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YY1–DNA interaction results in a significant change of electronic context as measured by capacitance

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Abstract

The detailed mechanism behind the processes of DNA-dependent RNA transcription initiation is largely unknown. When transcription initiation factors bind DNA, a significant change in the electrostatic state of the complex can result. Using electrical capacitance measurements of solutions of the YY1 zinc finger transcription initiation factor and the adeno-associated viral P5 promoter DNA, we observed a specific dielectric change when a protein–DNA complex was formed. We propose that complexation results in electrostatic changes that may trigger the markedly different electrical behavior, and offer a possible explanation for our results.

Keywords: Capacitance measurement of protein interactions; Specific protein–DNA interactions; YY1; Transcription initiation; Transcription factor; Adeno-associated viral promoter P5

1. Introduction

While much is known about the specific proteins involved in the transcription process of genetic information, relatively little is known about the mechanism through which the pre-initiation complex (PIC) begins the actual process of DNA transcription.

One important area of investigation is in the electronics of protein–DNA interactions. DNA possesses a varied electronic topology, with two highly charged phosphate backbones sandwiching aromatic bases. When a protein with charged side chains forms a specific complex with DNA, a significant change in the electrostatic state of the system can result. This may be a potential source of energy for the physical and chemical changes which are to follow in the transcription process. With this model in mind, the electrostatic environments of protein–DNA transcriptionally active complexes are worthy of detailed study.

In order to investigate the bulk electronic properties of a transcription initiation protein–DNA complex, it was necessary to select a functionally and structurally well-behaved and well-defined system. Here, we report results obtained from

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investigating the electronic properties of the eukaryotic protein transcription factor Yin–Yang 1 (YY1) alone and in the presence of DNA specifically recognizing YY1. YY1 is a 68 kDa protein with four zinc fingers which selectively bind to promoter DNA sequences, causing either repression or activation of the transcription rate, depending on the promoter context [1,2]. The YY1–DNA complex influences expression at the level of transcription in a wide variety of cellular and viral genes [1–3]. The adeno-associated virus (AAV) P5 promoter has a structurally and functionally well-characterized YY1 binding consensus sequence located at its transcription initiation start site, and is viable for basal transcription in the presence of YY1 and other basal transcription factors [3,4]. The three-dimensional crystal structure of the YY1-AAV P5 initiator complex has been solved [5].

Capacitance measurements on biological systems have historically focused on bulk systems, such as tissue or groups of cells [6,7], but they have recently been shown to be extremely sensitive and effective in measuring the presence of DNA, even in one single cell [8]. Here we report that the formation of a specific YY1–DNA complex in solution leads to dielectric values, as measured by capacitance, that are clearly different from those of the non-binding DNA and YY1 solutions. Increasing the measurement frequency of the alternating current applied resulted in a scaling of the values, but the frequency–capacitance curves for YY1 and DNA alone and in a complex were still distinguishable, suggesting that the electronic context around the promoter DNA changes significantly when protein–DNA interaction is active.

2. Experimental

2.1. Measurements

The capacitance of solution samples was measured on a capacitor-on-a-chip device that was assembled as illustrated in Fig. 1. The fabrication of the chip has been previously described [9]. It consisted of a silicon substrate passivated with a thermally grown SiO_2 layer. Electron beam lithography was used to define 200-nm-wide platinum

