Models of Antifreeze Proteins Bound to Ice by Tandem Repeat Motif

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Abstract

Antifreeze protein (AFP) can help organisms survive from freezing condition by thermal hysteresis activity to control ice growth and by recrystallization inhibition activity to inhibit recrystallization of ice granules, respectively. By bioinformatics methods especially protein docking, the newly developed models of surface complementarity were used to obtain the molecular models of AFPs and hence to interpret the binding mechanism between AFP and ice. The molecular models of relatively perfect surface complementarity were obtained basing on the three-dimensional structures of AFPs, and among the forces of models of surface complementarity, van der waals force and hydrophobic interaction is the key role. The docking result showed that the tandem repeat motif of each AFP can form the basic framework to support the firm binding between the ice binding sites of AFP and ice surfaces, providing useful insights to understand the molecular mechanism of the binding between AFP and ice.

Key words: Antifreeze protein; Protein docking; Bioinformatics; Surface complementarity

1. Introduction

Antifreeze protein (AFP) exists abroad in cold-resistant organisms including fishes, microbes, insects and plants from polar region or Frigid Zone, and can protect organisms from damage in freezing conditions by controlling the growth of ice and inhibiting the recrystallization between ice granules, which were termed thermal hysteresis (TH) activity and recrystallization inhibition (RI) activity, respectively [1]. AFP had been separated from fishes, insects, plants and bacteria respectively, which were correspondingly called fish AFPs consisting of AFGP (antifreeze glycoprotein) and type I-IV, insect AFPs including TmAFP (Tenebrio molitor), CfAFP (Choristoneura fumiferana) and DAFP (Dendroides canadensis), plant AFPs including DcAFP (Daucus carota) and LpAFP (Lolium perenne), and bacterium AFPs [2]. The structural characteristics of fish AFPs and insect AFPs were deeply interpreted, plant AFPs secondarily, and bacterium AFPs just initiatively [3].

AFP has the capability of inhibiting ice growth by a process of absorbing to the ice faces, which will result in the lowering of the freezing point non-colligatively while leaving the melting point unchanged, which is termed thermal hysteresis [4]. Besides the TH activity, AFP also possesses of another important antifreeze activity, recrystallization inhibition, which can effectively inhibit the recrystallization in organism cells against damage [5]. AFPs from fishes and insects were characterized by high TH and low RI, but plant AFPs were distinct for the characteristic of low TH and high RI [6]. For fishes and some insects whose body fluid should be unfrozen, it is lethiferous for the freezing in inner or outer of cells, thus a strong TH activity is necessary to inhibit the ice growth in their body fluid [7]. However, RI should be more important for hardy plants. The TH values of plant AFPs are usually 0.1~0.6 °C, which is not enough to avoid the ice growth and lower the freezing point, therefore, RI plays the primary role in prevent from damages in freezing condition by inhibiting the recrystallization of small ice granules [4]. Moreover, RI can be easier achieved and controlled than TH, for a small quantity of plant AFPs can bring high RI activity [8].

Because of the complexity of interaction between AFP and ice crystal, and lack of directly observable instrument, the molecular mechanism of AFP is still not convincingly interpreted though lots of researches on AFP structures were deeply carried out [9]. Several

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molecular models were brought forward to explain the AFP-ice interaction [10], however, these models are effective to only one or some AFPs, but ineffective to other AFPs [11]. Therefore, it is very necessary to develop a universal molecular model which can explain the molecular mechanisms of all AFPs. In this paper, bioinformatics methods, especially protein docking, were used to molecularly simulate the interactions of all structure-solved AFPs basing on the mode of surface complementarity, presenting molecular models for deeply interpreting the mechanisms of AFPs.

2. Material and methods

2.1 Three-dimensional structures of AFPs

PDB formats of all AFPs whose three-dimensional structures were solved by X-ray crystallography or Nuclear magnetic resonance (NMR) were obtained from the PDB website (http://www.rcsb.org/pdb), including AFGP, AFP I-IV, TmAFP and CfAFP. LpAFP was molecularly modeled to obtain its theoretical structure (PDB format) with software SYBYL. DcAFP was homologically modeled to obtain its theoretical structure (PDB format). PDB formats were used to analyze the structural characteristic by softwares.

2.2 Protein structure analysis

Protein Explorer was used for all protein structure manipulations, calculations of conservation scores and electrostatic potential distribution, and generation of the figures. Protein surface docking was carried out using program 3D-DOCK.

