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Microcanonical analysis of association of hydrophobic segments in a heteropolymer

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Using the replica-exchange multicanonical Monte Carlo simulation, the intra-association of hydrophobic segments in a heteropolymer was numerically investigated by the microcanonical analysis method. We demonstrated that the microcanonical entropy shows the features of one or multiple convexes in the association transition region depending on the number and distribution of hydrophobic segments in the chain. We found that one or multiple negative specific heats imply a first-order-like transition with the coexistence of multiple phases.

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I. INTRODUCTION

Microcanonical analysis is a promising method for investigation of a system where it becomes cool upon absorbing energy. In the transition region, where the energy E is treated as an intensive external control parameter, the microcanonical entropy $S(E)=k_B \ln n(E)$ shows a convex function of E, where $n(E)$ is the density of states. Correspondingly, the specific heat $C_V(E)=\partial E/\partial T(E)=-(\partial S/\partial E)^{-1}/(\partial^2 S/\partial E^2)$ is negative, and the inverse caloric temperature $T^{-1}(E)=\partial S/\partial E$ curve exhibits an S-shaped feature. This effect has been observed in an astrophysical system [1], where energy can be added to a star or star cluster which then cools. There is a large number of other isolated finite model systems for evaporation and melting effects [2,3]. A similar phenomenon is seen for melting of atomic clusters [4,5], nuclei fragmentation [6–8], and spin models on finite lattices that experience first-order transitions in the thermodynamic limit [9,10]. Experimental evidence was found that a cluster containing exactly 147 sodium atoms does indeed have a negative microcanonical heat capacity near its solid to liquid transition [11]. In particular, this negative specific heat effect was observed by Junghans *et al.* in the aggregation transition of heteropolymer chains [12]. They demonstrated that the aggregation transition is a phase separation process, and the loss of entropy due to the existence of a phase boundary leads to a negative specific heat. Since on a coarse-grained scale, a protein can be regarded as a heteropolymer chain consisting of both hydrophobic and hydrophilic segments in a special sequence. The success of microcanonical analysis in the interaggregation of heteropolymers prompted us to extend to the analysis the observation of intra-association among hydrophobic segments within a heteropolymer chain. The association among hydrophobic segments is recognized to have a crucial role in the protein folding. A deep understanding of association behavior would be very helpful for gaining insights into the general mechanism of protein folding.

II. MODEL AND SIMULATION

The conformation of a polymer chain of n segments is defined by n coordinates $r_1, r_2, \ldots, r_n$ of segments in three-dimensional space and a bond is used to connect every two adjacent segments. We chose the off-lattice Monte Carlo model developed by Gerroff *et al.* [13] and used in our group for observing the first-order transition of homopolymer and heteropolymer chains [14]. The total internal energy is the sum of respective Lennard-Jones terms between two nonbonded beads. The system has a Hamiltonian of $E=4\varepsilon\sum_{i=1}^{n-2}\sum_{j=i+2}^{n}[(1/r_{ij})^{12}-(1/r_{ij})^{6}]$, where r_{ij} represents the distance between two nonbonded segments (i and j), and ε is the parameter setting energy scale. The bond length is set to unity. The replica-exchange multicanonical (REMUCA) Monte Carlo simulation developed by Mitsutake *et al.* [15] was used in this study to guarantee an ergodic scan of the conformational space.

A 62 segment heteropolymer chain with two end hydrophobic segments was used in the model. The interaction parameters are chosen as $\varepsilon_{pp}=0.25$, $\varepsilon_{hp}=2.5$, $\varepsilon_{hh}=25$; the subscripts p and h represent polar (hydrophilic) and hydrophobic segments, respectively. These two hydrophobic segments have a tendency to associate with each other while the chain collapses. For the simulations of this system, we performed 200 multicanonical iterations in REMUCA. A total number of 3×10^{12} updates was generated. The primary result of these simulations is the density of states $n(E)$ is determined up to an unimportant constant.