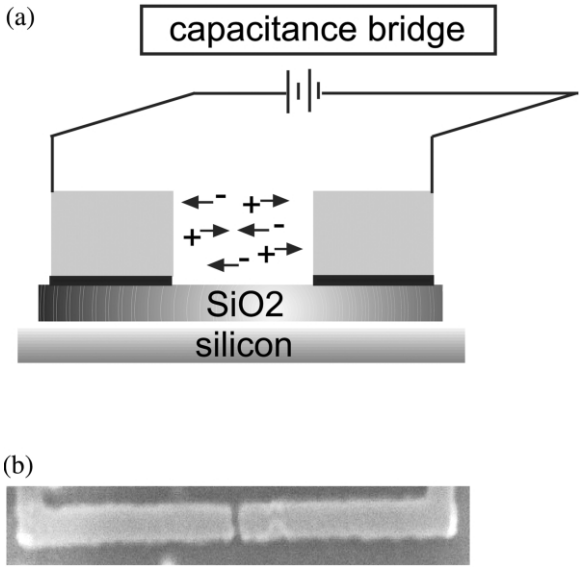

Fig. 1. Schematic illustration of the capacitor device. (a) A view of the entire device including the connection of the electrodes to the capacitor plates, made of Pt (platinum). They are placed on a Ti (titanium) layer, shown in black; silicon was used as a substrate, on top of which a SiO_2 layer was thermally grown. The distance between the electrodes is 80 nm; the solution containing the buffer system and DNA, YY1 or the DNA–YY1 complex was placed drop wise on the chip. The +/− symbols represent the positively and the negatively charged ions and dipoles in the solution. (b) SEM picture of the Pt leads with an 80-nm gap between the two electrodes.

electrodes atop a titanium adhesion layer. The distance between the electrodes was 80 nm. To measure the capacitance, 0.5 μl of YY1, DNA or complex in solution was placed on the capacitor. The device was connected to a commercial capacitance bridge (6440A Precision Component Analyzer, Wayne Kerr Inc.) and a driving force of 0.5 V was applied with alternating current using variable frequency. Capacitance was measured varying the frequency from 10 kHz to 3 MHz in 20 kHz intervals. All measurements were performed at ambient temperature and pressure, and repeated three times.

2.2. Preparation of DNA, YY1 and DNA–YY1 complexes

The 20 bp adeno-associated virus (AAV) P5 promoter oligomer was produced and purified as

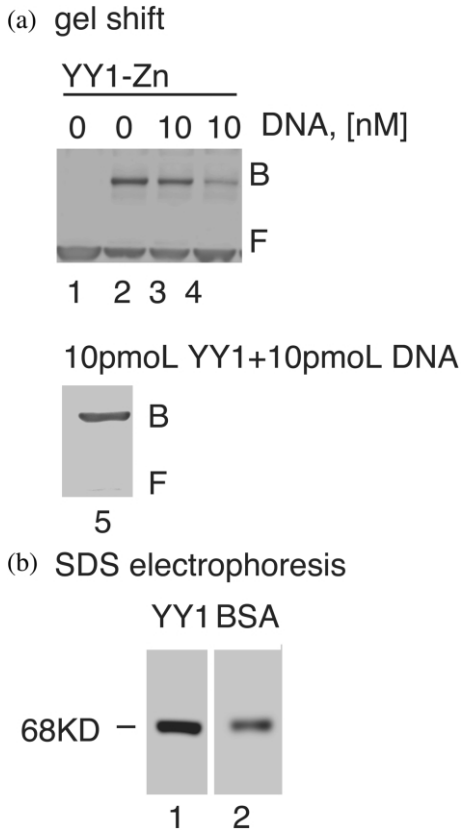

Fig. 2. Gel-shift assay for YY1-P5 promoter DNA complex formation in the buffer conditions that were used for capacitance measurements. (a) All reactions utilized 50.0 fmol of [^{32}P]-labeled AAV P5 DNA oligomer. The reaction in lane 1 received buffer, DNA and BSA (30 fmol); lane 2—reaction with DNA and 30 fmol of YY1; lane 3—the same as lane 2 but in the presence of a concentration of non-binding competitor oligomer as shown above the lanes; lane 4—cold P5 oligomer DNA was used as a competitor. The reaction in lane 5 contained 10 pmol each of DNA and YY1. To be able to monitor the complex formation, 1 fmol of [^{32}P]-labeled P5 DNA oligomer was added. The position of the free DNA (F) and the bound DNA (B) are indicated to the left of the panels. (b) 0.5 μg of YY1 and 0.2 μg of BSA were separated by SDS gel electrophoresis, and their positions verified by silver staining. The migration of the BSA marker (68 kDa) is indicated to the right of the panel. Lane 1—YY1; Lane 2—BSA.

previously described [5]. Recombinant YY1 protein was expressed in *E. coli*. The protein was isolated and it was purified near homogeneity (> 99%, Fig. 2b, lane 1). Mass spectroscopy (not shown) and SDS gel electrophoresis separation (Fig. 2b) with silver staining were used to estimate protein purity [3,5]. A mutated P5 promoter DNA oligomer previously shown not to bind to YY1 was used as a non-binding DNA [4].

