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Protein Structure and Dynamics in Ionic Liquids. Insights from Molecular Dynamics Simulation Studies

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We present in this work the first molecular simulation study of an enzyme, the serine protease cutinase from *Fusarium solani* pisi, in two ionic liquids (ILs): 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-butyl-3-methylimidazolium nitrate ([BMIM][NO_3]). We tested different water contents in these ILs at room temperature (298 K) and high temperature (343 K), and we observe that the enzyme structure is highly dependent on the amount of water present in the IL media. We show that the enzyme is preferentially stabilized in [BMIM][PF_6] at 5−10% (w/w) (weight of water over protein) water content at room temperature. [BMIM][PF_6] renders a more nativelike enzyme structure at the same water content of 5−10% (w/w) as previously found for hexane, and the system displays a similar bell-shape-like dependence with the water content in the IL media. [BMIM][PF_6] is shown to increase significantly the protein thermostability at high temperatures, especially at low hydration. Our analysis indicates that the enzyme is less stabilized in [BMIM][NO_3] relative to [BMIM][PF_6] at both temperatures, most likely due to the strong affinity of the $[NO_3]^-$ anion toward the protein main chain. These findings are in accordance with the experimental knowledge for these two ionic liquids. We also show that these ILs "strip off" most of the water from the enzyme surface in a degree similar to that found for polar organic solvents such as acetonitrile, and that the remaining waters at the enzyme surface are organized in many small clusters.

1. Introduction

Ionic liquids (ILs) are organic salts that are liquid at room temperature and are composed solely of an organic cation and an organic or inorganic anion. Through the combination of different cation and anion species, it has been possible to build a considerable collection of ILs that are being synthesized and characterized.[1] They stand today as potential replacements for volatile organic compounds employed in bio/chemical processes since they have negligible vapor pressure, are nonflammable, and are chemically and thermally stable.

Since 2000 there has been a comprehensive study of their application as solvents for proteins,[2] particularly on enzyme-catalyzed reactions[3−5] (see Moon et al. for a recent review[6]). Enzymes have long been shown to be active in other media than water, namely organic solvents, supercritical fluids, gases, and now, also ILs. Many enzymes have shown enhanced stability and activity in organic solvents. Research on this field has matured to a point of importance and applicability that are well recognized.[7] The application of ILs as solvents of proteins poses questions similar to those in the past when organic solvents were first employed. Of importance to our study, we point to some experimental facts showing that ILs and organic solvents have some relevant features in common. As in conventional organic solvents, it has been shown that some enzymes traditionally employed in nonaqueous enzymology, such as lipases and proteases, are active in some ILs and inactive in other ILs. ILs containing anions such as $[BF_4]^-$, $[PF_6]^-$, and $[Tf_2N]^-$ render active enzymes while $[NO_3]^-$, $[CF_3CO_2]^-$, and $[CF_3SO_3]^-$ inactivate enzymes,[6] suggesting that enzyme stability is mainly dependent on the type of the anion species.[8] ILs, in some cases, also provide an increased structural stabilization against thermal denaturation relative to the aqueous or organic solvent media.[9−11] Ulbert et al. has reported that *Candida rugosa* lipase in [BMIM][PF_6] was catalytic active with an half-life time of 10.2 h at 50 °C, while with the use of conventional organic solvents, namely hexane, dibutyl ether, or benzene, half-lives were on the order of 1.5−2.0 h.[11] The same report also points to higher enantioselectivity when using ILs, namely [BMIM][PF_6]. Furthermore, a point of significant importance is the fact that it was observed in some cases that enzyme activity or enantioselectivity is maximized at a specific hydration level.[11−16] This type of enzyme behavior was previously observed on several enzymes in conventional organic solvents[17−19] and also by our molecular modeling studies.[20,21] A bell-shape profile related to the effect of water activity and solvents on the catalytic activity and enantioselectivity of *Candida rugosa* lipase in ILs and organic solvents was reported by Ulbert et al.[11,15] This stresses the fact that, as in conventional organic solvents, enzyme hydration seems to have a fundamental role in the structure and function of enzymes in ILs. It is clear that, in this respect, it is important to discern

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how water is partitioned between the enzyme surface and the bulk IL solution. Spectroscopic techniques have also been used to study the structural properties and activities of enzymes in ILs.[9,22,23] It is becoming clear from these studies that some ILs have an exceptional ability of stabilizing enzymes as evidenced by the increase of melting temperature and heat capacity relative to other conventional media. Greater compactness of the enzyme structure and great enhancement of β-strands due to the presence of the ILs have also been shown.

