Peptides in Ionic Solutions: A Simulation Study of a Bis(penicillamine) Enkephalin in Sodium Acetate Solution

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Received February 24, 1993

Abstract: The bis(penicillamine) enkephalin, a small zwitterionic pentapeptide, has been studied in explicit 1.0 M sodium acetate solution with use of the molecular dynamics technique. During the simulation the association of multiple acetate ions with the positively charged N terminal region was observed. In addition, but to a far lesser extent, sodium ion binding to backbone carbonyl groups close to the negatively charged C terminus also occurred. Interesting individual events, such as the simultaneous binding of an acetate to a hydrogen of the N terminus and to backbone NH groups, were observed. Most often acetates only associated with the N terminal hydrogens. Subtle conformational changes in the peptide backbone were found as a consequence of acetate binding. These mechanistic observations are consistent with, and may be rationalized by, the known salting in and salting out properties of these ions and their relative positions in the Hofmeister (lyotropic) series.

I. Introduction

It is well-known that the addition of salts to solutions of peptides and proteins can have pronounced effects on the solubility and stability of these molecules.[1-4] The solubility of peptides can be increased (salting in) or decreased (salting out) depending on the exact nature of the salt. The relative stability of the native and denatured states of proteins is also affected by the addition of different salts. Experimentally it is observed that ions with large salting out characteristics also display a tendency to stabilize the native over the denatured state.

Qualitative experimental trends describing the effects of different anions and cations on proteins have been established for some time and form the basis of the lyotropic or Hofmeister series.[3,5] However, the exact underlying physical processes involved are still poorly understood. This is partly due to the lack of any direct methods for studying the interactions between peptides and most salt ions as well as to the problem of decoupling effects associated with individual anions or cations from certain anion–cation combinations.[6]

Current literature suggests that the influence of individual salts is determined by a delicate balance between two major effects. The first effect is a direct interaction between the peptide and the ions, *i.e.* ion binding or association.[7-13] The extent of this effect is dependent on the solvation and desolvation characteristics of both the peptide and the ions involved.[2] The other effect is related to the structure making/breaking properties of ions on the water structure in the vicinity of the peptide. In characterizing salts, a thermodynamically based concept, preferential exclusion, has been used and carries the microscopic connotation of both distance and number of ions in terms of structural models. In this model the monovalent ions such as Na^+ have a strong first solvation shell which is not easily disrupted. The result is often a preferential exclusion of these ions from the vicinity of the peptide, as inferred from thermodynamics and biochemical behavior. As a peptide aggregate has a smaller volume of exclusion, compared to the same number of isolated peptides, less solvent disruption occurs and the net result is aggregation which leads to precipitation of the peptide, *i.e.* a salting out effect.[4,14,15] For example, in order of decreasing preferential exclusion we have $SO_4^{2-} > MeCO_2^- > Cl^- > Br^- > ClO_4^- > I^-$ for anions and $NH_4^+ > Na^+ > Ba^{2+} \approx Ca^{2+} \approx Mg^{2+} >$ guanadinium$^+$ for cations.[2,14] Anion and cation effects are usually additive and preferential exclusion dominates over ion binding.[14] A corresponding stabilization of the native form follows from the idea that the loosely packed random coil denatured state has a larger volume of exclusion compared to the more compact native folded form.[14] The fact that the divalent cations are less preferentially excluded compared to sodium is a reflection of the fact that these ions have been found to bind weakly to peptides. A clear microscopic picture based on the direct observation of salt and solvent molecules and their relative interaction energies is needed to understand the balance between distance and number of species bound in a series of preferentially excluded salts.

Recently, we have been studying the effects of NaCl on the structure and dynamics of a small zwitterionic pentapeptide in aqueous solution using the molecular dynamics technique.[16-18] In these studies we observed a strong direct association between the peptide N terminus and the chloride ions which alters the conformational properties of the peptide in solution.[17,18] However, no direct binding of sodium ions was observed. These results presented a new microscopic picture of the structures involved.

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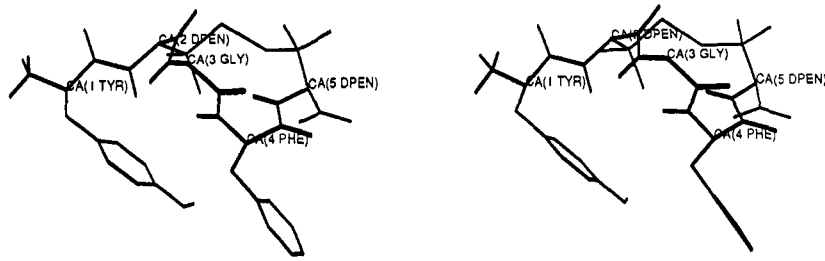

Figure 1. Stereoview of the initial conformation of DPDPE (taken from ref 19).