3. Results and discussion

3.1 Surface complementarity Model

Four models, including “adsorption inhibition model”, “dipole-dipole model”, “lattice occupancy model”, and “lattice matching model”, were ever used to explain the molecular mechanisms of AFPs. The same of above four models is that AFPs should bind to ice faces by hydrogen bond, but the number and mode are different. Therefore, we classed the four models as hydrogen-bonding matching model. Although this model can effectively explain the mechanism of only one or some AFPs, but is distinctly unsuitable for other AFPs [12]. A new model of receptor-ligand was brought forward, and this model shows that AFP is receptor and ice is ligand during their interaction [13].

We further developed receptor-ligand model into surface complementarity model, whose core is that the molecular surfaces of ice-binding site of AFPs can form surface complementarity with faces of ice crystals. Moreover, many forces (mostly including van der waals force, hydrophobic interaction, and hydrogen-bonding) are involved with this complementarity, and the area of complementarity is positively related to the intensity of interaction. The surface complementarity leads to the irreversibly binding AFP to ice crystal, for it is almost kinetically impossible to disconnect these forces simultaneously. Surfaces of different AFPs can bind to different faces of ice crystals. The reason is that discovered AFPs displays large evolutional difference, and ice crystal possesses many different faces whose topological structure and space of oxygen atom are totally different, hence any AFP can inhibit the ice growth by binding to a proper face [14]. Therefore, just like a ligand, ice crystal can selectively bind with AFP by proper surface complementarity. After analyzing solved or modeled structures of main AFPs, we consider that surface complementarity model is suitable for all discovered AFPs, and that van der waals force and hydrophobic interaction may be the key roles, hydrogen-bonding is subsidiary. Moreover, any mutation which changes the surface of ice-binding sites will leads to the fall of complementarity intensity between AFP and ice, hence corresponding reduction of antifreeze activity, especially TH.

3.2 Binding models of typical AFPs with ice

Four typical AFPs, including fish AFP (Type AFI), insect AFP (TmAFP) and plant AFP (DcAFP and LpAFP), were used to analyze the binding models between AFPs and ice.

3.2.1 Fish AFP I. Early research results suggested that the hydrogen bond of Thr of AFP I ice-binding sites is important to the interaction between AFP I and ice, however, more results revealed that other residues (especially those exposed to the outer) besides Thr may play key role in the binding of AFP I to ice, hence hydrogen-bonding matching model is doubted increasingly. Recent results suggested that the ice-binding sites should be the hydrophobic surface consisting of conserved Ala and near Thr [15]. After four Thr residues were substituted with Val, mutants showed a same conformation as the wild, suggesting that the hydrophobicity formed by Val is important to stable the interaction between AFP I and ice. We used the PDB format (1WFA) to analyze the structural characteristic (Fig 1A) and to model the binding of AFP I to ice (Fig 1B). Our model reveals that conserved Ala and immediate Thr are the ice-binding
sites, and form a special hydrophobic surface, which can interact with ice faces by van der waals force, hydrophobic interaction and hydrogen bond synchronously to bring a compact surface complementarity (Fig 1C).

Figure 1. Model of surface complementarity between fish AFP I and ice
A: three-dimensional structure of fish AFP I (PDB ID: 1WFA); B: fish AFP I binds to {2021} plane of ice crystal; C: Cross-section of fish AFP I showing positions of conserved repeat motif of Ala and near Thr, which is considered as the ice binding sites.

3.2.2 TmAFP. Since the discovery, TmAFP attracted interests for its high TH activity. All TmAFP isoforms possess 7-8 tandem repeats (TCTXSXCCXXAX), which form a regular right-handed β-helix consisting of 7-8 loops, in which regularly arrayed residues (TCT) are considered the perfect ice-binding sites [16]. The model of surface complementarity (Fig 2A) we obtained by protein docking is very suitable to explain the ice-binding mechanism of TmAFP (Fig 2B). The main forces to form surface complementarity is van der waals force and hydrophobic interaction, while hydrogen bond is subsidiary, so the number change of hydrogen bond will not seriously affect the total interaction, thus TmAFP mutants can not be desorbed from ice faces (Fig 2C). Moreover, any mutation at ice-binding sites will damage the complanarity of ice-binding surface, thus lead to the untight complementarity between mutant and ice and the lessen of effective area of complementarity, hence lead to the corresponding decrease of TH activity. Furthermore, after binding to faces of ice crystal, ice-binding surface of TmAFP will keep on a rigid conformation, which is in favor of tight binding of TmAFP to faces of ice crystal by perfect surface complementarity.