In all capacitance measurements we used 0.5 μl of solution. As controls we measured the capacitance of the 50 mM NaCl and 5 mM HEPES general buffer solution containing 10 pmol of highly purified bovine serum albumin (BSA, Gibco) as a carrier, the P5 promoter and YY1, also dissolved in the general buffer. BSA is a well-characterized protein long studied in dielectric dispersion experiments [10]. Both BSA and YY1 are basic proteins with a molecular mass of 68 kDa. To test for YY1–DNA complex capacitance in solution, DNA (10 pmol) and YY1 (10 pmol) were mixed in a reaction volume of 10.0 μl and incubated for 15 min at 22.0 °C prior to measurement. The measurements were recorded at a temperature of 22.0 ± 0.1 °C.

The YY1–DNA complex formation in the presence of 50 mM NaCl and 5 mM HEPES at pH 7.6 was verified by gel shift as previously described using [^{32}P]-labeled DNA oligonucleotides [3]. The gel shift reactions were visualized by scanning the gels on a Molecular Dynamics PhosphorImager.

The protein concentrations were estimated by applying the Bradford method [11]. The DNA concentration was estimated by UV absorption at 260 nm.

Capacitance measurements were performed with three different batches of purified YY1 and synthetic oligonucleotide. Three different chips have been tested and showed similar quantitative results. All measurements were performed three times, and we present averaged data over the three runs in our plots.

3. Results and discussion

We have used a capacitor device (Fig. 1) to compare the capacitance of the DNA–YY1 complex solution with the capacitance of DNA, the YY1 protein with non-binding DNA, non-binding BSA with the P5 promoter DNA, and the buffer solution systems alone. We first verified the binding of YY1 to the P5 promoter DNA in the

presence of the buffer system chosen for the capacitance measurements. Through a gel-shift assay, we determined that the YY1-P5 promoter DNA oligonucleotide form a complex specific with the P5 promoter sequence in the chosen buffer system (Fig. 2a). This was determined by the inhibition of complex formation in the presence of cold P5 promoter DNA oligonucleotide as a competitor in the reaction mixture (Fig. 2a, lanes 1–3). Next we determined that reactions with 1 nmol of YY1 and 1 nmol of DNA yielded more than 99% of the DNA in a complex with YY1 (Fig. 2a, lane 5). We applied these reaction conditions in our protein–DNA complex capacitance measurements. Aliquots of 0.5 μl of the reaction mixture containing non-radioactive P5 oligonucleotide and YY1 were placed on the capacitor and after a 5-min incubation period, the capacitance was measured.

The frequency–capacitance curves were obtained by measuring the buffer alone, the YY1 protein in solution with non-binding DNA, non-binding BSA protein with the P5 DNA oligonucleotide and the YY1-P5 DNA complex in solution (Fig. 3a). Repeated measurements with new samples resulted in almost identical curves for each of the reaction assemblies, with a standard deviation of 8×10^{-12}. We also examined the behavior of the dissipation factor, or the dielectric loss factor under these conditions (Fig. 3b). For these curves, we observe similar slopes at low frequencies, but divergence at higher frequencies. The S.D. for the dissipation factor was 8×10^{-2}.

Our capacitance measurements reveal characteristic behaviors for DNA, protein and protein–DNA complex in solution. The P5 promoter in buffer solution gives the lowest capacitance values for all frequencies tested, while the protein solution is much higher, close to the values for pure water. The solution containing protein–DNA complex yielded capacitance values that were clearly different from the controls. Increasing the frequency of the alternating current resulted in a scaling of the capacitance values, but the curves were still distinguishable. The capacity response to the frequency is mainly due to changes in the dipole moments of the solute molecules in modulus and orientational relaxation.

