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Electric Field Destabilizes Noncovalent Protein−DNA Complexes

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Abstract: Noncovalent protein−DNA interactions are involved in many vital biological processes. In cells, these interactions may take place in the environment of an electric field which originates from the plasma and organelle membranes and reaches strengths of 1 MV/cm. Moreover, protein−DNA interactions are often studied *in vitro* using an electric field as strong as 1 kV/cm, for example by electrophoresis. It is widely accepted that an electric field does not affect such interactions. Here we report on the first proof that an electric field of less than 1 kV/cm can destabilize the protein−DNA complexes through increasing the monomolecular rate constant of complex dissociation.

Noncovalent protein−DNA interactions are involved in many vital biological processes such as gene expression, DNA replication, DNA integrity control, DNA damage repair, and immune response.[1−3] In cells, such interactions may take place in the immediate proximity of the plasma and organelle membranes,[4,5] which generate a nonalternating electric field (simply electric field below) of up to 10^6 V/cm.[6,7] Moreover, protein−DNA interactions are often studied *in vitro* using an electric field as strong as 10^3 V/cm, for example by electrophoresis.[8,9] An electric field, thus, accompanies protein−DNA interactions in both nature and bioanalytical technologies; hence, it is important to understand how it can influence such interactions. Here we report on the first proof that an electric field can destabilize protein−DNA complexes through increasing the monomolecular rate constant, k_{off}, of complex dissociation. We showed at the quantitative level that other electric-field-associated phenomena, such as temperature increase, cannot account for the observed change in k_{off}, which reaches as much as 7-fold for an electric-field strength increasing from 0 to 600 V/cm. Our results suggest that the effect of an electric field on protein−DNA interactions must be taken into consideration while studying these interactions by electric-field-based techniques, such as electrophoresis. More intriguing, these results indicate that intracellular electric fields originating from mitochondrial membranes are certainly strong enough to affect protein−DNA interactions[6] and can, therefore, potentially contribute to the regulation of cellular processes.

To study the effect of the electric field on k_{off}, (i) the complex has to be placed in an electric field, (ii) free protein and DNA need to be continuously removed from the complex surroundings to allow for complex dissociation, and (iii) the kinetics of complex dissociation have to be followed. Interestingly, performing step one can facilitate steps two and three. There is a method, termed Non-Equilibrium Capillary Electrophoresis of Equilibrium Mixtures (NECEEM) that uses an electric field applied to capillary ends (step 1) to (i) continuously remove free protein and DNA from the complex (step 2) and (ii) record the kinetics of complex dissociation in a form of a NECEEM electropherogram (step 3) as schematically depicted in Figure 1A.[10] NECEEM was a method of choice in this work. Classical NECEEM utilizes a constant electric field and cannot measure k_{off} when the field strength is equal to zero. We developed "stopped-field" NECEEM to allow such measurements. In essence, stopped-field NECEEM starts with classical NECEEM, which is run for some time to separate the complex from free protein and DNA. The field is then stopped (turned off) for time τ, which is shorter than the equilibration time, to allow for complex dissociation without the field (see section 2 in the Supporting Information for more details on the equilibration time). Finally, the field is reintroduced to record the electropherogram. Complex dissociation during τ occurs without the electrophoretic displacement of protein and DNA and is manifested as a small peak on the exponential part of the NECEEM electropherogram (Figure 1B). Figure 1 illustrates how k_{off} with and without the electric field can be determined using simple algebraic formulas, which utilize only the values of four areas (A_1, A_2, $A_{1'}$, and $A_{2'}$) and one migration time (t_C) obtained directly from the electropherograms (see section 2 in the Supporting Information for derivations).

