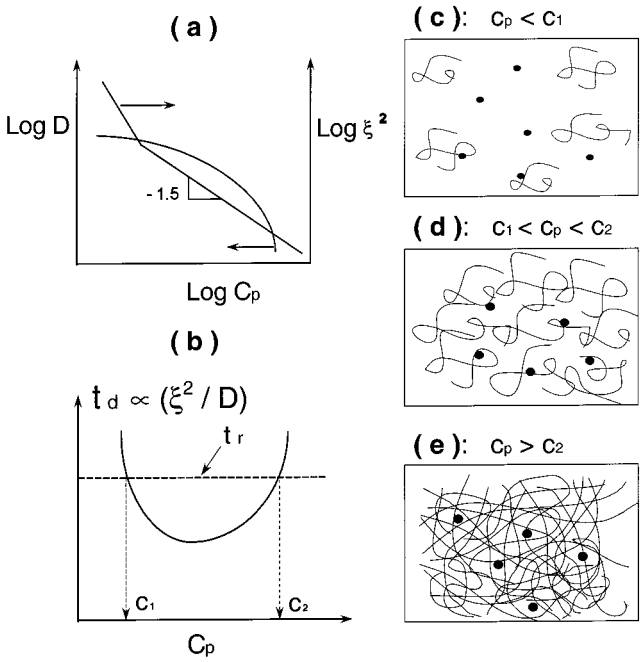

Figure 8. (a) Changes of the enzyme diffusion coefficient (D) and polymer mesh size (ξ) with the matrix polymer concentration (c_p). (b) Changes of the enzyme diffusion time with polymer concentration. The reaction time has a constant value at given conditions, and is shown as the dashed line. (c) Dilute regime ($c < c_1$): diffusion time $t_d \gg$ reaction time t_r. The black dots denote enzyme molecules. (d) Intermediate regime ($c_1 < c < c_2$): $t_r \gg t_d$. (e) Concentrated regime ($c > c_2$): $t_d \gg t_r$.

Parts a and b of Figure 8 qualitatively explain the competition between diffusion and reaction. The diffusion coefficient decreases with polymer concentration c_p as a stretched exponential, while mesh size ξ changes with c_p as a power law through eq 7. In the dilute range (Figure 8c), the average distance between polymer chains is so large that the diffusion time t_d is long for the enzyme to diffuse from one chain to another. At low enough concentration, t_d is bigger than t_r (an intrinsic property of the enzyme), and the reaction is enzyme diffusion controlled. In the dilute solution regime, as the concentration increases, the average distance between chains decreases as a power law. However, the diffusion coefficient of the enzyme, D, does not decrease significantly because the enzyme molecule diffuses through essentially solvents. As a result, t_d decreases, and reaction rate increases with concentration. With increasing concentration t_d decreases until $t_d \sim t_r$ which we denote as concentration c_1. For concentrations greater than c_1, t_d becomes smaller than t_r, and the reaction is reaction-controlled. Therefore, in the intermediate range (Figure 8d), the reaction rate is determined by the enzyme cleavage kinetics and is independent of polymer concentration (zero order). As the polymer concentration continues to increase, the stretched exponential function drops faster than a power law (Figure 8a). So D decreases faster than ξ^2, and t_d begins to increase again. Eventually, at some high concentration c_2, t_d will reach the value of t_r. When $c > c_2$, the reaction rate will decrease, and the reaction becomes diffusion-limited again (Figure 8e).

A simple scaling estimation can be constructed to give insight into the high concentration regime. The correlation length ξ can be calculated by eq 7. Both R_g and c^* for guar have been measured by previous studies. Taking $R_g \sim 150$ nm and $c^* \sim 0.1$ wt %,[21] we can calculate that the correlation length at 5 wt % guar concentration is about 8 nm. The β-mannanase has a molecular weight of 45 000.[15] By assuming the enzyme molecule is a sphere in solution and its density is $\rho = 0.7$ g/mL, which is a standard value for density of globular protein in solution,[30] we can estimate the size of the enzyme molecules. The diameter of the enzyme is approximately 6 nm. So at 5 wt % guar concentration, the correlation length of the polymer mesh is about the same as the diameter of the enzyme molecule. This gives rise to the large decrease in the effective diffusion coefficient of the enzyme, corresponding to a decrease of the degradation reaction rate.