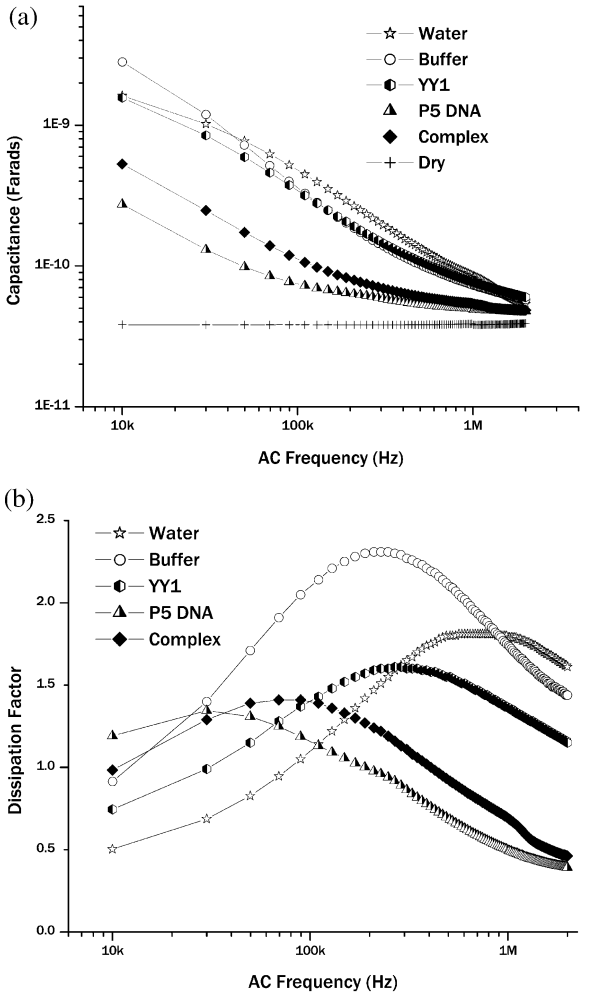

Fig. 3. (a) Frequency vs. capacitance averaged measurements for samples as labeled. (b) Frequency vs. dissipation factor averaged measurements. Aliquots of each sample were placed in the surface of the capacitor and subjected to alternating current of increasing frequency. The identity of each curve is given in the accompanying legend.

The capacitance of any capacitor is altered by a change in the dielectric constant of the medium between the charged plates. In our apparatus, the solution sample is acting as a dielectric material for the capacitor on the chip, and therefore altering the measured capacitance. The ability of a dielectric to change the capacitance in a capacitor system relative to a vacuum may be defined as the dielectric constant.

