Formation of Mesoglobular Phase of Amphiphilic Copolymer Chains in Dilute Solution: Effect of Comonomer Composition

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ABSTRACT: Homopolymer poly(*N*,*N*-dimethylacrylamide) (PDMA) is soluble in water, but poly(*N*,*N*-diethylacrylamide) (PDEA) and poly(*N*-ethylacrylamide) (PEA) are soluble only in cold water. The copolymers made of DEA and DMA or DEA and EA are hydrophilic at $T < \sim 32$ °C, but become amphiphilic in the higher temperature range. The synthesis of two series of P(DEA-*co*-DMA) and P(DEA-*co*-EA) copolymers with a similar chain length, but different compositions (30 and 50 mol % of DMA and 40, 60, and 80 mol % of EA, respectively), enabled us to study the composition dependence of their association in water by laser light scattering. Our results showed that a limited number of these neutral copolymer chains could associate together to form stable mesoglobules existing between single-chain collapsed globules and macroscopic precipitation. Besides thermodynamical consideration, the formation of such mesoglobules can also be attributed to the competition between intrachain contraction and interchain association as well as the viscoelastic effect. Increasing the hydrophilic DMA or EA content leads to a larger average aggregation number, but increasing the heating rate results in smaller, but less dense, mesoglobules consisting of many loosely associated small single- or pauci-chain globules. For the copolymers with higher EA contents, we unexpectedly found that interchain association can gradually relax to intrachain association as time elapses.

Introduction

The self-assembly of amphiphilic block copolymers has been extensively studied in recent years because of its fundamental importance and industrial and biomedical applications. The self-assembly in a selective solvent normally results in spherical polymeric micelles with different sizes and structures in dilute solution.[1] Such formed micelles are commonly used in drug delivery, detergency and emulsification.[2] For instance, poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) (PEO−PPO−PEO) can form micelles in warm water and one of its potential applications is to protect cells from drugs.[3,4] However, most commercially available amphiphilic copolymers are prepared by a random copolymerization of two or more kinds of monomers with different chemical and physical properties. The association of these random copolymers in a selective solvent has also been extensively studied, but is much less understood, because of its complicated nature. For example, what is the effect of comonomer composition and comonomer distribution on their association behaviors? On the other hand, some biopolymers, such as proteins, can generally be considered as amphiphilic copolymers. Their special sequence of amino acids often leads to specified folding/association in biological system.

It has been known that association of copolymers in solution can be induced by varying a range of experimental conditions, such as solvent, polymer concentration, ionic strength and pH. In particular, for a thermally sensitive copolymer, in which at least one monomer component has a lower or higher critical solution temperature (LCST or UCST), we can conveniently use temperature to adjust the degree of its amphiphilicity. For examples, Miyazaki et al.[5] synthesized a series of poly(*N*,*N*-dimethylacrylamide-*co*-*N*-phenyl-acrylamide) (P(DMA-*co*-PA)) polymers with different amounts of PA by free radical copolymerization. They showed that the LCST of such copolymers determined from a sharp change of the solution turbidity was controllable and decreased with increasing hydrophobic PA content. The transition was thermally reversible.

Qiu et al.[6] studied poly(*N*-isopropylacrylamide-*co*-acrylic acid) P(NIPAM-*co*-AA)) with different amounts of ionic AA. They also showed that the controllable LCST increased with increasing hydrophilic AA content. Moreover, they demonstrated that the copolymer with a very small amount of ionic AA could form stable nanoparticles in water at temperatures higher than its LCST. The stabilization was attributed to static repulsion from anionic AA groups on the periphery of such nanoparticles. Itakura et al.[7] studied the solvent composition dependence of the association of poly(*N*,*N*-dimethylacrylamide)-*graft*-poly(methylmethacrylate) (PDMA-*g*-PMMA) in a methanol-and-water mixture and found that a quick change of solvent quality from good to poor could lead to smaller aggregates with a unimodal size distribution, while slowly changing the solvent quality resulted in larger aggregates with a bimodal size distribution. Qian et al.[8] studied the association of ethylene−vinyl acetate (EVA) random copolymers with a UCST in two different thermal processes. If the solution was quickly quenched from 70 to 0 °C, the aggregates were mainly made of individual collapsed chains. However, when the solution was slowly cooled

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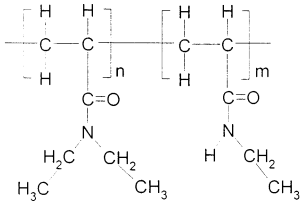
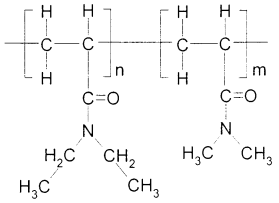
from 70 °C to room temperature and annealed for 24 h before further quenching to 0 °C, large microgels and fiberlike aggregates were formed via the interchain winding.

