pH-Induced Helix−Coil Transition of Amphipathic Polypeptide and Its Association with the Lipid Bilayer: Electrostatic Energy Calculation

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Received August 12, 2005
Revised Manuscript Received October 20, 2005

Introduction

Amphipathic helix is a helical polypeptide, one face of which is hydrophilic and the opposite face is hydrophobic. Amphipathic helices are involved in many membrane-dependent processes, since they can form an interface between aqueous and hydrophobic environments.[1] Recently, many synthetic polypeptides have been designed to mimic viral fusion peptides sequences for delivery of gene and drug into cells. Among them, GALA is a synthetic peptide designed to interact with lipid bilayers preferentially at acidic pH. GALA is composed of 30 amino acids with a sequence of WEAALAEALAEALAEHLAEALAEALEALAA.[2−5] GALA shows a helix-to-coil transition as the pH is increased from 5 to 7. It has been observed that the binding of GALA to vesicles made of POPC or egg PC becomes strong at low pH due to the stabilization of the amphipathic helix of GALA.[4] Interestingly, a leakage experiment with lipid vesicles entrapping compounds of different molecular weights ranging from 445 to 2000 g/mol reveals that the leakage induced by the binding of GALA to the vesicle proceeds via an all-or-none mechanism, i.e., the vesicles either leak their entire content or do not leak at all.[5] Therefore, it has been suggested that a specific number of GALA peptides are embedded into a vesicle to induce the leakage of its contents. Further experiments and the mathematical description for the kinetics of leakage provide the evidence that the rate-limiting step for the leakage is the formation of an aggregate composed of 10 ± 2 peptides in the bilayer.[2,5] In this note, the factors affecting the pore formation of GALA peptide in a lipid bilayer and the origin of the specific aggregation number are theoretically discussed in terms of the solvation free energy of peptide and the free energy of pore formation. For theoretical analysis of the pH-induced GALA insertion into a lipid bilayer, the pH-dependence of the stability of the amphipathic helix is first calculated by using the finite Poisson−Boltzmann method. Second, the preferential orientation of a single GALA in the lipid bilayer is investigated, and finally the change of free energy associated with the pore formation by aggregation of GALA peptide is calculated as a function of the aggregation number of the polypeptide.

Computational Method

Calculation of pH-Dependent Stability of Amphipathic Helix. The structure of a GALA helix is generated by using the CHARMM program.[6] The change of electrostatic free energy as a function of pH is calculated according to the method developed by Honig and co-workers.[7,8] If a protein has N ionizable residues, a given ionization state n, where $n = 1$ to 2^N, can be defined in terms of the vector $\delta_n(i)$, $i = 1$ to N. $\delta_n(i)$ is equal to zero when the group i is neutral and unity when it is charged. A reference state of zero free energy is defined as the state corresponding to all ionizable groups in their neutral form. The pH-dependent free energy of the nth state in the GALA helix G^n(helix) is given as

$$G^n(\text{helix}) = 2.3kT\sum_{i=1}^{N}\delta_n(i)\gamma(i)(\text{pH} - \text{p}K_{ai}^{\text{int}}) + \sum_{i=1}^{N-1}\sum_{j=i+1}^{N}\delta_n(i)\delta_n(j)\Delta G^{ij} \quad (1)$$

where $\text{p}K_{ai}^{\text{int}}$ is the $\text{p}K_a$ of ith residue in the protein when all other ionizable residues are in their neutral state, ΔG^{ij} is the electrostatic interaction between residues i and j in their charged state, and $\gamma(i) = -1$ or 1 for an acidic and basic residue, respectively. Therefore, the total free energy of the ionizable residues in the helix can be obtained from the statistical mechanical expression

$$G^{\text{ion}}(\text{helix}) = -kT\ln(Z^{\text{H}}) \quad (2)$$

where Z^{H} is the partition function for 2^N ionization states of the helix

$$Z^{\text{H}} = \sum_{n=1}^{2^N}\exp[-G^n(\text{helix})/kT] \quad (3)$$

The above equations are used for determining the GALA helix structure. An identical set of equations can be derived for coil sate, but no single conformation is defined for coil state. It is most likely that all ionizable residues are exposed to solvent and do not interact with one another electrostatically in coil state (8). Therefore, the $\text{p}K_a$s of ionizable groups in coil state are assumed to be identical to those of isolated amino acids in solution. The pH-dependent free energy of the nth state in coil state G^n(coil) is given as

