A Molecular Dynamics and Quantum Mechanics Analysis of the Effect of DMSO on Enzyme Structure and Dynamics: Subtilisin

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Abstract: This report describes a 745-ps molecular dynamics simulation of the enzyme subtilisin Carlsberg in a periodic box of dimethyl sulfoxide (DMSO). The starting coordinates for subtilisin and crystallographic waters were taken from the aqueous X-ray crystal structure. Although the overall structure of subtilisin is stable throughout the simulation, some structural perturbations are observed. The five N-terminal residues of the protein migrate away from the rest of the protein; whether this movement is the start of partial unfolding in DMSO is unclear. We compare these findings with recent simulations of this enzyme in CCl_4, acetonitrile, and water. The simulation results indicate that DMSO is capable of stripping waters and metal ions away from the protein surface. The total number of intraprotein hydrogen bonds is increased in DMSO compared to in water. Eighteen DMSO binding sites were identified based on root-mean-square fluctuations, most of which were hydrogen bonded to a protein amide hydrogen atom. Quantum mechanics calculations were used to investigate the hydrogen bonding strength between DMSO and protein amide hydrogen and between DMSO and water. These results are discussed in view of the known protein-dissolving property of DMSO.

Introduction

Information concerning the structure and dynamics of enzymes in nonaqueous solvents is essential toward acquiring a mechanistic understanding of enzyme reactions in these solvents.[1] Recently, several experimental techniques have been used to elucidate protein structure and dynamics in nonaqueous environments. X-ray crystallographic studies have been used to solve the crystal structures of proteases such as subtilisin in acetonitrile[2] and chymotrypsin in hexane.[3] In both cases, the crystal structures in water and nonaqueous solvents are very similar. This is not surprising due to the way crystals were prepared. Since proteins are not soluble in most organic solvents, it would be very difficult to crystallize proteins from organic solvents and indeed this approach has not been successful so far. An alternative approach is to grow a crystal in aqueous solution and then soak the crystal in organic solvents. In this way, some of the interstitial water molecules could be washed away by the nonaqueous solvent; even if the solvent is not water-miscible like hexane, by soaking, solvent molecules can still diffuse to the protein surface. Therefore, the resulting crystal is only a slightly perturbed "aqueous crystal". If large changes do occur, the crystal will probably crack and the structure would not be solved. On the other hand, spectroscopic studies on lyophilized powders are very controversial. Some studies suggest that lyophilization causes little structural change,[4] but others indicate that partial unfolding occurs during lyophilization.[5]

It is well-known that the kinetics of hydrogen isotope exchange can provide valuable information concerning protein dynamics and stability. Recent progress in NMR spectroscopy has made it possible to measure hydrogen−deuterium exchange rates for individual amide hydrogens in small and medium-sized proteins.[6] This technique has been used to probe transient protein folding intermediates,[7] equilibrium intermediates,[8] and protein−ligand interactions.[9] NMR studies have also been carried out to probe protein structure and dynamics in organic solvents by Desai and Klibanov on BPTI[5b] and Wu and Gorenstein on cytochrome C.[10] However, interpretation of the amide hydrogen exchange results in organic solvents remains a difficult problem. The amide hydrogen exchange results were interpreted as an indication of partial unfolding by Desai and Klibanov,[5b] but as an indication of enhanced flexibility in organic solvent by Wu and Gorenstein.[10]

Molecular dynamics (MD) simulations have also been used to address solvent effects on the structure and dynamics of

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proteins in organic solvents.[11−13] Recently, we have compared molecular dynamics simulations of subtilisin in water, acetonitrile, and carbon tetrachloride.[12,13] Here we report the results from MD simulation of subtilisin in dimethyl sulfoxide (DMSO). DMSO is a dipolar, aprotic solvent with a dielectric constant of 46.45.[14] It can compete with protein hydrogen bond acceptors for hydrogen bond donors. This is similar to acetonitrile, but DMSO is a much better hydrogen bond acceptor and is one of the few protein-dissolving solvents. DMSO has been widely used as an alternative solvent to water, due to the high solubility of peptides and proteins in it.