The examination of literature shows that the formation of stable aggregates made of neutral copolymer chains without any stabilizer has been proposed and predicted,[5−8] but not experimentally established yet. The effect of comonomer composition on the chain association has not been systematically investigated. This is partially because this kind of studies requires a challenging synthesis to control all the parameters so that only the comonomer composition is varied. In the present study, two series of thermally sensitive random copolymers, poly(*N*,*N*-diethylacrylamide-*co*-*N*-ethylacrylamide), P(DEA-*co*-EA), and poly(*N*,*N*-diethylacrylamide-*co*-*N*,*N*-dimethylacrylamide), P(DEA-*co*-DMA), with similar chain lengths, but different EA and DMA contents, were synthesized. Homopolymer PDEA changes from hydrophilic to hydrophobic when the temperature increases to higher than 32 °C, while PEA remains hydrophilic as long as the temperature is not higher than ∼82 °C and PDMA is always soluble in water. Heating the solution temperature to the range 32−82 °C makes these copolymers become amphiphilic so that they can associate in water through hydrophobic interaction between the PDEA segments. The association of these copolymers in water under different heating rates was studied by laser light scattering (LLS).



Chemical structure of poly(*N*,*N*-diethylacrylamide-*co*-*N*-ethylacrylamide)



Chemical structure of poly(*N*,*N*-diethylacrylamide-*co*-*N*-dimethylacrylamide)

Experimental Section

Sample Preparation. Poly(*N*,*N*-diethylacrylamide-*co*-*N*-ethylacrylamide), P(DEA-*co*-EA), and poly(*N*,*N*-diethylacrylamide-*co*-*N*,*N*-dimethylacrylamide), P(DEA-*co*-DMA), respectively, with 40, 60, and 80 mol % of EA and 50 and 70 mol % of DMA were synthesized by free radical polymerization.[9] P(DEA-*co*-EA) copolymers were prepared in THF with 2,2′-azobis(isobutyronitrile)) (AIBN) as initiator (1 mol %). The solution was bubbled with dry nitrogen for 15 min prior to polymerization. The temperature was gradually raised to 68 °C in a period of 2 h. and maintained for ∼18 h. Each reaction mixture was precipitated in ether or hexane. P(DEA-*co*-DMA) copolymers were prepared in methanol in a similar fashion. The copolymer compositions determined by ^{1}H NMR spectra were very close to the feed ratio of monomers prior to polymerization. The nomenclature used hereafter for these copolymers is P(DEA-*co*-x/*y*), where x is EA or DMA and *y* denotes the mol % content of x. Before the association study, the copolymers were characterized by laser light scattering, and the results are summarized in Table 1. The copolymer solutions (6.0×10^{-4} g/mL) were clarified with 0.45 μm Millipore Millex-LCR filter to remove dust before the LLS measurement. The resistivity of deionized water used was 18.0 MΩ cm.

Laser Light Scattering. A slightly modified spectrometer (ALV/DLS/SLS-5022F) equipped with a multi-τ digital time correlation (ALV5000) and a cylindrical 22 mW Uniphase He−Ne laser ($\lambda_0 = 632$ nm) as the light source was used. In static LLS,[10] we can obtain the weight-average molar mass (M_w) and the *z*-average root-mean square radius of gyration ($\langle R_g^2\rangle_z^{1/2}$ or written as $\langle R_g\rangle$) of scattering objects in a dilute solution from the angular dependence of the excess absolute scattering intensity, known as Rayleigh ratio $R_{vv}(q)$, on the basis of