III. RESULTS AND DISCUSSIONS

In order to make a microcanonical analysis, the parameters, microcanonical entropy $S(E)=\ln n(E)$, inverse caloric temperature $T^{-1}(E)=\partial S/\partial E$, specific heat $C_V(E)=\partial E/\partial T(E)$ $=-(\partial S/\partial E)^{-1}/(\partial^2 S/\partial E^2)$, canonical energy distribution $P(E)$, and average hydrophobic segment-to-hydrophobic segment distances $\langle R(E)\rangle$ are plotted as functions of energy E in Fig. 1. The convexity in microcanonical entropy together with the S-shaped feature of inverse caloric temperature and negative specific heat in the energy range from $E=-12.24$ to -42.56 displayed in Figs. 1(a)–1(c) are assumed to be contributed by the associations of these two hydrophobic segments. The ca-

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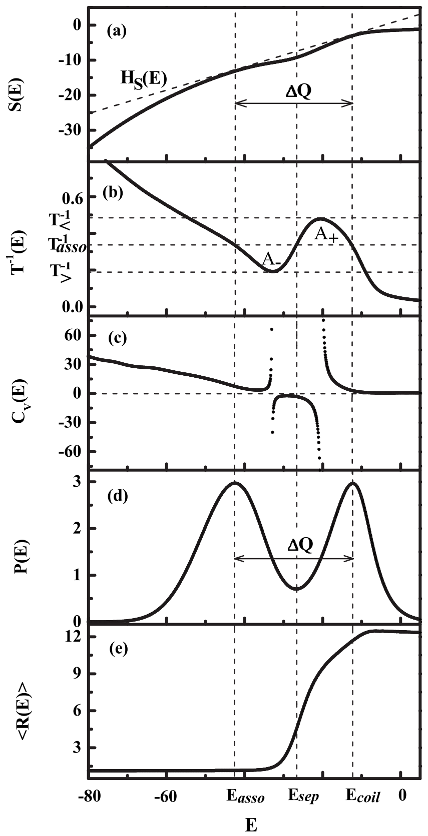

FIG. 1. Microcanonical results for the 62 segment heteropolymer with two end hydrophobic segments: (a) microcanonical entropy $S(E)$, (b) inverse caloric temperature $T^{-1}(E)$, (c) specific heat $C_V(E)$, (d) canonical energy distribution close to the association temperature $P(E)$, and (e) average hydrophobic segment-to-hydrophobic segment distance $\langle R(E)\rangle$.

nonical energy distribution $P(E) \sim n(E)\exp(-E/k_B T)$ is shown in Fig. 1(d), and its bimodality indicates the coexistence of two phases. Detailed analysis based on the conformations of the chain measured by the average hydrophobic segment-to-hydrophobic segment distance $\langle R(E)\rangle$ in Fig. 1(e) reveals the phase at higher energies, the $P(E)$ peak at $E=-15$ in Fig. 1(d), corresponds to the nonassociation of the two hydrophobic segments, having a larger value of $\langle R(E)\rangle \sim 12$, while the phase at lower energies, the $P(E)$ peak at $E=-42$ corresponds to the association of two hydrophobic segments having a smaller value of $\langle R(E)\rangle \sim 1$. These findings indicate the negative specific heat effect is, indeed, from the result of the association of two hydrophobic segments.

Pursuing the origin of this negative specific heat effect is extremely attractive. From Fig. 1 we understand that the configurations of the chain are switched between two structures in the transition temperature region, i.e., two phases coexist. One is a circlelike structure with association of the two end hydrophobic segments, and another is a coil-like structure without this association. As the two hydrophobic segments associate, they will bring other hydrophilic segments around them to rearrange. Hence, the association of the two end hydrophobic segments will constrain the movement of other segments along the chain in this small system, leading to the sharp reduction of the entropy. This phenomenon, however, can be observed only in a small system. For a large system, a long chain, association of two-end hydrophobic segments can induce the constraint of a few segments, which has less contribution for the entropy change in this large system, can rarely be observed. This phenomenon cannot be observed on a homopolymer chain. Furthermore, when the interactions are too weak, i.e., interaction parameters are $\varepsilon_{pp}=0.25$, $\varepsilon_{hp}=0.75$, $\varepsilon_{hh}=2.25$, the configuration of two end hydrophobic segments association is not easy to form, and the negative specific heat feature disappears.