Computational Methods

Molecular Mechanics. All calculations were carried out using the AMBER 4.0 program.[15] The all-atom AMBER forcefield[16] was used unless indicated otherwise. Starting coordinates for all heavy atoms of subtilisin and crystal waters were obtained from the crystal structure from aqueous solution.[2] The protein, together with crystal waters, was immersed in a box of DMSO, and all solvent molecules closer than 3 Å or further than 10 Å in any Cartesian direction from the protein were eliminated. The initial solvent box dimensions are 70.68 × 69.52 × 62.06 Å containing 1822 DMSO molecules. The total number of atoms in the system is 11 481. Periodic boundary conditions were used and the calculations were carried out at 298 K and 1 atm. Four chloride ions were added to neutralize the protein charges. The TIP3P model was used for crystal water molecules. There are several models for DMSO.[17−19] We used the recently developed OPLS model for DMSO.[18] The internal geometry of each DMSO molecule was kept rigid during the MD simulation.

The protein−solvent system was optimized prior to the simulation. First, the protein was fixed and the solvents and counterions were allowed to move. Second, the solvents and counterions were kept fixed and the protein was allowed to adjust to the solvent environment. Finally, the whole system was minimized to a root-mean-square gradient <0.1 kcal/mol. The solute and solvent were coupled to separate constant-temperature heat baths and a constant-pressure bath.[20] All bonds were constrained using the SHAKE[21] algorithm with a tolerance of 0.0005 Å, allowing a time step of 1.5 fs. The nonbond pairlist was generated every 16 steps using a residue-based cutoff distance of 10 Å. Coordinates were saved every 200 steps. A 745-ps simulation was carried out.

Quantum Mechanics. Quantum mechanics methods were used to examine the hydrogen-bonding interactions between DMSO and water and between DMSO and an amide hydrogen, using formamide as a model for the amide. The hydrogen-bonding interaction between two water molecules has been extensively investigated both experimentally[22] and theoretically.[23] The 6-31G (D) level of theory gives good agreement with experiment, but correlated treatments at this level result in interaction energies that are too negative. Only when a very large basis set was used together with correlation up to the MP4 level did the agreement with experiment improve.[23c] Since we are only interested in the trend of hydrogen bonding, no attempt was made to do calculations using very large basis sets with high electron correlation. Ab initio quantum mechanics calculations were carried out using the Gaussian 92 program[24] at 6-31G(D) and MP2/6-31G(D) levels. Geometries for DMSO, formamide, DMSO−water, and DMSO−formamide complexes were fully optimized at both 6-31G(D) and MP2/6-31G(D) levels. The energies for water at both 6-31G(D) and MP2/6-31G(D) levels were taken from published results.[25]

Table 1. The Calculated Total Energies (au)

molecule	6-31G(D)	MP2/6-31G(D)
H_2O[a]	−76.010 746 5	−76.199 244 2
formamide	−168.930 702 7	−169.394 457 2
DMSO	−551.537 277 6	−552.112 330 1
DMSO−H_2O	−627.562 034 5	−628.327 085 4
DMSO−formamide	−720.481 406 -	−721.524 593 6

[a] Values taken from ref 25.

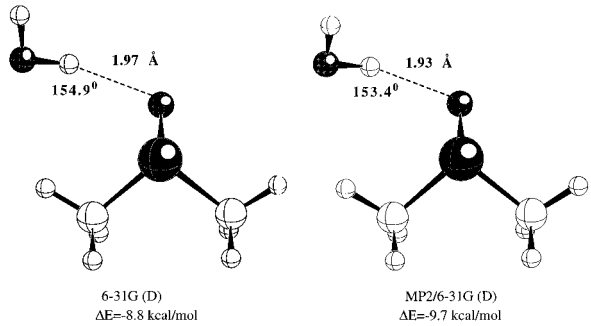

Figure 1. The calculated geometries and hydrogen-bonding energies for DMSO−H_2O complex at 6-31D(D) and MP2/6-31G(D) levels. The distance values are between the oxygen of DMSO and the closest hydrogen of water. The angle is that formed between the former two atoms and the oxygen of water. The same convention applies to Figure 2 except the NH_2 group of formamide replaces the water.

Results and Discussions

Quantum Mechanics Calculations. To examine the hydrogen-bonding interactions between DMSO and water, we carried out quantum mechanics calculations. The geometries of DMSO and DMSO−water complex were fully optimized without any geometrical constraints at both 6-31G(D) and MP2/6-31G(D) levels. As expected, DMSO adapts a pyramidal structure with a large inversion barrier. The calculated inversion barrier at the 6-31G(D) level is about 54 kcal/mol, which agrees well with experimental observation on phenyl *p*-tolyl sulfoxide.[26] Table 1 gives the calculated total energy for each molecule involved, while Figure 1 shows the optimized geometries and hydrogen-bonding energies of the DMSO−water complex at 6-31G(D) and MP2/6-31G(D) levels. As noticed before, the interaction energy at the MP2/6-31G(D) level of theory is larger

