RESEARCH ARTICLE | AUGUST 19 2016

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J. Chem. Phys. 145, 075101 (2016)
https://doi.org/10.1063/1.4961094

Structure of solvation water around the active and inactive regions of a type III antifreeze protein and its mutants of lowered activity

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(Received 23 June 2016; accepted 3 August 2016; published online 19 August 2016)

Water molecules from the solvation shell of the ice-binding surface are considered important for the antifreeze proteins to perform their function properly. Herein, we discuss the problem whether the extent of changes of the mean properties of solvation water can be connected with the antifreeze activity of the protein. To this aim, the structure of solvation water of a type III antifreeze protein from *Macrozoarces americanus* (eel pout) is investigated. A wild type of the protein is used, along with its three mutants, with antifreeze activities equal to 54% or 10% of the activity of the native form. The solvation water of the ice-binding surface and the rest of the protein are analyzed separately. To characterize the structure of solvation shell, parameters describing radial and angular characteristics of the mutual arrangement of the molecules were employed. They take into account short-distance (first hydration shell) or long-distance (two solvation shells) effects. The obtained results and the comparison with the results obtained previously for a hyperactive antifreeze protein from *Choristoneura fumiferana* lead to the conclusion that the structure and amino acid composition of the active region of the protein evolved to achieve two goals. The first one is the modification of the properties of the solvation water. The second one is the geometrical adjustment of the protein surface to the specific crystallographic plane of ice. Both of these goals have to be achieved simultaneously in order for the protein to perform its function properly. However, they seem to be independent from one another in a sense that very small antifreeze activity does not imply that properties of water become different from the ones observed for the wild type. The proteins with significantly lower activity still modify the mean properties of solvation water in a right direction, in spite of the fact that the accuracy of the geometrical match with the ice lattice is lost because of the mutations. Therefore, we do not observe any correlation between the antifreeze activity and the extent of modification of the properties of solvation water. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4961094]

INTRODUCTION

The organisms that live in sub-zero temperatures or are seasonally exposed to these temperatures are equipped with some tools that enable them to survive in these harsh conditions. One of them is antifreeze proteins (AFPs) that prevent the bodily fluids from freezing (in a limited range of temperatures). Their antifreeze activity is usually explained by the fact that they adsorb to the surface of the ice crystal. In the process of binding of AFPs to ice both hydrophobic and hydrophilic interactions appear to play a significant role.[1,2] Various AFPs are able to distinguish different crystallographic planes of ice and to adsorb to only one or two of them.[3–6] This ability is explained by a special spatial arrangement of the amino acids on the binding surface of AFPs.[7] The ability to bind to two ice planes, and especially to the basal one, has been proposed as a key prerequisite for an AFP to be hyperactive.[3] As a result of binding, the further growth of the crystal is suppressed because of the Gibbs-Thomson effect.[8–10] Although there is a consensus regarding the general outline of the process, some of the details are debatable. For example, a seemingly straightforward problem, i.e., whether the binding of AFPs with ice is a reversible or irreversible process, had not been unambiguously solved for a long time. Experimental results obtained recently[11–14] support the irreversibility hypothesis for both hyperactive and type III AFPs—this is the group to which the protein investigated herein belongs. Recently, it is often pointed out that the properties of solvation water are important for the process of binding of AFPs to the surface of ice. The water molecules are arranged in a way that makes them ice-like.[3,15,16] Thanks to that the protein can freeze onto the surface of ice along with some of its solvation water molecules. In our previous papers,[17,18] we discussed the elusive role of solvation water, the interaction of AFPs with the ice surface, and the proposed mechanisms of action of AFPs more thoroughly. Our previous results regarding the solvation of a hyperactive antifreeze protein from *Choristoneura fumiferana* (*Cf* AFP) do not support the hypothesis of a completely ice-like arrangement of solvation water molecules surrounding the binding plane, although some similarities to the structure of solvation water of ice can be found. Simultaneously, the structure and dynamics of solvation water of the ice-binding plane were significantly different from the properties of the solvation water of the rest of the protein. This was especially

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visible when the temperature changes of the structural and dynamical parameters were analyzed. These changes could be unambiguously connected with the biological function of the ice-binding plane. Because of that, we decided to broaden our investigations by discussing another problem—whether there is a connection between the biological activity of the protein (measured by the width of the gap between the melting and the freezing point of the solution) and the degree of the structural changes of the properties of solvation water.