$$\left(\frac{KC}{R_{vv}(q)}\right)_{C\to 0} \cong \frac{1}{M_w}\left(1 + \frac{1}{3}\langle R_g^2\rangle_z q^2\right) \quad \text{(Zimm plot)}$$

$$\text{for } \langle R_g^2\rangle^{1/2} q < 1$$

or

$$\left(\frac{KC}{R_{vv}(q)}\right)_{C\to 0} \cong \frac{1}{M_w \exp\left(-\frac{1}{3} R_g^2 q^2\right)} \quad \text{(Guinier plot)}$$

$$\text{for } \langle R_g^2\rangle^{1/2} q > 1 \quad (1)$$

where $K = 4\pi^2 n^2 (dn/dC)^2/(N_A \lambda_0^4)$ and $q = (4\pi n/\lambda_0)\sin(\theta/2)$ with N_A, dn/dC, n, and λ_0 being the Avogadro number, the specific refractive index increment, the solvent refractive index, and the wavelength of the light in vacuum, respectively. It is worth noting that our LLS spectrometer has an extraordinary small angle range down to 6.4°, which is vitally important in the investigation of large scattering objects.

In dynamic LLS,[11] the Laplace inversion of each measured intensity−intensity−time correlation function $G^{(2)}(q,t)$ in the self-beating mode can be related to a line-width distribution $G(\Gamma)$. For a diffusive relaxation, Γ is related to the translational diffusion coefficient D by $(\Gamma/q^2)_{C\to 0, q\to 0} = D$, so that $G(\Gamma)$ can be converted into a transitional diffusion coefficient distribution $G(D)$ or a hydrodynamic radius distribution $f(R_h)$ via the Stokes−Einstein equation, $R_h = (k_B T/6\pi\eta)D^{-1}$, where k_B, T, and η are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively. The cumulant analysis of $G^{(2)}(t)$ for a narrowly distributed sample can result in an accurate average line width ($\langle\Gamma\rangle$). In this study, we only measure dynamic LLS at a fixed low angle during the initial aggregation stage. Strictly speaking, such an obtained $\langle R_h\rangle$ is apparent. However, we are mainly interested in the comonomer composition-induced relative changes.

In the fast heating process, the copolymer solution at ∼25 °C was placed in the LLS cell holder which was preheated to a desired temperature with a precision of ± 0.05 °C. Time dependence of the scattering light intensity and intensity−intensity−time correlation function was measured during the association. Note that cooling the solution down to 4 °C can completely redissolve the association. In the slow heating process, the copolymer solution was heated slowly from 25 °C to the desired temperature inside the LLS cell holder at a rate of 0.3 °C/min. LLS measurement started only after the solution temperature reached the desired temperature.

Table 1. Laser Light Scattering Characterization of P(DEA-*co*-EA) with 40, 60, and 80 mol % of EA and P(DEA-*co*-DMA) with 30 and 50 mol % of DMA

samples	M_w/(g/mol)	$\langle R_h\rangle$/nm	T_{LCST}/°C	$T_{aggregation}$/°C
DEA-*co*-DMA/30	1.24×10^5	12.5	∼45	43.2
DEA-*co*-DMA/50	1.45×10^5	15.3	∼58	58.8
DEA-*co*-EA/40	1.70×10^4	5.6	∼35	35.7
DEA-*co*-EA/60	1.49×10^4	5.8	∼44	41.9
DEA-*co*-EA/80	2.11×10^4	9.6	∼55	55.5

Results and Discussion

Figures 1 and 2 show that for each copolymer studied, the average aggregation number (N_{agg}) and average hydrodynamic radius ($\langle R_h\rangle$) of the P(DEA-*co*-DMA/y) aggregates increase and then approach corresponding constants after a certain time, indicating the formation of stable aggregates, where N_{agg} was calculated from the

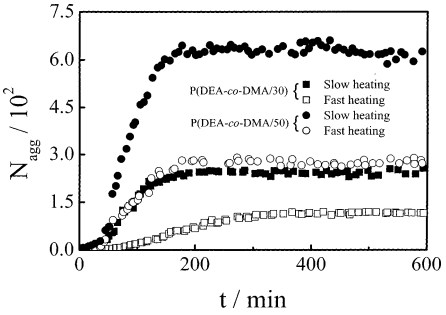


Figure 1. Time dependence of average aggregation number (N_{agg}) of P(DEA-*co*-DMA) mesoglobules formed under different heating rates.