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(18) Jorgensen, W. L. Unpublished. r(C−S) = 1.80 Å, r(S−O) = 1.53 Å, σ_s = 3.56 Å, σ_o = 2.93 Å, σ_c = 3.81 Å, ϵ_s = 0.395 kcal/mol, ϵ_o = 0.280 kcal/mol, ϵ_c = 0.160 kcal/mol, q_s = 0.139 e, q_o = −0.459 e, and q_c = 0.160 e. These give the correct density and ΔH_{vap} for DMSO at 25 °C and 1 atm.

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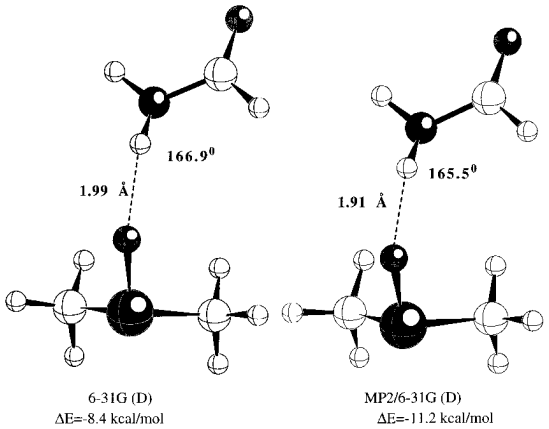

Figure 2. The calculated geometries and hydrogen-bonding energies for DMSO−formamide complex at 6-31G(D) and MP2/6-31G(D) levels.

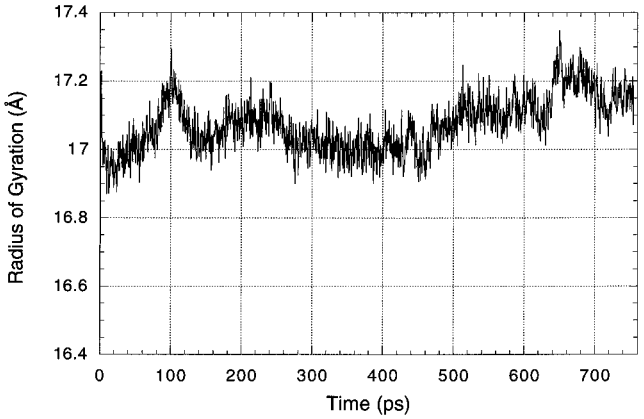

Figure 3. Plot of the radius of gyration of subtilisin in DMSO as a function of time.

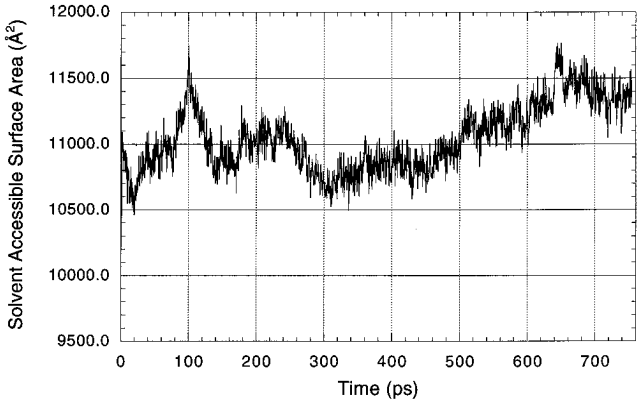

Figure 4. Plot of the solvent accessible surface area of subtilisin during the molecular dynamics simulation in DMSO.

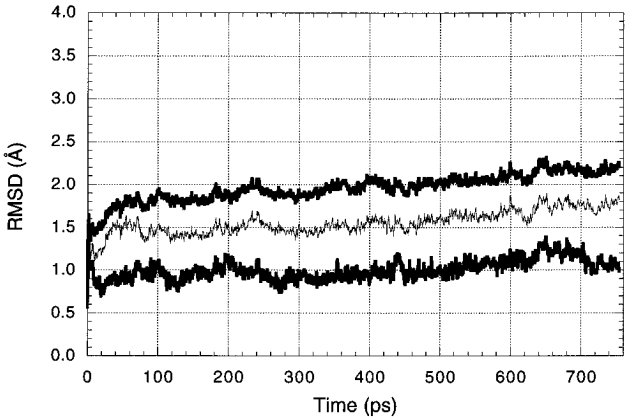

Figure 5. Plot of the calculated root-mean-square deviations (RMSD) of subtilisin in DMSO. The top curve is the RMSD for all heavy atoms, the middle for all C_α, and the bottom for C_α of secondary structural elements.

