Modeling the Phase Behavior of the Membrane Binding Protein Annexin V

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The bulk thermodynamic properties of proteins originate from a varied and complex combination of interactions. We propose a simple model for the formation of ordered two-dimensional aggregates based on the interactions between pairs of annexin V molecules. Simulations of this model are shown to reproduce the experimental observations of a honeycomb ($p6$) and a triangular ($p3$) crystalline phase. The simulations indicate that the transition between these two phases is first order. While this model is extremely simple in that it relies only on hard body and short-range directional interactions, it nevertheless captures the essential physics of the interactions between the protein molecules and reproduces the phase behavior observed in electron microscopy and atomic force microscopy experiments.

I. Introduction

The phenomena of protein crystallization, aggregation, liquid−liquid phase separation, and self-assembly are of great importance in the field of protein structure determination[1] and in the purification and stabilization of protein solutions. A detailed knowledge of the complex intra- and intermolecular interactions is required in order to fully understand the biological properties of individual proteins or protein complexes. In contrast, greatly simplified models for the protein−protein interaction can be used to model the phase behavior and aggregation form of large numbers of proteins. Such a simplification is, in fact, essential when using computer simulations to predict the phase diagram of a protein solution. Here, we take such a point of view to construct a simplified model for the intermolecular interactions of annexin V, a phospholipid binding protein,[2] and use computer simulation techniques to determine the phase behavior of the model system.

The most reliable methods for crystallizing proteins in two dimensions rely either on the incorporation of membrane proteins within lipid bilayers[3] or on the specific anchoring of soluble proteins to lipid monolayers at the air−water interface[4] or lipid bilayers at solid−liquid interfaces.[5] The association of a protein to a planar substrate seems to be sufficient for triggering self-assembly into two-dimensional (2D) crystals. This technology has been highly successful for annexin V, due to its property of binding specifically to negatively charged phospholipids such as phosphatidylserine in the presence of Ca^{2+} ions.[6,7] Annexin V molecules are monomeric in solution but, after binding to a membrane, spontaneously form tightly bound trimers. The process of trimerization is extremely rapid, and electron crystallography evidence suggests that there is no significant change in the molecular conformation upon binding to the membrane.[8]

The membrane-bound trimers self-organize into two types of 2D crystals.[9] Electron microscopy density maps probing the structure of these two phases are shown in Figure 1. These phases are based on the same building block, namely, the trimer, and show either $p3$ or $p6$ symmetry. Experiments on lipid monolayers using electron microscopy (EM) and lipid bilayers using atomic force microscopy (AFM) indicate that $p6$ is the stable phase at low surface coverage.[10,11] The $p6$ crystals are formed by

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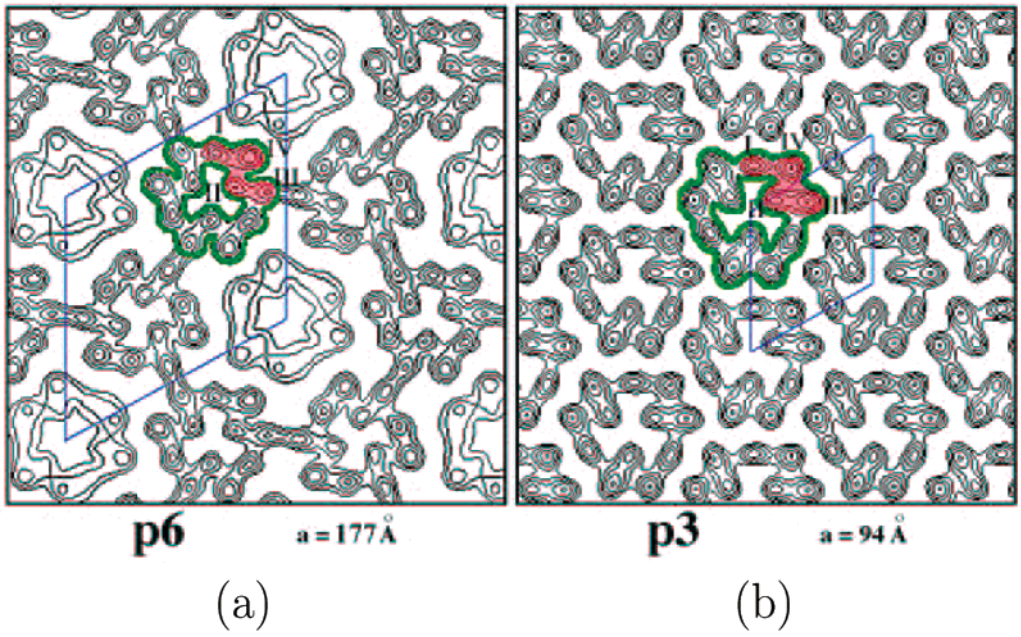