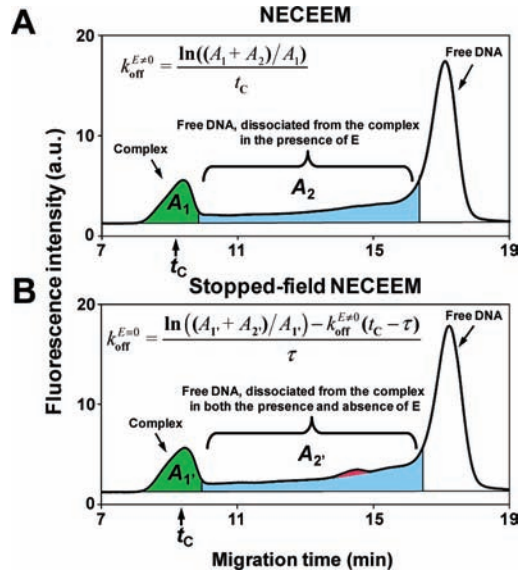

Figure 1. Electropherograms of classical NECEEM (A) and stopped-field NECEEM (B) for an equilibrium mixture of 100 nM MutS and 50 nM MutS aptamer, at 400 V/cm electric field. Classical NECEEM allows the determination of k_{off} in the presence of an electric field ($E = 0$) while stopped-field NECEEM facilitates the determination of k_{off} in the absence of an electric field ($E = 0$). A_1, A_2, $A_{1'}$, and $A_{2'}$ are the areas of corresponding colored features on the electropherograms, and τ is the time period during which $E = 0$. The red area represents the amount of DNA dissociated from the complex during the $E = 0$ period.

Using the method of k_{off} determination illustrated in Figure 1, we examined whether or not a detectable effect of the electric field on k_{off} can be observed. As our experimental model, we used a MutS protein and its DNA aptamer.[11] The experimental procedures

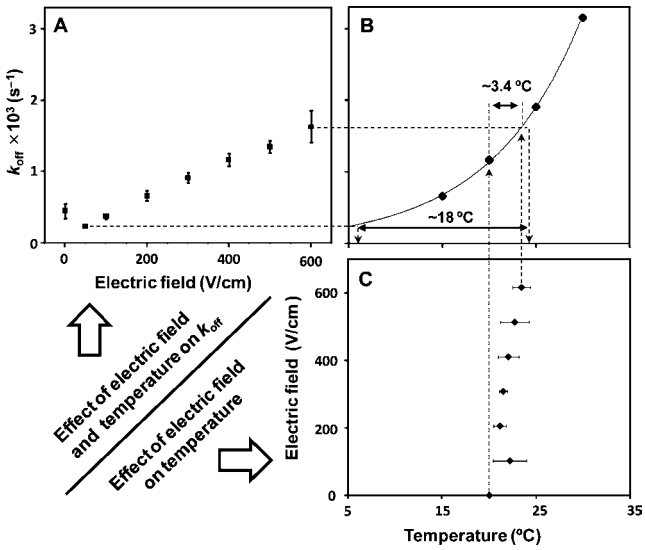

Figure 2. Effects of electric field and temperature on the rate constant of complex dissociation (k_{off}). (A) NECEEM-measured k_{off} values at different electric fields for the MutS-Aptamer complex. The zero-electric-field points were obtained by stopped-field NECEEM, while the nonzero-electric-field points were obtained using classical NECEEM. The experiments were performed at 20 °C. (B) NECEEM-measured k_{off} values for MutS-Aptamer complex at different temperatures and a constant electric field of 400 V/cm. (C) Temperature increase due to electric-field-associated Joule heating. The capillary coolant solution was thermostabilized at 20 °C. Temperature measurements were conducted using a diffusion-based method described elsewhere.[16] The electrophoresis buffer was 50 mM Tris-acetate pH 8.3.

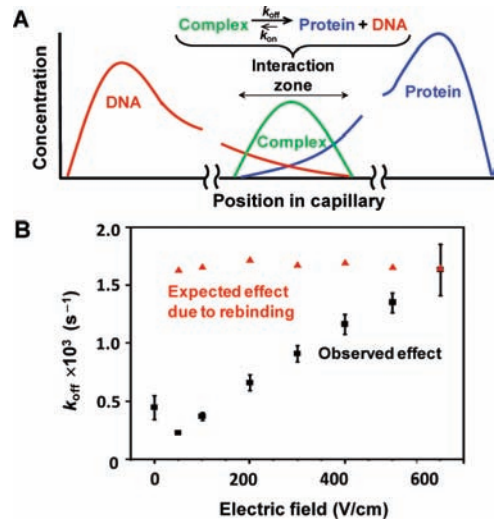

Figure 3. Effect of complex reassociation on k_{off} calculations in NECEEM. (A) Schematic showing the interaction zone where both dissociation and reassociation occur. The complex dissociates and releases protein and DNA, which move in opposite directions with respect to the complex. However, while still in the "interaction zone", the components have a chance to reassociate. (B) Illustration of the calculated maximum impact of complex reassociation on apparent k_{off} values (red triangles), which is negligible in comparison to the experimentally observed effect (black squares) (see section 4 of the Supporting Information for the method of calculations).

