Salt Effects on Peptide Conformers: A Dielectric Study of Tuftsin

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ABSTRACT Four 1-ns molecular dynamics computer simulations of tuftsin, Thr-Lys-Pro-Arg, are analyzed: (1) *cis* tuftsin in water, (2) *trans* tuftsin in water, (3) *cis* tuftsin in 1 M NaCl, and (4) *trans* tuftsin in 1 M NaCl. Independently of the salt concentration, the *trans* conformer has a higher dielectric constant than the *cis* conformer because the former exhibits a more widely distributed charge distribution in space. Independently of the peptide conformation, the presence of salt reduces the dielectric constants of both the peptide and the solvating water molecules because ions, on binding, restrict the motion of other atoms. In contrast to the dielectric constants, neither the peptide conformation nor the salt concentration shows a significant influence on the dielectric relaxation time of water molecules.

INTRODUCTION

The dielectric response of biomolecules is a property of fundamental importance in biochemistry and biophysics (Grant et al., 1978). The dielectric constant inversely scales the strength of electrostatic interactions that play a crucial role in the formation, stability, and function of nucleic acids, proteins, peptides, biological membranes, lipid bilayers, and micelles.

The so-called dielectric "constant" is actually a spatially varying and frequency-dependent quantity in most systems. One striking example of an inhomogenous and anisotropic system is the bacterial photosynthetic reaction center. Two possible pathways for electron transfer are nearly equivalent in structure, as discovered by x-ray crystallography (Deisenhofer et al., 1984, 1985), but are different by a factor of ~3 in dielectric constant, as determined by Stark-effect spectroscopy (Steffen et al., 1994). The experimentally observed unidirectionality of the light-driven electron transfer in the photosynthetic reaction center is believed to be caused by the dielectric asymmetry in the protein complex (Steffen et al., 1994). The dielectric constant influences the barrier height for quantum-mechanical tunneling of electrons in a number of biological systems (Steffen et al., 1994; DeVault, 1984).

One should note, however, that the dielectric constant (or the zero frequency and wave vector limit of the dielectric response) is fundamentally a macroscopic concept, which is most meaningful for systems large enough to reach the thermodynamic limit (Jackson, 1975). Therefore, the use of a dielectric continuum model on the molecular level is an approximation (Pollock et al., 1980; Rogers et al., 1985). Aspects of the approximation of considering a protein as a uniform dielectric medium with a single dielectric constant have been discussed in the literature (Churg and Warshel, 1986). In recent studies, the concept of the dielectric constant of a protein still remains somewhat unclear (Simonson and Perahia, 1995b).

Despite the ambiguities associated with the concept, the dielectric constant is the basis of a number of computationally convenient approximations for free energies in condensed phases. Consequently, a number of experimental and theoretical estimates have been made for the dielectric constant of a protein, with suggested values ranging from 2 to 100. Values toward the lower end of the range have been estimated from atomic polarizabilities (Pethig, 1979), normal mode analysis (Gilson and Honig, 1986), and Stark-effect spectroscopy (Steffen et al., 1994), whereas values greater than 20 have been extracted from dielectric dispersion at radio frequencies (South and Grant, 1972; Grant et al., 1978) and from measurements of changes in redox potentials (Rogers et al., 1985) and of values of pK_a of ionizable groups (Russell and Fersht, 1987; Goldenberg, 1992) in site-directed mutagenesis.

Site-directed mutagenesis experiments characterize the dielectric constant of a protein by an energy reduction factor for charge–charge interactions in the system (Rogers et al., 1985; Russell and Fersht, 1987; Goldenberg, 1992). At least some of the dielectric effect measured in this way is believed to be coming from water around the protein and not from the interior of the protein, regardless of whether there is protein or water between the two groups involved in mutagenesis (Churg and Warshel, 1986; Russell and Fersht, 1987). The value for the effective dielectric constant thus obtained is typically thought to be larger than the actual contribution from the protein itself.

Alternatively, one can formally view the dielectric constant of a protein as related to its dipole fluctuation (Kirkwood and Shumaker, 1952), which can be computed from simulations (Smith et al., 1993; Simonson and Perahia, 1995a). Such calculations are formally based on the relaxation properties of the protein, i.e., on its induced polarization shift on introduction of a vanishing external field or on its relaxation in response to perturbing charges (Simonson and Perahia, 1995b). According to this Fröhlich–Kirkwood-type theory of dielectrics, a low dielectric constant of a

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protein core emphasizes that the core is nonpolarizable but not necessarily nonpolar. This is in contrast to a dielectric analysis of charge distributions at equilibrium of an unrelaxed protein (Churg and Warshel, 1986).

