Dynamic Equilibria of Short-Range Electrostatic Interactions at Molecular Interfaces of Protein–DNA Complexes

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Supporting Information

ABSTRACT: Intermolecular ion pairs (salt bridges) are crucial for protein–DNA association. For two protein–DNA complexes, we demonstrate that the ion pairs of protein side-chain NH$_3^+$ and DNA phosphate groups undergo dynamic transitions between distinct states in which the charged moieties are either in direct contact or separated by water. While the crystal structures of the complexes show only the solvent-separated ion pair (SIP) state for some interfaceal lysine side chains, our NMR hydrogen-bond scalar coupling data clearly indicate the presence of the contact ion pair (CIP) state for the same residues. The 0.6-μs molecular dynamics (MD) simulations confirm dynamic transitions between the CIP and SIP states. This behavior is consistent with our NMR order parameters and scalar coupling data for the lysine side chains. Using the MD trajectories, we also analyze the free energies of the CIP–SIP equilibria. This work illustrates the dynamic nature of short-range electrostatic interactions in DNA recognition by proteins.

Ion pairs (also known as salt bridges) of electrostatically interacting cationic and anionic moieties are important for many proteins and nucleic acids to perform their function. Despite their importance, ion pairs of biological macromolecules are not well understood in terms of dynamics. The vast majority of experiment-based knowledge on the dynamic properties of ion pairs is limited to those for small compounds. One can distinguish two major states of the ion pairs, which are typically minima in the potentials of mean force: the contact ion-pair (CIP) state, in which a cation and an anion are in direct contact; and the solvent-separated ion-pair (SIP) state, in which one or more solvent molecules intervene between the electrostatically interacting cation and anion.$^{1–3}$ For small molecule compounds, kinetics and thermodynamics of the CIP and SIP states have been experimentally studied by time-resolved absorption spectroscopy since the 1980s.$^{4–7}$ By contrast, despite the wealth of solution NMR methods for investigating protein dynamics,$^4–12$ experimental studies on the dynamic properties of ion pairs in biological macromolecules remain very rare.

Recently, several groups, including some of us, developed NMR methods for investigating dynamics of charged moieties of protein side chains.$^{13–18}$ In particular, lysine (Lys) NH$_3^+$ groups have proven to be extremely useful probes for NMR studies of protein side-chain dynamics involving hydrogen bonds and ion pairs.$^{16–21}$ Owing to very slow relaxation of in-phase single-quantum $^{15}$N coherence of NH$_3^+$ groups, extensive characterizations are feasible for Lys side chains forming hydrogen bonds and ion pairs. For example, a recent study on the HoxD9-DNA complex showed that the Lys NH$_3^+$ groups forming intermolecular ion pairs with DNA are highly mobile despite the presence of short-range electrostatic interactions and hydrogen bonds.$^{18}$ However, the origin of this high mobility was unclear with the NMR methodology alone, largely because structural and energetic details were not available for this system.

In our current study, we resolve this problem and delineate the ion-pair dynamics by integrating NMR spectroscopy and molecular dynamics (MD) simulations for structurally well-characterized DNA complexes of the fruit fly Antennapedia (Antp) homeodomain and human Egr-1 (also known as Zif268) zinc-finger proteins. These proteins, representing two major classes of eukaryotic transcription factors, are well characterized by biophysical means.$^{22–26}$ The crystal structures of the specific DNA complexes of the Antp homeodomain and Egr-1 zinc-finger proteins are available at 2.5 and 1.6 Å resolutions,$^{22,23}$ respectively, and provide structural details around the ion pairs. For these systems, we investigated the dynamics of the short-range electrostatic interactions involving Lys side chains that are important for molecular association of protein and DNA.

By NMR spectroscopy, we characterized the Lys side chains forming the intermolecular ion pairs with DNA in these complexes at pH 5.8. In the Lys-selective $^1$H–$^{15}$N HISQC
s spectra\(^1\) (Figure 1A), the Antp homeodomain–DNA and Egr-1–DNA complexes exhibited \(^1\)H–\(^{15}\)N correlation signals from Lys79 exhibited sizable \(h_3\)J\(_{\text{NP}}\) signals due to coupled HSQC spectra\(^1\) clearly indicated that these Lys side-chain phosphate \(^31\)P nuclei, as previously described.\(^1\) Only CIP states involving a direct hydrogen bond can exhibit a sizable \(h_3\)J\(_{\text{NP}}\) coupling, whereas SIP states cannot. The \(\text{NH}_3^+\) groups of the Antp homeodomain Lys46, Lys55, and Lys57 and Egr-1 zinc-finger Lys79. The spin–echo \(h_3\)J\(_{\text{NP}}\) modulation difference constant-time HSQC spectra\(^1\) show signals only from \(\text{NH}_3^+\) groups that exhibit hydrogen-bond scalar coupling between Lys \(^{15}\)N and DNA \(^{31}\)P nuclei across CIP. (B) Crystal structures showing only SIP states for Lys\(\text{NH}_3\) in the Antp-DNA complex (PDB 9ANT)\(^2\) and Lys79 in the Egr-1-DNA complex (1AAY).\(^2\) The distances from the \(\text{N}_{\text{z}}\) atoms (blue) to the closest DNA phosphate oxygen atoms are 4.7 Å for Lys46 and 4.2 Å for Lys79.

