Scaling and Continuum Percolation Model for Enzyme-Catalyzed Gel Degradation

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Enzyme-catalyzed gel degradation is inherently controlled by diffusion of enzymes in the gel. We report kinetics measurements on the gelatin-thermolysin system, varying solvent viscosity as well as gel and enzyme concentrations. Scaling relations and reduced variables are proposed which are shown to account for the experimental results. Finally, we argue that the nontrivial experimental dependence on enzyme concentration for the degradation time demonstrates that enzyme random walk is self-attracting, leading to a continuum percolation model for gel degradation.

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The extracellular matrix is a gel made of various macromolecules—mainly proteins such as collagen—that isolates organs. In tumor dissemination, invasive cells liquefy this gel by producing proteolytic enzymes [1]. The biochemistry of these proteinases is well studied but not the physical aspects of their actual role in cell invasion: proteinases, by hydrolyzing peptide bonds between gel cross-links, catalyze a phase transition from a solid gel to a liquid. Some concepts from soft-matter physics such as percolation and gelation can, however, help in understanding the physical mechanism of this transition [2].

A key feature must first be considered: *in vivo*, the number of enzyme molecules per network mesh is much smaller than 1. Enzymes must therefore diffuse within the gel in order to significantly damage it. Sparse random breaking of peptide bonds would realize a simple percolation transition, but here enzyme diffusion introduces space correlations that control the gel-degradation mechanism [3] and its universality class. Note that this does not imply that the degradation kinetics is diffusion limited as enzyme-catalyzed chemical reaction may be the limiting step without affecting space correlations. Monte Carlo simulations were performed [4] that are based on a "Pacman percolation" model in which a proteinase is a random walker that cuts all bonds over which it passes. A new universality class was found for the critical gel-degradation mechanism, in agreement with the only critical exponent reported in the literature [2]. A model system consisting of gelatin (denatured collagen) and thermolysin as proteinase was studied [3]. To account for gel-degradation kinetics, it has been suggested that enzyme diffusion is anomalous and non-Brownian.

In this Letter, using the same model system as in Ref. [3], we report results on gel-degradation kinetics at different gel and enzyme concentrations. By varying solvent viscosity, we give a direct evidence, which was missing until now, that the degradation kinetics is diffusion limited. We propose a scaling relationship with reduced variables that account for the experimental results. Finally, we argue that the nontrivial experimental dependence on enzyme concentration for the degradation time demonstrates that the enzyme random walk (RW) is self-attracting. This RW leads to a continuum percolation model for the gel degradation ("Swiss cheese") that has important features towards the understanding of the motion of invasive cells.

Materials and methods.—Thermolysin (E.C. 3.4.24.27, Sigma) was prepared in 50 mM tris-HCl buffer $p\mathrm{H} = 7.4$. Thermolysin activity was determined following Ref. [5]. Its stability in gelatin solution was checked using a 186 nM thermolysin in 10% gelatin solution that was aged up to 600 h at 14 °C: no decrease of thermolysin activity was measured to within 1%. Solvent viscosity was increased by the addition of glycerol. The relative increase η_r of the viscosity with respect to glycerol-free solution was measured by quasielastic light scattering on a very dilute solution of latex particles. For a solution with 22.5% glycerol at 14 °C: $\eta_r = 2.02 \pm 0.02$. The addition of glycerol does not affect the gel [6,7], but decreases thermolysin activity. This latter effect was measured on azocasein in dilute solutions under stirring [5]. The relative activity, defined as the ratio of the reaction rates in 22.5% glycerol and in glycerol-free solution, is found to be $A_r = 0.390 \pm 0.006$. Gelatin of type *B* from bovine skin (Sigma G9382, 225 Bloom) was diluted in 50 mM tris-HCl buffer at $p\mathrm{H} = 7.4$ or in 22.5% w/w glycerol solution in the same buffer. Solutions were stirred at 60 °C for 1 h. For the measurement of gel-degradation kinetics, 120 μl of thermolysin solution was added to 1200 μl of gelatin solution at 60 °C, i.e., while the gelatin solution is still liquid. This solution was stirred for a few seconds, then put in a watertight tube containing a 2 mm diameter polyacetal ball (density 1.4), then cooled to 4 °C for 1 h to obtain rapidly a strong gel while slowing down the enzyme activity (from activation energy [8], the thermolysin activity is 2.7 times smaller at 4 °C than at 14 °C). Once the gel formed, the tube was inverted and placed at $T = 14$ °C for gel-degradation kinetics measurement characterized by the time t_c at which the sample liquefies under the action of proteinases. We take this as the time at which the ball begins to fall.