Chloride ions have been thought to be preferentially excluded ions, based on thermodynamic modeling, and have even been categorized as inert by some.[1] In this paper we investigate the effects of a polyatomic anion on the structure and dynamics of the same peptide. We have changed the anion from chloride to acetate. In so doing we have moved to an anion that is no longer spherical and thus has quite different interactions with both the solvent and the peptide. From the Hofmeister series, it is known that acetate ions are better salting out agents than chloride ions and should have a higher degree of preferential exclusion. In addition, the use of acetate means that our simulation more closely resembles the experimental conditions of a previous NMR study of the same peptide.[19]

Central to all these simulations is a realistic treatment of the long-ranged electrostatic interactions in these systems. The use of cutoffs and switches in these highly polar systems can lead to many unwanted artifacts.[20] In our simulations we have employed the Ewald technique to calculate the electrostatic interactions.[17,21] By doing so we hopefully remove the artifacts associated with cutoffs and switches.[22,23]

The peptide of interest is the zwitterionic pentapeptide Tyr-c[(D)Pen-Gly-Phe-(D)Pen], or DPDPE. It is a cyclic enkephalin derivative with high potency and δ opioid receptor selectivity.[24-26] DPDPE has been studied extensively by NMR,[19,26,27] molecular modeling,[19,26,28] and molecular dynamics[16,17,19,29] techniques. The peptide is thought to fold into a family of amphiphilic structures with the carbonyls on one face and the amide hydrogens on the other face of the macrocycle.[16,19] This proposed structure is shown in Figure 1 and is the initial configuration used for the simulation.

II. Experimental Method

The molecular dynamics simulation of DPDPE was performed in a cubic box of length 24.17 Å. The box contained DPDPE, 402 water molecules, 9 sodium ions, and 9 acetate ions and corresponds to a 1.0 M solution of sodium acetate. To generate an initial configuration we placed DPDPE into a previously equilibrated box of water and removed any waters within 2.3 Å of the peptide. The initial conformation of DPDPE corresponded to that obtained from previous quenched high-temperature molecular dynamics[19] and is displayed in Figure 1. Sodium ions were inserted by randomly replacing water molecules. Due to their larger size, acetate ions were inserted by replacing random waters and the nearest neighboring water molecule. The system was then relaxed with 100 steps of steepest descent minimization.

The peptide and ion force field incorporated the latest OPLS[30,31] nonbonded parameters in combination with the CHARMM[32] bonded parameters. A flexible SPC water model[33] was used for the solvent with a correspondingly conservative time step of 0.5 fs and the velocity Verlet algorithm for the integration of the equations of motion. All electrostatic interactions were calculated by using the Ewald procedure,[21,34] thereby avoiding the problems associated with the use of electrostatic cutoffs. More details of our implementation of the Ewald procedure for these calculations can be found in the Appendix of ref 16.

Initial velocities were assigned from a Maxwell–Boltzmann distribution at 300 K. The system was allowed to evolve for 20 ps with intermittent reassignment of velocities. An additional 80 ps of equilibration were then performed in the microcanonical (NVE) ensemble. Finally, 650 ps of production were performed, making a total of 750 ps.

Table I. Simulation Details

no. of peptide atoms	52
no. of waters	402
no. of NaAcO	9
total no. of atoms	1303
box length, nm	2.417
total simulation time, ps	750
averages	
total PE, kJ/mol	−25680
total KE, kJ/mol	5053
water KE, kJ/mol	4677
DPDPE KE, kJ/mol	200
ion KE, kJ/mol	176
interwater PE, kJ/mol	−15190
intrawater PE, kJ/mol	2851
DPDPE PE, kJ/mol	−37
DPDPE–water PE, kJ/mol	−801
DPDPE–ion PE, kJ/mol	−2248
ion–water PE, kJ/mol	−7514
ion–ion PE, kJ/mol	−2740
water temp, K	311
DPDPE temp, K	308
ion temp, K	313
total temp, K	311
Na^+ diffusion const, $m^2\ s^{-1}$	0.7×10^{-9}
AcO^- diffusion const, $m^2\ s^{-1}$	0.6×10^{-9}