As the object of our investigations we selected a type III AFP from *Macrozoarces americanus* (eel pout). It is a protein that expresses a medium antifreeze activity (the width of the hysteresis gap is equal to about 0.35 K at a concentration 1 mg/ml[19]). Part of its ice-binding surface binds to the pyramidal plane of ice, and the other binds to the primary prism plane.[20]

This choice of the protein was motivated by two main reasons. First of all, we previously investigated a hyperactive *Cf* AFP, therefore now our choice was a protein of lower activity, for comparison purposes. Secondly, for the protein in question a series of mutants of lowered activity was prepared and analyzed.[7,19,21] The significance of specific amino acids for the biological activity of the protein was investigated by introducing various mutations. The structures of the mutants were determined, and their antifreeze activities (the widths of the hysteresis gaps) were measured. Using these data, we wanted to investigate whether the activity can be somehow correlated with the changes of the structure of solvation water. Therefore, except from the wild type, we also investigated the solvation of three mutants with different activities. The first one, T15V, maintains 54% of the wild type activity.[21] The amino acid number 15 is changed from threonine to valine. The second one, T18N, maintains only 10% of the wild type activity.[21] The amino acid number 18 is changed from threonine to asparagine. The third one, N14SQ44T, also maintains 10% of the wild type activity.[21] The amino acid number 14 is changed from asparagine to serine, and the amino acid number 44 is changed from glutamine to threonine. Figure 1(a) shows the locations of the mutations in the protein molecule.

METHODS

Simulation procedure

The results were obtained using computer simulations. They were carried out with the molecular dynamics package Amber12[22] and ff12SB force field suitable for proteins.[23] A preliminary equilibration of each investigated system was performed in *NpT* conditions and lasted about 2.0 ns. The temperature (300 K) was kept constant by the weak coupling to an external bath (τ_T = 1.0 ps) using the Berendsen thermostat.[24] The pressure (1 bar) was kept constant by the weak coupling method (τ_p = 1.0 ps). The particle-mesh Ewald method was used for electrostatic interactions, and the lengths of chemical bonds involving hydrogen atoms were fixed using SHAKE. A cutoff of 1.2 nm for nonbonding interactions was used. After the equilibration, the starting coordinates for the final simulations at each temperature (from interval 260 to 300 K) were obtained from a two-step procedure.

During the first step, we performed 10 subsequent simulations in *NpT* conditions, each of them was 500 ps long. The starting coordinates were the final coordinates from the preliminary equilibration period. The initial velocities were generated from the Maxwell distribution, independently for each run. Then, final coordinates from these simulations were used as starting points to the second step. Each configuration file from the step one originated 10 new simulations, and the initial velocities were again generated from the Maxwell distribution. Therefore, after the second step, we got 100 starting conformations for 100 independent simulation runs in *NVE* conditions. The *NVE* simulations were 1.38 ns long, but the length of the analyzed trajectory was equal to 1.2 ns—the first 180 ps was treated as an equilibration period (transient period between *NpT* and *NVE* conditions) and therefore was excluded from the analysis. Trajectories were saved every 10 fs. For each 1.2-ns-long trajectory, the values of the parameters describing the properties of water were calculated. The results presented in the article are means obtained from all of these trajectories. The summed length of the analyzed trajectory was equal to 120 ns for each system.