Smith et al. recently pointed out, through studies using molecular dynamics (MD) computer simulations, that, whereas the protein backbone gives rise to a dielectric constant of approximately 2–3, the polar and charged sidechains increase the overall dielectric constant of a protein to ~30 (Smith et al., 1993). Such results were based on simulations on the nanosecond time scale, which is much longer than those in earlier simulation studies of protein dielectric properties (King et al., 1991; Treutlein et al., 1992) and more nearly comparable with time scales used for water dielectric studies (Neumann, 1986). Those findings were later qualitatively confirmed by nanosecond MD simulations of other proteins (Simonson and Perahia, 1995a). Similarly, Yang et al. found for nucleic acids that, whereas the bases and sugars give rise to a dielectric constant of approximately 2–3, the charged phosphates increase the overall dielectric constant of a triplex DNA molecule to ~16 (Yang et al., 1995).

In continuum model studies of biomolecules in solution using a variant of the Poisson–Boltzmann (PB) method (Kirkwood, 1934; Tanford, 1957; Gilson, 1995; Madura et al., 1995; Honig and Nicholls, 1995), one typically assigns a dielectric constant for the solute volume or region and another for the solvent region. For example, the dielectric constants of the solute and the solvent can be set to 1 and 80, respectively, such as in a recent study of alanine dipeptide (Marrone et al., 1996). Considering the recent developments in MD simulations (Smith et al., 1993; Simonson and Perahia, 1995a), it has been suggested that the protein bulk be treated as a low-dielectric medium and the charged surface groups be treated either as part of the solvent region or as a third region intermediate between the solute and the solvent regions (Simonson and Perahia, 1995a). Whether such a scheme of assigning dielectric constants in the PB equation will increase the accuracy of existing predictions for biomolecular systems is an open question. However, the role of the dielectric constant in the stability of folded proteins (Murphy, 1995; Lins and Brasseur, 1995) and DNA helices could be more conveniently studied with approximate theories based on the continuum representations if accuracy could be improved.

Methods of incoporating nonlinearities in the solvent response constitute an avenue for the improvement of continuum theory predictions. This was recognized and addressed as early as in the 1950's by Booth (1951) and Schellman (1957). Nonlinear effects arise from the saturation of the orientational polarizability of the solvent dipoles, a phenomenon referred to as dielectric saturation, and from electrostriction, which refers to the crowding of polar solvent molecules close to a charged solute. Attempts to account for dielectric saturation within the continuum scheme abound in the literature (Beveridge and Schnuelle, 1975; Abraham et al., 1979; Ehrenson, 1987). Whereas dielectric saturation effectively decreases the dielectric constant, electrostriction has the opposite effect. The work of Jayaram et al. (1989) explored the magnitudes of the roles of these two phenomena in the context of ion hydration. Although we recognize the relevance of an analogous analysis for peptide hydration, convergence issues prevented us from addressing nonlinearities in the solvent response in the present study.

Another issue that complicates the dielectric study of biomolecular systems is the treatment of ions; ions are often necessary in physiological systems. The finite contribution of water molecules to the overall dielectric constant of an ionic solution must be carefully separated from the infinite contribution of the ions (Jackson, 1975; Stillinger and Lovett, 1968; Yang et al., 1995). After the infinite contribution from the ions is removed, the static dielectric constant of water in pure electrolyte solution can be measured experimentally (Franks, 1973).

One important finding of the experiments (Franks, 1973) is the so-called "dielectric decrement," i.e., the decrease of the dielectric constant of water as the ionic strength increases. This decrease occurs because ions restrict the motion, thus reducing the dipole fluctuation, of water molecules. Extended Debye–Hückel theories (Harned and Owen, 1958) and integral equation theories (Levesque et al., 1980; Kusalik, 1987; Perkyns and Pettitt, 1992) have successfully reproduced the trends of the dielectric decrement of water in the presence of ions. However, the phenomenon of the dielectric decrement of water as a result of the addition of ions in a solution has not been quantified in computer simulations of salt water.