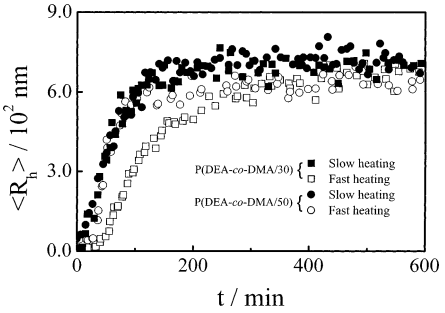


Figure 2. Time dependence of average hydrodynamic radius ($\langle R_h \rangle$) of P(DEA-*co*-DMA) mesoglobules formed under different heating rates.

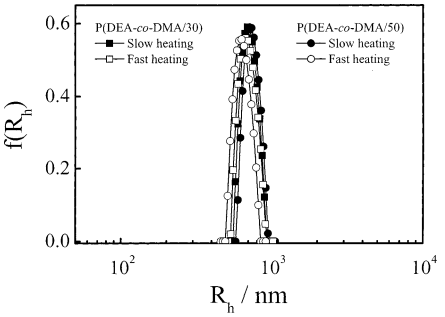


Figure 3. Typical hydrodynamic radius distributions ($f(R_h)$) of resultant P(DEA-*co*-DMA) mesoglobules formed under different heating rates.

ratio of the weight-average molar masses of the interchain aggregates and individual copolymer chains measured in static LLS. The point should be addressed that these aggregates were so stable that no change in the scattering intensity was observed over months. It is also helpful to note that the stabilization in water was reached without the addition of any ion or surfactant. The formation of such stable aggregates has been described as the mesoglobular phase in which a limited number of chains are associated together to form stable colloid particles existing between single-chain collapsed globules and macroscopic precipitation.[12−14] The stabilization is due to the concentration of hydrophilic DMA segments on the periphery of the aggregates during microphase separation. It has been found that $\langle R_h \rangle$ is slightly increases with q in the low scattering angle range. The resultant stable mesoglobules are narrowly distributed with a relative width less than 0.05, as shown in Figure 3. This is understandable because the association is an average process.

A combination of Figures 1 and 2 shows that for a give heating process, N_{agg} increases with the DMA content. On the other hand, for a given copolymer, the

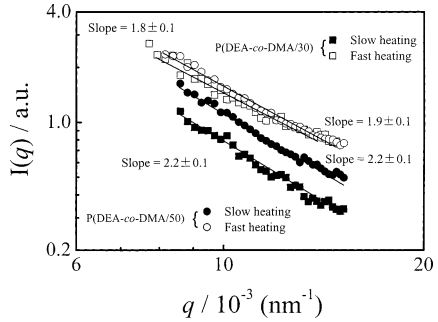


Figure 4. Scattering vector (q) dependence of scattered light intensity (I) of resultant P(DEA-*co*-DMA) mesoglobules formed under different heating rates.

fast heating results in a much smaller N_{agg} with a slightly larger size and the ratio of $R_g/R_h \sim 1$, indicating that they must have a loose structure. In comparison, the aggregates formed in the slow heating have the ratio of $R_g/R_h \sim 0.8$. Furthermore, it can be seen that, at the very initial stage of the microphase transition, N_{agg} remains a constant but $\langle R_h \rangle$ slightly decreases (not so obvious in Figure 2) in the fast heating process, reflecting that intrachain contraction appears before interchain association. As expected, intrachain contraction must force the hydrophilic DMA segments to stay on the periphery to minimize the surface energy and slow interchain association, resulting in a slower kinetics and smaller mesoglobules presumably consisting of many loosely packed small single- or pauci-chain collapsed globules. It is helpful to note (not straightforward) that intrachain contraction ("folding") can lead to a chain density lower than interchain aggregation (interpenetration) because no chain is infinitely flexible. This partially explains why the mesoglobules formed in the fast heating have a lower chain density.