According to Figure 6, the degradation rate begins to decrease at a polymer concentration of 5 wt %. Therefore, this is approximately the concentration c_2 where $t_d \sim t_r$. The reaction time t_r can be evaluated from the catalytic constant of the enzyme, k_{cat}, which is defined as

$$k_{cat} = V_{max}/c_e \tag{8}$$

This quantity is also known as the turnover number of an enzyme because it is the number of reaction processes (turnovers) that each active site catalyzes per unit time. The reaction time of the enzyme molecule, $t_r \sim 1/k_{cat}$. The enzyme concentration c_e used in the reaction is approximately 10^{-10} M. On the basis of eq 8, t_r is calculated to be ~0.1 s. At 5 wt %, the mesh size $\xi \sim 10$ nm, and the diffusion coefficient of the enzyme molecule is on the order of 10^{-8} cm^2/s, according to diffusion experiments.[28] The diffusion time for one collision between the enzyme molecule and polymer chain is approximately $\xi^2/D = 10^{-4}$ s. Hence, we can estimate the collision efficiency, α, to be about 1000. This suggests that there are nearly 1000 collisions between the enzyme and the polymer for each reaction (effective collision) to occur.

Effect of Substrate Structure. During the enzymatic hydrolysis process, the binding event involves molecular recognition between the enzyme and the substrate. A change of structure of the substrate changes the ability of enzyme to recognize and attach to the substrate, which will cause an alteration in the degradation rate. Six different systems were chosen to study the effect of substituent groups on guar polymer chain: guar, HPG (MS = 0.18), HPG (MS = 1.53), CMG (DS = 0.1), CMG (DS = 0.6) and HPTMAG (DS = 0.14). For HPG, the addition of hydroxypropyl groups to guar is described by the term molar substitution (MS) which is defined as the average number of moles of hydroxypropyl groups substituted per mole of anhydro sugar units and thus is a measure of the total number of moles of propylene oxide which have been added to the guar polymer chain. For CMG and HPTMAG, the addition of substituting groups to the guar polymer chain is described by the term degree of substitution (DS), which is the average number of carboxymethyl groups substituted per sugar unit. All reactions are run at a pH of 7. Therefore, HPG is neutral in solution. HPTMAG is positively charged, and CMG is negatively charged. The β-mannanase enzyme we used in the experiment has a pI of 3.5. So in the polymer solution at pH of 7, the enzymes are negatively charged.

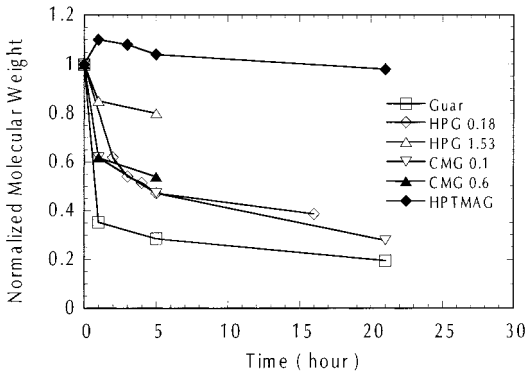

Figure 9. Normalized molecular weight changes with time during the degradation reaction for natural guar, HPG with molar substitutions of 0.18 and 1.53, CMG with degrees of substitution of 0.1 and 0.6, and HPTMAG. The concentration of β-mannanase is 0.0002 units/mL polymer solution (pH = 7).