This work follows our preceding research on the study of structure and function of enzymes in nonaqueous solvents[20,21,24,25] where we investigated enzymes in different organic solvents with different hydration conditions using molecular modeling methodologies. We recently developed a parametrization of two model ILs, 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-butyl-3-methylimidazolium nitrate ([BMIM][NO_3]), in the framework of the biomolecular GROMOS force field.[26] In this paper we are presenting the first application of these parametrized ILs and the first simulation of an enzyme in ILs. Our objective is to provide a molecular description of the structure and dynamics of a model enzyme named cutinase, a serine protease from *Fusarium solani* pisi, in two ILs, [BMIM][PF_6] and [BMIM][NO_3], at 298 and 343 K, with different hydration levels.

2. Materials and Methods

2.1. System Setup. We performed several simulations of molecular systems composed of one model enzyme hydrated with increasing amounts of water and solvated in two ionic liquids at two different temperatures. This approach is similar to our previous studies using the same enzyme in organic solvent with increasing amounts of water.[20,21] The 1.0 Å cutinase enzyme structure of Longhi was used,[27] and the protonated state of charged residues was estimated using a methodology previously described.[28] The selection of different amounts of water hydrating the enzyme was done as explained in detail elsewhere[20] for 11 different water percentages (w/w, weight of water over weight of protein)—0%, 2.5%, 5%, 7.5%, 10%, 15%, 25%, 35%, 50%, 60%, and 75%—and five replicate systems. In the system at 0% water 15 water molecules were kept. These water molecules are internalized inside the enzyme, and they were kept not only because they have an important structural role but also the experimental extensive drying of enzymes has shown the inability of removing this type of internal waters.[29] Each system was placed in a cubic box with a size of $6.5 \times 6.5 \times 6.5$ nm full of an equilibrated solution of the ionic liquids [BMIM][PF_6] and [BMIM][NO_3].[26] Five replicate systems of the enzyme in a full hydrated system were also simulated in a cubic box of the same size. The distance between the protein and box wall exceeds half the cutoff employed in the simulation, and the system was ensured to be neutral.

2.2. Simulation Details. Molecular dynamics/molecular mechanics simulations were performed with the GROMACS 3.1.4 package[30] using the GROMOS96 43A1 force field.[31,32] The SPC model of water was used.[33] Force field parameters for the [BMIM][PF_6] and [BMIM][NO_3] ionic liquids were taken from our previous parametrization work.[26] From our previous simulations[26] and experimental data[34] employing this type of ionic liquids, it has been shown that they have dynamic processes with relaxation times in the subnanosecond time scale at room temperature. A simple approach to improve the sampling of the configurational space can be attained by scaling down the mass of all heavy atoms in the system, making the atomic masses of heavy atoms and hydrogens more equal. We scaleddown by 1/10 the mass of all the heavy atoms. This type of procedure allows the redistribution of the total mass of the molecular system, reducing the range of the motional frequencies observed. The dynamic properties of the system are obviously affected, but the equilibrium thermodynamic properties of the system are not affected.[35,36] With this approach we significantly increase the mean square displacement of the solvent and solute atoms; as an example, the anions with normal and scaled atom masses display mean square displacements of 0.005×10^{-5} and 0.013×10^{-5} cm^2/s, respectively. The bond lengths of the protein and ionic liquids molecules were constrained with LINCS[37] and the ones of water with SETTLE.[38] The system was simulated with periodic boundary conditions. Nonbonded interactions were calculated explicitly up to 9 Å, and long-range electrostatic interactions were treated with PME[39,40] with a grid spacing of 1.2 Å and a fourth-order order interpolation. Neighbor searching was done up to 9 Å and updated every five steps. A 2 fs time step of integration was used.

For the aqueous and ionic liquid systems, the initial configurations were energy minimized (EM) for 5000 steps using the steepest descent method with all protein heavy atoms and waters harmonically restrained with a force constant of 10^6 kJ mol^{-1} nm^{-2}, followed by 5000 steps of energy minimization with positional restrains applied to the Cα protein atoms, and finally 5000 steps of unrestrained system energy minimization. The aqueous and ionic liquid systems were simulated at two temperatures, 298 and 343 K. The systems were initialized in the canonical ensemble during 500 ps (100 ps for the aqueous systems) with all protein heavy atoms harmonically restrained with a force constant of 10^6 kJ mol^{-1} nm^{-2}, followed by 100 ps with positional restraints applied to the Cα protein, and finally 100 ps with no restraints applied. The equilibration phase was continued for 20 ns (6 ns for the aqueous systems) in the isothermal−isobaric ensemble, in order to ensure the full equilibration of all system properties. The pressure control was implemented using the Berendsen barostat[41] with a reference pressure of 1 atm, 1.5 ps (0.5 ps for the aqueous systems) relaxation time, and isothermal compressibility of 4.23×10^{-5} bar^{-1} for the ionic liquid systems[42] and 4.6×10^{-5} bar^{-1} for the aqueous systems. Temperature control was set using the Berendsen thermostat.[41] The protein, waters, and ionic liquid were separated in individual heat baths with temperature coupling constants of 0.01 ps in the initialization steps and 0.1 ps in the equilibration phase. Five replicate simulations of each system at 300 and 343 K were done by the assignment of different initial velocities taken from a Maxwell−Boltzmann distribution. Properties were calculated on the last 10 ns (3 ns in the aqueous systems) simulation of each run. When error bars are presented on figures, they are estimated from the standard error of the five replicates.