Such a lower chain density can also be viewed from the scaling between the scattered light intensity (I) and the scattering vector (q) for resultant stable mesoglobules formed in different heating process (Figure 4). It has been known that the scaling exponent α in $I \propto q^{-\alpha}$ is the fractal dimension in the scaling between molar mass and size, i.e., $M \propto R^{\alpha}$. The increase of α from 1.8−1.9 to 2.2 indicates that the association changes from a diffusion-limited process to a reaction-limited process.[15] In a reaction-limited process, many collisions only results in a sticking (association), while in a diffusion-limited process, each collision leads to a sticking. Therefore, in a reaction-limited process, each coming particle or cluster has a much higher chance to penetrate into the "fiords" of the existing aggregates before they stick together,[15] which results in a higher chain density. This explains why α is higher for the mesoglobules formed in the slow heating.

As for P(DEA-*co*-EA/y) copolymers, P(DEA-*co*-EA/40) with a lower EA content can form stable mesoglobules in both the fast and slow heating process, but P(DEA-*co*-EA/60) can only form stable mesoglobules in the fast heating process. In the fast heating, intrachain contraction related to the initial induction is more evident because both N_{agg} and $\langle R_h \rangle$ remain as constants. The resultant stable mesoglobules have a larger $\langle R_h \rangle$, but a much smaller N_{agg}; namely, they are made of many loosely packed small single- or pauci-chain collapsed globules with a much lower chain density. On the other hand, in the slow heating, Figures 5 and 6 reveal an unexpected initial sharp increase of both N_{agg} and $\langle R_h \rangle$

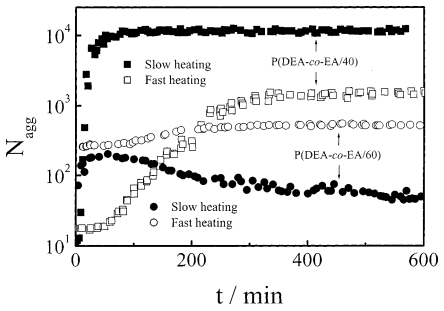


Figure 5. Time dependence of average aggregation number (N_{agg}) of P(DEA-*co*-EA) mesoglobules formed under different heating rates.

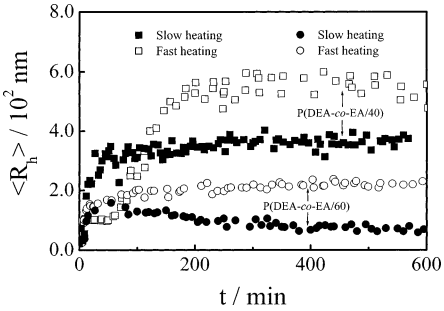


Figure 6. Time dependence of average hydrodynamic radius ($\langle R_h \rangle$) of P(DEA-*co*-EA) mesoglobules formed under different heating rates.

for P(DEA-*co*-EA/60) in the slow heating, followed by a gradual decrease, indicating that the interchain association formed in the initial stage is not stable. One possible explanation for the unexpected decrease of N_{agg} and $\langle R_h \rangle$ is as follows.

Poly(*N*-ethylacrylamide) (PEA) homopolymer has a much higher lower critical solution temperature (~82 °C) than poly(*N*,*N*-diethylacrylamide) (PDEA) homopolymer (~32 °C). Increasing the EA content makes P(DEA-*co*-EA) more hydrophilic so that its LCST becomes higher. For the three P(DEA-*co*-EA) copolymers studied, the aggregation temperatures are well below 80 °C, at which the PDEA segments become hydrophobic, but the PEA segments remain hydrophilic. The copolymer chains with a higher EA content are so hydrophilic that short hydrophobic PDEA segments are not able to provide a sufficiently strong hydrophobic association. As soon as the temperature is raised higher than ~32 °C, the association of short PDEA segments simultaneously leads to intrachain contraction and interchain association, resulting in an increase of N_{agg} and $\langle R_h \rangle$. During this process, long hydrophilic PEA segments are forced to stay on the periphery so that further interchain association are prevented. Inside each mesoglobule, the weak association and dissociation of short PEA segments should be in a dynamic equilibrium. Thermodynamically, when two chains are associated together, there is a penalty in both translational and conformational entropy, while for an intrachain association, there is no loss in translational entropy. Therefore, for a very weak interaction, intrachain association of a flexible chain should be more favored. In this way, interchain association inside each mesoglobule will eventually relax into intrachain association to reduce its free energy. This might explain the decreases of N_{agg} and $\langle R_h \rangle$. Such a relaxation from interchain to intrachain association can be better viewed for the association of P(DEA-*co*-EA/80) with a much higher EA content at

