

A Mechanism for Stimulation of Biosynthesis by Electromagnetic Fields: Charge Transfer in DNA and Base Pair Separation

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Electrons have been shown to move in DNA, and a specific DNA sequence is associated with the response to EM fields. In addition, there is evidence from biochemical reactions that EM fields can accelerate electron transfer. Interaction with electrons could displace electrons in H-bonds that hold DNA together leading to chain separation and initiating transcription. The effect of charging due to electron displacement on the energetics of DNA aggregation shows that electron transfer would favor separation of base pairs, and that DNA geometry is optimized for disaggregation under such conditions. Electrons in the H-bonds of both DNA and the surrounding water molecules fluctuate at frequencies that are much higher than the frequencies of the EM fields studied. The characteristics of the fluctuations suggest that the applied EM fields are effectively DC pulses and that interactions extend to microwave frequencies.

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How weak electromagnetic (EM) fields interact with DNA to stimulate protein synthesis is currently not well understood. An important clue, however, is the identification of a specific DNA sequence on the gene promoter that is associated with the response to EM fields. When this sequence is transfected into the promoter of a reporter gene, previously unresponsive to EM fields, this gene is now EM field-responsive. Previous research showed that EM field induction of the HSP70 gene involved signaling pathways that could respond to feedback information from the DNA interaction mechanism. An EM field sensitive DNA sequence suggests that EM fields may interact both directly and indirectly with DNA. The initial interaction could involve the displacement of electrons in the H-bonds that hold DNA together, thereby causing chain separation and initiating transcription and translation. Electrons have been shown to move in DNA and data from biochemical reactions indicate that EM fields can accelerate electron transfer. Interaction with electrons could account for activation of DNA by weak low frequency EM fields as well as the more energetic high frequencies. It has also been shown using multi-subunit proteins that charging leads to disaggregation. A simple model of the effect of charging due to electron displacement on the energetics of DNA aggregation shows that electron transfer would favor separation of base pairs, and that DNA geometry is optimized for disaggregation under such conditions. Electric fields exert comparable forces on electrons and also stimulate biosynthesis, as expected. The proposed mechanism suggests that there could be a maximum frequency for the EM field response, and that modifications of the charge on DNA affect the response.

EM Field Stimulation of Transcription

The interplay between experiment and theory usually catalyzes scientific development, but thus far, studies of EM field interactions with biological systems have not led to a generally accepted explanation of established biological effects. Theoretical approaches have been proposed, based on cyclotron resonance of ions (Liboff, 1985) and related approaches (e.g., Lednev, 1991; Blanchard and Blackman, 1994), the forced vibration of ions (Panagopoulos et al., 2002), and effects on electron transfer (Blank and Goodman, 2002, 2004;

Blank, 2005). The very low energy of the fields that are reported to be effective have even led to theoretical papers that question the validity of the experiments themselves (Valberg et al., 1997; Weaver et al., 1998). The lack of success in defining a mechanism may be due to trying to find a single over-arching principle that would describe a variety of experimental observations. For example, cyclotron resonance undoubtedly applies to charges in DC and AC fields in a vacuum, but would not be expected to apply to hydrated ions in membrane channels. In all cases, the low energies that are effective need to be explained, especially in activating the signaling pathways in the stress response.

Theoretical approaches to EM field mechanisms would probably do better to focus on a single well characterized biological effect and the low energy processes that could be involved. Two such attempts consider effects on electron transfer in a mechanism for EM field-DNA interactions that initiate transcription (Blank and Goodman, 2004), as well as for EM field acceleration of the Na,K-ATPase that leads to ion pumping (Blank, 2005). Elson (2006) has also considered the possibility that charge transfer in DNA may be important in affecting the rate of development of living systems. These approaches incorporate experimentally observed processes as links in a causal chain. This paper proposes the following processes in DNA activation of transcription:

EM fields displace electrons in DNA. This causes transient charging of small groups of base pairs. At the charged sites, disaggregation forces overcome H-bonds. Disaggregation of the two chains at those sites enables transcription.

Abbreviations: EM, electromagnetic; Hz, hertz; ELF, extremely low frequency; RF, radio frequency.