$$G^n(\text{coil}) = 2.3kT\sum_{i=1}^{N}\delta_n(i)\gamma(i)(\text{pH} - \text{p}K_{ai}^{0}) \quad (4)$$

where $\text{p}K_{ai}^{0}$ is the $\text{p}K_a$ value of an isolated amino acid in solution. G^{ion}(coil) can also be defined similarly to eqs 2 and 3. The total free energy of unfolding $\Delta G^{\text{C}\rightarrow\text{H}}$ can be written as

$$\Delta G^{\text{C}\rightarrow\text{H}} = \Delta G^{\text{neutral}} + G^{\text{ion}}(\text{helix}) - G^{\text{ion}}(\text{coil}) = \Delta G^{\text{netural}} + \Delta G^{\text{ion}} \quad (5)$$

where $\Delta G^{\text{neutral}}$ is the denaturant energy for the protein with all neutral residues, and only ΔG^{ion} is pH-dependent. More details are well described in the references by Honig and co-workers.[7,8] The electrostatic energies in the above equations are calculated by using the finite difference Poisson−Boltzmann method implemented in the PBEQ of CHARMM.[6]

Model of an Implicit Lipid Bilayer. The insertion of a single peptide into a lipid bilayer is investigated by using the continuum model for protein-membrane association.[9,10] The total

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10.1021/bm050579z CCC: $33.50
Published on Web 11/17/2005

free energy difference (ΔG_{tot}) between peptides in lipid bilayer and in the aqueous phase is decomposed into electrostatic contribution (ΔG_{elec}), nonpolar contribution (ΔG_{np}), lipid perturbation effect (ΔG_{lip}), peptide immobilization effect (ΔG_{imm}), and contribution from conformational change of peptide (ΔG_{con})

$$\Delta G_{tot} = \Delta G_{elec} + \Delta G_{np} + \Delta G_{lip} + \Delta G_{imm} + \Delta G_{con} = \Delta G_{solv} + \Delta G_{lip} + \Delta G_{imm} + \Delta G_{con} \quad (6)$$

where ΔG_{solv} is the free energy of transfer of a solute from water to a bulk hydrocarbon phase.[9] Since the contributions from ΔG_{lip}, ΔG_{imm}, and ΔG_{con} are not significant as compared with that of ΔG_{solv}, only ΔG_{elec} and ΔG_{np} are considered in the insertion of a single peptide. The electrostatic contribution to the free energy for transfer of a peptide molecule from water to lipid bilayer along the z axis (ΔG_{elec}) is calculated with the PBEQ in CHARMM. The continuum model for a lipid bilayer is represented as a planar slab with thickness of 40 Å corresponding to the distance between phosphate groups in an egg PC bilayer.[5] Dielectric constants (ϵ) are assigned to have $\epsilon = 80$ for bulk water, $\epsilon = 2$ for lipid bilayer, and $\epsilon = 1$ for the protein.[10] The nonpolar contribution (ΔG_{np}) is assumed to be related to the water-accessible surface of the peptide

$$\Delta G_{np} = -\gamma \sum_i S_i^{np} \begin{cases} e^{-(|z_i|-z_0)2/\Delta z} & \text{if } |z_i| \geq z_0 \\ 1 & \text{if } |z_i| < z_0 \end{cases} \quad (7)$$

where z_i is the z position of atom i, S_i^{np} is the water-accessible surface of the ith atom, and $\gamma = 0.033$ kcal/mol/Å^2 is the surface tension coefficient.[10] The position of the interface (z_0) and its width (Δz) are set to be 17.5 and 2.5 Å, respectively. White and co-workers[11] calculated the transfer free energy of amino acid by using the Monte Carlo simulation when the pentapeptide as a model peptide is transferred from aqueous medium to octanol phase, whereas in the present study, the transfer energy of amino acid has been calculated when a GALA helix is inserted into lipid bilayer. When the above two cases are compared with each other, it reveals that there is a difference of the free energy between two cases. This is probably because the solvent-accessible surface area of GALA helix is much different from that of the pentapeptide.

