Viscoelastic Effect on the Formation of Mesoglobular Phase in Dilute Solutions

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ABSTRACT: In dilute heteropolymer solutions, a limited number of the chains, just like proteins, could collapse and associate to form a stable/metastable mesoglobular phase between single-chain globules and macroscopic precipitates. Recently, we found that inserting more hydrophobic comonomers into a thermally sensitive chain backbone surprisingly led to the formation of smaller mesoglobules in water. This apparent contradiction to our conventional wisdom can be satisfactorily explained in terms of the overlooked viscoelastic effect; namely, hydrophobic association inside each mesoglobule increases the chain relaxation time (τ_e). When it becomes much longer than the interaction time (τ_c) of two colliding mesoglobules, each mesoglobule behaves like a tiny nonadhesive "glass" ball. This stabilization mechanism is completely different from thermodynamic consideration in which one normally tries to make the particle surface hydrophilic so that τ_c is reduced.

Associating copolymers normally consist of a water-soluble hydrophilic chain backbone and a few molar percentages of hydrophobic comonomers incorporated as a long block or randomly distributed segments or grafted chains. The association of diblock and triblock amphiphilic chains in dilute solutions in water or selective solvent to form stable polymeric core−shell-like micelles has been extensively studied.[1,2] The telechelic associating copolymers with a hydrophobic end or comb-type associating copolymers with short or long grafted hydrophobic chains can also form micelle-like or rosette-like clusters.[3] Recently, it has been shown that even random nonionic amphiphilic copolymers can form stable aggregates, a mesoglobular phase between individual collapsed single-chain globules and macroscopic precipitation.[4]

Thermodynamically, the stabilization is attributed to the formation of a more hydrophilic periphery during the chain association in microphase separation. Hydrophobically modified thermally sensitive poly(*N*-isopropylacrylamide) (PNIPAM) is a special kind of amphiphilic copolymer. Its degree of amphiphilicity in water can be conveniently adjusted by a simple change of the solution temperature because the PNIPAM backbone can change from hydrophilic to hydrophobic at a lower critical solution temperature (LCST) of ∼32 °C. The LCST can be increased (decreased) by its copolymerization with hydrophilic (hydrophobic) comonomer.[5,6] When a PNIPAM aqueous solution is heated over its LCST, two processes simultaneously occur, namely, (1) intrachain contraction, leading to the collapse of individual chains from a swollen coil to a compact globule, and (2) interchain association, resulting in aggregation and macroscopic precipitation.[7,8]

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It is easy to understand that copolymerization of the hydrophilic comonomer with PNIPAM can lead to the formation of stable mesoglobules at temperatures higher than the LCST because the hydrophilic components as stabilizers have a tendency to stay on the periphery during the microphase separation. On the other hand, conventional wisdom tells us that copolymers with a higher hydrophobic content should form larger and less stable aggregates. However, our recent experiments showed an opposite and surprising result; namely, PNIPAM copolymer chains with a higher hydrophobic content form smaller mesoglobules, which can be attributed to the viscoelastic effect because solvent and long chains are dynamically asymmetry. The detail is reported as follows.

First, different amounts of bioactive hydrophobic 2′-methacryloyl-amino-ethylene-3α,7α,12α-trihydroxy-5β-cholanoamide (MACA) were coploymerized with PNIPAM, which are hereafter denoted as PNIPAM-*co*-x-MACA, where x is the molar percent of MACA. The synthetic detail can be found elsewhere.[9−11] The effects of MACA content, heating rate, and copolymer concentration on the formation of mesoglobular phase were studied by a combination of static and dynamic laser light scattering (LLS). It is helpful to note that poly(MACA) homopolymer itself is insoluble in water at room temperature.[9,10] As expected, the solubility of these copolymers in water decreases with an increasing MACA content or temperature. To start with individual single chains, we kept each dilute solution in a refrigerator for more than 1 day to ensure a complete dissolution. The solution was clarified by a 0.45 Millipore (Hydrophlic Millex-LCR, PTFE) filter and then kept in refrigerator again before the LLS measurements.