Figure 1. Two-dimensional projection maps of the structure of (a) the *p*6 and (b) the *p*3 phases of annexin V, at 20 Å resolution. In each plot, one trimer is outlined and one annexin V molecule is shaded. The four domains are numbered according to Huber et al. (ref 22). The figure also indicates the lattice cell dimension *a*. Note that in the *p*6 phase, the presence of the central trimer enclosed within the 6-fold symmetry cage depends on the surface coverage.

a rigid hexagonal lattice of trimers, in which each cell can contain a rather mobile central trimer (see Figure 1). This phase is named *p*6 due to its apparent *p*6 symmetry although, in fact, the symmetry is lower since not every cell contains a "captured" trimer and, if it is present, the mobile central trimer does not exhibit 6-fold symmetry. At a higher density of binding sites, another crystalline form becomes more stable, where the trimers are packed more tightly, so that each particle is surrounded by nearest neighbors arranged in a *p*3 symmetry class.[9] The transition to the higher density structure is induced experimentally by stressing the system, for example, by transferring the films from an air−water interface to an EM grid (equivalent to increasing the lateral pressure) or by increasing the concentration of the lipid ligand in solution (equivalent to increasing the surface density of trimers). There is experimental evidence which suggests that the *p*6−*p*3 transition is first order, and its reversibility has been monitored using AFM; the transition is characterized by an increase in surface density of trimers of about 15%.[12]

Two-dimensional aggregation of membrane proteins has been approached with simple models since the 1990s. Sperotto and Mouritsen have applied Monte Carlo simulations and mean field calculations to a statistical mechanical lattice model of lipid−protein interactions in membranes in order to investigate the phase equilibria.[13,14] A good summary of protein aggregation in membrane systems has been given by Bruinsma and Pincus.[15] They focused on aggregation in membranes induced by membrane-mediated elastic deformation and concluded that aggregation may occur if the protein inclusion suffers a thickness mismatch, induces a local change in membrane stiffness, or acts as gap junction pinching together the membranes. The effect of lipid−protein interactions in the protein aggregation has been reviewed more in detail by Gil and co-workers.[16] On the basis of a variety of theoretical considerations and model calculations, the nature of lipid−protein interactions is considered both for a single protein and for an assembly of proteins that can lead to aggregation and protein crystallization in the plane of the membrane. The phenomena include lipid sorting and selectivity at protein surfaces, protein−lipid phase equilibria, lipid-mediated protein−protein interactions, wetting and capillary condensation as means of protein organization, mechanisms of two-dimensional protein crystallization, and nonequilibrium organization of active proteins in membranes. More recently, Dommersnes and Fournier[17] have studied the collective behavior of membrane inclusions in a flexible fluid membrane. In these calculations, the long-range attractive interactions between inclusions are found to be sufficiently strong to induce aggregation. However, as we shall see, the main result of our study is that the long-range interactions do not need to be included to reproduce the *p*3 and *p*6 phases for annexin V.

Before we describe the mode and simulation of annexin V, we note that there are other examples of proteins giving periodic phases in two dimensions, for example, the purple phase of aggregated bacteriorhodopsin,[18] where the geometry consists of hexagonal close packing. Although the model we describe is valid for annexin V, the general treatment is valid for any two-dimensional system with