3. Results and Discussion

We focus our attention on three main observed facts reported in the literature, which are the following: (1) Some ILs stabilize enzymes at room and high temperatures, whereas other do not.[9−11,43−46] (2) In the same way as happens for some enzymes in some organic solvents, a certain amount of water in the IL media is necessary to maximize enzymatic activity.[11,14,16] (3) ILs and organic solvents share some common features in the way they modulate the enzyme structure and how they partition water between bulk IL phase and the enzyme surface.[25] Our aim is to understand these experimental observations at the molecular level.

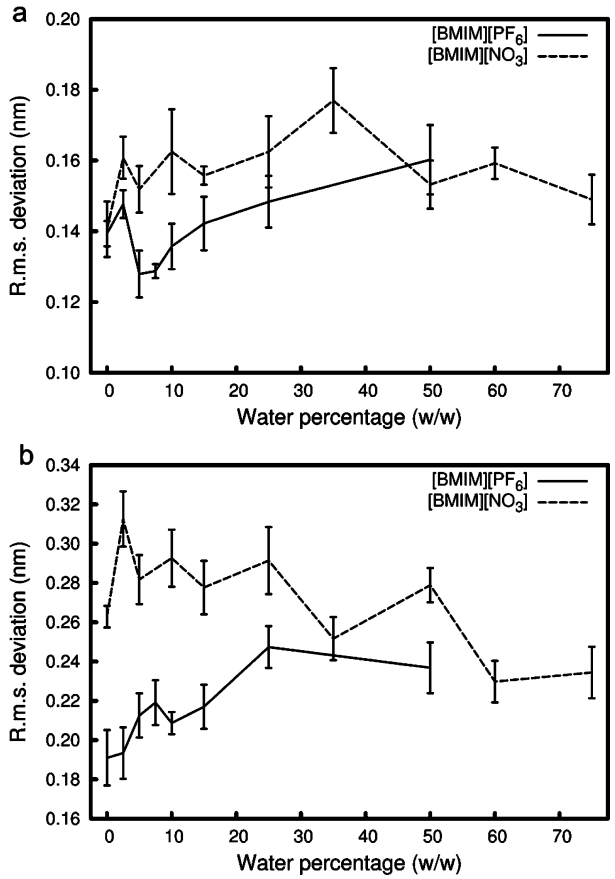

Figure 1. Average rmsd of the Cα atoms of cutinase from the X-ray structure in [BMIM][PF_6] and [BMIM][NO_3] at (a) 298 and (b) 343 K.

3.1. Structural Stabilization and Destabilization. The root-mean-square atomic positional deviation (rmsd) relative to the X-ray structure of the enzyme in the two ILs with increasing water content at 298 and 343 K is shown in Figure 1. At room temperature the rmsd's of the enzyme in both ILs at all hydration conditions are within the range or even lower than the rmsd value of 0.16 nm observed for the enzyme in the pure aqueous medium. The rmsd comparison of the two ILs at room temperature and different water percentages (Figure 1a) shows that the [BMIM][PF_6] medium is able to render an average enzyme structure closer to the X-ray than in the case of the [BMIM][NO_3], especially at low water percentages. The only exception for this behavior is at 0% water, where the rmsd in [BMIN][NO_3] is as low as in [BMIM][PF_6], suggesting that the protein is equally stable in both ILs. One possible explanation for this may be that, the protein is kinetically trapped in both ILs due to the absence of water. However this very low hydration condition might not be experimentally within reach, since, except in very controlled conditions, these ILs cannot be dried completely and also the complete dehydration of enzymes is not possible, as said before. At a higher temperature (Figure 1b), the difference in rmsd between the case where the enzyme is solvated by [BMIM][PF_6] and [BMIM][NO_3] is even more marked at low hydration conditions. Both plots show that the two ILs generate two distinct behaviors. The IL [BMIM][PF_6] clearly renders enzyme conformations similar to the reference X-ray structure, in particular when the system is simulated at 298 K and low hydration conditions (5−10%).

