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Scaling Behavior and Cluster Fractal Dimension Determined by Light Scattering from Aggregating Proteins

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The size distribution $n_i(t)$ for systems with Smoluchowski aggregation kinetics is shown to have the dynamic scaling form $n_i(t) \sim t^{-\theta} g(1/t^z)$, with $\theta = 2$ and $z = 1$. This gives a light-scattering intensity $\langle I \rangle \sim t^z$, and an effective hydrodynamic cluster radius $\langle R \rangle \sim t^{z/D}$ where D is the cluster fractal dimension. We find that human immunoglobulin (IgG) monomers exhibit Smoluchowski aggregation kinetics with $z = 1$, and $D = 2.56 \pm 0.3$, upon heating to 47–62 °C.

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The aggregation of monomers and the clumping of clusters is of central interest in biology, immunology, polymer and colloid chemistry, metallurgy, and in all kinds of nucleation phenomena at phase transitions.

Studies by Forrest and Witten of smoke-particle aggregates,[1] and computer simulations,[2-6] make it clear that aggregates are characterized by a Hausdorff or fractal dimension[7] D, by the relation $N = (r/R_0)^D$. Here N is the number of particles inside a radius r from the center of the aggregate and R_0 is the monomer radius. It follows that the characteristic radius R of an aggregate is related to the number of particles, i, in the cluster by $R_i = R_0 i^\beta$, with the cluster exponent, β, given by $\beta = 1/D$. By electron microscopy one finds $D = 1.5$–1.6 for Fe, Zn, and SiO_2 clusters,[1] $D = 2.3$ for carbon-black aggregates,[8] and $D = 1.7$ for gold colloids.[9] Optical microscope observation of two-dimensional clusters of small spheres[10] gives $D = 1.6$. Neutron diffraction[11] from silica particles gives $D = 2.6$. We show below that immunoglobulin aggregates induced by heating form clusters with a fractal dimension $D = 2.56 \pm 0.3$. The observed fractal dimensions $D(d)$ are significantly less than the spatial dimension d.

Witten and Sander[2,3] simulate diffusion-limited aggregation by letting "particles" do random walks on a lattice. The particles stick irreversibly on contact. Such simulations[2-6] give $D(2) = 1.7$ and $D(3) = 2.5$. Generalizations where many monomers are present initially and where the clusters also may diffuse[12-16] generate ramified clusters with $D(2) = 1.4$–1.45 and $D(3) = 1.75$–1.8.

Smoluchowski[17] introduced the equations for aggregation kinetics:

$$\frac{dn_k}{dt} = \sum_{i+j=k} n_i A_{ij} n_j - 2\sum_j n_k A_{kj} n_j, \qquad (1)$$

where $n_i(t)$ is the i-mer concentration, normalized to the initial monomer concentration c_0, and $\sum_i i n_i = 1$. The reduced time is $t = \gamma_S \hat{t}$, where $\hat{t}$ is the time, and the Smoluchowski diffusion-limited

collision rate constant is $\gamma_S = 8\pi D_0 R_0 c_0 \epsilon$. Here the monomer diffusion constant is D_0 and R_0 is the hydrodynamic radius. The probability that two colliding clusters will stick irreversibly is ϵ. Smoluchowski gave the collision matrix as $A_{ij} = (D_i + D_j)(R_i + R_j)/4D_0R_0$. Using the Einstein-Stokes expression for the diffusion constant, $D_i = kT/6\pi\eta R_i$, with viscosity η and $R_i = R_0 i^\beta$, we obtain

$$A_{ij} = \tfrac{1}{4}(i^{-\beta} + j^{-\beta})(i^\beta + j^\beta). \quad (2)$$

Here we have assumed that the effective hydrodynamic radius of an aggregate is the same as the radius used for the definition of the fractal dimension. No general solution of (2) exists. The kernel A_{ij} is only weakly dependent on β, and nearly a constant. Smoluchowski made the simplification $A_{ij} = 1$ for all i and j, and gave the exact solution of (1) when only monomers are present initially:

$$n_i(t) = t^{i-1}/(1+t)^{i+1} \sim t^{-\theta} g(i/t^z). \quad (3)$$

We have also given in (3) the asymptotic form easily obtained from the exact distribution in the limit of large times. Smoluchowski's solution has the exponents $\theta = 2$, $z = 1$, and the scaling function $g(x) = \exp(-x)$. The scaling form of the size distribution expresses the fact that with $A_{ij} = 1$, there is no intrinsic characteristic cluster size. The scaling form for the size distribution is the phenomenological form expected to be valid for the more general case where density fluctuations are important. The average cluster size, $i^* = \sum_i i^2 n_i = 1 + 2t \sim t^z$, increases with time and is the size scale of the aggregation problem. Vicsek and Family[18] found that two-dimensional simulations give $\theta = 2$, and $z = 1.4 \pm 0.2$, indicating that density fluctuations may lead to deviations from results obtained from the classical kinetic equations.