than that at the 6-31G(D) level.[23,25] The calculated interaction energy at the 6-31G(D) level is about 8.8 kcal/mol for a DMSO−water hydrogen-bonded complex. Compared to water−water interaction, DMSO−water interaction is stronger, which is in agreement with the experimental observation.[27] The calculated interaction energy at the 6-31G level for the DMSO−water complex is also stronger than that for the hydrogen-bonding interactions (1) between a backbone carbonyl oxygen and amide hydrogen, (2) between a carbonyl oxygen and water, and (3) between an amide hydrogen and water.[25] Our calculation seems to provide an explanation as to why DMSO is capable of stripping away water molecules from the surface of a protein. We have also investigated the interaction between a DMSO−amide complex as modeled by DMSO−formamide; the calculated geometries and interaction energies are shown in Figure 2. As can be seen, the interaction of DMSO with an amide hydrogen is about the same strength as the DMSO−water hydrogen bond at the 6-31G(D) level, but is stronger at the MP2/6-31G(D) level. DMSO−amide hydrogen interactions also seem to be stronger than hydrogen-bonding interactions between a backbone carbonyl and an amide hydrogen.[25] The strong hydrogen-bonding capability of DMSO may, at least in part, be responsible for previous reports that DMSO is a protein-dissolving solvent and can readily denature proteins.[28]

Molecular Dynamics Simulations. Several properties were monitored to assess the quality of the simulation. The temperature of the protein and solvent is stable during the last 650 ps, and so are the potential and total energy (data not shown). The radius of gyration and solvent accessible surface area of subtilisin Carlsberg (Figures 3 and 4) are relatively stable throughout the simulations.

Structural Changes. Organic solvents, especially hydrophilic solvents, could have great impact on the structure and stability of peptides and proteins. Hydrophilic solvents such as methanol are known to strip away water molecules from the surface of a protein and can disrupt tertiary structure.[29] Trifluroethanol has been shown to induce secondary structure formation.[30] Figure 5 shows the root-mean-square deviations (RMSDs) during our simulation in DMSO of subtilisin Carlsberg of all heavy atoms, all C_α, and C_α's of secondary structural elements from the initial aqueous crystal structure. The RMSDs remain relatively stable throughout the entire simulation. The RMSD of all C_α carbons of secondary structure elements remains at about 1 Å; the RMSD of all C_α carbons of subtilisin is about 1.5 Å during the first 500 ps and increases to about 1.8 Å. The all heavy-atom RMSD is about 2 Å during the first 640 ps and then increases to about 2.2 Å. Overall, the structure of subtilisin in DMSO after 700 ps is still not far away from the initial aqueous crystal structure. Figure 6 shows an α-carbon trace of the time-averaged structure from the last 100 ps of the simulation superimposed on the initial aqueous X-ray structure. The N-terminus moves away from the rest of the protein, an

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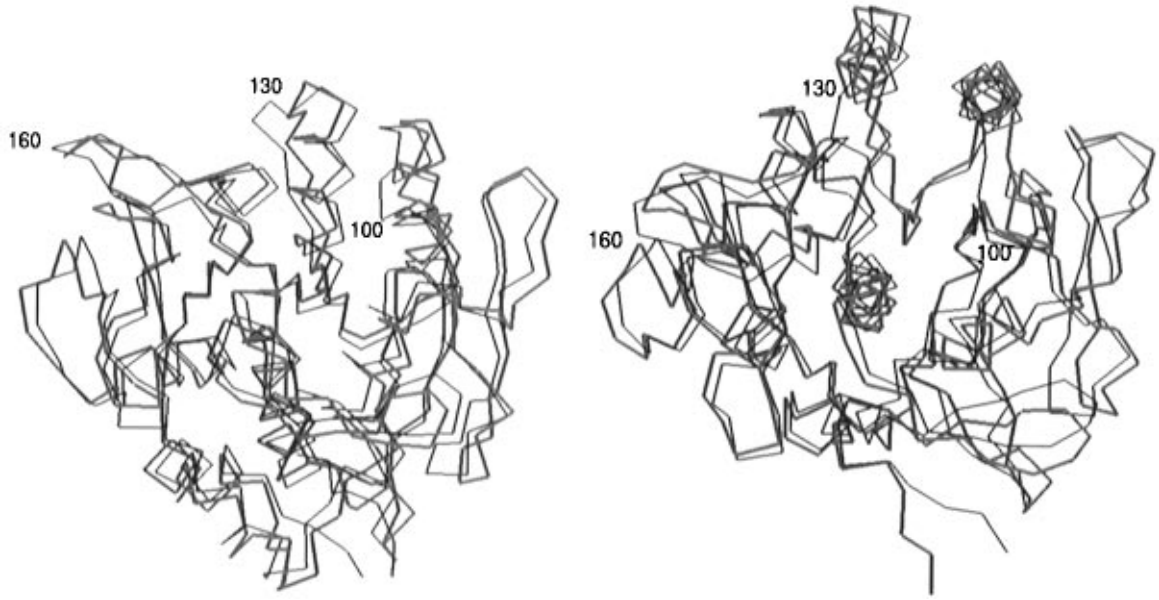