The use of [BMIM][NO_3] clearly leads the enzyme to higher rmsd's relative to what is observed with [BMIM][PF_6], demonstrating its poor enzyme-stabilizing properties. The enzyme rmsd in this IL is even higher than the 0.24 nm rmsd value observed in the pure aqueous conditions, demonstrating its destabilization properties, as described later. The two ILs share the same cation molecule and differ in the anion species, $[PF_6]^-$ in one case and $[NO_3]^-$ in the other. It has been suggested, and our results also seem to point in that direction, that the anion has a primary role in the stabilization/destabilization of the enzyme, while the cation plays a secondary role. In this particular case it has been also observed experimentally that enzymes are usually active in ILs containing the $[PF_6]^-$ anion, whereas ILs containing the $[NO_3]^-$ anion were responsible for loss of enzyme activity.[2,6,8,23,44,47,48]

3.2. Dependence on Hydration Conditions. What these results also reveal is that the amount of water present in the IL media has a clear effect on the average rmsd of the enzyme from the X-ray structure. Both plots of Figure 1 show that the structural properties of the enzyme when solvated by these two ILs are dependent on the amount of water present in the media. It is observed that in the case of [BMIM][PF_6] at room temperature there is an optimal water percentage (in the range of 5−10% content) that makes possible the existence of an enzyme with a very low rmsd. Below and above this optimal water range, the enzyme shows an increase in rmsd related to the absence or excess of water, respectively, resembling a bell-shape curve. The complete absence of water at the enzyme surface seems to impair the enzyme from reaching a more nativelike structure as seen with the 5−10% water percentage; one possible explanation for this is to consider the system to be kinetically trapped at 0% water content. The small addition of 5−10% water in [BMIM][PF_6] seems to be a necessary condition to permit the enzyme to reach a more nativelike structure. This bell-shape behavior due to the presence of different water percentages is a feature well-known from nonaqueous enzymology in conventional organic solvents[18−21,25,49−51] and also in room-temperature ionic liquids.[11,14,16] The effect of the increasing amounts of water in the media seems to perturb some localized regions of the enzyme as shown next. In Figure 2a we show the individual rmsd of each residue for two simulations in [BMIM][PF_6], one at 5% water content (with the lowest rmsd) and one at 50% water content (with the highest rmsd).

It is possible to see that the region that undergoes the highest displacement relative to the enzyme X-ray structure is an α-helix comprised by residues 63−70. Other significant structural displacements are found involving residues from loop regions involving residues 139−145.

In the case of [BMIM][NO_3] at room temperature, we do not observe the same structural rmsd trend related to the amount of water present (Figure 1a). It seems that 35% water percentage has a maximum detrimental effect on the enzyme structure. This is significantly higher than the 0.16 nm rmsd that is obtained in pure aqueous medium. At higher and lower water percentages this negative effect seems to be reduced, and the rmsd observed is similar to the aqueous solution. However it is still significantly higher than [BMIM][PF_6]. These higher rmsd values at all hydration conditions suggest that this IL is not as good as [BMIM][PF_6] in stabilizing the enzyme structure, as indicated by the experimental reports mentioned previously.

This is effectively seen if we analyze the individual residue rmsd's of two representative simulations of the enzyme in the two ILs at 2.5% water percentage (Figure 2b) at high temperature. In these conditions almost all regions of the enzyme, except the β-sheet regions, suffer a significant displacement