Free Energy for Pore Formation in Lipid Bilayer. The change of free energy upon pore formation is calculated as a function of the aggregation number. The total free energy is decomposed to electrostatic contribution (ΔG_{elec}), nonpolar contribution (ΔG_{np}), contribution due to immobilization of helix into lipid bilayer (ΔG_{imm}), and contribution due to pore formation in the lipid bilayer (ΔG_{pore})

$$\Delta G_{tot} = \Delta G_{elec} + \Delta G_{np} + \Delta G_{imm} + \Delta G_{pore} \quad (8)$$

The electrostatic contribution (ΔG_{elec}) to the free energy of transfer of an aggregate from water to lipid bilayer along the z axis is calculated with the PBEQ in CHARMM. Since the cylindrical pore in the bilayer is exposed to water, the dielectric constant in the pore is set to 80 using the cylindrical boundary condition in the PBEQ of CHARMM. The nonpolar contribution (ΔG_{np}) is calculated according to eq 7. Since the loss of free energy due to immobilization of a single helix in a lipid biayer is reported to be 5 kcal/mol,[9] the total immobilization free energy of an aggregate of polypeptides (ΔG_{imm}) can be calculated by multiplying the aggregation number by 5 kcal/mol. The free energy associated with the pore formation in the lipid bilayer can be expressed as the sum of the surface free energy and the chain stretching energy.[12] When the pore is absent, the free energy of the bilayer can be written as

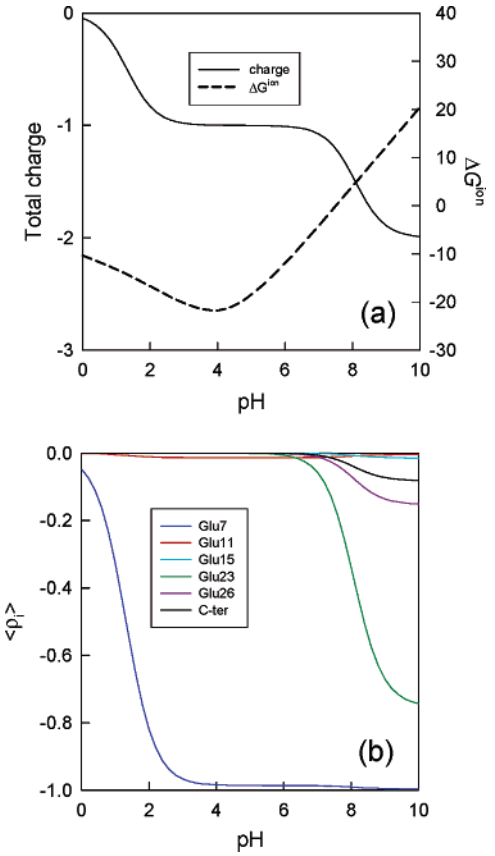

Figure 1. (a) Electrostatic free energy and the total charge plotted against pH, where the average charge of each residue $\langle \rho_i \rangle$ is defined as $\langle r_i \rangle = \sum_{n=1}^{2^N} \delta_n(i)\gamma(i) \exp(-\Delta G^n/kT)/Z_F$ and the total charge is the sum of $\langle \rho_i \rangle$ over all residues. (b) The average charges of ionizable residues are plotted against pH. N-terminal, Glu2, His16, and Glu19 are fully charged in the whole range of pH examined in the present study.

$$G(R_{pore} = 0) = 2\gamma S + 2\rho SkT(\pi^2/24)(L/2)^2/r_0^2 \quad (9)$$

where γ is the water/lipid interfacial tension, S is the surface area of the bilayer, ρ is the surface density of lipid alkyl chain, L (= 40 Å) is the thickness of the bilayer, and r_0 is the RMS end-to-end distance of a single lipid molecule in unimer state. In the right-hand side of eq 9, the first term and the second term correspond to the surface free energy and the lipid stretching energy, respectively.[12] In the formulation of eq 9, the bending effect due to formation of a vesicle is neglected, since the vesicle radius (typically ≈ 100 nm) is much larger than the thickness of bilayer L. The surface area per a lipid molecule and γ are 63.5 Å^2 and 8 mN/m, respectively.[13] Since a lipid molecule has two alkyl chains, ρ becomes 2/63.5 Å^{-2}. The end-to-end distance of an unperturbed lipid molecule r_0 can be calculated by the rotational-isomeric-state (RIS) model. In this calculation, the lipid tails are modeled as a fully saturated hydrocarbon, -$(CH_2)_n$-CH_3, with $n = 10$, and r_0 is estimated to be 10.5 Å according to the RIS model. When a pore of radius R_{pore} is formed, the free energy can be written as