The difficulties associated with the infinite contribution of ions mentioned above complicate the calculation of the dielectric constant of water in ionic solutions from computer simulations. Such formal obstacles associated with ions can be removed by use of the concept of group dielectric constants in multicomponent systems (Yang et al., 1995). By the decomposition of the overall dielectric constant of a system into approximate group dielectric constants one can obtain the intrinsic dielectric properties of the water molecules in an ionic solution. In fact, individual chemical species can be further categorized by proximity criteria (Mehrotra and Beveridge, 1980) for the distribution functions. It was found from computer simulations that the solvating water molecules that surround a triplex DNA in ionic solution have a much lower dielectric constant than that of pure water (Yang et al., 1995). However, from such a combined effect of the DNA and the ions it is difficult to identify the net effect that is due solely to the ions.

Here we continue to study the dielectrics of biomolecules in ionic solutions from a microscopic perspective. One interesting problem is the isolation of the local dielectric response near charged residues in proteins and polypeptides. In large proteins composed of long amino acid chains, however, the fraction of charged residues is typically small. Within this already small fraction of charged residues, ones very close to each other in sequence space are not common, thus increasing the statistical difficulty for the above-men

tioned isolation of local effects. To focus on local properties around charged amino acid residues we select a high-charge-density peptide to study the dielectric response near it in solution. Our results should also provide hypotheses about the dielectric response near charged residues in larger proteins and other systems.

The peptide tuftsin was modeled as a zwitterion and has an amino acid sequence of Thr-Lys-Pro-Arg. It is a biologically essential fragment of leukokinin (residues 289 to 292). More-detailed descriptions of the biological significance of tuftsin can be found in the primary literature (Valdeavella et al., 1995) and in a recent review (Nishioka et al., 1996).

We present a comparative study of distinct conformations of tuftsin in different salt environments to illustrate the conformation dependence of and the salt effects on peptide dielectrics. The dielectric response in the peptide–water–ion complex is examined by computer simulations at the atomic level. In the next section we briefly review the dipole fluctuation formulas used and the concept of group dielectrics. The simulation protocols are also sketched. In the third section the results of our study of four separate 1-ns simulations are presented. We follow with discussions and conclusions.

METHOD

We define the dielectric constant of the kth group (ϵ_k) within a system surrounded by an infinite dielectric medium that has a reaction-field dielectric constant of ∞ (conducting tin-foil boundary) as (Smith et al., 1993; Yang et al., 1995)

$$\epsilon_k = 1 + \frac{\langle \mathbf{M}_k^2 \rangle - \langle \mathbf{M}_k \rangle^2}{3\epsilon_0 V_k k_B T}, \quad (1)$$

where

$$\mathbf{M}_k = \sum_{i=N_{k-1}+1}^{N_{k-1}+n_k} q_i \mathbf{r}_i. \quad (2)$$

Here $\mathbf{M}_k$ and V_k are the dipole moment and the effective partial molar volume (Yang et al., 1995), respectively, of the kth group of particles in the system. ϵ_0, k_B, and T are the electric permittivity of vacuum, the Boltzmann constant, and the absolute temperature of the system, respectively. In Eq. 2, q_i and $\mathbf{r}_i$ are the partial charge defined in the atomic-level computer simulation and the position vector of the ith particle, respectively. The summation in Eq. 2 is over the n_k particles in the kth group. The subtotal of particles in the first k groups is $N_k = \Sigma_{l=1}^{k} n_l$. For $k = 1$, Eq. 2 requires the value of $N_{k-1} = N_0$, which is defined to be zero. This is an extension of the formulas for a pure fluid (Neumann et al., 1984). Cross-terms must be shown to be small for any particular decomposition a postiori (Yang et al., 1995).

To obtain the total dielectric constant of an inhomogeneous system composed of several chemical components, we then simply calculate $\epsilon_{k=1}$ in Eq. 1 while defining the entire system as one group in Eqs. 1 and 2.

Four MD simulations are analyzed in this study: 1) *cis* tuftsin in water (0 M NaCl), 2) *trans* tuftsin in water (0 M NaCl), 3) *cis* tuftsin in 1 M NaCl, and 4) *trans* tuftsin in 1 M NaCl (Valdeavella et al., 1995). The 49-atom (united-atom model) tuftsin molecule was placed in a (25-Å)^3 periodic box in all simulations. There were 468 water molecules in both of the 1 M NaCl simulations (9 Na^+ ions and 11 Cl^- ions). There were 475 and 457 water molecules, respectively, in the *cis* and *trans* simulations in 0 M NaCl (2 Cl^- ions only).