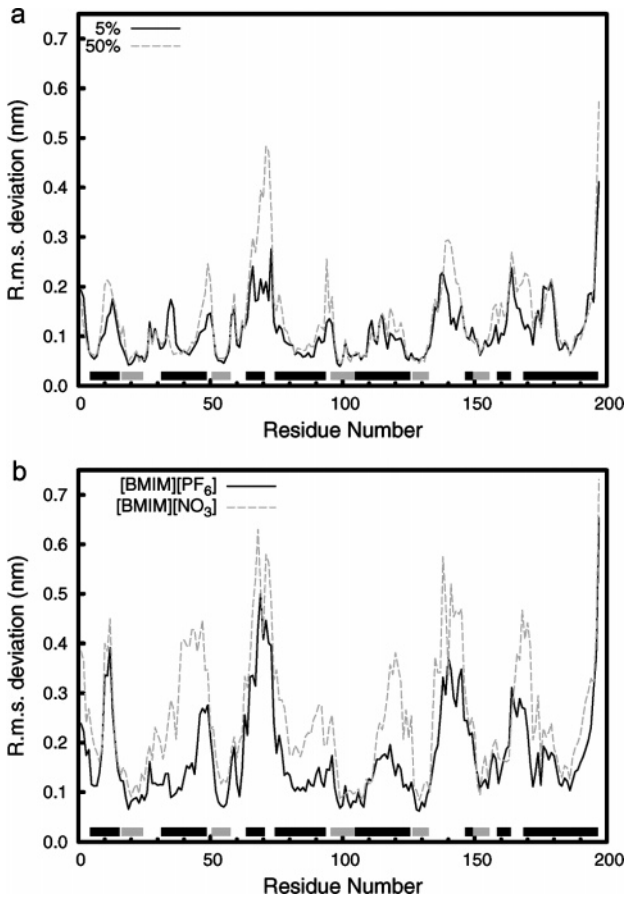

Figure 2. rmsd of each Cα residue atom of cutinase from the X-ray structure of (a) cutinase in [BMIM][PF_6] at 298 K with 5% and 50% water percentage, and (b) cutinase at 343 K with 2.5% water percentage in [BMIM][PF_6] and [BMIM][NO_3]. Bottom horizontal bars indicate secondary structure regions of the X-ray structure: black are α-helix segments and gray are β-sheet segments.

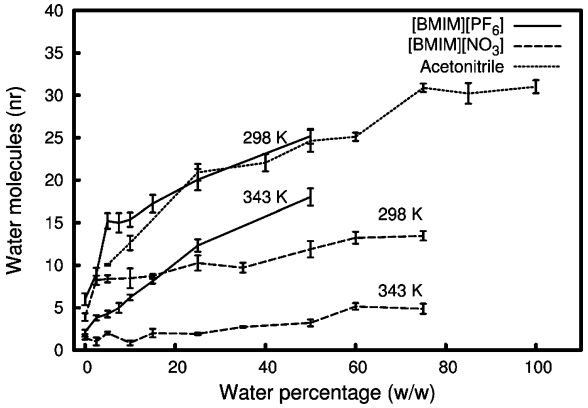

Figure 3. Average number of water molecules at a distance inferior to 0.25 nm from the enzyme surface in [BMIM][PF_6] and [BMIM]-[NO_3] at 298 and 343 K and in acetonitrile at 298 K from Micaelo et al.[25]

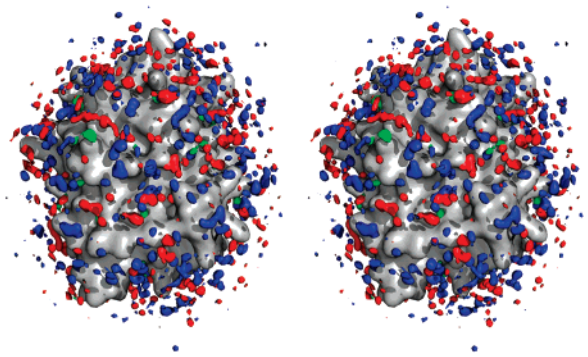

Figure 4. Stereo picture of the spatial distribution density of water, $[BMIM]^+$, and $[PF_6]^-$ in the system with 5% water content at 298 K from all the replicate simulations in these conditions. The molecular surface corresponds to the average structure of cutinase from the 10−20 ns period. The contours enclose regions with a probability density above 3.4×10^{-5} Å^{-3} for water (green contour), 5.2×10^{-6} Å^{-3} for $[BMIM]^+$ (blue contour), and 6.6×10^{-6} Å^{-3} for $[PF_6]^-$ (red contour).

when compared to the same enzyme with the same water amount but solvated with [BMIM][PF_6].

3.3. Thermal Stability. Some ILs have been successfully employed as solvents in biocatalytic reactions at high temperatures, allowing enzyme catalytic activity half-lives higher than in conventional organic solvents.[9−11,43−46] Our results (Figure 1b) shows that, at low water percentages, [BMIM][PF_6] minimizes the effect of temperature increase in the rmsd of the enzyme structure. This stabilization is effective when compared to the observed enzyme rmsd of 0.24 nm in a pure aqueous medium also at 343 K. We show that [BMIM][PF_6] has a effective role in increasing the protein thermostability provided that the enzyme is found in a low hydration state. The increase in the amount of water in the [BMIM][PF_6] IL has a direct consequence in the destabilization of the enzyme structure. We observe that the enzyme rmsd reaches values similar to those observed in the pure aqueous medium at this temperature when the water content in the IL is higher than 25%. The [BMIM]-[NO_3] IL at high temperature has a very high destabilizing effect on enzyme structure, which is not surprising, due to the evidence already found at room temperature for this IL. The temperature increase has just enhanced its native destabilizing properties. The detrimental effect of this IL on the enzyme structure seems to be much more significant than what we observe in a pure aqueous medium. The enzyme rmsd at low water contents in this IL is significantly higher than the 0.24 nm in a pure aqueous medium. This shows that the high rmsd observed in [BMIM]-[NO_3] is in fact due to the enzyme destabilization properties of this IL. Contrary to what we observed with the [BMIM][PF_6] at high temperature, the addition of water to the system seems to reduce the detrimental effect of the high-temperature effect in this IL.