III. Results

Properties and thermodynamic averages obtained from the simulation are presented in Table I. Most noticeable was the large peptide–ion interaction, which far exceeded the peptide–water (solvation) energy. The main reason for this large interaction energy was the association of several ions, in particular

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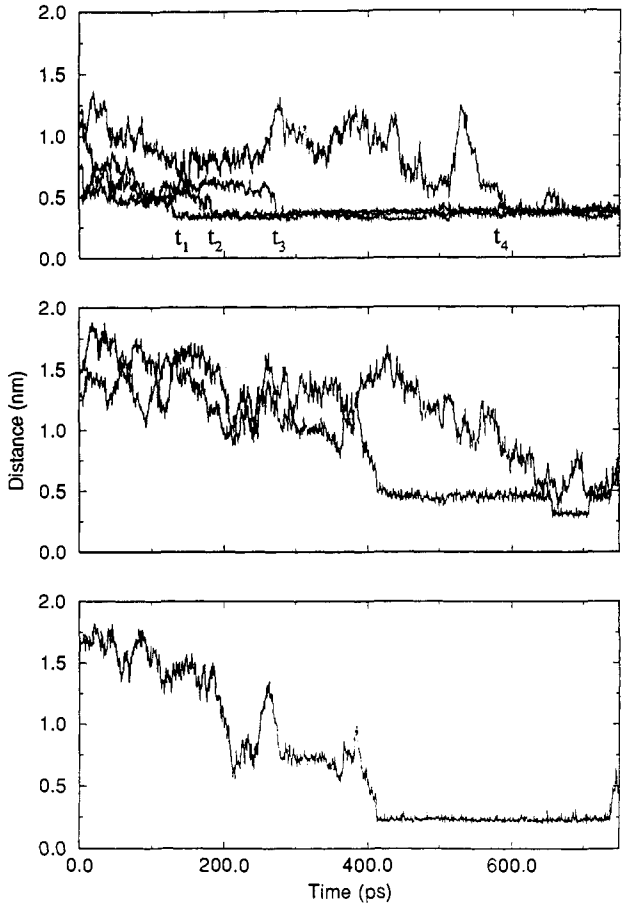

Figure 2. Distance time histories: (a, top) selected acetates to the N terminus (binding of four acetate ions to the peptide occurred at t_1, t_2, t_3, and t_4); (b, middle) selected sodiums to the C terminus (significant interaction with the peptide occurred between 400 and 730 ps, with direct binding to one of the C terminal oxygens occurring after 650 ps and lasting for 60 ps); (c, bottom) selected sodium to the carbonyl oxygen of Phe 4 (binding of sodium ion to carbonyl oxygen occurred around 400 ps; the ion remained bound for over 300 ps and then dissociated from the peptide around 730 ps).

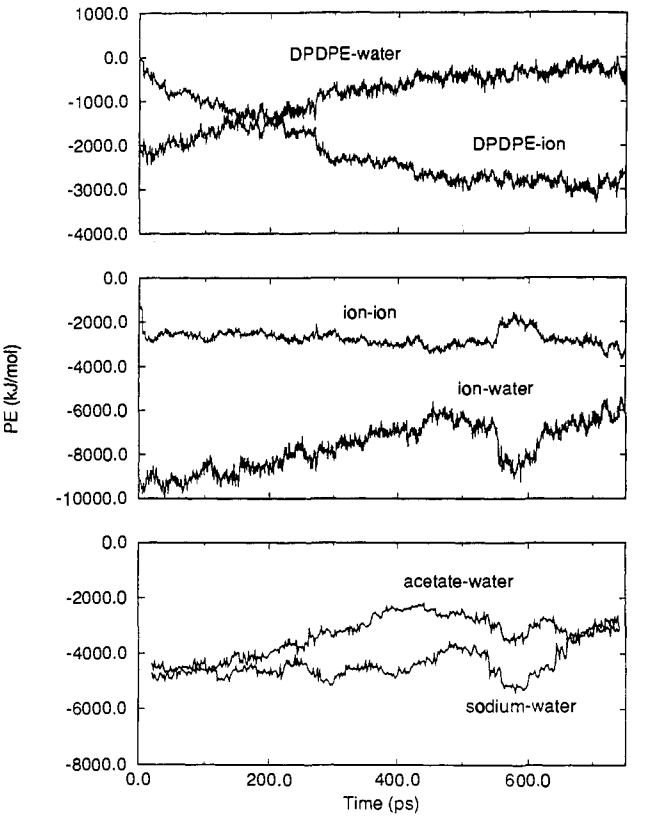

Figure 3. Potential energy time histories. Association of the fourth acetate ion occurred between 550 and 620 ps (b, middle). Corresponding changes in sodium and acetate ions' solvation can be seen in part c (bottom).

acetate ions, to various groups of DPDPE. In this respect the simulation was very similar to a previous simulation employing sodium chloride as the added salt,[17] and in which several chlorides bound to the peptide.