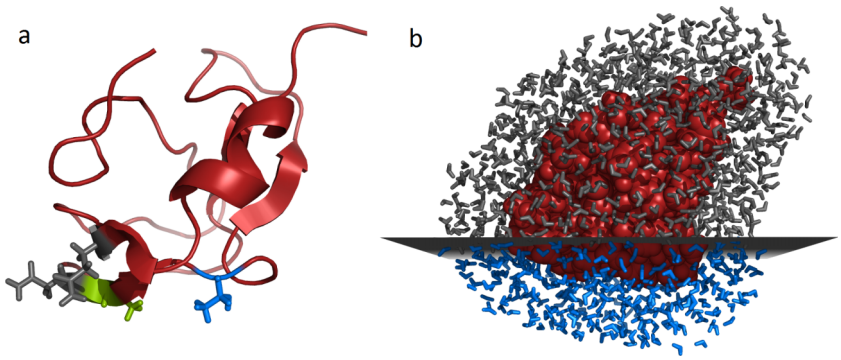

FIG. 1. (a) The molecule of a type III AFP from *Macrozoarces americanus* (wild type, in red). The changed residues are presented as sticks. The mutations are T15V (in green), T18N (in blue), and N14SQ44T (in grey). All of the mutants have very similar structure. The rmsd (root mean square deviation of atom positions) between the wild type and the mutants T15V, T18N, N14SQ44T is equal to about 1.50 Å, 1.51 Å, 1.57 Å, respectively (all structures immersed in TIP4P/Ice water and equilibrated at 260 K). (b) Definition of the solvation shells around the active and inactive regions on the protein surface. The plane that divides the solvation shell was defined using the positions of three alpha carbon atoms of the residues: LEU 10, LEU 19, and SER 42. The water molecules within the 1 nm distance from the protein are presented as sticks. The active side is the bottom side.

We used the TIP4P/Ice water model, this choice is discussed and justified below. Our investigations covered the temperature range from 260 to 300 K, with a 10 K interval between isotherms.

Selection of water model

The chosen water model was TIP4P/Ice.[25] It has got a freezing temperature that is very close to the experimental value, 272 K. The argument of the similarity of the freezing temperature was also used, for example, by Nutt and Smith,[15] to justify the choice of the model of water (in their case—TIP5P). The authors of the TIP4P/Ice water model convincingly argue that their model "greatly improves the melting properties of previous potentials. But, contrary to the case of TIP5P, the improvement in the melting properties is done without deteriorating the other computed quantities."[25] The TIP4P/Ice model had already been successfully used in simulations of interactions between an AFP molecule and ice.[26]

Systems setup

Five different systems had to be constructed, as follows:

1. system consisting of the wild type AFP molecule (Protein Data Bank (PDB) code: 1MSI) immersed in water.
2. system consisting of T15V mutant molecule (PDB code: 1MSJ) immersed in water.
3. system consisting of T18N mutant molecule (PDB code: 9MSI) immersed in water.
4. system consisting of N14SQ44T mutant molecule (PDB code: 8MSI) immersed in water.

   Each of the systems contained more than 20 000 water molecules.
5. system consisting solely of liquid water (12 665 molecules).

Definition of solvation layers

Our aim was to analyze the properties of water contained within 1 nm thick solvation shell. It is comprised of water molecules whose distance from the protein surface does not exceed 1.0 nm. This way of defining the solvation shell was used by us previously.[27–29] The active surface is flat, which allowed us to divide the solvation shell of the protein into two parts (active and inactive) using a plane, which is illustrated in Figure 1(b).

The parameters describing the structure of water

Short-ranged radial ordering

The parameters used to describe radial correlations were radial distribution function, $g_{OO}(r)$, and local structure index (LSI).[30,31]

The oxygen-oxygen radial distribution function was calculated according to the well-known relation (N and V symbolize number of molecules and volume, respectively)

$$g(r) = \frac{V}{N}\frac{dN}{dV} = \frac{1}{4\pi r^2 \rho_s}\frac{dN}{dr}, \tag{1}$$

and the results were averaged over all water molecules enclosed in the solvation layer (up to 1 nm from protein surface).