3.4. Enzyme Solvation by Water. Enzyme hydration is a fundamental aspect in nonaqueous enzymology, since water, particularly the water in close contact with the enzyme, determines to a great extent the structural and dynamic properties of the enzymes. In ILs it has been shown that, as in conventional organic solvents, water content or water activity is an important factor in enzyme catalysis.[14,52] We show in Figure 3 the number of waters retained at the enzyme surface in the two ILs at different water percentages.

This figure clearly shows that only a fraction of the total water present in the system is located at the enzyme surface plus some internal waters. The number of waters observed at 0% corresponds to these internalized water molecules that remained inside the structure of the enzyme (see section 2.1). It is also observed that, at both temperatures, as we add water to the system, the enzyme is able to retain only a fraction of that water on its surface. A molecular picture of how water is distributed at the enzyme surface is shown in Figure 4. In this figure is depicted the spatial probability distribution of water (rendered as a green contour) for the case of [BMIM][PF_6] at 5% water content at room temperature.

The highest water density regions are scattered over the enzyme surface. The excess water molecules are found on bulk

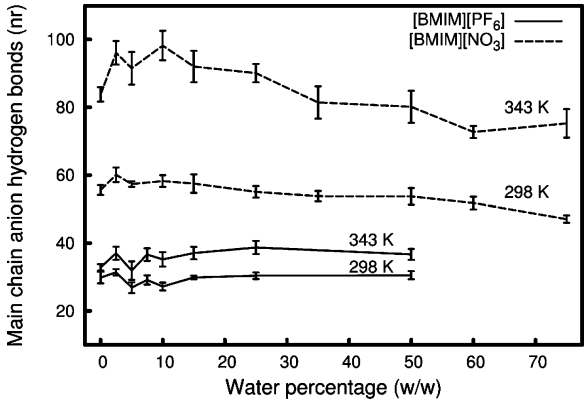

Figure 5. Average number of hydrogen bonds between the anions in solution and the enzyme main chain in [BMIM][PF_6] and [BMIM]-[NO_3] at 298 and 343 K. A hydrogen bond is considered to exist in one conformation if the distance between the hydrogen atom and the acceptor is less than 2.5 Å and the angle formed by donor−hydrogen−acceptor is less than 135°.

solution interacting preferentially with the anion species of the IL via hydrogen bonds (data not shown), as previously suggested by Diego et al.[9] and Cammarata et al.[53] Another observation is that the two ILs behave differently in their ability to strip water from the enzyme surface: it is observed that [BMIM][PF_6] allows the retention of more water at the enzyme surface than [BMIM][NO_3]. In this respect, [BMIM][PF_6] seems to retain similar amounts of water at the enzyme surface like what is found for acetonitrile[25] (Figure 3) and supports the evidence of the polar nature of these ILs (see next section). In the case of [BMIM][NO_3], this IL replaces almost the totality of the water at the enzyme surface, which may be the reason for the destabilization of the enzyme. Such an extensive removal of water from the enzyme surface may be critical to its function, as pointed by Laszlo et al.[14] Therefore, we see that [BMIM]-[PF_6] is able to preserve an essential water hydration level, even at very low hydration conditions, which allows the conservation of the enzyme native structure and function, whereas [BMIM]-[NO_3] does not. Increasing the temperature of the system leads to a significant reduction of water at the enzyme surface, but nevertheless keeping the same increasing trend.