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We know that power frequency (ELF) fields alter RNA transcription patterns (Goodman et al., 1983), induce upregulation of the early response genes, *c-fos* (Rao and Henderson, 1996) and *c-myc* (Lin et al., 1994, 1996), and the stress response gene HSP70 (Goodman et al., 1994; Lin et al., 1997; Goodman and Blank, 1998). Radio frequency (RF) fields have also been shown to induce stress response genes (Kwee et al., 2001; Leszczynski et al., 2002; Shallom et al., 2002; Weisbrot et al., 2003). Additional studies that support EM field interaction with DNA are electron conduction in DNA (Wan et al., 1999, 2000) and EM field-induced DNA single and double strand breaks (Lai and Singh, 1997, 2004; Diem et al., 2005; Ivancsits et al., 2005; REFLEX Project Report, 2004).

However, not all cell types respond to EM fields. A series of recent studies using both ELF and RF fields found, in addition to DNA strand breaks, cell type specific genotoxic effects from exposures to ELF fields (Sarimov et al., 2004; Winker et al., 2005; REFLEX Project Report, 2004). One effect of ELF fields on DNA that has been repeated many times in different laboratories is that 12 mG fields interfere with the ability of Tamoxifen to inhibit the growth of MCF7 breast cancer cells at low thresholds (2–12 mG; Liburdy, 2003).

The ‘dilemma’ of the cell line that does not respond to EM fields and the inability to ‘replicate’ positive reports have plagued this area of research for many years, and has led to mistrust of data. One such controversy concerned two cell lines of HL60 cells obtained from different sources. The discrepancy was resolved when it was shown that the two cell lines in question had dramatically different growth rates as well as differences in response to EM field exposure (Jin et al., 1997). These results show that cell lines that have been maintained for a long period of time in different laboratories must be characterized before using them in EM field experiments: for example, number of passages; whether they are transformed; and their genomic and proteomic composition. Effects of ELF and RF have been shown to differ depending upon a number of factors including different human donors and how long a cell line has been maintained in a specific laboratory. Natural selection takes place at each cell passage and eventually the genome of the cell line is permanently altered. Inability to replicate published data can be due to any number of factors. One such factor could be the presence or absence of the EM-field sensitive DNA sequence on the promoters of some of their genes, as described below.

Biochemical Signaling Pathways

In the absence of EM fields, an important series of cellular signaling events normally occurs prior to upregulation of gene expression. These events are controlled by members of the mitogen activating phosphokinase (MAPK) family. Transcription factors in the p38 MAPK pathway are involved during both ELF and RF exposures (Leszczynski et al., 2002, 2004). Increased phosphorylation of specific transcription factors has also been shown when cells and tissues are exposed to EM fields (Jin et al., 2000; Leszczynski et al., 2002; Weisbrot et al., 2003). In considering how EM fields affect DNA and the regulation and control of gene expression, it is important to take into account that the chain of events coming into the cell from outside is comprised of a large number of transcription factors that are regulatory proteins. Some of these enter the nucleus and bind to specific recognition sites on the DNA of the promoter.

How these ongoing events may be affected by EM field stimulated processes in the DNA is currently unknown, but the biochemical signaling pathways are inter-connected much like the intermediary metabolism charts, and they connect with the products of DNA transcription (Lin et al., 1996). The EM field can initiate DNA transcription by itself once the DNA

sequences in the promoter transduce the field energy, and this sets in motion the inter-connected processes that are activated in the stress response. Figure 1 shows a diagram linking activation of DNA with activation of the biochemical pathways. By this mechanism DNA stimulation can occur directly via the DNA molecule itself, as well as indirectly via the biochemical pathways, without necessarily involving interaction with the cell membrane.

The stress response, characterized by synthesis of stress proteins (e.g., hsp70), can be induced by elevated temperatures (‘heat shock’) as well as EM fields, but the stimuli act on distinctly different parts of the promoter. See Figure 2. Upregulation of the HSP70 gene by EM fields occurs in the absence of elevated temperature. The promoters of both HSP70 and another EM field-sensitive gene, *c-myc*, have multiple copies of a specific nucleotide sequence that responds to EM field exposures. This consensus sequence, nCTCTn (shown as the MYC binding sites in Fig. 2), is upstream on the promoter relative to the transcription initiation site from a different nucleotide sequence that is associated with the heat shock response (Lin et al., 1994, 1999, 2001). EM field exposure of HSP70 deletion constructs, linked to a CAT or Luciferase reporter genes and containing all three nCTCTn binding sites, showed more than a three-fold increase in CAT and Luciferase activity (Lin et al., 1998, 1999, 2001). The presence of even one nCTCTn binding site is sufficient for a 1.5-fold increase. To demonstrate EM field specificity and sensitivity, nCTCTn sequences were mutated one by one. The CAT and Luciferase assays showed that the ability of an EM field to induce hsp70 protein disappears as the sequences are mutated (Lin et al., 1998, 1999). Since the nCTCTn sequences have low electron affinities and electrons are easily displaced, these data support the idea that EM fields could interact with electrons in the promoter of the gene.