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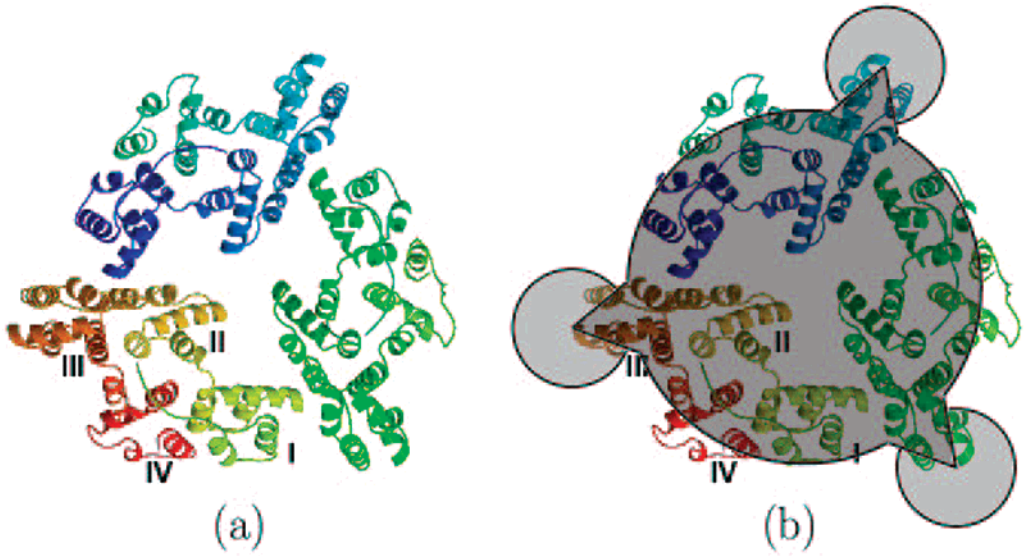

Figure 2. (a) Molecular structure of annexin V trimer (ref 23) and (b) the simplified model, with the approximate location of the domains of the real trimer numbered according to Huber et al. (ref 22). The light gray regions indicate the location and range of the attractive interactions which occur between the domains III in pairs of the real protein, and the darker gray central region indicates the excluded area "hard body" of the model potential.

specific, localized interactions, that is, when the relevant protein−protein interactions can be described by (highly directional) short-range forces. There are cases where long-range forces are important. This could be the case for other protein inclusions which strongly modify the elasticity of the supporting membrane.

II. Simplified Model for the 2D Crystallization of Annexin V

The trimer structure of the surface-bound proteins (see Figure 2) and the organization of these within the *p*6 and *p*3 phases (see Figure 1) suggests the essential features of a simplified model potential. The electron microscopy map of the *p*6 phase shows clearly that the trimer−trimer interactions are extremely localized between adjacent domains III of neighboring molecules and that these domains must be almost perfectly aligned. In other words, the interactions should be extremely localized, short ranged, and orientation dependent. Consideration of the *p*3 phase, on the other hand, suggests that at higher density the trimers need to rearrange in order to accommodate particles in a more efficient space-filling way, rotating such that the domains III of a triplet of trimers interact at 120° as opposed to 180° for a pair of trimers in the *p*6 phase.

These observations lead us to formulate a simplified version of the trimers which is pictured in Figure 2. The internal structure of the trimer is taken to be frozen; thus, the shape of the trimer is taken to be a rigid hard body model composed of an equilateral triangle and a hard disk sharing the same center, with the diameter of the circle the same as the height of the triangle $\sigma = h$. The three vertexes of the triangle represent the location of the three domains III in the real trimer. Short-range attractions $r = h/6$ are located at the vertexes, which are modeled as a simple linear ramp increasing from $-\epsilon$ to zero. As discussed earlier, directionality of the attractive interactions is important, as it singles out two preferred orientations at 120° (*p*3 phase) and 180° (*p*6 phase). The actual functional form of the angle dependence is not important for our model so long as these two angles are preferred, but the EM maps and molecular mechanics calculations suggest that three main ingredients should be included: preferred interaction angles between the domains III at 120° and 180°, comparable interaction energies when two trimers interact at 180° (*p*6 phase) and three interact at 120° (*p*3 phase), and a relative energy penalty for any other angles, especially less than 90°. We choose the angular-dependent potential to be

$$u_2(\theta) = \begin{cases} 1 & \theta < 90° \\ \cos\theta + \frac{1}{2}(1 - \cos 6\theta) & 90° < \theta < 270° \\ 1 & \theta > 270° \end{cases} \quad (1)$$

Full details of the potential and the simulations, as well as a theoretical study of the *p*6 phase, will be given elsewhere.[19] The important point here is that as the potential is somewhat generic, any potential of similar form should reproduce the experimental phase behavior, so long as the correct physics enters the potential model.