These simulations employed CHARMM 20 bonded (Brooks et al., 1983) and OPLS nonbonded parameters (Jorgensen and Tirado-Rives, 1988) for the peptide (united-atom model), OPLS parameters for the ions (Chandrasekhar et al., 1984), and SPC/E parameters for the water molecules (Berendsen et al., 1987). We integrated Newton's equations of motion, using a 2-fs time step in the velocity Verlet algorithm coupled with SHAKE bond constraints and the Ewald method for calculating long-range electrostatic interactions (Allen and Tildesley, 1987). The initialization (random placement of ions) and equilibration (50 ps) procedures and other details of the simulations can be found elsewhere (Valdeavella et al., 1995). The constant-energy production runs lasted ~1 ns in each simulation.

For the tuftsin simulations the particles can be partitioned into four groups: k = 1, 2, 3, 4 for the neutral (neutral part of peptide), charged (charged part of peptide), water, and ion groups, respectively. For the charged group we include the four charges in the peptide (Thr-Lys-Pro-Arg in the zwitterion form): +e from the amine terminus attached to Thr, +e from the sidechain of Lys, +e from the sidechain of Arg, and −e from the acidic terminus attached to Arg. This amounts to five atoms (CNH_3) from the Thr residue, five atoms (CNH_3) from the Lys residue, and eighteen atoms from the Arg residue (i.e., the entire residue) when we use the partial charge assignments in the force field employed. Thus, the total number of atoms in the charged group is 28. The remaining 21 atoms in tuftsin are sorted into the neutral group.

RESULTS

Group partitioning of the total dipole moment fluctuations

For the simulation of the *trans* tuftsin in 1 M NaCl we calculated, and present in Fig. 1, cumulative fluctuation of the group dipole moments, i.e., $\langle \mathbf{M}_k^2 \rangle - \langle \mathbf{M}_k \rangle^2$ in Eq. 1 as a function of the amount of time used to calculate the fluctuation. As shown in Fig. 1 (*A–C*), the dipole fluctuations of the neutral part of the peptide, the charged part of the peptide, and water are more-or-less converged near the end of the simulation. To quantify approximately the statistical errors of such fluctuations, we calculated the root-mean-square deviations to be 1.2%, 3.0%, and 1.3% of the averages for the last segment of the trajectory between 1 and 1.225 ns and display the results in Fig. 1 (*A*) (neutral), (*B*) (charged), and (*C*) (water). The dipole fluctuation of the ions exhibits the expected diverging behavior, with a root-mean-square deviation of 11.0% of the average between 1 and 1.225 ns (Fig. 1 *D*), because ions are capable of forming infinitely polarizable cation–anion pairs by conduction (Yang et al., 1995). Fig. 1 (*D*) also includes a curve for the dipole fluctuation of all particles, labeled *All*. It is clear that the overall dipole fluctuation of the entire system is dominated by the contribution from the ions, as illustrated in Fig. 1 (*D*).

Table 1 presents the components of the mean-square deviation of the dipole fluctuation for the *trans* tuftsin simulation in 1 M NaCl. It is shown in Table 1 that the cross-term between two groups is much smaller than the larger of the two self-terms (diagonal terms) of the two groups involved. For example, the cross-term between the neutral group and the charged group (−1.2 for the mean-square deviation) is much smaller than the self-term of the