Following Junghans *et al.* [12], in Fig. 1(a) the phase coexistence with convex behavior of entropy is observed in the range $E_{asso}=-42.56<E<-12.24=E_{coil}$; the subscript *coil* represents the chain in coil conformation without association between two hydrophobic segments. The association temperature $T_{asso}=2.978$ is calculated from the slope of the line tangent connection of $S(E_{asso})$ and $S(E_{coil})$. The interval $\Delta Q=E_{coil}-E_{asso}=T_{asso}[S(E_{coil})-S(E_{asso})]\approx 30.32$ is the latent heat required to separate the two end hydrophobic segments at the association temperature. E_{sep} is defined as the energy at which the difference $\Delta S(E)=H_S(E)-S(E)$ is a maximum, and the corresponding maximum deviation is the interfacial entropy, $\Delta S_{surf}=\Delta S(E_{sep})$. On the basis of the definition given by Junghans *et al.* [12], we have $T_{>}\approx 5.213$ and $T_{<}\approx 2.092$; the association of two end hydrophobic segments is carried out in the temperature range $T_{<}<T<T_{>}$. The two end hydrophobic segments should associate completely at temperatures $T<T_{<}$, but remain totally separate at temperatures $T>T_{>}$. The Maxwell line $T_{asso}^{-1}=0.335$ divides the $T^{-1}(E)$ curve into two equal parts in area, $A_{+}=T_{asso}^{-1}(E_{sep}-E_{coil})-[S(E_{sep})-S(E_{coil})]=1.4$ and $A_{-}=T_{asso}^{-1}(E_{sep}-E_{asso})-[S(E_{sep})-S(E_{asso})]=1.4$, which determines the interfacial entropy $\Delta S_{surf}=A_{+}=A_{-}$ [9].

The negative specific heat cannot be observed with two adjacent hydrophobic segments along the chain. Thus, how the hydrophilic length between two hydrophobic segments influences the association is worthy of study. For this purpose, we set the hydrophilic length as 1, 4, or 30 segments between hydrophobic segments on the 62 segment chains, i.e., two hydrophobic segments do not locate on two ends of the chain again. Calculated from Fig. 2, the latent heats $\Delta Q=8.16$, 25.28, and 30.72, association temperatures $T=9.009$, 5.25, and 3.075, temperature gaps $\Delta T=T_{>}-T_{<}=0.1$, 3.352, and 3.625 indicate that increasing the hydrophilic length between the two hydrophobic segments leads to three results in the association of two hydrophobic segments: first, it produces more latent heat; second, it happens at a lower temperature; and third, it is accomplished in a broader temperature range, $T_{<}<T<T_{>}$, i.e., broader transition region. The first two facts imply that it is difficult for two hydrophobic segments far apart in the chain to associate (or inversely, difficult to separate); and the last fact means a larger interfacial entropy $\Delta S_{surf}=A_{+}=A_{-}$ [9], i.e., more entropy loss. As we mentioned in previous sections, the association of two hydrophobic segments can force the aggregation of other hydrophilic segments. If two hydrophobic segments live far away along the chain, they are relatively difficult to meet together. Reasonably, it should happen at

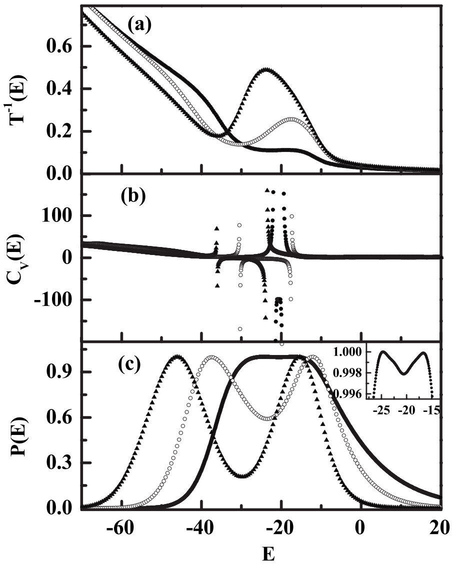

FIG. 2. Microcanonical results for the 62 segment heteropolymer chains with different hydrophilic lengths between two hydrophobic segments: (a) inverse caloric temperature $T^{-1}(E)$, (b) specific heat $C_V(E)$, and (c) canonical energy distribution close to the association temperature. The circle, hollow circle, and up triangle represent 1, 4, and 30 hydrophilic segments between two hydrophobic segments, respectively. An enlargement of the circle curve is shown in the inset at the top right corner.

