Progr Colloid Polym Sci (2000) 115:117–120

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Study of mesoglobules in solutions of amphiphilic heteropolymers

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Abstract We study the conformational states in solutions of amphiphilic copolymers by means of the Gaussian variational theory and lattice Monte Carlo simulation. We find that due to a delicate balance of microphase separation and entropic effects under the connectivity constraints macromolecular clusters consisting of a few distinct chains may become thermodynamically stable in some narrow regions of the phase diagram within the conventional two-phase coexistence region. These are characterised by a relatively monodispersed size distribution, with the mean size of these mesoscopic globules related to a characteristic scale of the microphase separation. Thus, in contrast to the homopolymer case, here collapse and aggregation can compete with each other, producing thermodynamically stable particles with a predominantly hydrophobic core and a hydrophilic shell. These were recently observed experimentally as, at least, rather long lived final states in the kinetics after quenching beyond the spinodal in aqueous solutions of poly(*N*-isopropylacrylamide) with some of the monomers hydrophobically modified.

Key words Mesoglobule · Heteropolymer · Solution · Phase separation

Introduction

The physical properties of polymers in dilute solutions have been a matter of intensive study using various experimental techniques. Much of the previous experimental work has been carried out near the upper critical solubility temperature of polystyrene in cyclohexane. In recent years there were also numerous works devoted to water-soluble polymers near the lower critical solubility temperature. Popular systems include poly(oxyethylene)–poly(oxypropylene) block copolymers and poly(*N*-isopropylacrylamide) (PNIPAM) homopolymer. Generally, experiments in this area are quite difficult as they are obscured by interchain aggregation phenomena. Although contraction of chains in the poor solvent regime has been observed many times, compact isolated globules have probably never been seen at equilibrium for homopolymers in pure solvent [1].

Recently, it has been discovered experimentally that at temperatures above the transition point dense spherical mesoscopic globules composed of a number of polymer molecules are formed [2] in dilute aqueous solution of PNIPAM. Clearly, the process of formation of these rather stable and monodispersed particles with sizes in the 50–500 nm range involves some kind of competition between the collapse of single globules and aggregation, which thus take place simultaneously. We called these particles mesoglobules because of their size being intermediate between that of a single globule and a macroscopic aggregate. To make this notion more precise we shall use the term mesoglobules only when talking about equally (or nearly equally) sized globules composed of several distinct chains.

It is our intention here to explain by theory and simulation this experimental observation. In Ref. [3] we conjectured that for copolymers the mesoglobules are

stabilised by the microphase separation and could really become thermodynamically stable. A number of recent experimental works [4], in which, however, ionomers were also included, seem to indicate that this might be indeed the case.

Here, we shall concentrate on trying to understand the issue of the monodispersity of the mesoglobules. First, we shall use the Gaussian variational theory to investigate which of the clusters obtained by association of distinct chains are most stable and thus possess the lowest free energy among other possible local minima. Second, to understand the fluctuations beyond the mean-field approximation, we shall use lattice Monte Carlo simulations to obtain the histograms of the cluster size and mass distributions directly. Knowing the widths of these distributions would allow us to characterise quantitatively the monodispersity property of the mesoglobules.

We should also mention perhaps that block and random heteropolymers in solutions and melts have been attracting a great deal of interest as they exhibit ordered microphase-separated and disordered glassy phases [5]. Block copolymers are often used as surfactants in ternary mixtures of two otherwise immiscible liquids, such as water and oil, and these mixtures produce many sophisticated structures, such as micelles and lamellae [6].

Numerical results from the variational theory

First of all, we have to perform a careful numerical analysis of the free energy obtained from the Gibbs–Bogoliubov variational principle with a generic quadratic Hamiltonian [7].

It is reasonable to expect that in the simple case of diblock copolymers mesoglobules are nothing but ordinary polymer micelles. Clearly, in a poor solution, hydrophobic units would tend to escape from unfavourable contacts with the solvent, but the connectivity of each chain seriously restricts their freedom to do so. However, for arbitrary heteropolymer sequences simple micellar structures are not possible due to the connectivity of the chains. For heteropolymers with an essential heterogeneity along the chain there are many competing interactions and the free-energy profile is very rugged; therefore, some new free-energy minima may appear due to a specific compensation of the interaction terms and the entropy.