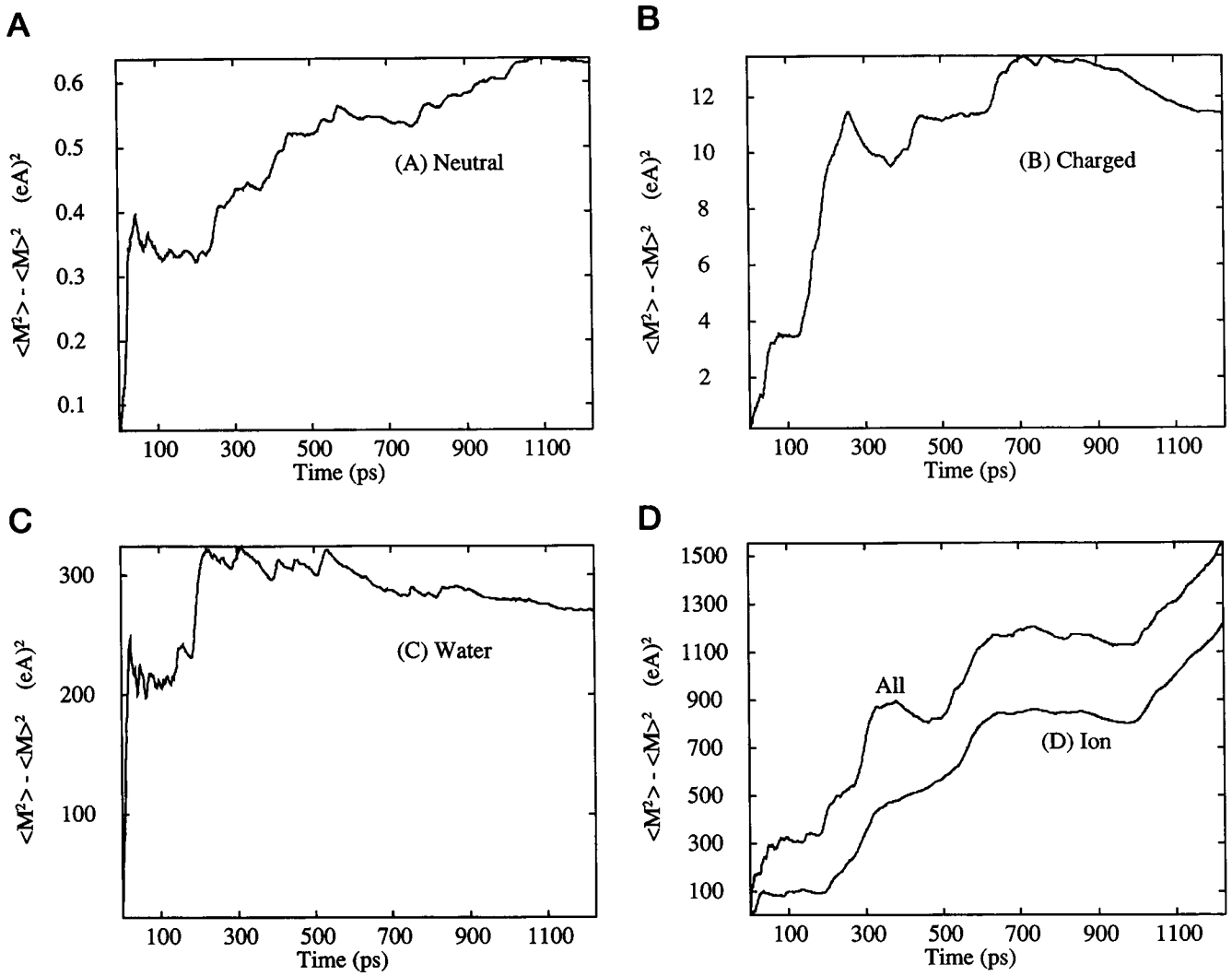

FIGURE 1 Cumulative mean-square deviation of the dipole moment as functions of time (*A*) for the neutral part of the peptide, (*B*) for the charged part of the peptide, (*C*) for the water molecules, and (*D*) for the ions and all particles in the *trans* tuftsin simulation in 1 M NaCl.

charged group (11.4). Therefore, it is reasonable to partition the combined dipole fluctuations of two groups and neglect the cross-terms. This observation on the relatively small size of the cross-terms is crucial in justifying the separation of the total dipole fluctuation according to groups or components. Thus, our partitioning of the system into the four groups (neutral, charged, water, and ion) chosen seems appropriate for the tuftsin–water–ion complex. The reader is referred to Yang et al. (1995) for more detailed discussions of the decomposition of dipole fluctuations in inhomogeneous systems.

The analysis of our simulation data for dielectric saturation can be achieved by further decomposition of the overall solvent response into contributions by solvent vicinal to the solute and the bulk solvent. This was not attempted here because of the lack of convergence of the dipole moment fluctuations of the solvent molecules in the vicinity of the peptide within the length of the simulation. An examination of the solvent residence time and the time history of the orientation of the water dipoles around selected functional groups on tuftsin reveals a lack of simple correlation between the saturation of the solvent dipole orientations and functional group polarity (Valdeavella et al., 1996).