LSI is a measure of heterogeneity of radial distribution of water molecules within short distances (up to about 0.37 nm). It was defined by Shiratani and Sasai as follows. For each water molecule i, the rest of the water molecules is ordered depending on the radial distance r_j between the oxygen atom of the molecule i and the oxygen atom of molecule j: $r_1 < r_2 < \cdots < r_j < \cdots < r_n < r_{n+1}$, while n is chosen so that $r_n < 0.37$ nm $< r_{n+1}$. Then, LSI is defined as[31]

$$\mathrm{LSI} = \frac{1}{n}\sum_{J=1}^{n}\left[\Delta(j) - \overline{\Delta}\right]^2, \tag{2}$$

where $\Delta(j) = r_{j+1} - r_j$, and $\overline{\Delta}$ is the average value of $\Delta(j)$ (over all molecules). The distance 0.37 nm was used by the authors of this definition in order to include in the calculations some water molecules adjacent to the first solvation shell.

As we described previously,[17] LSI is helpful in characterizing the structure of water because its values depend on the degree of uniformity of the radial distribution of water molecules around a central one. Let us assume that we move from the center of some random water molecule and encounter four hydrogen-bonded and tetrahedrally arranged water molecules. The second layer of water molecules is hydrogen-bonded with the first layer and because of that it appears after a gap. Therefore, up to the limit of the first solvation shell, we find water molecules at very similar distances from the central one. But the distance to the first molecule in the second solvation shell is significantly different. This would have an impact on the value of LSI, since the mean distance between aligned molecules would be quite different from each distance on its own. On the other hand, if the molecules were arranged in such a way that they would be more uniformly distributed, and there would not be any gap after the first solvation shell, then we would not have one value that is significantly different from the others, and the mean value would be similar to each distance on its own. To sum up, a small value of LSI is an indicator of a more uniform radial distribution of surrounding molecules and, probably, more crowding at the border between the first and the second solvation shell of a molecule of water. A large value of LSI is an indicator of a non-uniform radial distribution and a gap between the first solvation shell and the second solvation shell.

Short-ranged angular ordering

In order to analyze the angular distribution of water molecules, we used the previously adapted[28] the probability distribution of angle α between vectors connecting the central water molecule with water molecules from its first solvation layer. This parameter measures the heterogeneity of angular distribution and can also depend on the crowding. It also takes into consideration the nearest neighborhood only (molecules present within the radius equal to 0.33 nm). As we discussed previously,[17] the characteristic features of the probability distribution of angle α are three extrema localized at angles

α equal to about 58°, 71°, and 109°. Neighboring water molecules arrange themselves around the central molecule roughly in a tetrahedral manner[32] (angles α would be then equal to about 109°). However, when the first hydration shell becomes more crowded, excess water molecules place themselves between the corners of a tetrahedron or above the middle of the faces, which can disturb the geometry of the original structure. These situations can be detected by examining the differential distribution of angle α (increase in the values of probability distribution of angle α around 58° and 71°, respectively[28,33]). Inversely, less extensive crowding of the solvation shell should be connected to a decrease in these values.

Long-ranged (0.58 nm) radial and angular ordering (local ordering parameter)

The calculations of the local ordering parameter of water were described by us in details in our previous papers.[17,34] Therefore, herein we present only a general draft. The local ordering parameter $s(R_c)$ was defined as the following integral of the two-particle correlation function:

$$s(R_c) = -k_B \frac{\rho W}{16\pi^2} \int_{r=0}^{r=R_c} \int_{\vec{\omega}} \left\{ g^{(2)}(r,\vec{\omega}) \ln\left[g^{(2)}(r,\vec{\omega}) \right] - g^{(2)}(r,\vec{\omega}) + 1 \right\} r^2 dr d\vec{\omega}, \quad (3)$$

where r represents distance between two water molecules, while $\vec{\omega}$ represents five angles describing their relative orientation. Parameter ρ_W represents the number density of bulk water, k_B is the Boltzmann constant, and R_c is the distance corresponding to the limit of the second hydration shell around a water molecule (equal to 0.58 nm[34,35]). The available data suggest that the range of the structural changes in solvation water of macromolecules does not exceed the range of the second solvation shell of a water molecule.[36–39] Moreover, following the idea of Lazaridis and Karplus,[40] we also decomposed this quantity into three parts, translational, configurational, and orientational, called s_{tra}, s_{con}, and s_{orient}. In this work we used $s_{ort} = s_{con} + s_{orient}$ as the overall measure of total orientational effects, as it was described previously.[27,34]