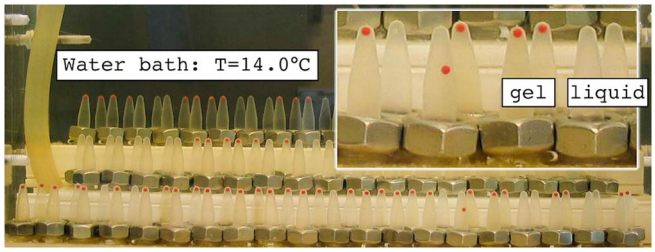

FIG. 1 (color online). Experimental setup for determination of the gel-sol transition time t_c: while the sample is still a solid gel, the red ball stays at the top of the tube (tubes 1, 4–6 from the left in the inset); at t_c the ball falls down (tube 3).

Photographing the device at time intervals allows t_c to be determined (Fig. 1) with sufficient accuracy. From the weight and size of the ball, the stress imposed to the gel is estimated to be about 5 Pa, smaller than in usual rheological measurements. Our method allows the simultaneous study of many samples (good statistics) over times as long as 40 days (no evaporation). Over 1000 distinct samples were involved in this study.

Results.—The gel-degradation time t_c was measured for different gelatin volume fractions ϕ_{gel} as a function of thermolysin concentration $[E]$. For a given ϕ_{gel}, we observed a standard deviation of the data of the order of 30%; for this reason, up to 10 samples were studied at each concentration. In Fig. 2, the mean degradation time is plotted as a function of $[E]$ for different ϕ_{gel}. For $\phi_{\text{gel}} = 1\%$, measurements performed using a cone-plate rheometer [9] give same results as the falling ball device, which gives confidence in neglecting shear effects due to the latter. In opposition, the present data differ slightly from those previously reported [3] that were obtained by quasielastic light scattering on latex particles trapped in the gel [10]. This discrepancy comes either from a different gelatin batch, from a different thermal history, or from the much better accuracy of the present study. For each ϕ_{gel}, t_c follows a power law dependence on $[E]$: $t_c([E]) = t_c(1)[E]^{-1/b}$. In Fig. 3, the best-fit values for $t_c(1)$ and $1/b$ are plotted as a function of ϕ_{gel}.

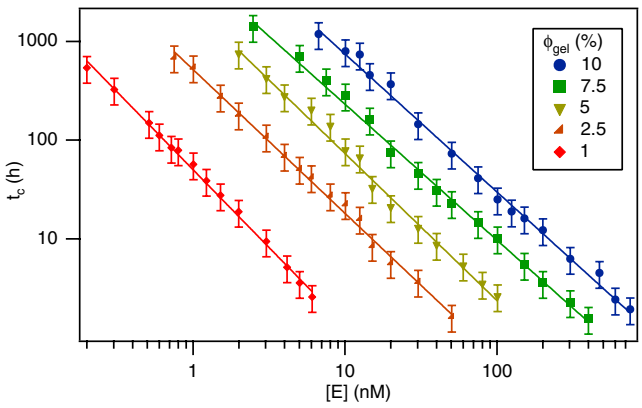

FIG. 2 (color online). Mean degradation time t_c vs thermolysin concentration $[E]$ for different gel volume fractions ϕ_{gel}. Lines are best fits $t_c = t_c(1)[E]^{-1/b}$.

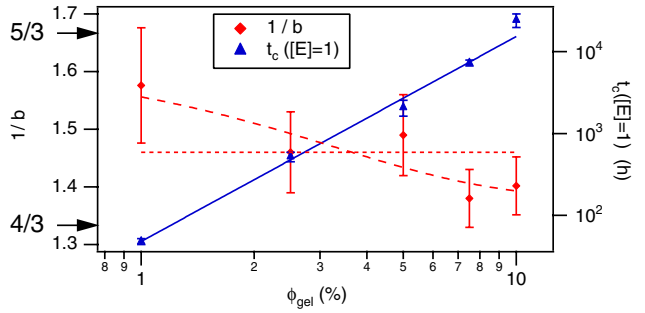

FIG. 3 (color online). Result of the power law fits to the data: $t_c([E]) = t_c(1)[E]^{-1/b}$ vs ϕ_{gel}. The solid line and the horizontal dotted line correspond to $(t_c/\text{h}) = (48 \pm 4)(\phi_{\text{gel}}/\%)^{2.50\pm0.05} \times ([E]/\text{nM})^{-1.46\pm0.07}$. The nonhorizontal dashed line is a guide for the eyes.