A commercial LLS spectrometer (ALV/DLS/SLS-5022F) equipped with a multi-τ digital time correlation (ALV5000) and a 22-mW UNIPHASE He−Ne laser (λ_0 = 632 nm) as the light source was used. The details of LLS instrumentation and theory can be found else-

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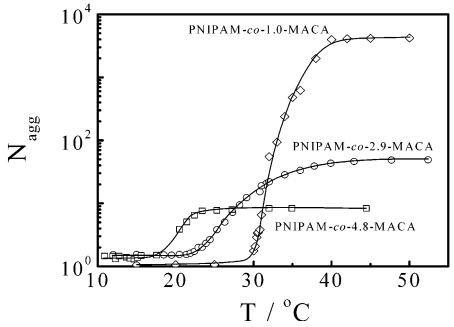

Figure 1. Temperature dependence of average aggregation number (N_{agg}) of PNIAPM-*co*-MACA mesoglobules formed in a gradual heating process, where $N_{agg} = M_{w,agg}/M_{w,chain}$ with $M_{w,agg}$ and $M_{w,chain}$, the weight-average molar masses of the copolymer aggregates and of the chains, respectively, and the copolymer concentration is $\sim 10^{-5}$ g/mL. Note that each data point was obtained after the temperature equilibrium was reached.

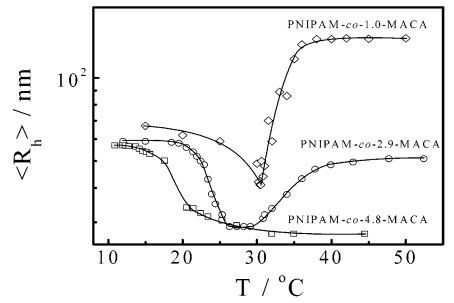

Figure 2. Temperature dependence of the average hydrodynamic radius ($\langle R_h \rangle$) of PNIAPM-*co*-MACA mesoglobules formed in a gradual heating process, where each data point was obtained after the temperature equilibrium was reached and the copolymer concentration is $\sim 10^{-5}$ g/mL.

where.[12] In static LLS, the angular dependence of the absolute excess time-average scattering intensity, known as the Rayleigh ratio $R_{vu}(q)$, leads to the weight-average molar mass (M_w), the root-mean-square radius of gyration $\langle R_g^2 \rangle_z^{1/2}$ (or written as $\langle R_g \rangle$), and the second virial coefficient A_2 of the scattering objects. In dynamic LLS, each measured intensity−intensity time correlation function $G^{(2)}(t,q)$ in the self-beating mode results in a characteristic line-width distribution $G(\Gamma)$. For diffusive relaxation, Γ is related to the translational diffusion coefficient (D) of a scattering object (polymer chain or colloid particle) in a dilute solution or dispersion by $(\Gamma/q^2)_{q\to 0, C\to 0} = D$ or to the hydrodynamic radius (R_h) by the Stokes−Einstein equation.

Figure 1 shows an expected decrease of the LCST with an increasing hydrophobic MACA content. For PNIPAM-*co*-1.0-MACA, a gradual heating of the solution from 10 to 50 °C leads to large mesoglobules with $N_{agg} \sim 4000$, whereas for PNIPAM-*co*-4.8-MACA, the same heating results in much smaller aggregates only with $N_{agg} \sim 10$, which clearly shows that N_{agg} decreases with an increasing MACA content. On the other hand, the initial decrease of $\langle R_h \rangle$ in the range T < LCST, as shown in Figure 2, reflects intrachain contraction because $N_{agg} = 1$ in the same temperature range. For PNIPAM-*co*-1.0-MACA, the increases of both N_{agg} and $\langle R_h \rangle$ in the range T > LCST reflect that interchain association becomes dominant. For PNIPAM-*co*-4.8-MACA, Figure 2 shows a continuous decrease of $\langle R_h \rangle$ before its leveling-off at ~32 °C, similar to the coil-to-globule transition of individual single chains.[13] However, the increase of N_{agg} in Figure 1 reveals that there still exists interchain association even thought intrachain contraction is dominant here. For PNIPAM-*co*-2.9-MACA, the conversion from intrachain- contraction dominant to interchain-association dominant is fairly clear. The leveling-off of N_{agg} and $\langle R_h \rangle$ for all of the samples at higher temperatures indicates that such resultant chain aggregates are stable. This is why they are described as a special mesoglobular phase between individual single-chain globules and macroscopic precipitation.[4,14]