Figure 2 displays some selected time histories for the distances between various ions and different atoms of the peptide. In Figure 2a the distance between the central carbon of the acetate ion and the nitrogen of the N terminus is plotted. Four of the acetates associated directly with the N terminus of DPDPE. Binding of an acetate ion occurred after 130, 180, 270, and 590 ps. Once bound, none of the acetates were exchanged over the period of the simulation.

The occurrence of ion pairs between acetate ions and the N terminus may be somewhat surprising considering the calculations of Jorgensen.[35] Jorgensen has computed the potential of mean force (pmf) between $MeNH_3^+$ and $MeCO_2^-$ in solution using the OPLS parameters for the two ions and the TIP4P model for water. In these calculations no stable contact minimum, or solvent separated minimum, was observed.[35] However, it should be noted that during the calculation the ion pair was constrained to adopt a linear MeN–CMe configuration. None of the ion pairs observed in the present simulation adopted such an arrangement.

In Figure 2b the distance between two of the sodium ions and the carbon of the C terminus is displayed. These two sodiums were the only sodiums that appeared to have an interaction with any of the atoms of DPDPE. Both of the sodiums diffused toward the negatively charged C terminus and one of them appeared to interact significantly with DPDPE between 400 and 730 ps. Between 650 and 710 ps this ion then interacted very strongly with the C terminus. To illustrate more clearly the ion–peptide structures involved, the corresponding distance between the sodium ion and the carbonyl oxygen of Phe 4 is displayed in Figure 2c. As one can see the sodium ion bound to the carbonyl oxygen. The ion adopted a linear C=O—Na arrangement, as observed for analogous complexes in the gas phase.[36] After 650 ps the same sodium also bound directly to one of the two terminal oxygens, being approximately 2.1 Å away from an oxygen of the terminal carboxyl group and 2.5 Å from the carbonyl oxygen. The sodium ion then dissociated from the peptide after 730 ps. In comparison, no sodium ions bound to any atoms of DPDPE during an equivalent sodium chloride simulation.[17]

The potential energy time histories describing the association process are shown in Figure 3. From Figure 3a it is clear that there was a gradual decrease in the degree of solvation of DPDPE by water, which was accompanied by a concomitant increase in the peptide–ion interaction energy. From this plot it also appears that convergence in the distribution of ions may require 500–1000 ps of simulation. In Figure 3b the corresponding ion–ion and ion–water potential energies are displayed. The ion–water energy gradually weakened as ions shed their solvation shells in order to interact more favorably with the peptide. The feature observed between 550 and 620 ps is particularly interesting and corresponded to the association of the fourth acetate ion (Figure 2a). A configuration possessing relatively poor ion–ion and relatively large ion–water interactions persisted for 70 ps and then collapsed. This configuration appeared to involve just ions and water as there was no noticeable variation in the peptide–ion

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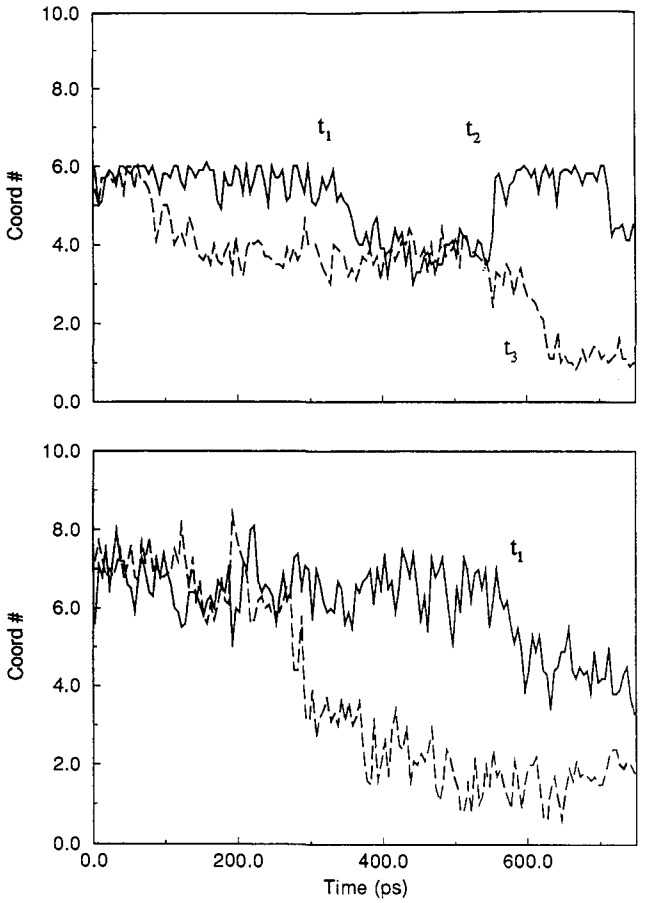