The "reference state" for structural parameters describing the properties of water within solvation shell

In order to detect small changes in the structure of solvation water, two series of simulations were performed. The first series was the standard simulation of the protein in water. The second one was the simulation of water alone, under identical pressure and at the same temperature. During the analysis, the (time-dependent) coordinates of the protein atoms from the first simulation were inserted into the box with bulk water. After the insertion, water molecules from the region occupied by the protein molecule were removed. As expected, we encountered the problem of choosing the proper cut-off value, r_{cut}, which is a minimum acceptable distance of any water molecule from the protein. This problem has been carefully examined previously (see supplementary material attached to our previous papers, Refs. 27 and 28). We concluded that the r_{cut} value does not influence the outcome of analysis as long as it is within a reasonable range (we tested the r_{cut} values in the range (0.15–0.19) nm). Thus, in this work we used r_{cut} = 0.17 nm, which is the same value as the one used in our previous publications.

As a result of this procedure, we obtain a protein molecule surrounded by water with the structural properties that correspond (by definition) to the properties of bulk water. Further treatment is the same as in the case of the standard system, i.e., after selection of water molecules belonging to fictitious solvation shells, the appropriate structural parameters of this solvation water are determined. These parameters describe the properties of water with bulk-like structure. Therefore, we obtain the "reference state" for further interpretation of properties of water enclosed within the real solvation shells. This procedure allows us to circumvent the problem of the space volume inaccessible to water molecules due to the presence of the protein (excluded volume). As a result, we obtain values $\Delta(X) = (X)_{solv} - (X)_{bulk}$, where X is a calculated parameter, and $\Delta(X)$ is the difference between the value of the parameter calculated for the real solvation shell and fictitious solvation shell filled with bulk water.

RESULTS

To investigate the changes in the structure of solvation water, we implemented some methods used by us previously to investigate the structure of solvation water of a hyperactive *Cf* AFP,[17] and outlined in the section titled Methods. The parameters measured short-ranged and long-ranged (in the range of the first and second hydration shell) radial and angular changes in the structure of water. The changes were measured as a function of temperature, separately for the solvation water of the active and inactive surfaces of the protein.

Short-ranged radial correlations and angular ordering of water molecules

The parameters used to describe radial correlations were radial distribution function, $g_{OO}(r)$, and local structure index (LSI).[30,31] In order to analyze the angular distribution of water molecules, we used the probability distribution of angle α.[28] These parameters allowed us to draw conclusions on the changes in density and tetrahedral ordering of water molecules.[17] In Figure 2 (wild type AFP and mutants T15V, T18N, N14SQ44T) we present the differential distributions (comparing to the bulk water, as described in the section titled Methods) of parameters describing the properties of solvation water. There are two main conclusions that can be drawn from these figures. First of all, there are visible differences between the properties of solvation water of the active and inactive surface of the protein. The general picture is in fact very similar to what was observed by us previously for the hyperactive *Cf* AFP.[17] The differential distributions of the LSI and the angle α, according to the argumentation presented in the section titled Methods, can be interpreted as