Because of the complications of determining MW of polyelectrolyte polymers CMG and HPTMAG, steady shear rheometry was used to follow the degradation. Morris et al.[2] have shown that random coil polymers obey a universal $\log(\eta_{sp}) \sim \log(c[\eta])$ master curve, where η_{sp} is the specific viscosity, c is the solution concentration, and $[\eta]$ denotes the intrinsic viscosity. Because of the "hyperentanglements" between polymer chains, they showed that galactomannans (guar) fall on a master curve with a lower overlap concentration and somewhat greater concentration dependence thereafter. Therefore, based on the master curve by Morris et al. and the Mark−Houwink−Sakurada equation given by Robinson et al.,[18] we can calculate the molecular weight change with the reaction from the zero shear viscosity of the solution. Since the initial molecular weights of the polymers are different, we chose the normalized molecular weight as our basis of comparison. The normalized molecular weight is defined as the ratio of the molecular weight of the polymer solution incubated with enzyme to that of the initial, nondegraded polymer. Figure 9 shows the normalized molecular weight changes with time during the degradation reaction. After incubation with enzyme for the same time period, the average molecular weights of HPG and CMG are both higher than that of guar. The addition of hydroxypropyl groups on the polymer chain sterically hinder the attachment of enzymes to the polymer molecules, which decreases the total enzymatic degradation rate. For CMG at pH 7, the electrostatic repulsion between the enzyme and the carboxyl groups on the polymer backbone also slows down the degradation. Figure 10 shows the normalized molecular weight of guar as a function substitution level after reaction for 5 h. As the substitution level increases, the degradation rate decreases.

Surprisingly, for cationic guar, the molecular weight did not change with time: there was no enzymatic degradation. Since the cationic polymer and the enzyme have opposite charges, the ionic attractions immobilize the enzyme molecules on the polymer chain to form an enzyme−polymer

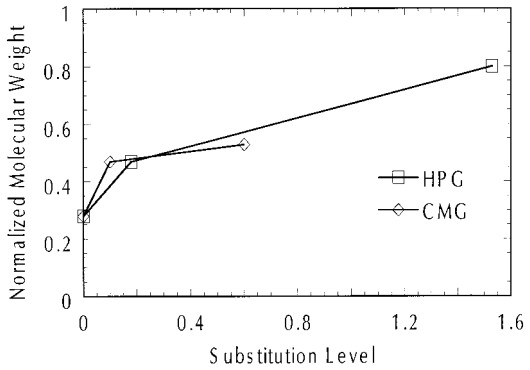

Figure 10. Normalized molecular weight of HPG and CMG as a function substitution level after incubated with enzyme for 5 h. As the substitution level increases, the degradation rate gets slower. The concentration of β-mannanase is 0.0002 units/mL polymer solution (pH = 7).

complex. This interaction is the basis for ion-exchange separations of proteins.[31] Because enzymes have both carboxyl and amine groups, their net charges can be altered by changing the solution pH. Therefore, a novel triggered degradation mechanism is discovered by which we can turn on and off the reaction by shifting the solution pH or ionic strength. We have reported on these results in more detail elsewhere.[32] This should be an important trigger to control enzyme reactions with polyelectrolyte polymers.

Conclusion

The reaction kinetics and mechanism of enzymatic degradation of guar and substituted guar over a wide range of polymer (i.e., substrate) concentrations has been studied. Both GPC and steady shear viscometry were applied to follow the degradation process. In dilute and semidilute guar concentrations, a Michaelis−Menton kinetics model was used to analyze the results, and for native guar at pH = 7, the Michaelis−Menton parameters are $K_m = 0.6$ mM and $V_{max} = 7.8 \times 10^{-10}$ mol/(mL s). As predicted by the kinetic model, in the very dilute regime, the reaction rate is first-order with substrate concentration. In the intermediate regime, the enzyme is saturated and the degradation kinetics is zero-order. Our new observation is the onset of diffusion-controlled reaction at higher polymer concentrations. The reaction rate decreases by 50% between 5% and 7.5% polymer. We proposed a reaction−diffusion model to describe the enzymatic degradation in all substrate concentration ranges. The onset of diffusion control is shown to occur when the enzyme size is comparable to the polymer mesh size.

The influence of substrate structure on the degradation kinetics was also explored. The degradation rate was found to be greatly affected by the type of substituent groups added and the degree of substitution. Increasing either carboxymethyl or hydroxypropyl groups cause a 3-fold decrease in enzymatic degradation rate. Steric and electrostatic blocking of enzyme docking onto the polymer chain accounts for the decrease. The triggering mechanism was found for the enzymatic hydrolysis of cationically derivatized guar solutions. At high pH the enzyme is complexed with the guar and inactive; at low pH the enzyme is released and activated. This mechanism holds promise for the controlled degradation of electrolyte biopolymers in industry, and provides a model for studying the enzyme−polymer interactions.

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