Figure 6. The superimposition of the time-averaged structure (gray) with the X-ray (dark) structure in two different views.

Table 2. Summary of the Calculated Root-Mean-Square Deviation (RMSD) of C_α and Standard Deviation for Each Individual Secondary Structural Element of Subtilisin in DMSO

element	residues	RMSD (Å)	standard deviation
H1	6−11	0.22	0.07
H2	12−19	0.36	0.08
H3	63−74	0.36	0.09
H4	103−117	0.44	0.12
H5	132−146	0.40	0.08
H6	220−238	0.65	0.16
H7	242−253	0.52	0.13
H8	269−274	0.26	0.09
sheet[a]		0.84	0.11

[a] The sheet consists of residues 43−50, 89−95, 26−32, 120−124, 148−153, 175−180, 197−220, and 265−268.

effect not seen in simulations in water, acetonitrile, or carbon tetrachloride.[12,13] Previously, Desai and Klibanov suggested that BPTI undergoes partial unfolding in DMSO.[5b] Whether what we observed in the present simulation of subtilisin in DMSO is the beginning of protein partial unfolding is not clear; it is likely that only minor unfolding occurs on the nanosecond time scale. The movement of the N-terminal residues is responsible for the increase in RMSDs after 640 ps and the rise in both radius of gyration and solvent accessible surface area (see Figures 3, 4, and 5). As shown in Figure 6, the time-average structure and the initial X-ray structures are very similar with a RMSD of 1.64 Å for backbone atoms. Even in the loop regions the differences are not as apparent as in the simulation in CCl_4. Since DMSO is much more polar than CCl_4, it is not surprising to see that the structure of subtilisin in DMSO resembles the aqueous structure more than the structure in CCl_4. The RMSDs were also determined for each secondary structural element (Table 2). The RMSD is calculated individually for each helix. All secondary structural elements remain intact, although helices 4, 6, and 7 show larger deviation compared to the other five helices. The β-sheet displays larger RMSD than the helices, which is expected.

Flexibility. To assess the flexibility of subtilisin in DMSO, we performed a structure averaging and calculated the RMS fluctuation about the time-averaged structure for the last 100 ps of the simulation. Figure 7 shows the calculated RMS fluctuation of each amino acid residue for simulations in DMSO and water. Overall, the RMS fluctuations in DMSO are shifted by 0.2 Å and the locations of peaks and valleys are in good agreement. The first five residues in DMSO simulation show

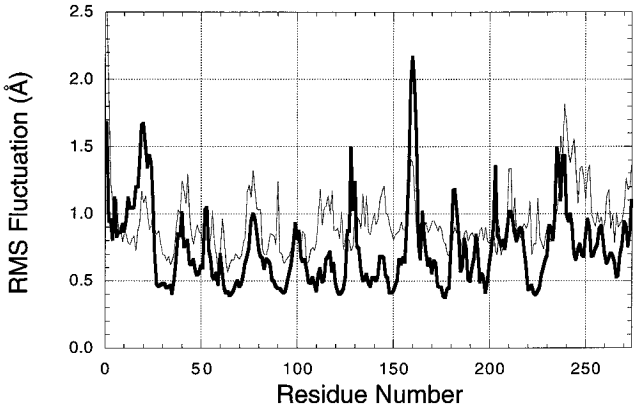

Figure 7. Plot of the calculated RMS fluctuations for subtilisin in DMSO (gray) and in water (dark).

very large RMS fluctuations (higher flexibility), while residues around 20−30, and 130, 160−170 display higher flexibility in water.[12] However, it is difficult to say whether the protein is more flexible in DMSO than in water. Compared with a previous simulation in CCl_4, clearly, subtilisin is more flexible in DMSO than in CCl_4.[13] Since the calculated RMS fluctuation depends on how the structure averaging was done, those data should only be viewed qualitatively. In protein-dissolving solvents, the protein may undergo partial unfolding, it is very difficult to know whether a larger RMS fluctuation indicates greater flexibility or partial unfolding as exemplified by previous NMR studies on cytochrome *c*[10] and BPTI.[5b] The unusual behavior of subtilisin in acetonitrile might be partly due to the different number of metal ions and waters in the structures (discussed below).