EM Fields Interact With Electrons in Biochemical Reactions

One expects electric (E) and magnetic (B) fields to interact most strongly with electrons, because of their unusually high charge to mass ratio. In quantum theory, this basic assumption, known as the Born–Oppenheimer Approximation, applies to sub-atomic reactions. Electrons are assumed to respond instantaneously compared to protons and heavier atomic nuclei

Signaling Pathways

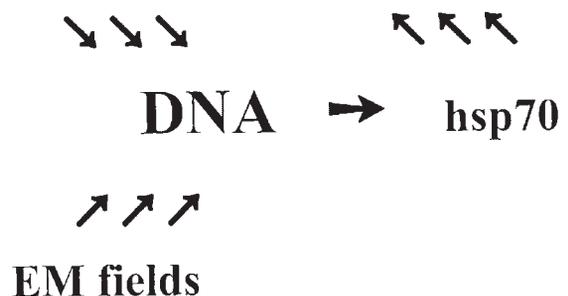


Fig. 1. A diagram showing interaction of EM fields and the biochemical signaling pathways with DNA leading to synthesis of the stress protein hsp70. The stress protein hsp70 acts as a negative feedback agent in controlling its own synthesis. We assume this to be characteristic of feedback mechanisms in the signaling pathways of EM field activated mechanisms.

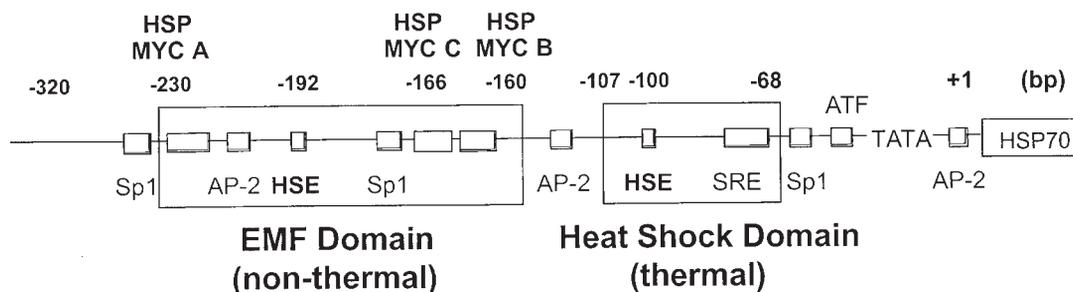


Fig. 2. A map of the EM field and thermal ('heat shock') domains on the HSP70 promoter. Binding sites within the EM field domain are indicated (HSE, AP-2, Sp1). The DNA consensus sequence that interacts with EM fields is nCTCTn and is at the three MYC binding sites (A,B,C) shown as boxes. All the locations are indicated by the numbered sequence of bases at the top of the diagram.

because of their much smaller mass, and electronic responses are assumed to be essentially complete before the heavier atomic nuclei start to react. It is, therefore, reasonable to expect EM fields to interact initially with electrons in biological systems, including DNA.

Interaction of electric and magnetic fields with electrons was indicated in studies of the Na,K-ATPase, the membrane enzyme that transports Na⁺ and K⁺ ions across membranes against electrochemical gradients (Blank, 2005). Low frequency electric and magnetic fields were shown to affect enzyme function differently, but both fields accelerated the reaction when the enzyme was relatively inactive. We assumed that the same force was needed at the threshold for acceleration by each field, and calculated the velocity (*v*) of the charge (*q*) that is affected in the two fields by equating the electric with the magnetic force,

$$F = qE = qvB. \quad (1)$$

It follows that $v = E/B$, the ratio of the threshold fields. The measured thresholds (Blank and Soo, 1992, 1996) were $E = 5 \times 10^{-4}$ V/m and $B = 5 \times 10^{-7}$ T (0.5 μ T), giving $v = 10^3$ m/s, a speed similar to that of electrons in DNA (Wan et al., 1999).