lower temperature with more latent heat. But once they associate, it will cause more hydrophilic segments aggregated, more hydrophilic segments' movement constrained, and more entropy loss. Correspondingly, the rearrangement of the larger number of segments should be accomplished on a broader temperature scale.

We put one more hydrophobic segment in the middle of this 62 segment chain and considered the case of three hydrophobic segments along the chain. The features of S-shaped caloric temperature and negative specific heat were observed in two energy ranges. Correspondingly, the energy distribution at a chosen temperature is characterized by three peaks, as shown in Fig. 3, indicating the coexistence of three phases. The feature with multiple S-shaped caloric temperatures was observed by Junghans *et al.* in the aggregation of peptide chains [12]. Detailed analysis of the conformations of the chain corresponding to individual peak reveals these features originate from the association of a different number of hydrophobic segments. We set a threshold value $r=1.4L$ ($L=1.0$ is bond length); two hydrophobic segments closer than this value are assumed to be in contact. It was found that the peak corresponding to the highest energy is contributed by chains with no association among the three hydrophobic segments, the peak at the lowest energy, however, is contributed by chains with association among all three hydrophobic segments, and the middle peak is contributed by those having two of three hydrophobic segments in contact, i.e., 15% from two end hydrophobic segments and about 85% from one middle hydrophobic segment with one of the two end hydrophobic segments [Fig. 3(e)]. Using four hydrophobic segments along the chain, we observed four coexisting phases at association temperatures.

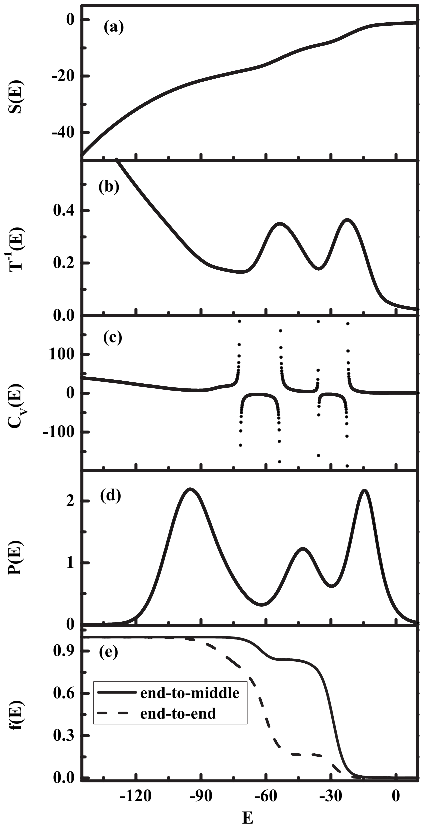

FIG. 3. Microcanonical results for a 62 segment heteropolymer with two end hydrophobic segments and one middle hydrophobic segment: (a) microcanonical entropy $S(E)$, (b) inverse caloric temperature $T^{-1}(E)$, (c) specific heat $C_V(E)$, (d) canonical energy distribution close to the association temperature, and (e) the number fraction for chains having a conformation with every two hydrophobic segments apart at $r \leq 1.4$. The curves (d) and (e) are measured at temperature $T=4.032$.

IV. CONCLUSION

We have used the hydrophobic-hydrophilic heteropolymer chain model to demonstrate that microcanonical analysis is very promising for studying the intra-association of hydrophobic segments. In the small system, the association transition has been verified as a phase separation process. The loss of entropy that leads to negative specific heat is due to the reduction of freedom of the chain induced by the association among hydrophobic segments. The features of multiple S-shaped caloric temperatures and negative specific heats are indicators for the existence of multiple phases, which cannot be discriminated in canonical analysis with temperature as the extensive external control parameter.

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