Similar measurements were performed after increasing the solvent viscosity through the addition of glycerol. We observed an increase in t_c by a factor that was independent of both $[E]$ and ϕ_{gel}. Actually, whatever ϕ_{gel}, the data can be superimposed to those obtained without glycerol by taking into account both the decrease of the enzyme activity A_r and the increase of the solvent viscosity η_r and plotting t_c/η_r as a function of $[E]A_r$ as shown in Fig. 4 for $\phi_{\text{gel}} = 7.5\%$. Similar results were obtained for the other ϕ_{gel} values of Fig. 2. This gives an evidence that the gel-degradation kinetics is diffusion limited. The results can be summarized as follows:

$$t_c \propto \eta_r \phi_{\text{gel}}^{2.50\pm0.05}[E]^{-1.46\pm0.07}. \quad (1)$$

Scaling.—Let us model the gel prior to any degradation as a semidilute solution. Only one length, the mesh size $\xi_0 = a\phi_{\text{gel}}^{-\nu_F/(3\nu_F-1)}$ [11], is pertinent to describe the gel, where a is the length of the statistical chain segment and

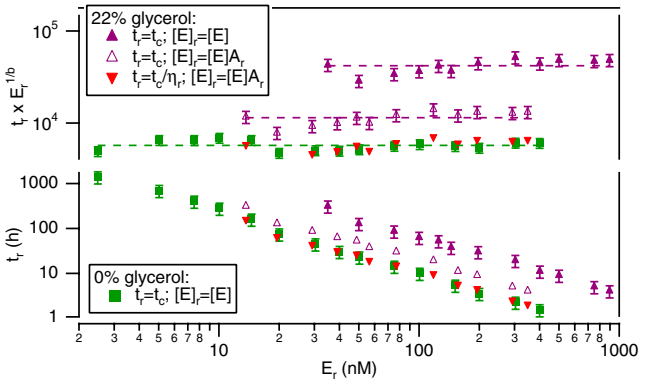

FIG. 4 (color online). The effect of glycerol on t_c for $\phi_{\text{gel}} = 7.5\%$. Bottom: t_r vs $[E]_r$. Top: $t_r \times [E]^{1/b}$ vs $[E]_r$. t_r is either t_c or t_c/η_r, depending on symbols, with η_r the relative solvent viscosity. $[E]_r$ is either $[E]$ or $[E]A_r$, depending on symbols, with A_r the relative enzyme activity.

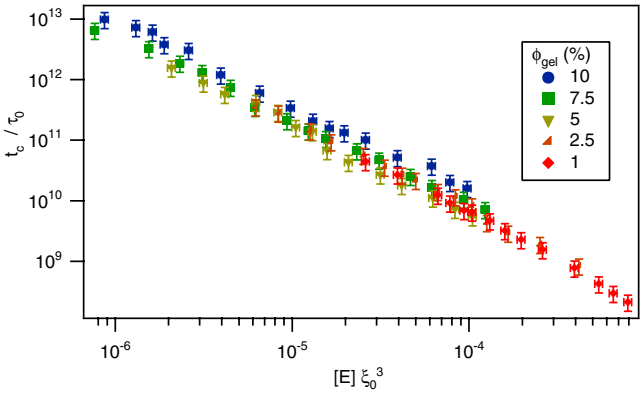

FIG. 5 (color online). Same data as in Fig. 2 using the reduced variables of Eq. (2) with $\xi_0 = a\phi_{\mathrm{gel}}^{-1}$ ($\nu_F = 1/2$). Adimensional quantities are obtained with $a = 0.7$ nm [19] and $D_0 = 83\ \mathrm{nm}^2/\mu\mathrm{s}$ [3].