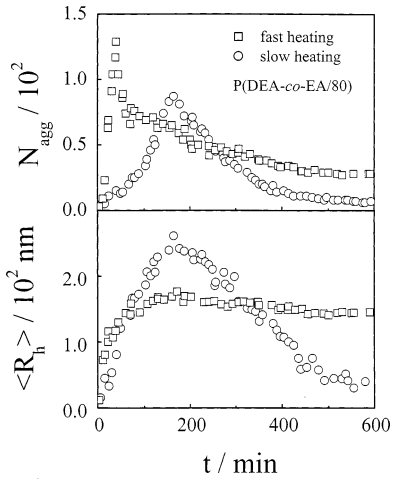


Figure 7. Time dependence of average aggregation number (N_{agg}) and average hydrodynamic radius ($\langle R_h \rangle$) of P(DEA-*co*-EA/80) mesoglobules formed under different heating rates.

55.5 °C (Figure 7) because here the hydrophilic association is even weaker.

Besides thermodynamic consideration, the formation of stable mesoglobules can also be discussed from a kinetic and viscoelastic point of view. It can be visualized that as the solvent quality changes from good to poor, i.e., as the temperature increases, the degree of amphiphilicity of the copolymer chain increases. The aggregation of insoluble PDEA segments leads to intrachain contraction and interchain association. It is known that, for two particles to merge into one larger particle, they have to interact (contact) for a sufficient long time (τ_C) during which the copolymer chains inside each particle have to relax and diffuse into each other, which must take a certain time (τ_D). If $\tau_C \ll \tau_D$, each particle will act as an elastic glass ball so that two approaching particles will collide and bounce away. In this case, they are stabilized by the viscoelasticity.

As expected, intrachain contraction increases the copolymer concentration inside each mesoglobule, which slows down the chain relaxation so that τ_D increases. On the other hand, the concentration of hydrophilic PDMA or PEA segments on the periphery reduces τ_C to a great extent. This is why only amphiphilic copolymer chains can form stable mesoglobules in solution. Therefore, we need to reduce τ_C and increase τ_D in order to form smaller mesoglobules. The incorporation of more hydrophilic comonomers into a chain backbone is one way to decrease τ_C; however, for a given composition, the segmentation of hydrophilic comonomer on the chain backbone is another way because they will be less likely trapped inside during intrachain contraction and interchain association. Moreover, one can promote intrachain contraction and suppress interchain association by a quick change of the solvent quality or a dilution of the solution. It is helpful to note that for a given copolymer concentration (*w*/*V*), using long copolymer chains can also enhance intrachain contraction, resulting in smaller mesoglobules or even single-chain globules.[16]

Conclusion

Neutral linear random copolymers, poly(*N*,*N*-diethylacrylamide-*co*-*N*,*N*-dimethylacrylamide) or poly(*N*,*N*-diethylacrylamide-*co*-*N*-ethylacrylamide, are soluble in cold water, but become amphiphilic when the temperature is higher than the lower critical solution temper-

ature (∼32 °C) of PDEA. The association of such copolymers with a proper comonomer composition in the range 32−80 °C in which water is a selective solvent, can form stable mesoglobules existing between single-chain collapsed globules and macroscopic precipitation. The formation and structure of such stable mesoglobules are essentially controlled by a competition between intrachain contraction and interchain association. The intrachain contraction forces hydrophilic DMA or EA segments to stay on the periphery, which stabilizes resultant mesoglobules. Increasing hydrophilic DMA or EA content generally leads to small mesogloblues. It is interesting to find that increasing the heating rate can change the association from a reaction-limited process to a diffusion-limited process. In the fast heating process, intrachain contraction is dominate and the collapsed chains have less chance to undergo further interchain association. Therefore, the mesoglobules formed in the fast heating consist of loosely packed small single- and pauci-chain globules. Our results reveal that for the copolymers with short hydrophobic segments, interchain association is too weak to form stable mesoglobules, and there exists an interesting relaxation from interchain association to intrachain association as the time elapses.

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