Intra-Protein H Bonds. The total number of intra-protein hydrogen bonds of each instantaneous configuration is plotted as a function of time (Figure 8). Again, the trend in DMSO is different from what was observed for the simulation in water, but it is consistent with what we saw for other organic solvents.[12,13] The calculated average numbers of intra-protein hydrogen bonds from the last 100 ps are 214 ± 7, 247 ± 6, 259 ± 7, and 264 ± 7 for simulations in water, DMSO, acetonitrile, and CCl_4. Clearly, there are more intra-protein hydrogen bonds for subtilisin in DMSO than in water. However, the total number of intra-protein hydrogen bonds is lower in DMSO than in CCl_4, which correlates well with the fact that CCl_4 is nonpolar and DMSO is hydrophilic. It seems to be a

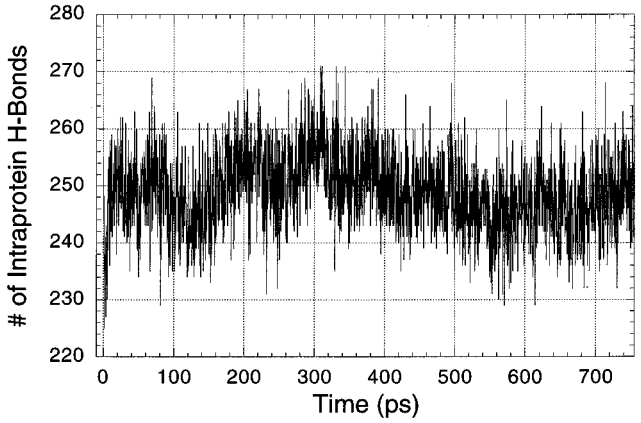

Figure 8. Plot of the total number of intra-protein hydrogen bonds.

general trend that there are more intra-protein hydrogen bonds for a protein in a nonaqueous environment than in aqueous environment. The difference in intra-protein hydrogen bonding may be responsible for the higher thermostability and reduced flexibility of proteins and enzymes observed in nonaqueous environments. The DMSO molecules that are close to the protein surface form hydrogen bonds with protein hydrogen bond donor groups.

Solvent Dynamics. During the simulation some water molecules left the protein surface. More water molecules leave the protein surface in the DMSO simulation than in the acetonitrile simulation,[12] while no water leaves the protein surface in the simulation in CCl_4.[13] A snapshot at 648 ps of subtilisin with bound waters of crystallization is shown in Figure 9. As discussed before, DMSO, a hydrophilic, water-miscible solvent, forms a stronger hydrogen bond with water than the hydrogen bond formed between water−water and water with neutral hydrogen bond donors of the protein. So, it is not surprising to see that during the simulation some water molecules leave the protein surface and move into DMSO solution. The ability of hydrophilic solvents to strip away water molecules from the protein surface has been demonstrated experimentally.[29]

Most of the DMSO molecules have very high mobility. To examine the interaction between DMSO and the protein in more detail, we calculated a time-averaged structure for the last 350 ps. The RMS fluctuations around the time-averaged structure were calculated for each atom in the system. We find 18 DMSO molecules that have an RMS fluctuation less than 2 Å, which is about the same magnitude as the heavy atoms of the protein. Each of these 18 DMSO molecules were examined carefully and the neighboring protein groups were identified. The "localized" DMSO molecules fall into three groups. The first group contains only one DMSO that is coordinated to a calcium ion with an average Ca^{2+}- - -O distance of about 2.5 Å (no. 6 in Table 3). The coordination of this DMSO molecule occurred at about 320 ps into the 745-ps simulation and remained coordinated to the calcium ion for the rest of the simulation. The second group of DMSOs interact with the protein mainly through hydrogen bonding. It is interesting to note that the hydrogen bonds are formed between the oxygen atom of DMSO and an amide hydrogen of an amino acid residue. There are 13 DMSO molecules in this category. The remaining four (nos. 7, 12, 14, and 16 in Table 3) DMSO molecules do not seem to have any specific interactions and interact with the protein mainly through van der Waals interactions.