Since electrons are affected by EM fields in the ELF range, there should be sufficient energy to stimulate electrons in DNA and other biochemical reactions. To test the effect of EM fields on reactions where we know that electrons are involved, we studied electron transfer from cytochrome *c* to cytochrome oxidase (Blank and Soo, 1998) and in the Belousov–Zhabotinsky (BZ) reaction, which is the oxidation of malonic acid (Blank and Soo, 2003). In all three reactions, EM fields:

- accelerate chemical reactions (including electron transfer reactions)
- compete with the intrinsic chemical forces driving the reactions, and are most effective when the intrinsic chemical forces are low.
- activate at low thresholds: Na,K-ATPase (0.2–0.3 μ T), cytochrome oxidase (0.5–0.6 μ T), BZ reaction (<0.5 μ T); the threshold for biosynthesis is below 0.8 μ T.
- show frequency optima for the two enzymes studied that are close to reaction turnover numbers (Na,K-ATPase, 60 Hz; cytochrome oxidase, 800 Hz), suggesting a tie-in with the molecular kinetics. This is not a resonance-like interaction because the optima are broad.

A study reporting no effect of EM fields on the BZ reaction (Sontag, 2006) actually strengthens the above interpretation. In

that study, the EM field was not applied until the reaction was well under way for about seven minutes. In our studies, the field was applied from time zero, that is, at the mixing of the reactants. This difference is critical. We have shown that all three reactions studied respond to EM fields only when the intrinsic chemical forces are relatively weak. EM fields accelerated the Na,K-ATPase reaction only when enzyme activity was low. The same was true for cytochrome oxidase, and also can be seen from the temperature dependence of the BZ reaction. EM fields are not magic. They exert a force in competition with other forces that affect chemical kinetics, and their effect is negligible when overcome by intrinsic chemical forces. To study effects of EM fields, one must select conditions where intrinsic chemical forces are weak and the EM field is strong enough to have an effect on the kinetics.

Studies of the three biochemical reactions, show that EM fields accelerate electron transfer, and that the EM forces ($\sim 10^{-20}$ N) at the low thresholds may be strong enough to displace electrons in DNA. The force due to interaction of an electron with a magnetic field is determined by the strength of the field and the velocity of the electron. Relative change of field or motion of electron is required. The force due to an AC magnetic field acting on a 'static' electron is due to the rate of variation of the B field and is usually much smaller. The largest force on an electron results when the magnetic field is changing, as in AC, and the electron is also moving. Significant movement would be expected due to the 'flickering' of H-bonds that occurs in water (Fecko et al., 2003). This also occurs at water interfaces (McGuire and Shen, 2006), and probably in the hydration layer of DNA.

In the experiments stimulating protein synthesis, an EM force of only $\sim 10^{-20}$ N was shown to activate DNA. This force can move an isolated electron ~ 1 nm in 1 nsec, a distance that is greater than the length of an H-bond (~ 0.3 nm). The displaced charge can create conditions that lead to disaggregation by overcoming the cohesive forces, including the H-bonds, and enabling water molecules to enter any gap created by the weakened bond. In principle, this process could occur at the site where the electron has added a net negative charge, or at the site where the electron came from and left an unbalanced positive charge.

A related mechanism probably occurs in the DNA of striated muscle, where the electric fields (not EM fields) associated with action potentials stimulate the nuclei to synthesize muscle proteins in vivo (Blank, 1995). That the effect is due to the electric field stimulus is shown by the relation between the muscle proteins synthesized and the frequency of the action potentials. Under normal physiological conditions, conduction of an action potential along the muscle membrane creates an electric field estimated at ~ 10 V/m (Blank and

Goodman, 2004). In striated muscle, this electric field drives the currents across the nuclei adjacent to the membrane and stimulates the DNA to synthesize different muscle proteins in response to the frequency of the action potentials. The magnitude of electric field provides a large safety margin in muscle, since fields as low as 3 mV/m stimulate HL60 cells (Blank et al., 1992), and the threshold electric stimulus for the Na,K-ATPase is even lower, at ~ 0.5 mV/m (Blank, 2005).