ν_F the Flory exponent. The evidence for a diffusion-driven kinetics allows us to define an elementary time as that of diffusion over this length: $\tau_0 = \xi_0^2/D_0$, with D_0 the diffusion coefficent of the enzyme in the solution. In Fig. 5, the kinetics measurements are plotted using the reduced variables: $[E]\xi_0^3$ (the number of enzymes per mesh) and t_c/τ_0, with ν_F as the only adjustable parameter. The data collapse to one curve, obtained over 4 orders of magnitude in time, with $\nu_F = 1/2$, the ideal-chain value. Equation (1) can be rewritten as:

$$t_c/\tau_0 = f([E]\xi_0^3) \quad \text{with} \quad f(x) \propto x^{-1/b}. \tag{2}$$

Gelatin is denatured collagen and behaves at high temperature as swollen polymer chains ($\nu_F = 3/5$). At low temperature, gelation is due to the formation of rodlike triple helices as cross-links between chains ($\nu_F = 1$). Far from the gel point and whatever ϕ_{gel}, such triple helices involve about 50% of the gelatin strands, as revealed by optical rotation measurements [6]. Similar measurements performed during gel degradation show that this fraction remains until $2t_c$. Thus the network can be viewed as rigid rods end-linked by random coils [7]. The value $\nu_F = 1/2$ deduced from the scaling of the data presumably accounts for this mixed composition rather than for an actual Gaussian structure.

Modeling.—The scale invariance [Eq. (2)] allows us to use a lattice model of unit spacing ξ_0, on which each enzyme is assumed to step from one bond to a neighbor at frequency τ_e^{-1}. Quite generally, the time τ_e spent by one enzyme in a network mesh is the sum of the diffusion time τ_0 and the reaction time τ_R needed for the enzymatic reaction of cleavage of one or more peptide bonds belonging to the network mesh. Let ψ be the fraction of cleaved bonds at time t. Because the reaction is of order 1 with respect to the enzyme, one has:

$$\frac{d\psi}{dt} = \sum_{i=1}^{E} \frac{d\psi_i}{dt}, \tag{3}$$

where ψ_i is the fraction of broken bonds due to a given enzyme i and E denotes the number of enzymes.

First, let us assume that at time t_c, the paths of the different enzymes completely overlap; i.e., the distance walked is much larger than $[E]^{-1/3}$. In this case, sufficiently long times are involved allowing us to consider degradation as homogeneous. As each elementary process is random, $d\psi_i/dt$ can be replaced by an averaged value k. The evolution rate of ψ is then:

$$\frac{d\psi}{dt} = Ek(\psi, t). \tag{4}$$

In the simplest case, $k = (1 - \psi)\tau_e^{-1}$. One may also consider a more general form $k(\psi)$ in order to take into account memory effects: the elementary process due to one given enzyme depends on whether the bond was already cut or not (by the considered enzyme or by another). This amounts to introducing enzyme interactions. All these forms lead to ψ being a function of the product Et and thus t_c proportional to E^{-1}, contrary to our experimental results. To avoid such a behavior, an explicit time dependence of k, through, for instance, $\tau_e \propto t^{\beta}$, could be invoked. However, we see no physical basis for this. Similarly—but not being accounted for by Eq. (4)—the possibility of a wide, anomalous distribution of τ_e should be mentioned. In this case, the enzymes undergo a subdiffusive motion. Such an explanation would require a wide time distribution extending at least up to t_c ($\simeq$1000 h), which is doubtful.

In the opposite case, if the different paths do not overlap, the individual evolution rates are independent, then:

$$\frac{d\psi_i}{dt} = k(\psi_i, t) \tag{5}$$

with possible individual memory effect. Then, $\psi(t) = E\psi_i(t)$ with $\psi_i(t_c) = \psi_c/E$. In this case, t_c is a function of ψ_c/E that may lead to a nontrivial dependence of t_c on the enzyme concentration. This may account for the experimental results provided that enzyme random walks (RW) remain insulated from others up to t_c. RW must be compact and thus self-attractive (SAtRW).