III. Computer Simulation of the Model Trimers

Systems of model timers were studied using grand canonical (constant chemical potential or osmotic pressure) Monte Carlo simulations.[20] Rather than using a fixed number of molecules, which is common for many simulations, the grand canonical ensemble uses a varying amount of molecules, but with the average amount governed by the chemical potential. This simulation technique is particularly attractive for this model, since it mimics the behavior at the surface in the real experiments during the formation of the *p*6 phase. Thus, by altering the chemical potential of surface trimers in the simulation, which is equivalent to controlling the concentration or osmotic pressure of the protein in solution in the real experiments, the surface coverage can be varied. The advantage of the grand canonical simulations over other common simulation techniques is that we can simulate an "open" system, in which the number of particles is not fixed. Our system is open in the sense that we are

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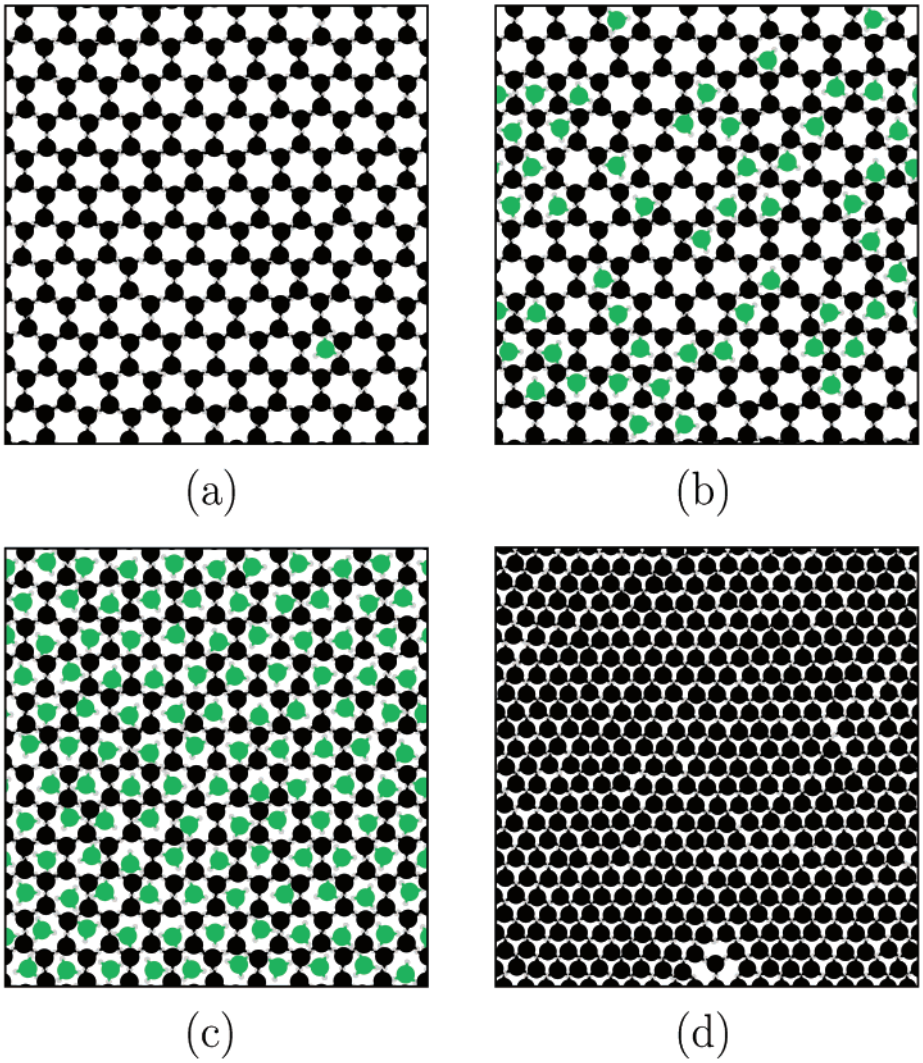