There are three metal ions (two calcium and one sodium) in the X-ray structure and all three were included in our simulation. All three are coordinated to protein ligands and/or crystallographic water in the X-ray structure. These metal ions may

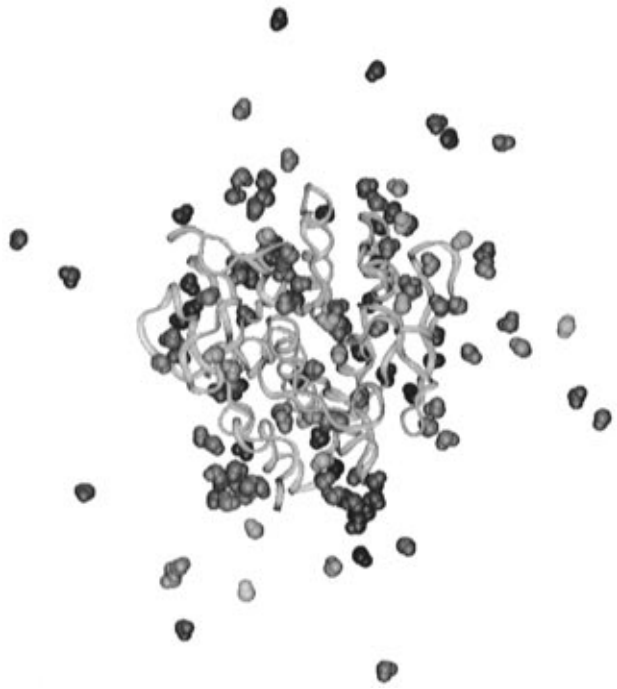

Figure 9. A snapshot of subtilisin with bound crystallographic water molecules.

Table 3. The Environments of 18 "Localized" DMSO Molecules

no.	RMS fluctuation of O of DMSO (Å)	amino acid environment
1	1.219	H-bonded to Arg 186
2	1.274	H-bonded to amide hydrogen of Phe 261
3	1.464	H-bonded to amide hydrogen of Ser 130
4	1.480	H-bonded to amide hydrogen of Gly 23
5	1.511	Asn 158
6	1.516	coordinated to Ca^{2+} ion
7	1.559	Asn 62, Gly 61, Ser 98, and Ser99
8	1.586	H-bonded to Phe 189
9	1.607	H-bonded to HN of Ala 243
10	1.609	H-bonded to HN of Asn 240
11	1.709	H-bonded to HN of Gly 154
12	1.764	Arg 145, Gly 146, Val 147, and Thr 115
13	1.807	H-bonded to HN of Asp 155
14	1.824	Ala 194, Lys 265, and Phe 261
15	1.849	H-bonded to HN of Ala 134
16	1.909	Thr 208, Tyr 209, His 39, and Asn 212
17	1.915	H-bonded to HN of Tyr 6 most of the time
18	1.995	H-bonded to HN2 of Asn 62

be important for the structural stability of subtilisin Carlsberg. In the DMSO simulation, we observed that one metal ion remains in the original position and coordinates to protein; one metal ion is sequestered by DMSO and starts moving away from the protein; the third one has one DMSO coordinated to it. The sequestering of metal ions will have some impact on the structure and dynamics of the N-terminal half of the protein. Previously, it has been shown that when the aqueous crystal was washed using pure acetonitrile, two metal ions were lost.[2a] Thus, there are three metal ions (one sodium and two calcium ions) in the aqueous crystal structure, but there is only one in the cross-linked crystal structure from acetonitrile. Since the presence of metal ions plays an important role in the structure and stability of an enzyme, and since in the previous simulations in water and acetonitrile the respective X-ray crystal structures were used as starting structures, the difference in the number of metal ions present in these two structures may cause some difference in the simulated results. Therefore, the high flexibility in acetonitrile solution for subtilisin may be to some extent due to this difference in the number of bound metal ions. The notion of organic solvent-dependent sequestering of metal ions

was not recognized prior to the current simulation. Its effect on protein structure and flexibility needs to be explored in future work.