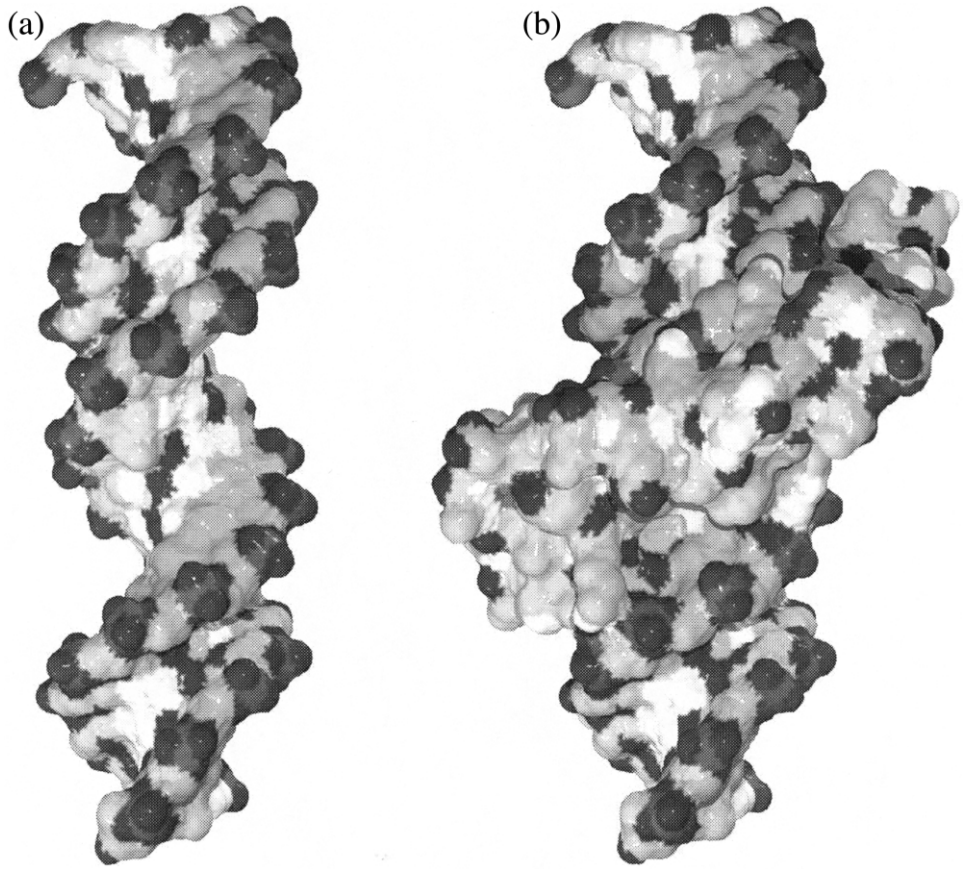

Fig. 4. Electrostatic Map of the P5 Promoter DNA and YY1-P5 Complex. The YY1 protein is less electronegative than the promoter DNA, and complex formation results in a completely new dipole moment 'fingerprint'. The crystal structure of YY1-AAV P5 promoter complex, in which DNA is pictured, with Poisson–Boltzmann calculated partial charges mapped on the molecular surface. The dark regions represent electronegative sites, while the lightest regions show areas of relative positive charge. (a) The crystal structure of the unbound AAV P5 promoter DNA. (b) The co-crystal structure of the zinc-finger domain of YY1 complexed with the P5 promoter is shown. Image created using the crystal structure data file PDB id: 1UBD, Swiss PDB Viewer and Mega-POV 0.7.

As most proteins have a dielectric constant of approximately 30, and DNA has a dielectric constant around 1, we can rationalize our capacitance measurements. The dielectric effect is primarily due to the alignment of dipoles to the electric field of the capacitor, so a stronger dipole should result in greater capacitance [12]. Solvating effects do dampen the impact of the dipole moments, however.

The dipole moment of the YY1-AAV P5 complex crystal structure is 486 Debye, slightly less that the average value for about 2300 proteins in the RCSB protein database [13]. It has a relatively strong quadrupole moment, brought about by the wrapping of YY1 around the major groove of the DNA, which is strongly negatively charged (Fig. 4). This juxtaposition of charge may dampen the dipole of the complex relative to unbound YY1, and result in the specific capacitance values reflected in our data. Essentially, the solution samples are acting as dielectrics for the capacitor.

Examination of the dissipation factor, which is related to the dielectric losses in the solution sample in the alternating electric field, also shows

differential behavior. This can be interpreted as the resistance of YY1 and DNA to reorienting to match the electric field. We observed similar behavior at different levels by all samples at low frequencies, but divergence at high frequencies. Consistently, protein solutions show the highest values, followed by the YY1–DNA complex, and finally DNA solution in the basic buffer. Dissipation factor and capacitance are the real and imaginary components of the complex response of the system to the alternating electrical field, and show a correlation to the dielectric constant.

By measuring the solution capacitance, we have been able to differentiate protein–DNA complexes from free DNA and unbound protein, using less than $\sim 10^6$ molecules in a volume of only 0.5 μl. The changes in the capacitance of the solution are due to the specific protein–DNA complexation. Though dependent on many contributing factors, we propose that capacitance measurements may form a basis for sensitive devices able to detect protein–DNA interactions. It is important to note that our measurements were sensitive for countable, sub-nanomolar quantities of protein–DNA complex in solution at ambient temperature. The readings were facile and extremely fast, possibly making the technique suitable in rapid assays for disease.