We have studied the aggregation kinetics of immunoglobulins[19] by quasielastic light scattering.[20,21] For clusters with $R_i^{-1} >> Q = (4\pi n/\lambda)\sin\Theta/2$, where the scattering vector Q is given by the scattering angle Θ, the index of refraction n, and the laser wavelength λ, the scattering intensity $\langle I \rangle$ may be expressed by

$$\langle I \rangle / I_0 = \sum_i n_i i^2 = (1 + 2t) \sim t^z; \quad (4)$$

here I_0 is the initial scattering intensity. The asymptotic form follows from (3). Note that $\langle I \rangle / I_0 = i^*$, the average cluster size. The intensity correlation function $C(\Delta t) = \langle I(0) I(\Delta t) \rangle$, where Δt is the delay time, measures the effective hydrodynamic radius $\langle R \rangle = kTQ^2/6\pi\eta\langle\Gamma\rangle$. The decay constant, $\langle\Gamma\rangle$, of the intensity correlation function is obtained by fitting an exponential to $C(\Delta t)$, or equivalently by using a cummulant expansion.[20,21] We find that $\langle R \rangle$ may be written as

$$\langle R \rangle / R_0 = \sum_i i^2 n_i / \sum_i i^{2-\beta} n_i \sim t^{z/D}, \quad (5)$$

where we also give the asymptotic scaling form. The asymptotic forms in (4) and (5) result when the scaling form of the size distribution is used in the evaluation of the sums. The precise result as $t \to \infty$ is $\langle R \rangle / R_0 \to [2/\Gamma(3-\beta)]\, t^\beta = \gamma_\infty t^\beta$, where $\Gamma(x)$ is the gamma function. In the limit $t \to 0$, we find $\langle R \rangle / R_0 = (1 + \gamma_0 t)^\beta$, with $\gamma_0 = 4(1 - 2^{-\beta})/\beta$, using a series expansion.[20] We note that γ_0 and γ_∞ differ at most by 5% for β in the range 0.3 to 1. There is no simple general expression for $\langle R \rangle / R_0$. However, the form

$$\langle R \rangle / R_0 = (1 + \gamma t)^{\tilde\beta} \quad (6)$$

is consistent with our asymptotic results, and approximates (5) well. We have fitted numerical results obtained by solving (1) with (2) for A_{ij}, in the range $0 \leq t \leq 5$ corresponding to our experimental range. We find $\tilde\beta = -0.0215 + 1.056\beta$, for β in the range 0.3–0.5. To better than a few percent $\langle I \rangle$ satisfies (4). If instead we use $A_{ij} = 1$, we find $\tilde\beta = \beta$. The growth rate constant γ is in the range γ_∞ to γ_0. We conclude that measurements of $\langle I \rangle$ and $\langle R \rangle$ as functions of time allow the direct determination of the exponent z and the cluster fractal dimension $D = 1/\beta$, for systems with Smoluchowski aggregation kinetics.

We prepared monomeric IgG from pooled human immunoglobulins (Gammaglobulin Kabi 16%, AB Kabi, Stockholm, Sweden) by gel filtration. The hydrodynamic radius of IgG monomers is $R_0 = 5.51 \pm 0.03$ nm, at 20 °C in water, and the molecular weight is 163 000. Samples with 2, 4, 8, and 16 mg/ml monomers in a 0.2*M* NaCl, *tris*-HCl buffer at *p*H = 7.6 were heated to 47, 53, 56, 59, and 62 °C, and $\langle I \rangle$ and $\langle R \rangle$ measured as functions of time. Heating causes these proteins to aggregate as seen by the increase in $\langle I \rangle$ and $\langle R \rangle$. The process is irreversible—it may be halted by cooling the sample, and it resumes when heated again. The experimental results can be fitted accurately with the forms $\langle R \rangle / R_0 = (1 + \Gamma_R t)^{\tilde\beta}$, and $\langle I \rangle / I_0 = (1 + \Gamma_I t)$. We obtain aggregation rates Γ_R and Γ_I, which increase rapidly with temperature. The experimental results as a function of the reduced time $\tau = \Gamma_R t$ and $\Gamma_I t$ are shown in Fig. 1. A very satisfactory data collapse of all our results demonstrates that a scaling form of the cluster size distribution is consistent with the experimental results. $\langle I \rangle$ increases linearly with time and $z = 1$. We find