Figure 3. Configurations from the simulations of model annexin V. The *p*6 phase at (a) low, (b) intermediate, and (c) high surface coverage and (d) the *p*3 phase. For clarity, the captured central trimers in the *p*6 phase have been colored differently to those forming the hexagonal lattice. Note the "*p*6-like" defect in the *p*3 phase. At low temperature, these are relatively uncommon; however, at high temperature they are more common and can frequently be observed along line defects between different regions of *p*3 structure.

simulating only the surface-adsorbed proteins and not the proteins in solution; this means that adsorption (or desorption) of a protein trimer onto (from) the surface can be modeled by the addition (or removal) of a model trimer from the simulation.

At low protein concentration, the surface coverage is low, and the adsorbed proteins hardly interact. If the chemical potential is increased past a certain threshold, a large jump in the surface density is observed. The resulting structure is shown in Figure 3a; the system clearly forms an open hexagonal structure of *p*6 symmetry. As the chemical potential is increased, the density is found to increase continuously up to a critical value. Similarly, if the chemical potential is decreased, the coverage decreases continuously down to that of the open hexagonal lattice. Example configurations at intermediate and higher coverage are shown in Figure 3b,c and are clearly comparable to that shown in Figure 1. We observe that the surface coverage increases simply by adsorbing trimers into the unoccupied cavities in the hexagonal lattice. Similarly, if the chemical potential is lowered, it is the central trimers which are desorbed, and the hexagonal lattice remains intact.

However, once the surface coverage reaches a certain limit, with all the hexagonal cavities filled, there appears to be no way of absorbing further trimers onto the surface. However, the simulations indicate that there is a coverage jump of 15% as the chemical potential is further increased. Figure 3d shows how this can occur. The hexagonal structure observed previously is destroyed, in favor of a structure with *p*3 symmetry, reminiscent of the *p*3 phase of annexin V (see Figure 1). This phase is more efficient at packing than the *p*6 structure and so is stable at higher densities. Note that, in our model, we choose the energy of a *p*6-type bond (180°) to be twice that of a *p*3-type bond (120°); since each trimer interacts with three others in the (empty) *p*6 phase and six others in the *p*3 phase, the potential energy of these phases will be equal. However, since $^1/_7$ of the trimers in the full *p*6 phase are noninteracting (the captured ones), the *p*3 phase is also slightly energetically favored at high density in comparison to the "filled" *p*6 phase. Thus, this transition is most probably partly energy and partly entropy (or packing) driven.

The simulations described are performed at a single finite temperature, $kT \sim \epsilon$, and the system is found to reproduce the experimentally observed phase behavior. In a simulation, we need not be constrained by the temperatures usually accessible for an experiment and so can study extreme limits for the model, which would not be accessible in the laboratory. However, limits such as

very high temperatures are usually not so interesting since these would correspond to conditions at which, for example, the individual trimers would behave differently and not form trimers or possibly even decompose. Therefore, here we only describe simulations at reasonable temperatures. However, we have computed the phase diagram as a function of temperature and this will be presented elsewhere, along with a simple theory for the behavior of the *p*6 phase.[19] We should also point out that while we use a simple potential defined by a given set of parameters, variation in the parameters should lead to very similiar phase diagrams so long as the essential physics of the interactions described in section II are upheld; that is, the potential must possess the correct 3-fold symmetry, the angular potential should favor the correct alignments, and the attractive interactions are short range. Indeed, if the range of the attractive interactions is chosen to be of the order of the trimer size, the phase behavior is dramatically modified, leading to a 2D liquid−vapor type transition, as we expect.[21]

IV. Conclusions

The highly simplified model for annexin V described here, based only on hard core interactions plus highly directional short-range interactions, is able to reproduce the qualitative phase behavior of such a complex membrane binding protein in a surprisingly good agreement with experiments. It is also able to reproduce quantitatively the change in surface coverage of 15% at the first transition between the *p*6 and *p*3 phases. These results indicate how relatively crude, generic models which strip away the complicated chemical detail but retain the essential physics of the interactions between large molecules can be used to understand the formation of phase structures such as the *p*6 and *p*3 phases of annexin V.

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