The dielectric properties of DNA in solution have been shown in previous studies to depend heavily on the concentration of DNA [14] and on the length of the oligomers [15]. However, both of these factors are well determined and held constant in our experiments, as can be verified by our reproducible curves for each solution sample.

4. Conclusion

It is clear that for YY1 and the P5 promoter element, the electrostatic context around the promoter DNA changes significantly when protein–DNA interaction is active. This change may have important ramifications for the transcription initiation event which is to follow. Detailed investigations are in progress to determine the exact effects of complexation on the system.

Acknowledgments

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References

[1] Y. Shi, E. Seto, L.S. Chang, T. Shenk, Transcriptional repression by YY1, a human GLI-Kruppel-related protein, and relief of repression by adenovirus E1A protein, Cell 67 (1991) 377–388.

[2] K.M. Galvin, Y. Shi, Multiple mechanisms of transcriptional repression by YY1, Mol. Cell. Biol. 17 (1997) 3723–3732.

[3] A. Usheva, T. Shenk, YY1 transcriptional initiator: protein interactions and association with a DNA site containing unpaired strands, Proc. Natl. Acad. Sci. USA 93 (1996) 13571–13576.

[4] A. Usheva, T. Shenk, TATA-binding protein-independent initiation: YY1, TFIIB, and RNA polymerase II direct basal transcription on supercoiled template DNA, Cell 76 (1994) 1115–1121.

[5] H.B. Houbaviy, A. Usheva, T. Shenk, S.K. Burley, Cocrystal structure of YY1 bound to the adeno-associated virus P5 initiator, Proc. Natl. Acad. Sci. USA 93 (1996) 13577–13582.

[6] K. Asami, E. Gheorghiu, T. Yonezawa, Real-time monitoring of yeast cell division by dielectric spectroscopy, Biophys. J. 76 (1999) 3345–3348.

[7] Y. Polevaya, I. Ermolina, M. Schlesinger, B.Z. Ginzburg, Y. Feldman, Time domain dielectric spectroscopy study of human cells. II. Normal and malignant white blood cells, Biochim. Biophys. Acta 1419 (1999) 257–271.

[8] L.L. Sohn, O.A. Saleh, G.R. Facer, A.J. Beavis, R.S. Allan, D.A. Notterman, Capacitance cytometry: measuring biological cells one by one, Proc. Natl. Acad. Sci. USA 97 (2000) 10687–10690.

[9] J. Moser, R. Panepucci, M.J. Naughton, E-beam fabrication of electrodes for transport measurements across single molecules, in Cornell Nanofabrication Facility 2000–2001 Research Accomplishments, (2001) pp. 132–133.

[10] E.H. Grant, Dielectric dispersion in bovine serum albumen, J. Mol. Biol. 19 (1966) 133–139.
[11] N.J. Kruger, The Bradford method for protein quantitation, Methods Mol. Biol. 32 (1994) 9–15.
[12] R.W. Hendricks, Dielectric Constant. (1997). Retrieved 24 April 2002, from Virginia Tech, Materials Science and Engineering Department, [http://www.mse.vt.edu/faculty/hendricks/mse4206/projects97/group01/solid-state/dielect.htm].
[13] C. Felder, J. Sussman, Web Server to Calculate Dipole Moments of Proteins. (2000). Retrieved 24 April 2002, from The Weizmann Institute, [http://bioinformatics.weizmann.ac.il/dipol/].
[14] S. Bone, R.S. Lee, C.E. Hodgson, Dielectric studies of intermolecular interactions in native DNA, Biochim. Biophys. Acta 1306 (1996) 93–97.
[15] A. Bonincontro, M. Matzeu, F. Mazzei, A. Minoprio, F. Pedone, Influence of defects on the electrophoretic, thermodynamic and dielectric properties of a 21 base pair DNA in solution, Biochim. Biophys. Acta 1171 (1993) 288–294.