are detailed in section 1 of the Supporting Information. We found that k_{off} increased by about 7-fold when the electric field changed between 0 and 600 V/cm (Figure 2A; see also Figure S3 and Table S1 in the Supporting Information). Note that the differences between k_{off} values measured here and in the previous works are due to different run buffers used. Some evidence that an electric field may affect protein−DNA complex stability was previously reported by Kennedy and co-workers, but the influence was explained by "side effects" accompanying the electric field.[12] The first suggested side effect was a temperature increase inside the capillary due to Joule heat produced by an electric current. The rate constant of the complex dissociation increases with increasing temperature according to the Arrhenius equation. The second suggested side effect was rebinding of the protein and DNA dissociated from the protein−DNA complex in NECEEM before they are separated. The efficiency of this process is inversely related to the electric-field strength. In addition to the above-mentioned phenomena, we also need to consider processes at the capillary wall that were not previously suggested as potential side effects. One example of such a wall effect is mechanical stress, which may be experienced by the complex on the interface between the immobile and mobile ion layers, due to the high velocity gradient ranging from zero to the velocity of the electroosmotic flow.[13] The velocity of the electroosmotic flow increases with increasing electric field, which can potentially lead to increased tension and accelerated complex dissociation. There may be other wall effects, for example, a different pH near the walls,[14] which can also be confused with the electric-field effect if the wall effects are electric-field-dependent.

To demonstrate that an electric field itself can affect complex stability, we had to prove that the above side effects, even if present, could not quantitatively account for the experimentally observed increase in k_{off} with increasing electric-field strength.

The temperature inside the capillary inevitably grows with the electric-field strength;[15] the extent of this growth depends on the efficiency of heat dissipation from the capillary. Since k_{off} increases with increasing temperature, the temperature effect on k_{off} can potentially be confused with the effect of the electric field. To understand the extent to which temperature increase can affect k_{off}, we measured k_{off} values for a complex of MutS and its DNA aptamer at different temperatures (Figure 2B). We, then, measured the temperature in the capillary as a function of the electric field; a recently developed diffusion-based method was utilized (Figure 2C).[16] We found that the temperature inside the capillary grew by 3.4 ± 0.9 °C when the electric field increased from 0 to 600 V/cm. The observed temperature increase could only lead to a 1.3 times increase in k_{off} (see the lines projected from panel C to panel B) and, thus, could not account for the observed 7-fold increase in k_{off} when the field strength changed from 0 to 600 V/cm (Figure 2A). The data in Figure 2 show that the temperature needed to grow by more than 18 °C to fully account for the 7-fold increase in k_{off} (see the lines projected from panel A to panel B). We can, thus, conclude that temperature alone cannot explain complex destabilization with growing electric field.

DNA and protein dissociated from the complex can rebind again with a finite probability due to a finite length of the complex zone in NECEEM. This probability decreases with increasing electric-field strength as the electrophoretic velocities of the protein and DNA grow with the increasing strength. This, in turn, leads to the decrease of the reactants' residence time in the interaction zone (Figure 3A). The decreasing rate of field-dependent complex reassociation leads to an increase of the apparent k_{off} value. The decreasing rate of reassociation can, thus, be confused with a genuine electric-field-caused increase in k_{off}. We calculated the maximum potential impact of the reassociation process on the k_{off} value using experimentally determined velocities (of protein, DNA, and the complex), length of interaction zone, and time of interaction (Figure 3B). The decrease of the electric-field strength from 600 to 50 V/cm can only cause a 3% increase in the calculated k_{off}. This effect is smaller than the experimental error of k_{off} measurements and, thus, can be neglected. Therefore, we conclude that the combined effect of the temperature increase and complex reasso-