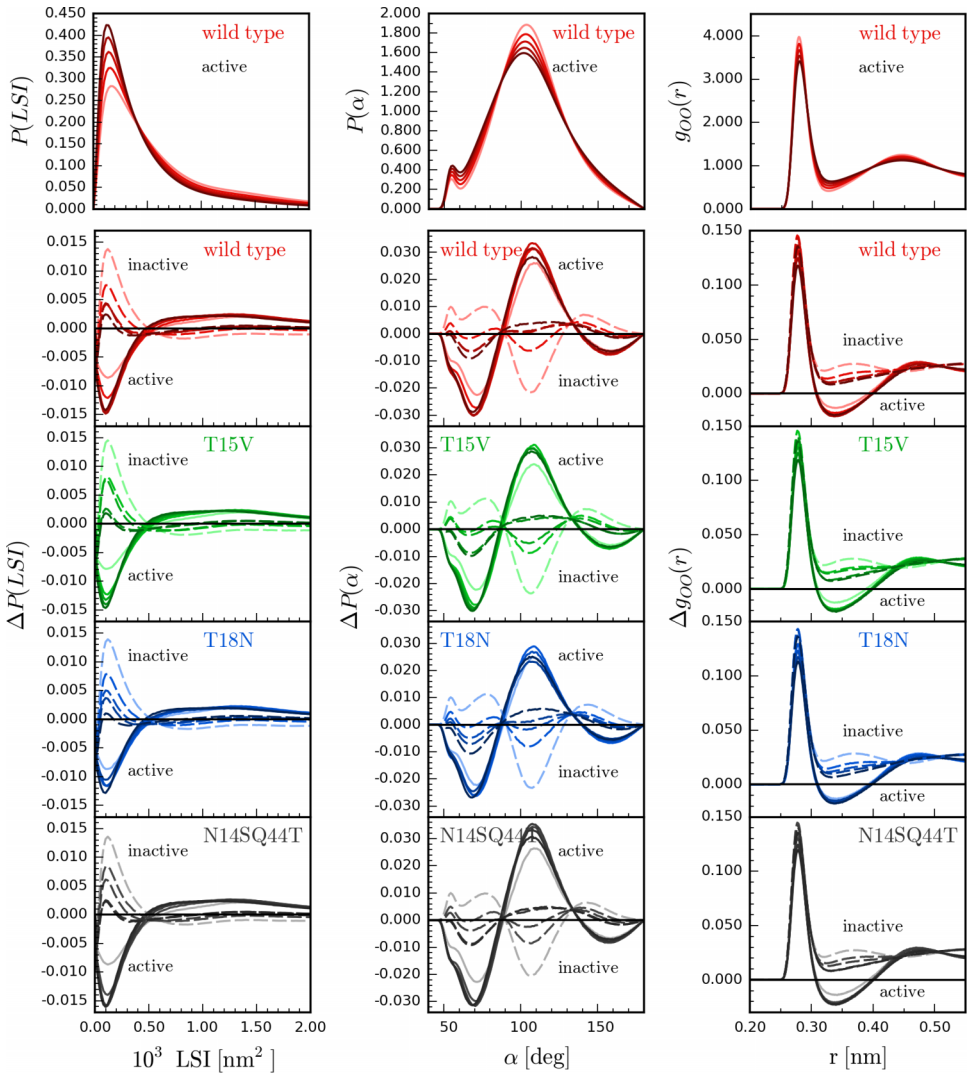

FIG. 2. The differential probability distributions ΔP(LSI), $\Delta P(\alpha)$, and Δg_{OO}(r) of the LSI, angle α, and radial distribution function (see text) for water in the solvation shells of the two surfaces (active—solid line and inactive—dashed line) of the type III AFP from *Macrozoarces americanus* and its three mutants, N14SQ44T, T15V, and T18N, determined at temperatures from the range 260 K–300 K. The data for the lowest temperature are presented as the lightest lines, and the data for the highest temperature are presented as the darkest lines. The top row depicts the original distributions P(LSI), $P(\alpha)$, and g_{OO}(r) for the solvation water of the active surface of the wild type.

a sign of an enhanced ordering of water around the active plane: better separation of the first and second solvation shell accompanied with a more tetrahedral arrangement. The conclusion regarding the increased gap between the first and second solvation shell can be supported by the analysis of the differential radial distribution function that displays a minimum above 0.30 nm. Moreover, the differential plots ΔP(LSI), $\Delta P(\alpha)$, and Δg_{OO}(r) (of the LSI, angle α, and radial distribution function) indicate that the solvation water of the active surface generally responds less profoundly to the temperature changes than the solvation water of the inactive part of the protein. We suspect that it also might be connected with the higher ordering of the solvation water of the active site than the inactive site. The active surface promotes a specific ordering of water molecules and because of that it reduces the influence of the temperature changes on the structure of the adjacent water. The solvent-ordering capabilities of the inactive surface are less manifested. This might explain why the structure of its hydration water changes more with temperature.