Figure 2. Model of surface complementarity between insect TmAFP and ice
A: three-dimensional structure of TmAFP (PDB ID: 1EZG); B: TmAFP binds to {1010} plane of ice crystal; C: Cross-section of TmAFP showing positions of conserved repeat motif “TCT”, which is considered as the ice binding sites.

3.2.3 DcAFP. DcAFP has a higher TH but lower RI than LpAFP [6], which are confirmed respectively by our results of prokaryotic expression and transgenic plant [4]. We modeled the theoretical three-dimensional structure of DcAFP by homology modeling (Fig 3A). The result of structure analysis revealed that DcAFP is a regular right-handed β-helix consisting 10 loops of 24-amino-acid leucine-rich tandem repeat (PXXXXXLXXLXXXLXXNXLXG) [3]. The surface docking of DcAFP and ice crystal suggested that only one putative ice-binding site displays imperfect surface complementarity to 1010 prism plane of ice (Fig 3B). The conservative asparagine residues in each β-loop within LRR motif are very important in maintaining the TH activity. DcAFP shows almost same distance between each loop and same space of residues in ice-binding site as TmAFP (Fig 3C). However, only one ice-binding site remains the RI of DcAFP unexplained.
3.2.4 LpAFP. The low molecular weight (118 residues for 13 kDa) LpAFP is characterized by very low TH and extremely high RI [17]. The theoretical three-dimensional structure (Fig 4A) suggested that LpAFP is a regular right-handed β-helix consisting of loops of 14-15 residues of two repeats XXNXVXG, in which two TVT arrays may be the putative ice-binding sites [18]. TVT array is similar to TXT array of TmAFP and CfAFP, however, the Thr conservation of LpAFP is relatively low [19]. The result of protein surface docking to faces of ice crystal showed that both two TVT arrays can form surfaces, which are untightly complementary with ice faces by van der waals force primarily and by hydrogen bond secondarily (Fig 4B). The distance 4.5 Å of each β-strand and space 7.4 Å of two Thr are accordant with those of oxygen atoms in ice faces, this facilitate the surface complementarity between LpAFP and ice (Fig 4C).

“Double-sided adhesive tape” was presented to explain the high RI of LpAFP. Two ice-binding sites on opposite sides of β-roll can simultaneously bind the ice fronts between two adjacent ice grains to stop ice boundary migration, hence to inhibit recrystallization. However, the low TH of LpAFP is still unexplained. According to molecular model of surface complementarity, we suggest that the low conservation (65% substitution) of TVT arrays leads to the untight surface complementarity with ice hence the decrease of TH. This is almost similar to mutants of fish and insect AFPs. Moreover, the opposite ice-binding surfaces can bind only two different ice crystals, not a same; therefore, the TH is actually from only one ice-binding site, hence significantly lower than those of fish and insect AFPs.

4. Conclusion and discussion

Above AFP models of fish, insect and plant shows that surface complementarity model can be used to effectively explain the strong interaction between AFP and ice. Forces involved in these models are mostly van der waals force, hydrophobic interaction and hydrogen bond. Therefore, compared with hydrogen-bonding matching model, surface complementarity model can keep down the characteristic of hydrogen bond, and also emphasize the significance of van der waals force and hydrophobic interaction. Under the cooperation of these forces, AFPs can form tight surface complementarity with ice faces hence display strong TH activity [20]. The low conservation of plant AFPs is the reason of low TH, for the imperfect complementarity can weaken interaction between AFP and ice, which is observable in mutants of fish and insect AFPs [21]. Two ice-binding sites are suitable to explain high RI of plant AFPs or other AFPs, but this need more experimental support.
Therefore, despite the distinct difference of structures and ice-binding sites, surface complementarity model is suitable to interpret the interaction between AFP and ice, hence can be developed into the universal mechanism, for this model may be suitable for unsolved AFPs from microbe and others [22]. However, more experiments and instruments which can be used to directly observe the AFP-ice interaction are needed to confirm the kinds, numbers and contributions of involved forces.

5. References