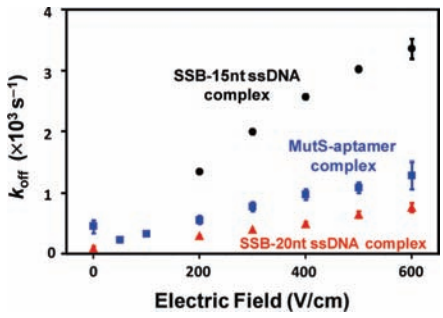

Figure 4. Effect of electric field on the rate of complex dissociation (k_{off}) for MutS with aptamer (blue squares), SSB with 15nt-ssDNA (black circles), and SSB with 20nt-ssDNA (red triangles). The effect of electric-field-associated temperature increase was subtracted using a procedure described in section 3 of the Supporting Information. For all experiments the capillary temperature was thermostabilized at 20 °C. Each point is represented by at least three different experiments, and an error is represented by 1 standard deviation.

ciation cannot account for the observed 7-fold increase in k_{off} with the field strength changing from 0 to 600 V/cm.

Finally, the rate of complex dissociation in the capillary volume may be different from the rate near the capillary walls due to various capillary-wall-associated effects (see above). To examine whether or not the wall effects can change k_{off}, we varied the capillary volume to surface ratio by changing its inner diameter. If the wall effects are measurable, a smaller-diameter capillary would have a higher k_{off} value. Experimentally, however, we observed that k_{off} dependence on the electric field was not distinguishable between capillaries with 50 and 75 μm inner diameters when the temperature effect was taken into account (see Figure S2 in section 3 of the Supporting Information). We can, thus, conclude that the combined influence of three side effects cannot account for the observed 7-fold increase in k_{off} with field strength increasing from 0 to 600 V/cm. Unless another unaccounted side effect exists, it is the electric field itself that destabilizes the protein−DNA complex.

To demonstrate that the observed electric-field effect on protein−DNA complex stability is not unique to the MutS-Aptamer complex, we also studied complexes of single-stranded DNA binding (SSB) protein with two different single-stranded (ss) DNA molecules (scrambled sequences of 20 and 15 nucleotides). The electric field accelerated the dissociation of the two SSB−DNA complexes, and the complex-destabilizing effect was different for all the studied protein−DNA pairs (Figure 4). We can, thus, conclude that the effect of the electric field on protein−DNA interactions depends on the interacting pair.

The mechanism for the effect of the electric-field strength on k_{off} is unknown; however it is quite logical to assume that due to the high negative charge of DNA, a protein−DNA complex is a strong dipole that tends to orient itself along an electric field. The electric field exerts an electrostatic force that pulls the protein and DNA away from each other. While this force may not be enough to break all the weak bonds at once, it can break a single bond without affecting others because of the flexibility of the DNA and protein. Other weak bonds can then be "opened" one-by-one like a "zipper". The suggested hypothetical mechanism is only one example of how an electric field can destabilize the complex.

Our finding has a number of important implications. First, a nonalternating and, likely, low-frequency alternating electric field can potentially affect cellular processes that involve protein−DNA complexes, e.g. gene expression, DNA replication, and DNA repair. Many other research groups reported different manifestations of the electric-field effect on cellular or animal models that can be potentially explained by the reported finding.[17−20] It is intriguing to learn whether or not endogenous electric fields, which always exist within cells, can regulate cellular processes and whether or not exogenous electric fields can inhibit these processes. Second, an electric field, which is routinely used in studies of protein−DNA interactions, can potentially lead to mistakes and misinterpretations. Electrophoresis is an example of a vulnerable technique, in particular, affinity capillary electrophoresis used for measuring equilibrium constants and NECEEM used for measuring k_{off}.[8,9] With the method developed in this work, one can study the effect of an electric field on specific protein−DNA interactions and take such effects into account.

To conclude, we outline our vision of future directions in this research area. It will be very interesting to experimentally test the ability of an electric field to affect the rate of formation of protein−DNA complexes (as opposed to the rate of complex dissociation studied in this work). Unfortunately, the method presented here is not suitable for such experiments. It is also very interesting to probe whether or not the electric field can inhibit the processes that rely on protein−DNA interactions *in vitro*. Examples of such processes include the polymerase chain reaction, *in vitro* transcription, restriction enzyme-catalyzed DNA digestion, and enzymatic DNA ligation. Progress in this area will also depend on the theoretical developments in the description of protein−DNA interactions and multibond affinity interactions in general. Finally, experiments must be designed to test the effect of an electric field on protein−DNA interactions in cells.

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Supporting Information Available: Supporting materials and methods and supporting results. This material is available free of charge via the Internet at http://pubs.acs.org.

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