Figure 4. Coordination number time histories for (a, top) sodium ions and (b, bottom) acetate ions. In part a the solid line corresponds to a sodium ion which formed a sodium–acetate ion pair in solution. Formation and dissociation of the ion pair occurred at t_1 = 350 ps and t_2 = 550 ps, respectively. The dashed line corresponds to the sodium ion which formed the $Na(OAc)_3^{2-}$ complex at around 590 ps (t_3) (also see t_1 in part b). In part b the solid line corresponds to the last acetate ion to bind to the peptide. The formation of the $Na(OAc)_3^{2-}$ complex occurred at t_1. The dashed line corresponds to the acetate which bound to both the N terminal hydrogens and the backbone NH groups.

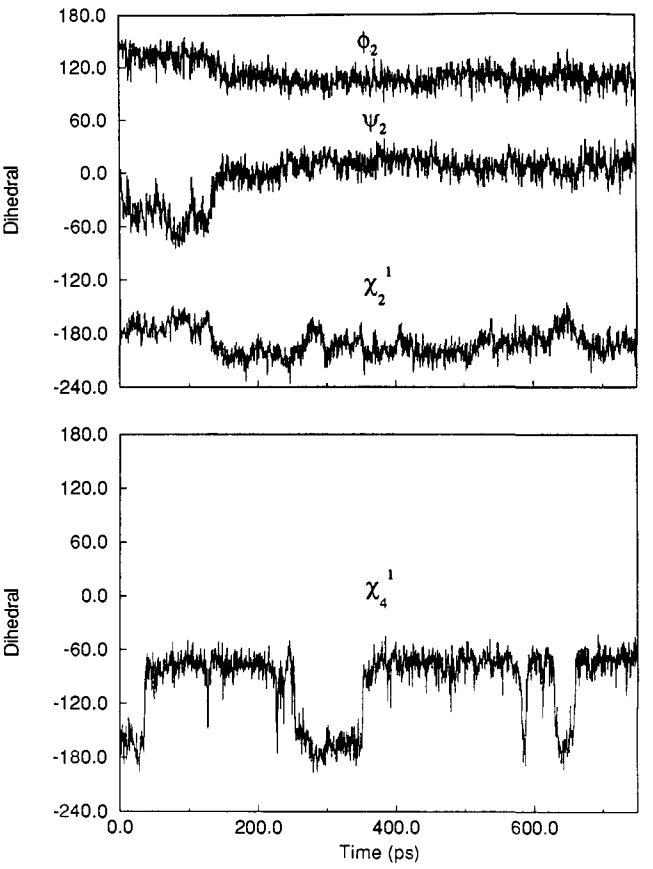

Figure 5. Dihedral angle time histories for DPDPE. (a, top) Rearrangement of the peptide structure near the N terminus upon binding of the first acetate can be seen at 130 ps. (b, bottom) Conformational mobility of the Phe 4 side chain.

interaction energy. From Figure 3c it is apparent that this configuration involved the rearrangement of both sodium and acetate ions which became more heavily solvated in the new configuration. The large change in the ion–water interaction energy appeared to be the result of two unconnected rearrangements which, purely by coincidence, occurred at the same time.

The first rearrangement involved the breaking of a sodium–acetate ion pair in the bulk of the solvent far away from the peptide. This is shown in Figure 4a, where we have plotted the coordination number of individual sodium ions as a function of time (defined as the number of water oxygens within a distance equal to the first minimum in the corresponding radial distribution function). The ion pair was initially formed around 350 ps and then broke up around 550 ps. The same sodium then diffused away and formed another sodium–acetate ion pair with a different acetate ion, around 700 ps. Obviously, when an unlike ion pair is destroyed the degree of ion solvation is increased and the ion–ion interaction is diminished.