SAtRW [12] was already studied by Monte Carlo simulations [13,14]. It is generated by allowing the walker to access a bond it already visited with a probability p^+ greater than that p^- of penetrating virgin area. There is a critical ratio $p_+/p_- = 6.8 \pm 0.2$ above which SAtRW collapses and is compact [14]. Then, each enzyme creates a compact bubble of damaged gel. Let us consider the growth of a so-damaged lattice bubble of volume v, surface s, and radius r, using ξ_0 as length unit. Reflections of RW at the bubble boundary occur at a frequency $\propto \tau_0^{-1} s/v$ [13] and are efficient for bubble growth with probability τ_0/τ_e (probability to damage a lattice mesh). As $v \propto \psi_i$, one gets:

$$\frac{d\psi_i}{dt} \propto \tau_e^{-1}\psi_i^{-1/3} \tag{6}$$

leading to: $r \propto (t/\tau_e)^{\nu_{RW}}$ with $\nu_{RW} = 1/4$. This asymptote is valid for a spherical bubble. At short times, surface roughness causes a still slower rate of growth. Then the actual ν_{RW} value would be closer to 1/5 [14]. Finally:

$$r \propto (t/\tau_e)^{\nu_{RW}} \quad \text{with} \quad 1/5 < \nu_{RW} < 1/4. \tag{7}$$

The time t_c corresponds to the overlap of bubbles: $r(t_c) = (t_c/\tau_e)^{\nu_{RW}} = [E]^{-1/3}/\xi_0$. Thus:

$$t_c/\tau_e = ([E]\xi_0^3)^{-1/3\nu_{RW}} \tag{8}$$

which accounts for the experimental dependence of t_c on $[E]$. A chain segment of size ξ_0 contains several sites of size a that are able to be cleaved by the enzyme. Let us denote $\tau_a = a^2/D_0$ and d_c the mean distance between two first neighbor cleavage sites. As $d_c < \xi_0$, d_c is independent of ξ_0 and ϕ_{gel}. At each step of duration τ_a, the enzyme RW meets a cleavage site with probability $\phi_c = a^3/d_c^3$. After ξ_0^2/a^2 steps, the total time spent for the chemical reaction is $\tau_R \propto (\xi_0^2/a^2)\phi_c\tau_r$, with τ_r^{-1} the rate constant of the chemical reaction. The total time $\tau_e = \tau_0 + \tau_R$ the enzyme spends per network mesh is

$$\tau_e = \tau_0(1 + \phi_c\tau_r/\tau_a). \tag{9}$$

Therefore, Eq. (8) accounts also for the scaling of our data [Eq. (2) and Fig. 5]. From Eq. (7), a smooth crossover bridges the regime $1/3\nu_{RW} = 5/3$ at short times to $1/3\nu_{RW} = 4/3$ at long times; we therefore expect a variation in the apparent exponents. Figure 5 is an experimental determination of this crossover function. From this figure, short reduced degradation times correspond to low gel concentrations. Then, the trend indicated by the dashed line in Fig. 3 is perfectly consistent with our data being in this crossover range. Note that in order to account for the experimental effect of the solvent viscosity, the quantity $\phi_c\tau_r/\tau_a$ in Eq. (9) must either be independent of the viscosity or much smaller than 1. This point is not yet understood.

In this model, the critical behavior corresponds to the continuum percolation model (''Swiss cheese'') [15]. Its static properties map onto classical bond percolation. As bubbles are randomly distributed (as enzymes), the equivalent bond percolation is not correlated, unlike for ''Pacman percolation'' [4]. However, critical exponents for transport properties differ in a continuum percolation model from the corresponding bond percolation model [16]. The major consequence of the ''Swiss cheese'' model is the existence in three dimensions of a permeation threshold occurring before gel degradation: bubbles can overlap leaving the complementary space still solid [17]. Note that this ''Swiss cheese'' model could be pertinent to describe the matrix degradation of bones by proteinase K in osteoporosis. With respect to cell invasion, permeation would allow cells to move through the gel even if it is macroscopically still solid. In this model, a single invasive cell would produce its own tunnel through the extracellular matrix. *In vivo*, invasive cells act as proteinases sources. This is not accounted for by our study that considers a homogeneous repartition of proteinases at $t = 0$. But rather to mimic the *in vivo* complexity, the present study aims to capture the physical mechanism of proteinases work. The major consequence of our model is that the proteases working in this manner do not diffuse through the matrix but degrade it locally in the vicinity of the cell. Owing to scaling, all the microscopic features have been overlooked even though they are important from a biochemical point of view: in particular, at a molecular level, the origin of the self-attraction for enzyme RW remains to be elucidated. This latter point is presumably not specific to our enzyme-gel system; e.g., Ref. [18] reports unexpected association kinetics of gelatinase A at the surface of gelatin gel conjointly with a constrained diffusion of gelatinase.

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