Based on Fig. 1 *A* and *C* and according to Eqs. 1 and 2, we calculated the group dielectric constants to be 4 for the neutral part of peptide and 46 for the solvating water molecules in the *trans* tuftsin simulation in 1 M NaCl. We then applied these calculated dielectric constants as initial inputs to the PB equation (Valdeavella et al., 1996). The dielectric constant of the neutral part of the peptide was used to represent the solute dielectric continuum because the charged part of the peptide was treated explicitly in the PB calculations (King et al., 1991; Simonson and Perahia, 1995a). On refining the input dielectric constants to the PB equation, we found that using 2 for the solute dielectric constant and 45 for the solvent dielectric constant gives the best fit between the PB- and the MD-generated electrostatic potentials in space (Valdeavella et al., 1996). Such a test

TABLE 1 Components of the mean-square deviation of dipole moment (M) in units of (eÅ)^2 obtained from a nanosecond molecular dynamics simulation of the *trans* tuftsin, a tetrapeptide, with two Cl^- ions and 1 M NaCl in water

Group A	Group B	$\langle M_A M_B \rangle - \langle M_A \rangle \langle M_B \rangle$	$\langle M_A M_B \rangle$	$\langle M_A \rangle \langle M_B \rangle$
Neutral	Neutral	0.6	0.8	0.1
Charged	Charged	11.4	13.7	2.3
Water	Water	269.3	280.0	10.7
Ion	Ion	1215.6	2716.3	1500.8
Neutral	Charged	−1.2	−1.0	0.2
Neutral	Water	−0.5	−0.1	0.4
Neutral	Ion	−4.0	−9.2	−5.2
Charged	Water	2.1	2.3	0.2
Charged	Ion	−6.8	−43.5	−36.6
Water	Ion	38.4	116.5	78.1

suggests that the group dielectric constants calculated from MD simulations are in reasonable ranges for the expected qualitative behavior of electrostatic field calculations.

Table 2 presents the group dielectric constants calculated by use of Eqs. 1 and 2 for all four simulations: *cis* and *trans* tuftsin in pure water (0 M NaCl) and *cis* and *trans* tuftsin in 1 M NaCl. The group dielectric constant of ions is formally infinite, although Eq. 1 gives a large finite value for finite simulation times (Yang et al., 1995). Therefore, in Table 2 we have exclude the direct contribution from the ions. The numerical uncertainties for the dipole fluctuations in Fig. 1 (*A*)–(*C*) (relative errors of less than 3%) are transferable to the calculated values of the dielectric constant. However, a more accurate error estimate (e.g., from time block averages) of the data in Table 2 cannot be made because of the limited simulation time. Within the peptide, the main contribution to the dielectric constant clearly comes from the charged part of the peptide. Almost independently of the conformation and salt concentration, the dielectric constant of the neutral part of the peptide is approximately 2–4. This is in accord with earlier indications from MD simulations that the protein interior (Smith et al., 1993; Simonson and Perahia, 1995a) and the DNA base-sugar region (Yang et al., 1995) have dielectric constants of approximately 2–3. Inclusion of the charged part of the peptide increases the overall dielectric constant of the peptide to the range of 10–50, depending on the peptide conformation and the salt concentration. This finding is similar to those of previous studies of proteins (Smith et al., 1993; Simonson and Perahia, 1995a) and DNA (Yang et al., 1995), as discussed above.

TABLE 2 The dielectric constant ϵ obtained from nanosecond molecular dynamics simulations of the *cis* and *trans* tuftsin, a tetrapeptide, with two Cl^- ions in 0 M (pure water) and 1 M NaCl solution

	0 M		1 M	
Group	*cis*	*trans*	*cis*	*trans*
Neutral part of peptide	2	2	2	4
Charged part of peptide	36	91	15	46
Peptide	20	49	9	23
Water	68	60	48	46
Peptide and water	65	58	45	45

Conformation and salt dependence of the solute dielectric response

Our results indicate that the effects of solute conformation and added salt on the solute dielectric constant are decoupled. As shown in Table 2, the *trans* conformer has a larger dielectric constant than the *cis* conformer at the same salt concentration (49 and 20 in water and 23 and 9 in 1 M NaCl, respectively). This is because the *trans* conformer has a more widely spread distribution of charges in space; i.e., it is more polar, based on the calculated values of the average dipole moment ($\langle \mathbf{M}_k \rangle$) of both the *trans* and the *cis* conformations of the peptide (8.8 and 4.2 eÅ in water and 1.7 and 0.5 eÅ in 1 M NaCl, respectively). A more pronounced polarity for the *trans* conformer, coupled with comparable degrees of thermal motion for both conformations, leads to a larger dipole fluctuation for the *trans* configuration of the peptide. Note, however, that the necessary condition for a biomolecule to have a large dielectric constant is not that the biomolecule need be very polar; rather, the biomolecule needs to be very polarizable according to Eq. 1. Clearly, conformational flexibility is a major component of the charge motions in small peptides.