$$G(R_{pore}) = 2\gamma S - 2\gamma\pi R_{pore}^2 + 2\gamma\pi R_{pore} L' + 2\rho_{eff} SkT(\pi^2/24)(L'/2)^2/r_0^2 \quad (10)$$

where L' is the thickness of the lipid bilayer after pore formation

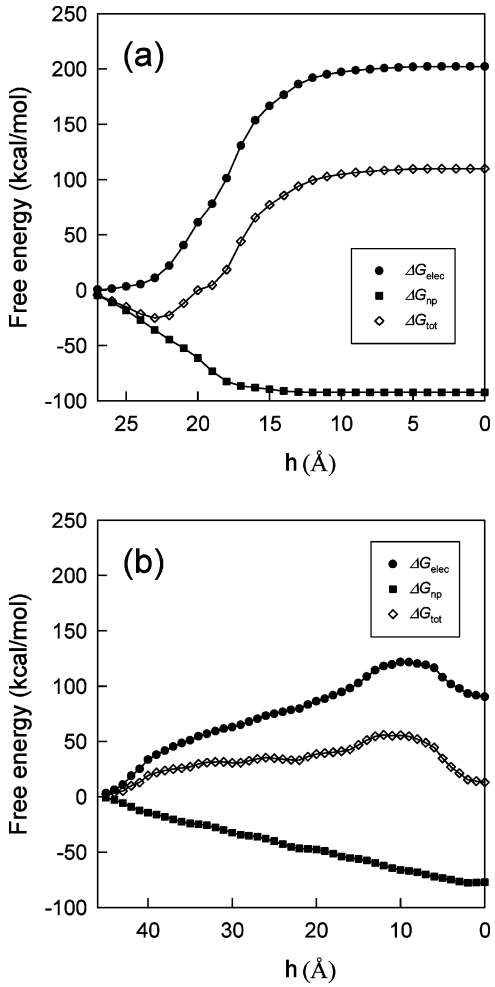

Figure 2. Change of free energy as a function of the distance between the center of lipid bilayer and the center of the helix as the polypeptide is inserted into a lipid bilayer horizontally (a) or vertically (b), where the distance between the center of mass of polypeptide and the center of lipid bilayer is denoted as *h*. All glutamate residues of GALA peptide are set to be neutral in the calculation, which corresponds to an acidic condition favoring a helical state of GALA peptide.

and ρ_{eff} is the effective surface density of a lipid alkyl chain. If the volume of the lipid bilayer is conserved, L' can be written as

$$LS = L'(S' - \pi R_{pore}^2) \tag{11}$$

and then ρ_{eff} can be defined as

$$\rho_{eff} = \rho S/(S - \pi R_{pore}^2) = \rho/(1 - \pi R_{pore}^2/S) \tag{12}$$

Therefore, the contribution to the total free energy from the perturbing effect of lipid due to the formation of a pore of radius R_{pore} can be represented as

$$\Delta G_{pore} = G(R_{pore}) - G(R_{pore} = 0) \tag{13}$$

Results and Discussion

Stability of GALA Peptide. When the change of the electrostatic free energy and the total charge of GALA are calculated as a function of pH, as shown in Figure 1a, it reveals that ΔG^{ion} decreases and becomes negative at pH < 7.4 as pH decreases, which means that the helical state is favored at pH < 7.4. This result agrees qualitatively with an experimental