The second ion rearrangement was far more intricate and involved many ions close to the peptide. The fourth acetate ion approached the N terminus as a sodium–acetate ion pair between 550 and 600 ps. On reaching the peptide N terminus the ions separated for approximately 50 ps (see Figure 4, a (dashed) and b (solid)). During this time the sodium ion penetrated the remaining solvation shells of the two acetate ions already bound to the N terminus. When it had done so, the sodium ion and the three acetate ions collapsed to form a $Na(OAc)_3^{2-}$ complex which remained bound to the terminus of the peptide. As this occurred toward the end of the simulation it is not clear whether this moiety was stable while bound to the N terminus or whether the ion complex would then have diffused away into the solution.

Also shown in Figure 4b is the coordination number of the single acetate which bound to both the N terminal hydrogens and the backbone NH groups. This occurred around 270 ps, and once the acetate was bound to the peptide it possessed virtually no waters of solvation.

In order to understand what happens to the peptide as a result of these specific ion interactions, we have displayed the time histories of the conformationally flexible dihedrals of DPDPE in Figure 5. On binding of the first acetate at 130 ps, there was a significant rearrangement of the peptide structure in the vicinity of the N terminus. The rearrangement corresponded not to any major dihedral transitions but rather to subtle conformational changes. The major result of these changes was to reverse the orientation of the N terminus so it moved from a position pointing directly out into solution (Figure 1) to one in which it pointed back toward the macrocycle (Figure 6). The rest of the macrocycle displayed no conformational transitions, in accord with the constrained nature of the ring[19] and previous dynamics simulations.[16,17] This conformation is similar to the conformations found in a quenched high-temperature dynamics conformational search.[29] There was still significant conformational mobility in the Phe 4 side chain as seen in Figure 5b.

The final structure of DPDPE after 750 ps of simulation in sodium acetate solution is displayed in Figure 6. A consequence of acetate binding appears to have been the shielding of the NH groups on one side of the molecule from potential solvating water molecules. By so doing, the molecule plus bound ions then possessed a polar face containing carbonyl groups and a nonpolar

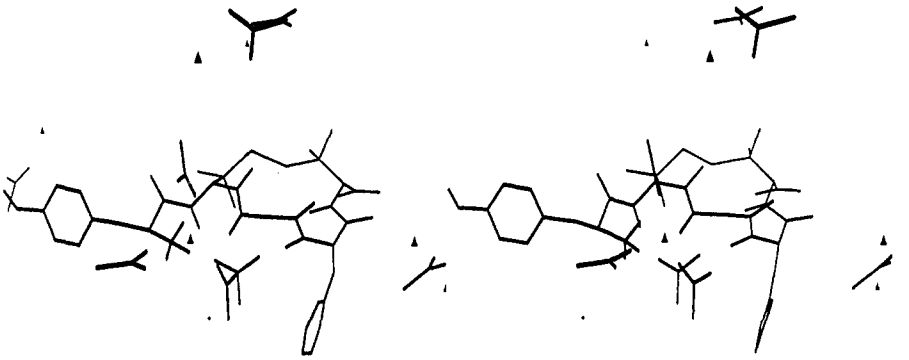

Figure 6. Stereoview of the final concentration after 750 ps. Waters are removed for clarity.

Table II. Radial Distribution Function Analysis

rdf	peak positions	integrated to	coord no.
WO–WO	2.72, 4.56	3.32	4.2
WO–WH	1.72, 3.24	2.40	1.7
WH–WH	2.36, 3.82	2.96	5.0
Na^+–WO	2.28, 4.36	3.08	5.2
Na^+–WH	2.96, 5.08	3.74	14.2
Me–WO	3.84		
Me–WH	3.76		
C–WO	3.48, 5.20	4.08	6.8
C–WH	2.48, 3.92	3.08	5.3
O–WO	2.60, 4.56	3.12	2.7
O–WH	1.60, 3.04	2.32	2.6

lower face containing the methyl of an acetate ion together with the phenyl ring of Phe 4 and the dimethyl groups of the two (D)Pen residues. A similar amphiphillic structure is seen in aqueous solution simulations and modeling,[19,29] albeit *via* a different mechanism for stability. This could have important consequences for the binding of DPDPE to the δ opioid receptor.