Now, let us discuss why those chains with a high content of hydrophobic MACA form smaller aggregates. Picarra and Martinho[15] showed that in the phase separation of a thin-layer dilute *homopolymer* solution on surface, the collision would not be effective as long as the collision (or contact) time (τ_c) is shorter than the time (τ_e) needed to establish a permanent chain entanglement between two approaching aggregates. Quantitatively, Tanaka[16] showed that τ_c and τ_e could be roughly characterized as

$$\frac{l_0}{\langle v \rangle} < \tau_c < \frac{l_0^2}{\langle D \rangle} \text{ and } \tau_e \sim \frac{a_m^2 N_m^3 \varphi_p^{3/2}}{D_m} \qquad (1)$$

where l_0 is the interaction range, $\langle v \rangle$ and $\langle D \rangle$ are the mean thermal velocity and transitional diffusion coefficient of the aggregates, respectively, ϕ_p is the average polymer concentration inside chain aggregates, and a_m, N_m, and D_m are the length, number, and diffusion coefficient of the monomer, respectively. Note that, in the study of homopolymer solutions,[17] the transient homopolymer aggregates were named as the "moving droplet phase" so that the concept of "viscoelastic phase separation" was introduced. However, it is questionable whether such slowly aggregated transient homopolymer "droplets" is a phase because viscoelasticity only slows down the aggregation. In other words, the time difference between τ_c and τ_e is not sufficiently large. In the present case, the aggregates made of amphiphilic chains are stable over months. This is because when $\tau_c \ll \tau_e$ two colliding aggregates have no time to stick together and they behave just like two tiny elastic nonadhesive "glass" balls. Such an effect is characteristic of the viscoelasticity of long polymer chains. Our previous study showed that ϕ_p in the collapsed state could be as high as 30 wt % even though the overall concentration was very low ($\sim 10^{-6}$ g/mL).[19] Therefore, the relaxation of long chains inside each aggregate is very slow. Such homopolymer aggregates are thermodynamically unstable because there is no hydrophilic stabilization group on the periphery to reduce τ_c.

Equation 1 shows that, for a given polymer solution under a fixed set of experimental conditions, l_0, a_m, N_m, and D_m are constants. One can only increase $\langle D \rangle$ and ϕ_p to ensure that $\tau_c \ll \tau_e$ in order to obtain a stable mesoglobular phase. Over the years, researchers always try to make the periphery of aggregates hydrophilic to prevent aggregation based on thermodynamics, which actually reduces l_0 and τ_c but overlooked the effect of increasing τ_e to keep $\tau_c \ll \tau_e$, i.e., playing the viscoelastic effect on the stabilization of mesoglobular phase. As expected, increasing the hydrophobic MACA content promotes both interchain association and intrachain contraction. In dilute solutions, enhancing intrachain contraction increases ϕ_p even though the overall macroscopic concentration remains. At the same time, τ_c decreases because the strong intrachain contraction reduces the initial size of the aggregates, leading to a

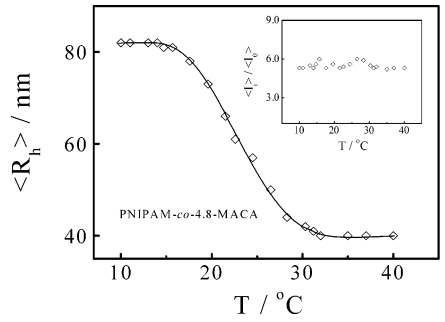

Figure 3. Temperature dependence of the average hydrodynamic radius ($\langle R_h \rangle$) of PNIAPM-*co*-4.8-MACA mesoglobules formed in a gradual heating process, where each data point was obtained after the temperature equilibrium was reached and the copolymer concentration is 10-times more dilute than that in Figures 1 and 2. The inset shows the temperature dependence of average scattering intensity ($\langle I \rangle / \langle I \rangle_0$), where $\langle I \rangle_0$ is the reference intensity.

higher $\langle D \rangle$. Moreover, hydrophobic association inside each mesoglobule significantly slows down the chain relaxation inside each mesoglobule and increases τ_e. Therefore, in some senses, it is not unreasonable to claim that the hydrophobic association plays a partial role in the stabilization of these mesoglobules.