The coordination of organic solvent molecules to metal ions is very interesting. Previously two groups reported that the presence of salt (or counterions) can have profound effects on the stability and activity of proteins in organic solvents.[31,32] Our simulation seems to provide a good explanation as to why salt ions could have such a profound effect on proteins in organic solvents. The presence of counterions, especially cations, will prevent direct contact between organic solvents and protein either by retaining more water molecules during lyophilization or by coordinating to solvent molecules to form complexes. Such metal ion−organic solvent complexes will shield the protein from organic solvent molecules since it is energetically costly for these charged complexes to leave the protein surface. It is noteworthy that if a counterion is too large, it will not form strong complexes and the counterion will tend to be very mobile. In such cases, the presence of large counterions will not be able to effectively shield the protein from the organic solvent molecules, which is consistent with the previous observation of subtilisin BPN′ lyophilized from Tris−HCl buffer and sodium phosphate and suspended in dipolar organic solvent.[31] In aqueous solution, since the counterions are hydrated and very mobile, no such profound effect has been observed.[31]

We also monitored the four chloride ions in the present simulations. They also stay close to the protein surface and hydrogen bonded either to water or to protein polar hydrogen bond donor groups. This is consistent with the nature of DMSO.

Conclusions

A molecular dynamics simulation was carried out for subtilisin Carlsberg in DMSO. The structure and dynamics of subtilisin Carlsberg in DMSO was examined and compared with previous simulations in other solvents. We found that even after 745 ps of simulation the protein structure is still not far away from the aqueous crystal structure. However, there are some major differences in the DMSO simulation compared to other solvents. During the simulation in DMSO, the five residues at the N-terminal end move away and have no contact with the rest of the protein. Previous reports indicated that DMSO is a protein-dissolving solvent and capable of denaturing proteins. A recent NMR study by Desai and Klibanov of BPTI in DMSO also suggested that DMSO causes partial unfolding of BPTI.[5b] Whether what we saw during our simulation in DMSO is the beginning of partial unfolding is not clear. The simulation has also shown that DMSO is capable of stripping away waters from the protein surface and this ability seems to be related to the hydrophilicity of the solvent. We saw more waters that left the protein surface in DMSO than in acetonitrile,[12] while in a hydrophobic solvent such as CCl_4,[13] no water left the protein surface during the simulation. This is in agreement with experimental observation.[29]

There are three metal ions in the system. During the molecular dynamics simulation, one of the metal ions got sequestered by solvent and started to move into solution and another one has one DMSO coordinated to it. The ability of polar solvent to sequester metal ions seems to be supported by the observation that when a subtilisin crystal grown from aqueous solution was washed using acetonitrile, two metal ions were lost.[2] The formation of a metal ion−solvent complex is very interesting, which provides a plausible explanation to previous reports that salts (or counterions) could have profound effects on protein stability and activity in organic solvents. The presence of these salt ions will prevent direct contacts between organic solvents and protein either by retaining more water molecules during lyophilization or by coordinating to solvent molecules to form complexes.

The calculated total number of intra-protein hydrogen bonds behaves differently in DMSO compared with that in water. The behavior in DMSO is similar to that in other organic solvents. It seems to be a general trend that there are more intra-protein hydrogen bonds in a protein in nonaqueous solution compared to in aqueous solution. The increase in total number of intra-protein hydrogen bonds is likely to be partially responsible for the increased thermostability of proteins in nonaqueous solutions.

Quantum mechanics calculations were performed to examine the strength of hydrogen-bonding interactions between DMSO and a protein amide hydrogen and between DMSO and water. Quantum mechanics calculations predict that the hydrogen bonding between DMSO and water is stronger than that between two water molecules, which is in agreement with experiment.[27] The calculations also indicate that hydrogen bonding between DMSO and an amide hydrogen is stronger than between a carbonyl oxygen and an amide hydrogen. Taken together our studies seem to provide a good explanation as to why DMSO is a protein-dissolving solvent and is capable of causing partial unfolding or denaturation of proteins.

From our simulation, we have also identified 18 DMSO binding sites. Most of the "localized" DMSO molecules are hydrogen bonded to an amide hydrogen of an amino acid residue. One of these 18 DMSO molecules is coordinated to a metal ion; the rest of these DMSO molecules do not appear to form any specific interactions. These predicted DMSO binding sites could be examined by X-ray crystallography or NMR techniques. Previously, it has been suggested that organic solvents may be used to map an interaction surface and to identify possible lead compounds for drug design.[2] This idea has been confirmed by a recent success in designing a new elastase inhibitor.[33] Molecular dynamics simulations could also play an important role here, especially when obtaining experimental molecular structures is difficult.

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