3.5. Enzyme Solvation by IL. We have seen that the optimal enzyme hydration for the case of [BMIM][PF_6] is achieved with 5−10% water content. However, this amount represents a very small fraction of the enzyme surface solvated by water. The majority of the enzyme surface is primarily solvated by the IL, and this evidence stresses the fact that these two ILs have a significant role in the solvation of the enzyme. Figure 4 shows the spatial contours enclosing high probability regions of finding water, $[BMIM]^+$ in blue and $[PF_6]^-$ in red, at the enzyme surface for the exemplificative case of [BMIM][PF_6] with 5% water at room temperature. The localization of the highest probability regions for the cation and anion are found at the enzyme surface. A more detailed analysis of enzyme solvation by the two ILs shows that the anion species dominates the nonbonded interactions with the enzyme, as judged by the number of hydrogen bonds observed between the enzyme and the cation and anion species of each IL (Figures 1S and 2S, Supporting Information). The ability of ILs to dissolve molecules depends mainly of the hydrogen bond accepting properties of the anion, as stated by Anderson et al.[54] In our particular case, it is found that the $[NO_3]^-$ anion is strongly interacting with the main chain of the enzyme through a hydrogen bond with the amide group (Figure 5).

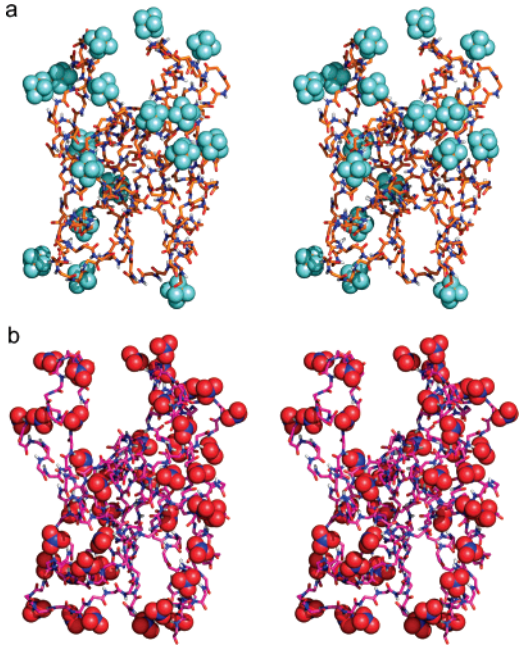

Figure 6. Stereo pictures of two final conformations of cutinase at 343 K with 2.5% water percentage in (a) [BMIM][PF_6] and (b) [BMIM]-[NO_3]. (The replicate simulations with the lowest and the highest enzyme rmsd's in [BMIM][PF_6] and [BMIM][NO_3] respectively are used here for comparison.) The main chain of each conformation is rendered using sticks. The figure also shows the anion molecules (a) $[PF_6]^-$ and (b) $[NO_3]^-$ rendered in spheres that are found at a minimum distance of 2.5 Å from the enzyme.

At room temperature, the number of hydrogen bonds between $[NO_3]^-$ and the enzyme is approximately twice the number found for $[PF_6]^-$. It seems that $[NO_3]^-$ interacts with the enzyme strongly enough to enter the protein interior and disrupt essential intraprotein hydrogen bonds, in particular in the α-helix regions. The interaction of this anion with the enzyme main chain seems to be reduced as the amount of water in the system increases at both temperatures.

The increase of temperature enhances the interaction of the anions with the protein main chain, especially in the case of $[NO_3]^-$ at low water percentages. As seen before, the increase of temperature in this IL is followed by a significant enzyme structural perturbation that can be attributed, in part, to the particular action of $[NO_3]^-$ with the enzyme. For the IL containing the $[PF_6]^-$ anion, the temperature increase has a minor effect in the interaction of this anion with the protein main chain.

A detailed molecular picture of the interaction between the anion species of the two ILs and the protein main chain is shown in Figure 6. This figure depicts the enzyme structure and the closest anions to the enzyme in the last configuration of a simulation run of the enzyme in [BMIM][PF_6] and [BMIM]-[NO_3] with 2.5% water content at 343 K. It is possible to observe that several $[NO_3]^-$ anions are forming hydrogen bonds with the enzyme main chain (Figure 6b). These numerous $[NO_3]^-$ anions significantly disrupt the native intra-main-chain hydrogen bonds (Figure 7) especially at low hydration conditions, except in the near absence of water at 0% where we see a high number of intra-main-chain hydrogen bonds as a response to the lack of water at is surface. As said before, this may be due to the possibility that the protein is kinetically trapped due to the absence of water. This strong interaction perturbs the enzyme secondary structure and can explain the experimental evidence of protein loss of structure and function in this IL.[6] In the case

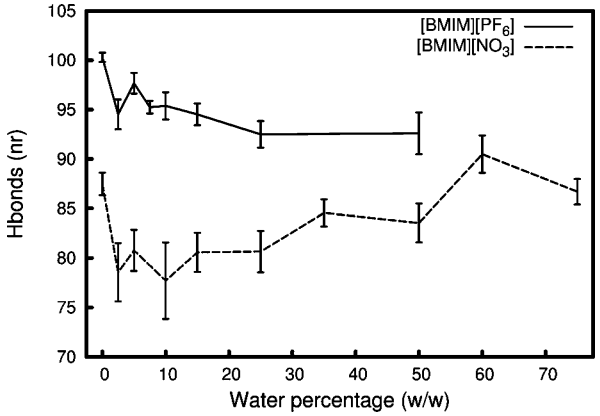

Figure 7. Average number of enzyme intra-main-chain hydrogen bonds in [BMIM][PF_6] and [BMIM][NO_3] at 343 K.

of the $[PF_6]^-$ anions this type of interaction with the enzyme main chain does not occur so significantly (Figures 6a and 7).