Following such a viscoelastic consideration, there are different ways to increase τ_e. For example, using long chains (larger N) should be very effective in increasing τ_e on the basis of eq 1. Previously, we did found that longer chains could form smaller stable mesoglobules if other conditions were kept the same,[8] but we did not attribute it to the effect of viscoelasticity by that time. Conventionally, we would think that the association of long chains should lead to larger aggregates. However, we would forget that long chains can reach the condition of $\tau_e > \tau_c$ much more easily in the earlier stage of the microphase separation than short chains so that further intergloblar chain entanglements become impossible in the experimental time scale. In other words, the viscoelastic effect "overwrites" thermodynamics and leads to a special metastable, but very stable, state in dilute solutions.

On the other hand, considering such a competition between intrachain contraction and interchain association as well as the viscoelastic effect, we dilute the copolymer solution to the limit of our LLS detection in order to suppress interchain association. Figure 3 shows that after ~10-time dilution, there is no observable change in the time average scattering intensity $\langle I \rangle$ over the entire temperature range studied. Note that $\langle I \rangle$ is proportional to the weight- average molar mass (M_w) for a given copolymer concentration (C) and M_w is further proportional to $\sum M_i^2 N_i$ by its definition, where M_i and N_i are the molar mass and number of the ith species, respectively. Therefore, $\langle I \rangle$ is extremely sensitive to interchain association; namely, the association of two chains doubles the scattering intensity. The constant value of $\langle I \rangle$ indicates that there is no interchain association. Figure 3 represents a pure intrachain coil-to-globule transition. It is helpful to note that, due to the help of "hydrophobic stabilization", the experimental realization of the folding of individual MACA-modified PNIPAM copolymer chains without interchain association is much easier than that of PNIPAM homopolymer chains.[18] Further, we have also found (data not shown here) that intrachain contraction is promoted if the condition of phase separation is suddenly reached for the copolymer solution to increase ϕ_p and increase τ_e.

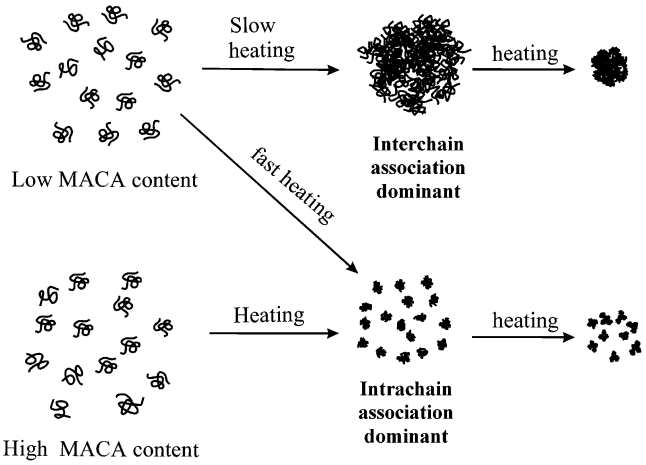

Figure 4. Schematic of competition between intrachain contraction and interchain association in formation of PNIAPM-*co*-MACA mesoglobules, respectively, made of the chains with low and high hydrophobic MACA contents.

The above discussion can be schematically summarized in Figure 4. The formation of the mesoglobular phase of amphiphilic heterocopolymer chains in dilute solution during the microphase separation inevitably involves of a competition between the intrachain contraction and interchain association. The resultant mesoglobules are stabilized not only by the well-thought concentration of hydrophilic components on the periphery but also by the overlooked hydrophobic association inside each mesoglobule. This can be attributed to the effect of viscoelasticity even in a dilute solution; namely, when the chain entanglement time (τ_e) between two colliding mesoglobules is much longer than their interaction time (τ_c), each mesoglobule becomes a tiny nonadhesive "glass" ball. Increasing the MACA content and chain length can increase τ_e. On the other hand, increasing the rate of microphase separation or diluting the solution can decrease τ_c because it can make intrachain contraction so dominate that the initial resultant mesoglobules are smaller with a larger diffusion coefficient. The more important point here is that we might have to reconsider the stabilization mechanism of globular proteins.

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