The free energy, $\mathscr{A} = \mathscr{E} - T\mathscr{S}$, obtained from the Gibbs–Bogoliubov variational principle, $\mathscr{A} = \mathscr{A}_0 + \langle H - H_0 \rangle_0$, as described in Ref. [7] has the following conformational "entropy" part $\mathscr{A}_0$,

$$\mathscr{S} = \frac{3}{2} k_{\mathrm{B}} \ln \det{}' R, \quad R_{\mathrm{AA}'} = \frac{1}{N^2 M^2} \sum_{\mathrm{BB}'} D_{\mathrm{AB},\mathrm{A}'\mathrm{B}'} \ , \qquad (1)$$

$$D_{\mathrm{AA}',\mathrm{BB}'} = -(1/2)(D_{\mathrm{AB}} + D_{\mathrm{A}'\mathrm{B}'} - D_{\mathrm{AB}'} - D_{\mathrm{A}'\mathrm{B}}),$$
$$D_{\mathrm{A\,A}'} = (1/3)\left\langle (\mathbf{X}_{\mathrm{A}} - \mathbf{X}_{\mathrm{A}'})^2 \right\rangle \ , \qquad (2)$$

where we have denoted by $\mathbf{X}_n^a$ the coordinates of the nth monomer in the ath chain, multi-index $A \equiv (a, n)$, and N and M are the number of monomers in a chain and the total number of chains, respectively. The mean energy part, $\mathscr{E} = \langle H \rangle_0$, is then written as follows [7]

$$\mathscr{E} = \frac{3k_{\mathrm{B}}T}{4L^2N^2M^2} \sum_{\mathrm{AA}'} D_{\mathrm{AA}'} \left(\frac{1}{M} - \delta_{aa'} \right) + \frac{3k_{\mathrm{B}}T}{2l^2} \sum_{n,a} D^a_{n\,n-1}$$
$$+ \frac{1}{(2\pi)^{3/2}} \sum_{\mathrm{AA}'} \frac{\bar{u}^{(2)} + \Delta(\sigma_{\mathrm{A}} + \sigma_{\mathrm{A}'})/2}{D^{3/2}_{\mathrm{AA}'}} + \frac{3u^{(3)}}{(2\pi)^3} \sum_{\mathrm{AA}'} D^{-3}_{\mathrm{AA}'}$$
$$+ \frac{u^{(3)}}{(2\pi)^3} \sum_{\mathrm{AA}'\mathrm{A}''} \left(D_{\mathrm{AA}'} D_{\mathrm{A}''\mathrm{A}'} - D^2_{\mathrm{AA}',\mathrm{A}''\mathrm{A}'} \right)^{-3/2} \ , \qquad (3)$$

where L is the box size, l is the statistical segment length, $\bar{u}^{(2)}$ is associated with the quality of the solvent, the amphiphilicity, Δ, characterises the difference in interactions of hydrophilic and hydrophobic units with the solvent and $u^{(3)}$ is the third virial coefficient[1]. The set $\{\sigma_n^a\}$ expresses the chemical composition, or the primary sequence of a chain. Here the variables σ_{A} take only two values: -1 and 1, corresponding to the hydrophobic a and hydrophilic b monomers, respectively. The free energy then has to be minimised with respect to the full set of mean-squared distances, $D_{\mathrm{AA}'}$.

We see from numerical analysis that there is indeed a large number of local free-energy minima. These correspond to conformations in which polymers form one or several clusters, each consisting of one or more chains. Let us consider the system composed of $M = 12$ chains of $N = 12$ monomers each for two different sequences, one periodic and the other random. Values of the free energy at local minima corresponding to equally sized (symmetric) clusters are presented in Table 1 for different values of $\bar{u}^{(2)}$ at a fixed sufficiently high Δ. All the asymmetric clusters considered were found to possess a higher value of the free energy than these symmetric ones. One can see that in some range of $\bar{u}^{(2)}$ the main (deepest) minimum is reached at a state corresponding to mesoglobules – clusters of equal size, such as 6×2, 4×3, 3×4 or 2×6. This means that such a state becomes thermodynamically stable there. We may also note from the table that, with all other parameters being fixed, the size of the stable mesoglobules increases with the concentration and with $|\bar{u}^{(2)}|$, but it is also very sensitive to the heteropolymer sequence. It is important to emphasise that mesoglobules can have the lowest free energy not only for heteropolymers with a periodic

[1] We chose the system of units such that $l = 1$, $k_{\mathrm{B}}T = 1$ and fix $u^{(3)} = 10\ k_{\mathrm{B}}Tl^6$.