![Figure 1.](https://example.com/1.png)

Figure 1. Discrepancy between NMR and crystallographic data with regard to the intermolecular ion pairs. (A) NMR evidence for the CIP states for Antp homeodomain Lys46, Lys55, and Lys57 and Egr-1 zinc-finger Lys79. The spin–echo \(h_3\)J\(_{\text{NP}}\) modulation difference constant-time HSQC spectra\(^1\) show signals only from \(\text{NH}_3^+\) groups that exhibit hydrogen-bond scalar coupling between Lys \(^{15}\)N and DNA \(^{31}\)P nuclei across CIP. (B) Crystal structures showing only SIP states for Lys\(\text{NH}_3\) in the Antp-DNA complex (PDB 9ANT)\(^2\) and Lys79 in the Egr-1-DNA complex (1AAY).\(^2\) The distances from the \(\text{N}_{\text{z}}\) atoms (blue) to the closest DNA phosphate oxygen atoms are 4.7 Å for Lys46 and 4.2 Å for Lys79.

To detect the CIP states, we measured the hydrogen-bond scalar coupling \(h_3\)J\(_{\text{NP}}\) between Lys side-chain \(^{15}\)N and DNA phosphate \(^{31}\)P nuclei, as previously described.\(^1\) Only CIP states involving a direct hydrogen bond can exhibit a sizable \(h_3\)J\(_{\text{NP}}\) coupling, whereas SIP states cannot. The \(\text{NH}_3^+\) groups of the Antp homeodomain Lys46, Lys55, and Lys57 and Egr-1 zinc-finger Lys79 exhibited sizable \(h_3\)J\(_{\text{NP}}\) coupling (Figure 1A; see also Table S1 in the SI). All of them are located at the protein–DNA interfaces in the crystal structures. For Lys55 and Lys57 of the Antp homeodomain–DNA complex, the sizable \(h_3\)J\(_{\text{NP}}\) couplings are consistent with the CIP states seen in the crystal structure. For the Antp homeodomain Lys46 and the Egr-1 zinc-finger Lys79, however, we found an intriguing difference between the crystallographic data and our NMR data. Although the crystal structures show only SIP states for these side chains (Figure 1B), our \(h_3\)J\(_{\text{NP}}\) data clearly indicate a major presence of the CIP states for their \(\text{NH}_3^+\) groups. Given this discrepancy, it is important to study the dynamic behavior of the intermolecular ion pairs.

To gain insight into the above-mentioned discrepancy, we performed 0.6 μs MD simulations for the Antp homeodomain–DNA and Egr-1 zinc-finger–DNA complexes solvated with TIP3P water molecules using the NAMD 2.9 software\(^3\) together with CHARMM31 force field parameters.\(^3\)–\(^5\) The protonation states of titratable residues were assigned according to their standard states at pH 7.0. This seems to be valid for the interfacial Lys side chains as well because Poisson–Boltzmann equations-based calculations with the APBS program\(^5\) for the crystal structures suggest that the interactions with DNA can lower their p\(\text{K}_{\text{s}}\) from the standard value (10.4) by no more than 1.7. We monitored the contacts between each Lys side chain group and any DNA phosphate group in the MD trajectories (Figure 2). For all the Lys \(\text{NH}_3^+\) groups that can directly contact DNA phosphate group, the N–O distances dynamically fluctuated between two ranges: one between 2.5–3.2 Å, corresponding to the CIP states, the other between 3.8–6.0 Å, corresponding to the SIP states. The transitions between the CIP and SIP states occurred typically on a pico- to nanosecond time scale. These dynamic transitions in the MD simulations between the CIP and SIP equilibrium states give an interpretation of the above-mentioned discrepancy between the crystallographic and NMR data. This also resolves the issue of why some protein–DNA crystal structures show intervening water while others do not for similar systems. Consideration of
any single structure is obviously inadequate to describe transitions between the CIP and SIP states.