3.6. Comparing ILs and Organic Solvents. We have been showing evidence that suggests that ILs seem to share similar properties with conventional organic solvents in the way they model the enzyme structural and catalytic properties, as also shown by experimental studies.[11,15] In the light of our modeling studies we have evidence that shows that the enzyme is preferentially stabilized in [BMIM][PF_6] and hexane[20,25] at similar low water contents, although, in absolute value, the enzyme has higher rmsd values in hexane than in the IL.

In both solvents the enzyme shows a similar rmsd dependence in a bell-shape-like behavior with the amount of water present in the media.[20,25] In each case, the enzyme is preferentially stabilized at around 7.5% water content. This fact is particularly curious given the significantly dissimilar properties of the [BMIM][PF_6] and hexane molecules. One is an IL that is able to interact with the enzyme via long-range electrostatic forces and short-range hydrogen bonds, while the other one is a very apolar molecule. On the other hand, the enzyme hydration profile observed with [BMIM][PF_6] is more similar to polarlike organic solvents such as acetonitrile.[20,25] We shown that in [BMIM][PF_6] only a small fraction of the total water present in the system is retained at the enzyme surface (Figure 3) that is organized in many small water clusters (Figure 4). This similarity is particularly evident in Figure 3, where we show that [BMIM][PF_6] and acetonitrile strip water from the same enzyme surface in a comparable way. In the case of [BMIM]-[NO_3] a greater removal of water from the enzyme surface is observed. These observations support the polar character of these ionic liquids.

4. Concluding Remarks

The use of ILs as solvents for biomolecular catalyzed reactions is one of the most nonphysiological environments for an enzyme. Our molecular modeling studies have shown that some ILs are able to render native enzyme structures at room temperature and high temperature, similar to what is observed in conventional organic solvents.[20,25] We show that [BMIM]-[PF_6] is a good medium for our model enzyme since our simulations show a conserved average structure similar to the reference X-ray structure. We see that the enzyme structure is more nativelike at optimal 5−10% water content. In a previous study we also found that the same enzyme in hexane had also structural properties more nativelike at low water percentages (7.5% (w/w)).[25] This suggests that some enzymes behave in a similar way both in ILs and in organic solvents, they can be structurally stable in both liquids, and they seem to display structural properties following a bell-shape-like behavior relative to the amount of water present in the media. In addition, we show that [BMIM][PF_6] is able to provide a relatively stable enzyme structure at a high temperature of 343 K, providing that the enzyme is found in a low hydration state. We also tested a second IL, [BMIM][NO_3], that has been shown to have a destabilizing effect on several enzymes. We show that, replacing the $[PF_6]^-$ anion by $[NO_3]^-$, we promote a significant perturbation on the enzyme structure. We suggest that the strong interaction of the $[NO_3]^-$ anion with the enzyme hydrogen donor groups, especially the amide group from the peptide bond in the α-helix and loop regions, is one of the main mechanisms of enzyme structure destabilization by this IL. Therefore, the design of optimum IL solvents for biomolecules seems to be mostly dependent on the correct choice of the anion species.

One of the key aspects of enzyme stabilization in nonaqueous media is the existence of an essential enzyme water hydration layer. An essential enzyme hydration layer in this medium seems to be attained in the presence of the [BMIM][PF_6]. We show that [BMIM][PF_6] strips water from the enzyme surface in a way very similar to that shown for acetonitrile,[25] a very polar organic solvent. The water that is added to the system is mostly found in bulk solution, but an essential fraction of this water is kept at the enzyme surface, allowing the enzyme to maintain its native structure and be fully active as shown experimentally. However, this does not hold true for [BMIM][NO_3], where extensive water stripping from the enzyme surface is observed compromising the native enzyme structure and activity, as also seen experimentally.

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Supporting Information Available: Additional ionic liquid and enzyme hydrogen bond analysis in Figures 1S and 2S. This information is available free of charge via the Internet at http://pubs.acs.org.

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