Table 1 Values of the specific free energy, $a = \mathscr{A}/MN$, at its various minima for the system of $M = 12$ heteropolymers of sequences $(a_3b_3)_2$ (*top half*) and $b_3a_2ba_2baba$ (*bottom half*) versus the mean second virial coefficient, $\bar{u}^{(2)}$. Here $L = 20$ and $\Delta = 30$. The value for the global (deepest) minimum is printed in *boldface*. Note that all rows except the first and last for each sequence correspond to the region where one of the mesoglobular states is stable

$\bar{u}^{(2)}$	12×1	6×2	4×3	3×4	2×6	1×12
$(a_3b_3)_2$						
0	**−3.02**	−2.81	−2.61	−2.45	−2.18	−1.65
−10	−8.12	**−8.37**	−8.34	−8.26	−8.08	−7.63
−15	−11.23	−11.78	**−11.85**	−11.83	−11.71	−11.33
−20	−14.74	−15.60	−15.80	**−15.84**	−15.79	−15.51
−30	−23.05	−24.61	−25.07	−25.26	**−25.39**	−25.32
−35	−28.00	−29.89	−30.48	−30.75	−30.97	**−31.03**
$b_3a_2ba_2baba$						
−10	**−7.78**	−7.77	−7.61	−7.43	−7.10	−6.40
−15	−10.93	**−11.23**	−11.16	−11.03	−10.77	−10.14
−20	−14.49	−15.11	**−15.17**	−15.11	−14.92	−14.37
−25	−18.47	−19.44	−19.63	**−19.65**	−19.54	−19.11
−35	−27.91	−29.60	−30.08	−30.27	**−30.36**	−30.18
−45	−40.47	−42.75	−43.26	−43.31	−43.59	**−43.67**

(block) structure, but essentially for many random sequences as well.

Results from lattice Monte Carlo simulation

It is interesting to check these predictions of the Gaussian variational method by Monte Carlo simulation on a lattice. We adopt the Metropolis technique in the lattice model of Ref. [8]. This model, apart from the connectivity and excluded-volume constraints, includes short-ranged pairwise interactions between lattice sites. The system is completely characterised by three Flory interaction parameters, χ_{aa}, χ_{ab} and χ_{bb}, along with N, M and the linear lattice size, L. In addition to local monomer moves [8], we include translational moves representing diffusion of chains. The latter moves are applied to all clusters of chains with a probability inversely proportional to the number of monomers within (Stokes law).

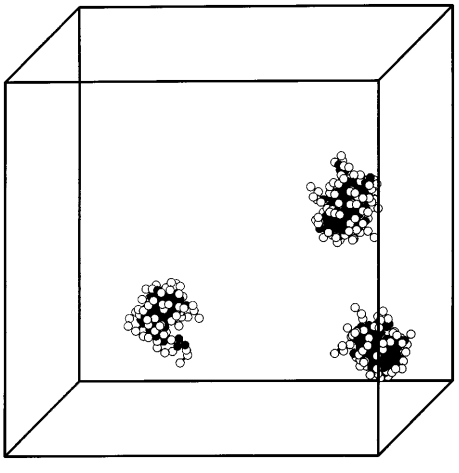

Fig. 1 Snapshot of a typical mesoglobular conformation of heteropolymer sequence $(a_3b_3)_4$. Here the equilibration time was 1.92×10^9 of attempted Monte Carlo moves and the other parameters were $L = 60$, $N = 24$, $M = 20$, $\chi_{aa} = 1$, $\chi_{ab} = 0.4$ and $\chi_{bb} = -0.2$. *Black circles* correspond to hydrophobic monomers and *white circles* correspond to hydrophilic monomers