Excess salt reduces the group dielectric constant of the peptide. Ions impose restrictions on the motions of the atoms of the peptide molecule, leading to a lowered dielectric constant of the peptide at a higher salt concentration (from 20 in water to 9 in 1 M NaCl for *cis* tuftsin and from 49 in water to 23 in 1 M NaCl for *trans* tuftsin in Table 2).

Solvent dielectric response

The dielectric constants of the water molecules solvating the *cis* and the *trans* isomers are similarly reduced by excess salt (Table 2). As previously mentioned, the dielectric decrement of water in ionic solutions has been studied exper

imentally and theoretically. Ions impose restrictions on the translational and rotational motions of water molecules, thus lowering the dipole fluctuation of the proximal water molecules. Table 2 demonstrates that, under otherwise identical conditions, the dielectric constant of water is indeed much reduced on the addition of 1 M NaCl (from 68 to 48 around *cis* tuftsin and from 60 to 46 around *trans* tuftsin). Therefore, the present MD study independently reproduces that characteristic behavior, i.e., dielectric decrement of water in ionic solutions.

Finally, we calculated the temporal correlation functions of the dipole moment of the water molecules in the simulations. Sample correlation functions for the simulation of *trans* tuftsin in 1 M NaCl are presented in Fig. 2. We consider only three of the five equally partitioned time intervals (*2nd*, *3rd*, and *4th* in Fig. 2) of the nanosecond MD trajectory that have intermediate dipole correlation times. Data from the other two intervals that have the largest (1st) or the smallest (5th) dipole correlation time were discarded. For each curve in Fig. 2 we obtained an individual dipole correlation time by performing an exponential fit to the dipole correlation function between 1 and 6 ps (Yang et al., 1995). The averaged dipole correlation time based on Fig. 2 is presented in Table 3 together with the estimated statistical error (τ_ω = 8.5 ± 0.6 ps). Other values in Table 3 were generated in the same fashion for the other three simulations. For all simulations, the value of τ_ω obtained from a single curve for the dipole correlation function by use of the entire nanosecond MD trajectory is close to the average and within the bounds presented in Table 3.

Table 3 shows only minor differences for the dipole correlation times of water between different salt concentrations and peptide conformations, all within the range of statistical errors. These minor differences in Table 3, however, seem to indicate two trends: 1) increasing the salt concentration slightly reduces τ_ω (9.9 to 8.8 ps for *cis* and 8.9 to 8.5 ps for *trans*) and 2) changing the conformation from *cis* to *trans* also slightly reduces τ_ω (9.9 to 8.9 ps in water and 8.8 to 8.5 ps in 1 M NaCl). Increasing salt concentration decreases the strength of the electrostatic interactions (e.g., by means of the exponential damping factor of the Debye–Hückel type in the limit of low ionic strength), thus reducing the hindrance, among water molecules. (However, the dielectric decrement works to oppose this change in some sense.) Consequently, this leads to a faster dipole relaxation of water, i.e., smaller τ_ω. Similarly, changing the peptide conformation from *cis* to *trans* also decreases the strength of the electrostatic interactions among water molecules (because of the larger dielectric constant in the peptide region for the *trans* conformer, which has a larger dipole moment, as discussed above); this also reduces the hindrance among water molecules and leads to a smaller τ_ω. However, the magnitude of the relaxation-time differences among the four simulations is small compared with the statistical errors in Table 3. Thus, the data in Table 3 cannot provide completely unambiguous support for our proposed mechanism. We should mention that the experimentally measured dipole relaxation time of pure water (Franks, 1973; Hasted, 1973), 9.3–9.4 ps, is also within almost all of the four ranges listed in Table 3. Thus, the dielectric relaxation time of water shows only a modest sensitivity to the environment.