Given these computational results, we compared observables that can be assessed both computationally by MD and experimentally by NMR. One of them was the three-bond scalar coupling between $^{14}$N$_C$ and $^{13}$C$_C$ nuclei ($J_{N,C}$) related to the Lys $\chi_4$ torsion angle. Because the $^{15}$N$_C$ atoms are within the Lys NH$_3^+$ cations, this observation is directly relevant to the dynamics of the intermolecular ion pairs. As described, we measured $J_{N,C}$ coupling constants for Lys side chains in these complexes (Table S1 in the SI). Figure 3A shows comparisons of the experimental and computed $J_{N,C}$ coupling constants for Lys side chains in these complexes (Table S1 in the SI). Figure 3A shows comparisons of the experimental and computed $J_{N,C}$. Two correlation plots are displayed. One plot compares the experimental data with the ensemble averages of $J_{N,C}$ coupling constants, $\langle J_{N,C} \rangle$, calculated from the MD configurational ensemble, whereas the other plot compares to those calculated from single crystal structures. For each structure, a $J_{N,C}$ constant was calculated from a $\chi_4$ angle using the Karplus equation together with the empirical coefficients for Lys side chains. The MD ensemble $\langle J_{N,C} \rangle$ shows excellent agreement with the experimental data, for which the root-mean-square deviation (rmsd) was 0.22 Hz. By contrast, the $J_{N,C}$ constants calculated from the single crystal structures exhibited bimodal distributions with two clusters corresponding to trans and gauche $\chi_4$ conformers and show poor agreement with the experimental NMR data (rmsd, 0.93 Hz). This remarkable difference between these plots is due to the presence of various different torsion angles sampled in the MD trajectories.

The other observables we used to validate the dynamics of the intermolecular ion pairs were the order parameters, $S^2$, for the Lys C$_r$-N$_i$ bonds. As previously described, we measured $^{15}$N relaxation parameters for Lys NH$_3^+$ groups at the $^1$H-frequencies of 800 and 600 MHz (Table S2 in the SI) and determined the order parameters $S^2$ for the two protein–DNA complexes (Table S3 in the SI). The order parameters were also calculated using autocorrelation functions for the internal motions of the C$_r$–N$_i$ bonds from the MD trajectories (Figure S2 and Table S4 in the SI). Figure 3B shows comparison of these computational and experimental $S^2$ data. Both the MD-derived and NMR-derived $S^2$ data show values less than 0.6, indicating the highly dynamic nature of the Lys NH$_3^+$ groups, even for those in intermolecular ion pairs. The correlation coefficient was 0.75 between the computational and experimental $S^2$ data for the Lys NH$_3^+$ groups. The MD-derived $S^2$ parameters tended to be smaller than NMR-derived $S^2$ parameters. The same tendency for other bond vectors has been noted and discussed by other groups. Nevertheless, the NMR $J_{N,C}$ and $S^2$ data for the Lys NH$_3^+$ groups collectively indicate that the intermolecular ion pairs are indeed essentially as dynamic as seen in the MD simulations.

Encouraged by the consistency between NMR and simulation, we extended our analysis of the MD trajectories to argue the energetics of the CIP and SIP states. Figure 4 shows the probability distribution and free energy profile of the contact distances from the Lys N$_i$ atom to the closest DNA phosphate oxygen atom. Vertical relative normalization is arbitrary between curves.
force-field parameters gave the same overall trends in PMFs, but with moderate variations (± ~0.5 kcal/mol) in calculated free energies of the CIP and SIP states. Charge transfer, which is not taken into account in the simulations, might also impact the CIP–SIP equilibria in the simulations. Nonetheless, it is worth mentioning that the free energies for the intermolecular ion pairs in the protein–DNA complexes are similar to previous experimental data for monovalent ion pairs of some small organic compounds [ΔG°(CIP → SIP) ≈ 1–2 kcal/mol] and computed PMF between free Lys→Glu– pairs in water [ΔG°(CIP → SIP) ≈ 0.7–1.6 kcal/mol; ΔG°(CIP → SIP) ≈ 0.8–3.4 kcal/mol].

In conclusion, our current study combining the experimental and computational approaches illustrates the dynamic equilibria of short-range electrostatic interactions at protein–DNA interfaces. Although crystal structures typically show either CIP or SIP state for each ion pair, our data show that the intermolecular ion pairs in the protein–DNA complexes undergo the dynamic transitions between the CIP and SIP states on a pico- to nanosecond time scale, which allows facile sampling of recognition events. Rapid breakage of CIPs might facilitate the proteins to slide on nonspecific DNA and to locate the target sites. The dynamic behavior of electrostatically interacting cationic and anionic moieties at molecular interfaces might facilitate molecular recognition and catalysis by proteins.

**EXPERIMENTAL METHODS**

The Egr-1 zinc-finger and Antp homeodomain proteins were expressed in *Escherichia coli*, and purified by cation-exchange, and size-exclusion chromatographic methods. DNA was chemically synthesized and purified by anion-exchange chromatography. NMR experiments for the protein–DNA complexes were performed using Bruker Avance III spectrometers operated at the 1H frequencies of 800, 750, and 600 MHz. Other experimental and computational details are given in the Supporting Information.

**ASSOCIATED CONTENT**

1. Supporting Information

Experimental and computational details; tables of NMR scalar coupling constants, 15N relaxation, and order parameters; a table summarizing the dynamic behavior of interfacial Lys NH3+ groups in the MD simulations; a figure of F1-H-coupled HSQC spectra; and a figure of autocorrelation functions. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.5b01134.

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**Notes**

The authors declare no competing financial interest.

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