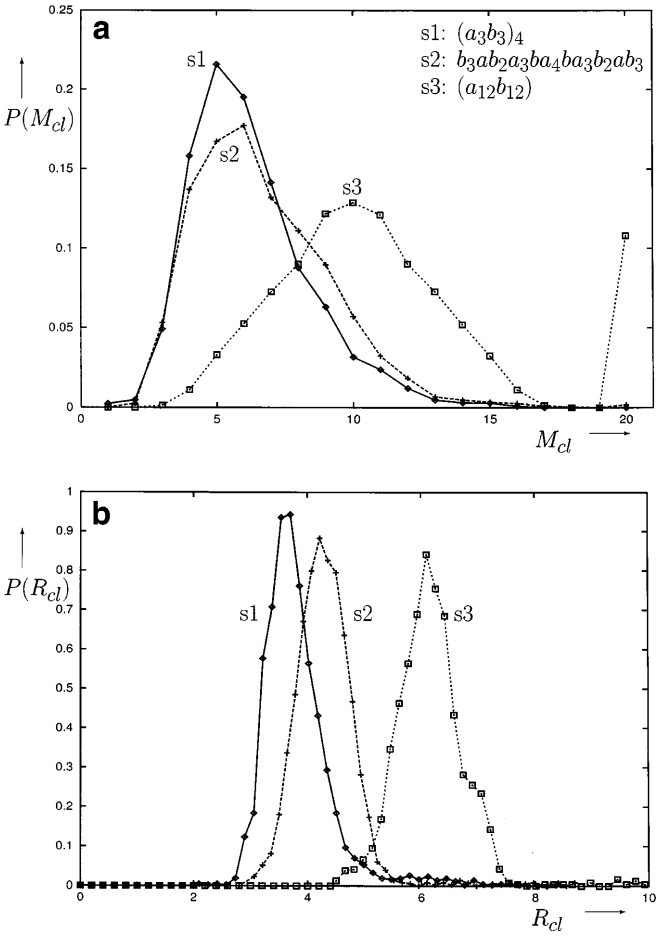

Fig. 2 Probability densities of **a** the number of chains (mass), M_{cl}, and **b** the radius of gyration (size), R_{cl}, for mesoglobules of different sequences. These results were obtained by analysing data for the ensemble size $Q = 1000$

We considered several concrete sequences consisting of strongly hydrophobic and slightly hydrophilic units. From the snapshot in Fig. 1 we can clearly see a number of distinct clusters there, each consisting of several chains. These tend to have a larger amount of hydrophilic (white) material on the outside. Strikingly, these clusters are of nearly equal size, something we have already noted from the above theory.

To address the question about the size polydispersity of the mesoglobules we obtained a large ensemble of

independent equilibrium states. The calculated histograms of the mass and size probability densities for the sequences considered are presented in Fig. 2. The most typical picture is seen for sequences s1 (intermediate-sized blocks) and s2 (a random sequence). These have a single well-distinguished peak in the mass and size distributions with a fairly narrow Gaussian-like shape; therefore, the mesoglobules are quite monodisperse with about 10–15% relative dispersion in size. Other sequences, however, such as s3 (diblock copolymer) have different distributions. The mass distribution of s3, in addition, has a large population of single aggregates, $M_{cl} = 20$. This is believed to be a finite-size effect as the characteristic mesoglobule mass is comparable to M here. Nevertheless, the size distribution for s3 still has a single peak.

Conclusion

Thus, the main conclusion from our analysis is that the mesoglobules become thermodynamically stable in a narrow region of the phase diagram for a wide class of periodic and random amphiphilic heteropolymers possessing hydrophobic and hydrophilic monomers. The size of the mesoglobules varies somewhat due to fluctuations transforming symmetric clusters into slightly asymmetric ones, although the barriers separating these structures are high. The mean size of the mesoglobules is determined by the characteristic scale of the microphase separation. Thus, the larger this scale (e.g. block size) the larger the mesoglobules produced. The size distribution of the mesoglobules is sufficiently monodisperse due to the thermodynamic preference for clusters to be of equal size. The two methods employed here give the upper and lower bounds on the variations in mesoglobule size. The variational method, being merely an optimised mean-field theory, predicts perfectly monodispersed mesoglobules. The Monte Carlo method, on the other hand, tends to overemphasise their polydispersity. This is because breaking away from an already-formed dense cluster is harder than joining it. It seems quite reasonable that even weak electrostatic repulsion may play a crucial role for further stabilisation of the mesoglobules and for improving their monodispersity.

We believe that these observations may shed some light on understanding the problems of competition aggregation versus folding in protein solutions and self-organisation of the quaternary structure in multimeric proteins. It appears that for the latter process to take place a considerable number of "sticky" hydrophobic amino acid residues should be exposed on the exterior of each of the folded subunits. The resulting association produces a well-defined quaternary structure, which is biologically functional as in hemoglobin, for example, rather than a disordered aggregate. In our view, this feature looks similar to the process of mesoglobule formation, but of course one should bear in mind the complexity of real proteins not represented in the current oversimplified model of heteropolymers. Hopefully, work in this direction would also help unravel the mechanism of association of so-called Bence–Jones proteins believed to be related to amyloid fibril formation.

Speaking of other possible applications, water-soluble polymers are widely used in chemical, pharmaceutical and food industries. Therefore, detailed understanding of the mechanism of mesoglobule formation is important for being able to select polymers from which nanoparticles of the required size and polydispersity can be prepared. The main advantage of this new approach is that nanoparticles can be formed reversibly in mild conditions from a prepurified polymer. Also, nanoparticles carrying functional groups properly located in the mesoglobule can be obtained and these could be further modified. Such nanoparticles with a well-controlled size distribution can find a broad range of applications in pharmaceutical, biotechnological and cosmetic industries, where technological control is one of the major problems.

Acknowledgements The authors acknowledge interesting discussions with F. Ganazzoli, G. Allegra, G. Raos and our colleague A.V. Gorelov. This work was supported by grant SC/99/186 from Enterprise Ireland.

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