TABLE 3 Collective dipole correlation time (τ_ω) of water molecules obtained from nanosecond molecular dynamics simulations of the *cis* and *trans* tuftsin, a tetrapeptide, with two Cl^- ions in 0 M (pure water) and 1 M NaCl solution

	0 M		1 M	
	cis	*trans*	*cis*	*trans*
τ_ω (ps)	9.9 ± 0.7	8.9 ± 0.6	8.8 ± 0.8	8.5 ± 0.6

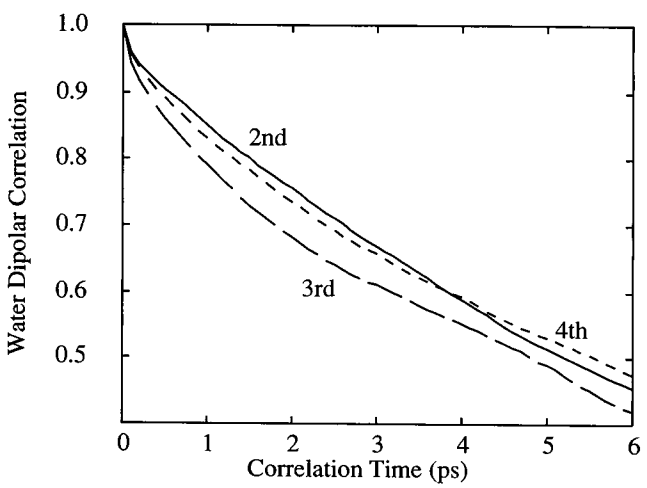

FIGURE 2 Temporal correlation functions of the dipole moment of water molecules in three of the five equally partitioned intervals (labeled *2nd*, *3rd*, and *4th*) of the 1225-ps *trans* tuftsin simulation in 1 M NaCl.

CONCLUSION

Group dielectric constants have been calculated for the peptide–water–ion complex by dipole fluctuations generated in atomic-level computer simulations. The overall dielectric constant of the peptide, tuftsin, is found to be in the range of 10–50, depending on the peptide conformation and the salt concentration. However, the group dielectric constant for the neutral part of the peptide remains in the range of 2–4 in all cases. Thus, the present study supports previously reported findings on the overall dielectric constant for a biomolecule and the dominance of the charged part of a biomolecule to its dielectric response. The values of group dielectric constants obtained in this study are reasonable based on tests that used the Poisson–Boltzmann method (Valdeavella et al., 1996).

Salt effects on the dielectric response of biomolecules and the solvating water molecules are significant. From atomic-level computer simulations we verified the experi

mental measurements and previous theories on the dielectric decrement of water molecules in ionic solutions. We found that the dielectric decrement that is due to ionic components applies not only to water molecules but also to charged biomolecules, independently of the conformation of the biomolecules in solution.

For a peptide conformation that has a more dispersed charge distribution in space, i.e., is more polar, the corresponding dielectric constant is larger. Note that the theory employed here requires only that the biomolecule be very polarizable, but not necessarily very polar, for there to be a large dielectric constant. The conformational variation of the dielectric constant is found to be independent of the ionic concentration, even though the absolute values are sensitive.

The dielectric relaxation time of water molecules can be calculated from simulations with a reasonable degree of accuracy. This conclusion is based on the relatively small statistical errors and the favorable comparison with available experimental data. Such a relaxation time, however, seems to be rather insensitive to the conformational change of the biomolecule or to the change in salt concentration on a modest scale.

Our choice of a short peptide enables one to identify the local dielectric response around charged residues, to perform multiple simulations on the nanosecond time scale with a modest demand for computer time, and produces information transferable to the studies of larger proteins and other biomolecules. The present study explores a conceptual dilemma in the concept of the dielectric constant of a biomolecule. On the one hand, the inhomogeneity within the biomolecule requires one to break the biomolecule into progressively smaller regions (fewer than 100 atoms) to describe the local dielectrics more accurately. On the other hand, the very concept of a dielectric constant dictates that each region of concern be individually large enough to reach the thermodynamic limit ($\omega = 0$, $\kappa = 0$); thus, a smaller region means less consistency with the concept of a dielectric constant and, consequently, a lowered reliability for conclusions reached by following such an approach. This should remain a concern for future studies of dielectrics of biomolecules when one is interpreting site-directed mutagenesis (and other experiments), molecular dynamics simulations, or Poisson–Boltzmann continuum modeling studies. Finally, we should note that our findings on the dielectric decrement of water and the dielectric relaxation time of water are unrelated to the above-mentioned dilemma.

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