On the other hand, the same parameters indicate to the increased crowding at the border between the first and second solvation shell of a water molecule in case of the inactive surface. When it comes to the density of water, the analysis of the radial distribution function indicates that the mean density of water is increased around the whole protein (see included supplementary material for the values of the integrals from the differential radial distribution function).

Secondly, the differences between the properties of solvation water of the three mutants are practically invisible. Therefore, the change of one (T15V, T18N) or two (N14SQ44T) amino acids in the active surface does not significantly influence the mean properties of solvation water, although it significantly decreases the antifreeze activity to 54% (T15V) or 10% (T18N, N14SQ44T).

Analysis of the full form of the two-particle correlation function: A local ordering parameter

Above, we analyzed parameters (LSI, angles α) that are able to measure only local structure of liquid (in immediate vicinity of a given water molecule). The analysis of the long-range structural ordering was performed using the concept of two-particle correlation functions, depending on the distance and mutual orientation of the two molecules. Thanks to that the structural changes can be characterized more thoroughly. In our previous papers,[17,34] we described in details the translational, s_{tra}, configurational, s_{con}, and orientational,

s_{orient}, ordering parameters. They are also briefly characterized in the section titled Methods. The values of these parameters depend on how molecules of water are distributed and oriented around each other, therefore even subtle structural changes in solvation water of proteins are expected to have an impact on their values.[27] These parameters were used to construct ordering maps, following some concepts from the papers by Truskett *et al.*[41] and Esposito *et al.*[42] Herein, we would like to construct the ordering maps in a modified form, as it was done previously for *Cf* AFP.[17] In our modified ordering maps we use Δs_{tra} and $\Delta s_{ort} = \Delta s_{con} + \Delta s_{orient}$ instead of s_{tra} and s_{ort} to eliminate the influence of excluded volume, which is inaccessible to water molecules because of the presence of the protein. Thanks to that the location of points on the modified map reflects changes in internal water structure (comparing to properties of bulk water). It is a sensitive, convenient, and illustrative way to visually present changes in the properties of solvation water of the investigated regions on the protein surface. The relations $\Delta s_{tra} = f(\Delta s_{ort})$ are depicted in Figure 3 for the wild type and the three mutants under investigation: T15V, T18N, and N14SQ44T.

The conclusions that can be drawn from Figure 3 are more far ranged than those from Figure 2. First of all, we can see a significant similarity of the properties of solvation water surrounding the three mutants. Moreover, this map is also quite similar to the map obtained previously[17] for the hyperactive *Cf* AFP molecule. This statement applies to the general pattern of the placement of the points on the map—points for active and inactive surfaces are grouped separately. It also applies to the way that the changes of temperature influence the values of Δs_{tra} and Δs_{ort} for the active and the inactive surfaces—the response of the solvation water of these two sites is opposite. The solvation water of the inactive site seems to follow the pattern characteristic for "ordinary" proteins—it is said that proteins impose a pseudoglassy character on their hydration spheres.[43] When the temperature drops, the absolute values of the parameters Δs_{tra} and Δs_{ort} diminish. It means that the degree of disorder resembles more and more the degree of disorder in bulk water at the same temperature. This resemblance is the highest for the supercooled fluid. However, this pseudoglassy state does not have to have the same structure as the bulk water. From the analysis of the data in Figure 2 and in the supplementary material, we remember that there is a density increase in the solvation water, and the difference in the density increases in lower temperatures. This increase influences the values of LSI, α, and $g_{OO}(r)$ and the shape of the differential histograms.