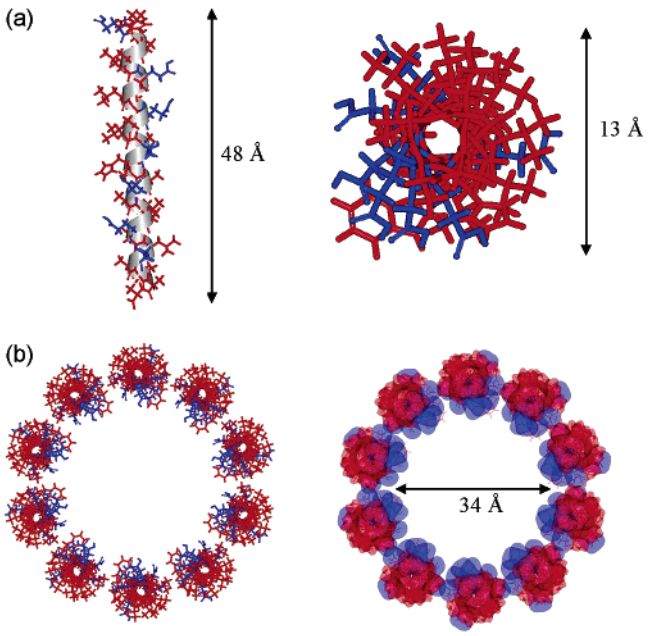

Figure 3. (a) Structure of GALA helix. (b) The structure of pore formed by aggregation of 10 GALA polypeptides. The polypeptides are arranged to minimize the nonbonded interactions between them by using the CHARMM program. The energy minimized pore structure is shown (top view): stick representation (left) and surface representation (right). The side chains colored as blue and red represent hydrophilic Glu residues and hydrophobic residues, respectively.

result[2] that the fraction of α helix increases from 19% to 69%, as pH is decreased from 7.0 to 5.0. When the GALA peptide forms an α helix, the Glu residues in GALA are positioned repeatedly every 4 residue and hence they make a hydrophilic face to generate an amphiphatic helix. Therefore, it is easily expected that the helical structure is destabilized as the Glu residues are charged negatively, because the charge−charge repulsion between Glu residues becomes large under the helical conformation. When the average charges of ionizable residues are plotted against pH, as shown in Figure 1b, it reveals that all Glu residues except Glu2, Glu7, and Glu19 are nearly neutral at pH < 7. This result leads us to conclude that there exist a helix-coil transition as pH decreases and that the helical structure is favored at low pH.

Orientation of GALA Peptide upon Insertion into Lipid Bilayer. To find a more probable orientation of GALA peptide when GALA is inserted into a lipid bilayer, we first calculated the free energy changes upon insertion of a single helix for two cases (vertical insertion and horizontal insertion) and compared the free energy changes with each other, as shown in Figure 2. It is obvious from Figure 2 that GALA is not thermodynamically stable when it is inserted fully into the lipid bilayer in both cases. Since the contribution of ΔG_{elec} is very large due to the free energy for transferring the seven glutamate residues in GALA from water to the center of lipid bilayer, the total free energy becomes positive although the hydrophobic interaction between the peptide and the alkane chains in lipid bilayer contributes to reduce the nonpolar free energy term. Close examination of Figure 2a reveals that the total free energy shows a minimum value (−25.03 kcal/mol) when the center of GALA is located at the interface between water and the lipid bilayer, whereas Figure 2b does not show a minimum with negative free energy. This indicates that the orientation parallel to the surface of the lipid bilayer is more probable than the vertical insertion. Indeed, this horizontal orientation has been predicted for other peptides.[14]