During the simulation (100–200 ps) we calculated radial distribution functions (rdfs) and velocity autocorrelation functions in order to characterize the ion–solvent medium. The rdfs between the ions and water are presented in Table II (WO = water oxygen, WH = water hydrogen). The ion–water rdfs compare very favorably with the original Monte Carlo parametrizations,[31,37] and the results from previous simulations.[16,17] The rdfs between the acetate ions and water are displayed in Figure 7. Again, these compare favorably with previous results,[31] even though the water model employed is slightly different (SPC *vs.* TIP4P). One interesting feature concerns the rdf between the central carbon of the acetate ions and both water oxygens and the water hydrogens (Figure 7b). This carbon carries a charge of +0.70 in the OPLS parametrization and yet the closest atoms of water to this center were the hydrogens which are also positively charged (+0.41). It appears that the solvation shell surrounding each of the carboxylate oxygens dominated the water distribution around the carbon center as well.

Figure 8 shows the normalized velocity autocorrelation functions of the ions as defined by

$$C(t) = \frac{\langle v(t)\cdot v(0)\rangle}{\langle v(0)\cdot v(0)\rangle} \qquad (1)$$

where $v(t)$ is the velocity of an atom at time t and the angular brackets denote an average over individual ions and time origins. The corresponding power spectra (spectral densities) are also displayed in Figure 8 and were obtained *via*

$$I(\omega) = \int_0^\infty C(t) \cos \omega t \, dt \qquad (2)$$

and subsequently normalized to unit area. The peaks observed

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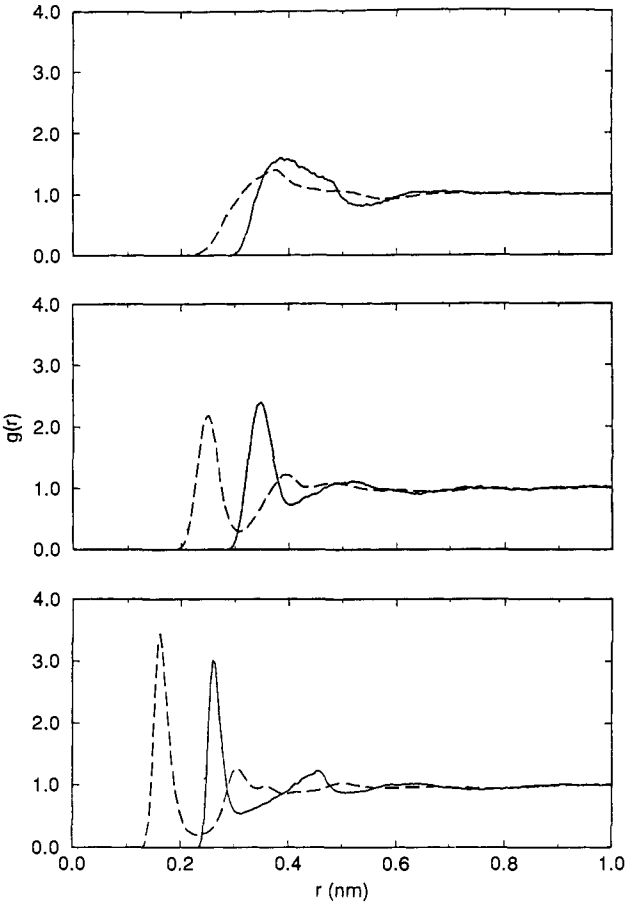

Figure 7. Radial distribution functions between acetate ions and water: (a, top) Me–WO (solid) and Me–WH (dashed); (b, middle) C–WO (solid) and C–WH (dashed); (c, bottom) O–WO (solid) and O–WH (dashed).

in Figure 8b at 1400 and 1530 cm^{-1} correspond to the bond stretching modes of the acetate ion, while the peaks at 570 and 770 cm^{-1} can be assigned to the angle bending modes.

IV. Discussion and Conclusions

A zwitterionic pentapeptide (DPDPE) has been simulated for 750 ps in 1.0 M sodium acetate solution. During the simulation four acetate ions associated closely with the N terminus of the peptide and several NH groups of the peptide backbone. Some binding of sodium ions to the backbone carbonyl oxygens close to the C terminus was also observed, but the majority of sodium ions remained either free or as sodium–acetate ion pairs in solution. The association of the acetate ions had a subtle but significant effect on the conformation of the peptide around the N terminus, and these types of effects should be considered when trying to interpret NMR observations for example.[18]