$$\Gamma_R = \Gamma_R^0 \exp[-\Delta H^*/(1/RT_0 - 1/RT)],$$

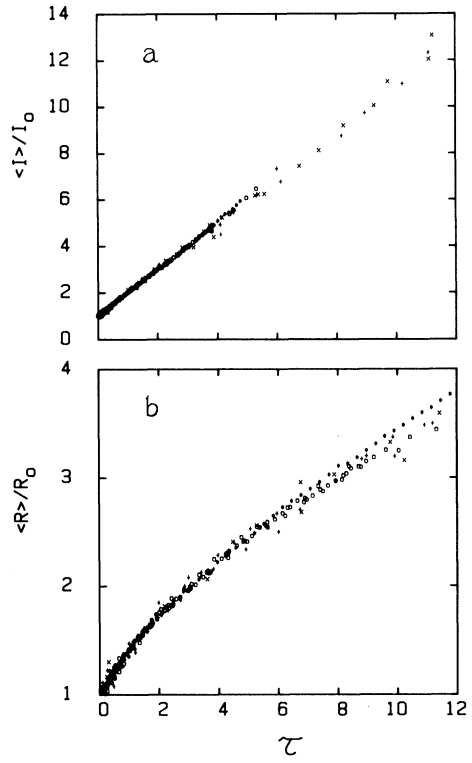

FIG. 1. (a) The reduced scattering intensity and (b) the effective hydrodynamic radius, as functions of the reduced time $\tau = \Gamma t$, for heat aggregation of IgG monomers at 2, 4, 8, and 16 mg/ml and at 47°C (circles), 53 °C (asterisks), 56 °C (squares), 59 °C (crosses), and 62 °C (pluses).

with an activation enthalphy for the aggregation process $\Delta H^* = 120 \pm 5$ kcal/mol. At the reference temperature $T_0 = 329$ K, the radius growth rate constant is $\Gamma_R^0 = \rho c_0$, with $\rho = 0.18 \pm 0.01$ ml/mg·h, which yields a very small value for the sticking coefficient $\epsilon = 1.1 \times 10^{-9}$. The exponent $\tilde{\beta} = 0.48 \pm 0.05$ must be corrected for the presence of nonaggregating species before the cluster exponent can be obtained. By heating monomeric samples to 62 °C for six hours the aggregation process produces white precipitates. However, gel filtration and ultracentrifuge studies show that (48 ±5)% of the IgC monomers form a heat-stable subclass, the *H* fraction, which remains as monomers in the experiments presented in Fig. 1. We have solved (2) and (3) numerically and evaluated $\langle I \rangle$ and $\langle R \rangle$ by adding 50% nonaggregating monomers in (4) and (5). The calculated results give a linear increase in $\langle I \rangle$ with time, and (6) fits the numeric results as well as before but $\tilde{\beta}$ is larger: $\tilde{\beta} = 0.0153 + 1.198\beta$. We conclude that the clusters may be described by a cluster exponent $\beta = (\tilde{\beta} - 0.015)/1.2 = 0.39 \pm 0.04$, or a fractal dimension $D = 2.56 \pm 0.3$. It would have been desirable to pursue the aggregation process to longer times in order to get a more precise value for *D*. However, with increasing $\langle R \rangle$ the condition $R_i Q \ll 1$ is finally violated and more elaborate expressions than (4) and (5) are required.

The coagulation equation (1), with various kernels A_{ij}, is widely used in the discussion of polymerization and gelation.[22] Some of these kernels, such as the multiplicative form $A_{ij} = (ij)^{D-1}$, lead to results qualitatively inconsistent with our experiments. The combined effect of an activated collision process as described by a statistical reaction rate K_{kj} and of the diffusion-limited process (2) is obtained by using $A_{ij}^* = K_{ij}/(1 + K_{ij}/A_{ij})$ as the kernel in (1).[22,23] We have found that $\epsilon = 1.1 \times 10^{-9}$, and conclude that the aggregation process is not diffusion limited. This is consistent with our classical value for *z*. Two recent papers[16,24] are relevant to the discussion of our results. The observed fractal dimension $D = 2.56$ is well above the value[16] $D(3) = 1.8$ for the diffusion-limited aggregation of clusters with clusters. No simulations are available for the case of very small sticking probabilities; therefore we cannot determine if the clusters are more compact than the existing simulations predict because of molecular interactions or because of the very low value of ϵ. Recently Chen, Deutch, and Meakin[24] generated clusters with a cluster exponent $\beta = 0.4$, and determined the hydrodynamic radius for the cluster, $R_h = R_0 i^{\beta_h}$, from the calculated friction coefficient, with the result $\beta_h = 0.47 \pm 0.01$. This result indicates that the observed *D* is less than the cluster fractal dimension.

We conclude that quasielastic light scattering can measure the exponents of aggregation kinetics directly. IgG aggregation proceeds as a Smoluchowski process with $D = 2.56$. For a better understanding of IgG aggregation, simulations with very small sticking coefficients are required, and for comparison with experiments the hydrodynamic radii for the aggregates should be evaluated.

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