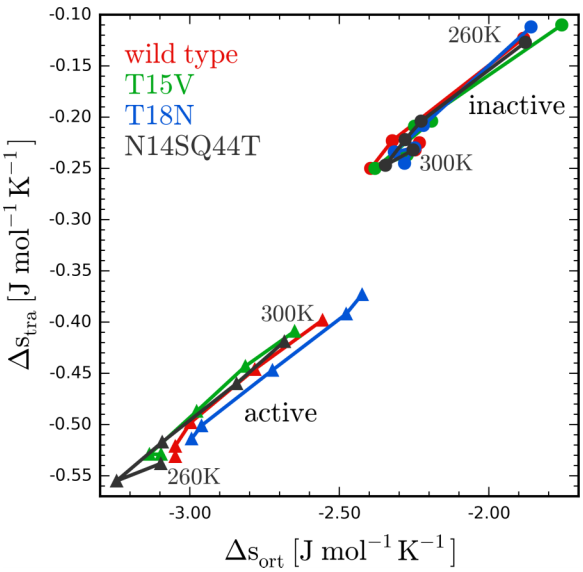

FIG. 3. Ordering map—temperature dependences of the relation $\Delta s_{tra} = f(\Delta s_{ort})$—determined for solvation water around two surfaces (active and inactive) of the type III AFP from *Macrozoarces americanus* and its three mutants: T15V, T18N, and N14SQ44T. The values of the parameters were determined at temperatures from the range 260 K–300 K.

In case of the solvation water of the active surface, the lowering of the temperature causes the degree of the ordering to increase relatively to the bulk water (the absolute values of the parameters Δs_{tra} and Δs_{ort} increase). As we demonstrated previously,[17] the same direction of the temperature changes can be observed for solvation water of ice. This occurs in spite of the fact that the density of solvation water of ice is lower than bulk, and the density of solvation water of the active site of the protein is, on average, a little higher than bulk.

These observations allow us to draw a conclusion that the modification of the structure of water is a necessary prerequisite for the protein to perform its function. The modification, generally, brings the properties of solvation water of the active plane and solvation water of ice closer together, although the solvation water of the active surface still significantly differs from the solvation water of ice. The main difference is the significantly lowered density of solvation water of ice,[17] while in the case of solvation water of the active surface of the protein we do not observe a density decrease.

However, this modification of water properties is not sufficient for the protein to perform its function. Once the properties of water are suitably adjusted, the geometrical match to the ice is required for the efficient adsorption.

CONCLUDING REMARKS

Our present results, combined with the results obtained previously,[17] strongly suggest that the proper modification of the properties of water solvating the active region of the AFP molecule is a necessary prerequisite for the protein to function properly. The modification of the properties of solvation water makes it partly similar to the solvation water of ice, what was also observed before.[17] However, it needs to be stressed that the properties of water are still different from the properties of solvation water of ice, what was also discussed previously.[17]

Simultaneously, we do not observe the correlation between the extent of the modification of the structure of water and the antifreeze activity. The modification of the properties of water is most probably a necessary prerequisite but is not sufficient for the proper functioning of the protein. Even small modification of the amino acid composition of the ice-binding surface can lead to a dramatic decrease of

antifreeze activity, while keeping the mean properties of solvation water relatively unchanged.

These observations can be connected with the mechanism of the process of adsorption of AFP molecule to ice that postulates the solidification of water present between an AFP molecule and the ice surface.[15,44] It results with the freezing of the AFP onto the surface of ice together with part of its solvation shell. It seems that this process leads to the irreversible adsorption of the protein.[11–14] For this model to work most efficiently, it would be highly desirable for the solvation water of the protein to be structured in a way that could facilitate its freezing once it is merged with the solvation water of ice. When the freezing is finished, the strong binding of the AFP requires the surface of the protein to be properly configured to geometrically match to the lattice of ice. This matching can be perturbed by changing one amino acid (threonine) into another (serine, valine).[21] It leads to reducing the strength of interactions between the ice and the protein. As a result of impaired bonding, the antifreeze activity diminishes.

To sum up, we believe that the specific structure and amino acid composition of the ice-binding surface evolved to obtain two independent goals: to induce the modification of the properties of water and to geometrically fit to the lattice of ice. The properties of water are still modified in the right direction (i.e., similarly to the solvation water of the wild type) in case of AFPs with significantly reduced activity. Therefore, this factor is probably essential but not sufficient for the protein to work properly.

SUPPLEMENTARY MATERIAL

See supplementary material for further information on the calculations of local density of water within the solvation layer.

ACKNOWLEDGMENTS

The calculations were carried out at the Academic Computer Center (TASK) in Gdańsk. This research was supported in part by PL-Grid Infrastructure.

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