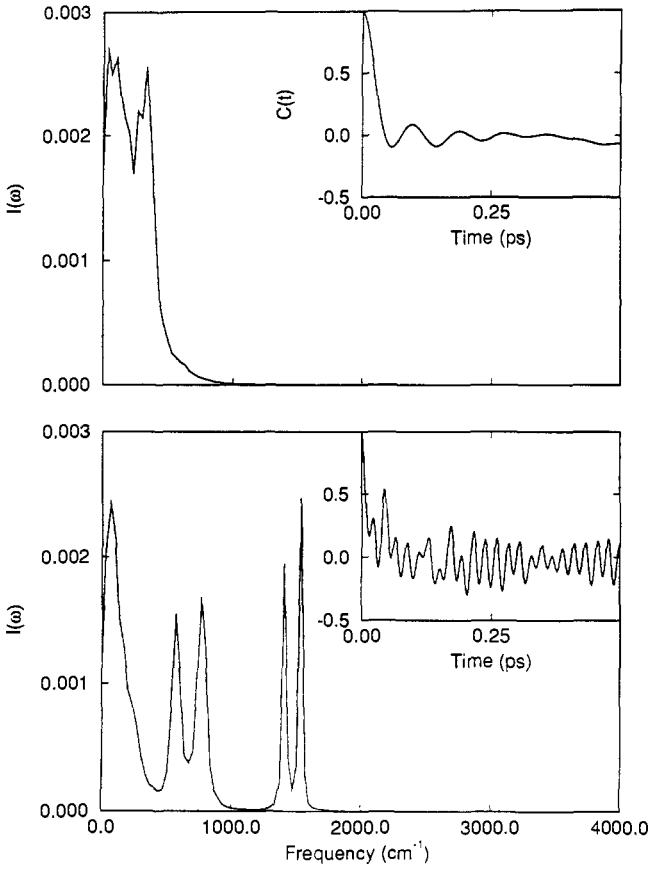

Figure 8. Power spectra and velocity autocorrelation functions (inset) for (a, top) sodium ions and (b, bottom) acetate ions. Spectra have been normalized to unit area.

In terms of the binding/preferential exclusion model discussed in the introduction, the sodium ions appear to be fairly well excluded from the peptide surface, although binding is not totally eliminated. In contrast, the acetate ions bind significantly to the peptide. In comparison with simulations involving NaCl,[17] the number of acetates bound to the peptide is reduced in accordance with the experimental observation that acetate ions have a larger preferential exclusion than chloride ions.[14] Obviously, this observation is relative as both ions demonstrate some appreciable equilibrium binding to the peptide with the current models.

Sodium is known to be a structure maker, diffusing with a bound solvation shell. Structure making increases with decreasing ionic radius for the larger ions. The larger acetate ions which do not bind water as tightly as the sodium ions are more willing to exchange interactions with water molecules for direct interactions with the peptide. This is similar to the case found in the analysis of the pair interaction energies in a recent peptide–NaCl simulation.[17]

It is not completely warranted to make too many direct comparisons of the experimental effects of salts on the solubility and stability of proteins as described by the Hofmeister series with these results. For instance, the observed binding of anions to DPDPE would be altered significantly if a non-zwitterionic form (*e.g.* at nonneutral pH) of the peptide was used for the simulations. While there are charged residues on the surface of globular proteins to which ions could bind, they do not often occupy such a high percentage of the available surface area as they do in the case of DPDPE. Our results suggest ion binding would still occur because of the observed strong affinity of the anions for the backbone NH groups and not just the N terminal hydrogens. Given interaction model uncertainties this is open to question. Another consideration is the relatively small size of DPDPE which may not be completely representative of many of the properties associated with globular proteins. In particular, all the backbone NH groups of DPDPE are initially exposed to solvent, whereas in most globular proteins, these groups usually participate in the secondary structure hydrogen bonding network. In this respect DPDPE might be closer to a denatured protein, and therefore the correspondingly weaker binding (larger preferential exclusion) available in the native form could explain why chloride and acetate ions stabilize the native form of proteins. Yet the constraining disulfide bond in DPDPE restricts the conformational space just as secondary structure restricts short loops exposed on the surface of proteins. Clearly, more work has to be done in order to separate the various effects before one can fully understand the complex processes at work in these systems.

Acknowledgment. The authors thank the Robert A. Welch Foundation and the National Institutes of Health for partial support. G.M. thanks the Keck Center for Computational Biology for a Keck fellowship. We are also grateful to the San Diego Supercomputer Center for generous use of their Cray Y-MP, the NSF chemical instrumentation program for local computing facilities, and the CRPC for experimental computing time and software advice.