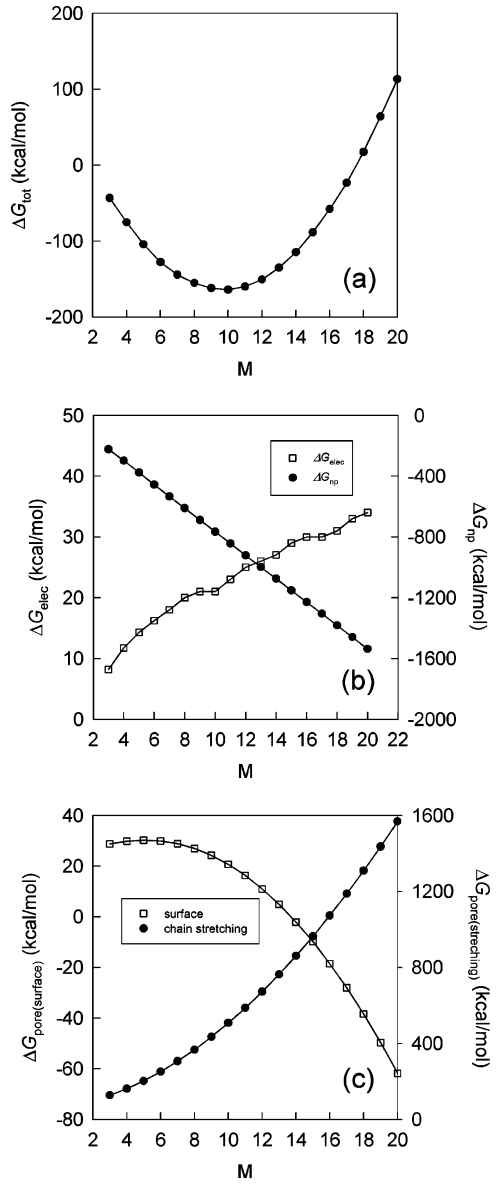

Figure 4. Change of the free energy upon pore formation as a function of the aggregation number of amphipathic GALA helix (*M*). All glutamate residues of GALA peptide are set to be neutral in the calculation, which corresponds to an acidic condition favoring a helical state of GALA peptide: (a) the total free energy; (b) the electrostatic (G_{elec}) and nonpolar (G_{np}) contributions; (c) the lipid perturbation term (the change of surface area + the effect of chain stretching).

Aggregation Number of GALA Peptide for Pore Formation in the Lipid Bilayer. It has been reported that an aggregate of 10 ± 2 peptides is formed and penetrates into the lipid bilayer to generate a pore as the concentration of polypeptide increases at the surface of the lipid bilayer.[2] For the purpose of elucidating the origin of the specific aggregation number of GALA for pore formation, model aggregates are first generated and then energy-minimized using the CHARMM program. Figure 3 shows a typical example for pore formation from 10 GALA peptides. The helices in the aggregate are arranged parallel to each other along the *z* axis, and the final structure forms a hollow cylinder, in which the hydrophilic residues form an inner surface of the cylinder, whereas the hydrophobic faces form an outer surface of the cylinder.

When the total free energy of pore formation is plotted against the aggregation number (*M*) of GALA helix, as shown in Figure 4a, it reveals that there exists a minimum of the total free energy at $M = 10$, which is very consistent with an experimentally observed value of 10 ± 2.[2] To analyze the origin of the specific aggregation number, each of free energy contributions is plotted as a function of the aggregation number in Figure 4, panels b and c. The electrostatic free energy increases with increasing *M*, whereas the solvation free energy (ΔG_{np}) due to hydrophobic interaction between the lipid alkyl chain and the hydrophobic face of the aggregate decreases with large negative values as *M* increases, as shown in Figure 4b. The change of free energy due to the change of surface area starts to decrease at $M = 6$ and becomes negative at $M = 13$, whereas the free energy due to chain stretching of lipid increases with large positive values as *M* increases, as shown in Figure 4c. Here, it should be noted that the contributions of the electrostatic free energy (ΔG_{elec}) and the surface free energy ($\Delta G_{pore(surface)}$) to the total free energy of pore formation are relatively very small. In short, as the aggregation number increases, the solvation free energy (Figure 4b) decreases whereas the free energy due to chain stretching increases. As a consequence, the balance between the solvation and chain stretching terms results in a minimum of the total free energy at $M = 10$.

Conclusion

The pH-dependent helix−coil transition of an amphipathic polypeptide GALA and its association with the lipid bilayer are investigated. The amphipathic helix is more stable at pH = 5 than at pH = 7 by 14 kcal/mol. At low concentration of GALA peptide, a GALA molecule lies parallel to the surface of lipid bilayer with its hydrophilic face exposed to water. As the aggregation number increases, the solvation free energy decreases, whereas the free energy due to chain stretching increases. The balance between these free energy terms results in a minimum of the total free energy at $M = 10$, which is very consistent with an experimental value of 10 ± 2.[2]

Acknowledgment. The authors thank the Korea Science and Engineering Foundation (KOSEF) for financial support through the Hyperstructured Organic Materials Research Center (HOMRC).

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BM050579Z