Differences in the frequency response between DNA and the enzymes provide some insight into the EM field interaction mechanism. For the two enzyme reactions studied in the ELF range, the peaks of the broad frequency optima are close to reaction turnover numbers (Na,K-ATPase, 60 Hz; cytochrome oxidase, 800 Hz) and appear to be related to the molecular kinetics. That is, the applied EM fields at those particular frequencies aid the electron transfer that occurs at that frequency. Unlike the case of the enzymes, the wide range of frequencies that stimulate stress protein synthesis indicates that the characteristics of the EM signal that activate DNA are probably unrelated to an ongoing biochemical reaction.

Electrons in DNA probably interact with the H-bonded water network, where bonds are in constant motion, and they move much faster than the changing EM fields that have been studied. Electrons would be expected to move at the \sim nanometer/picosecond 'flicker' rate of protons in H-bonded networks (Fecko et al., 2003), and one would expect a velocity of this magnitude. Comparing the 'flicker' rate (10^{12} Hz) to the power (60 Hz) and radio (10^6 Hz) frequencies in DNA studies, it appears that the EM fields hardly change while an electron is in motion; they are like repeated 'DC pulses.' For this reason, all frequencies in the range where EM fields act as DC pulses affect the electrons similarly, and even the weak power frequency fields exert sufficient force to move an electron. The characteristics of the fluctuations suggest that EM field interactions extend to microwave frequencies. The picosecond 'flicker' rate (10^{12} Hz) may also represent an upper limit in the ability of EM fields to affect DNA, because there may be insufficient time to move electrons at the higher frequencies.

EM Fields Interact With Electrons in DNA

DNA is composed of two single strands in the form of a double helix or twisted ladder that has 'rungs' formed by pairs of complementary molecular bases, AT and GC. There are π -electron orbits within the base pairs that extend above and below each 'rung' of the ladder, and these overlap with their counterparts from neighboring rungs, thus creating a electron pathway through the molecule that enables charge migration/transport. Wan et al. (2000) have used a well-characterized duplex DNA consisting of a fluorescent charge donor and a charge acceptor, bridged by varying numbers of intervening base pairs. They found indications of a decrease in charge transfer rate as a function of bridge distance. Several groups have shown that DNA can transfer electrons and that electron transfer can chemically repair a thymine dimer, that is, when two adjacent thymines on the same DNA strand bond together. They have shown that cells can modulate the electrical properties of DNA using an enzyme, methyltransferase, and that electron transfer can be interrupted by inserting an insulating chemical group in the π -electron stack.

Electron migration in DNA is complicated, and the debate on the nature of the conductivity of DNA has been controversial and contentious. One model that has been used to explain charge migration is hole hopping between local amino acid sites driven by the torsional motions of the 'floppy' ribose-phosphate backbones. This model has been used to analyze experimental results for sequence-dependent long range hole transport in DNA (Ratner, 1999; Giese and Spichty, 2000; Berlin et al., 2001). Porath et al. (2000) have made direct

electrical transport measurements on DNA, and have shown that DNA behaves as a linear conductor. Shao et al. (2005) have demonstrated sequence dependence on charge transport through DNA domains. DNA charge appears to be remarkably sensitive to DNA sequence and structure. The unique DNA sequence on both the HSP70 and *c-myc* promoters, an nCTCTn domain, responds to EM fields and induces upregulation of the genes. The responsiveness is dependent on the number of nCTCTn present (Lin et al., 2001). It is clear that electrons can move in DNA and that some DNA sequences are associated with the response to EM fields.

Separation of Biopolymer Chains Due to Charging

In the proposed mechanism, DNA chain separation is initiated by charging of the chain segments where electrons are displaced. The disaggregation that follows is not simply the result of electrostatic repulsion, since a large part of the energy change is associated with hydration of the newly exposed chains to the aqueous solvent. The extent of disaggregation is determined by the balance between electrostatic and hydration forces, with H-bonds between the base pairs also contributing to the bonding energy.

Biopolymer disaggregation has been studied primarily in proteins in solution, where the emphasis has been on interaction with the aqueous medium. Lauffer (1975, 1989) focused almost entirely on the hydration energy. He used the term 'entropy driven' to describe aggregation of protein subunits in aqueous media, where the large increase in entropy was due to release of many bound water molecules when subunits aggregate. The term 'entropy driven' indicates that aggregation is spontaneous (i.e., the free energy change is negative), and that it occurs with a production of heat (i.e., a positive enthalpy). The negative free energy together with a positive heat production results in a large positive entropy change.

Characterizing protein aggregation as 'entropy driven' has caused many to overlook the importance of charge. Proteins disaggregate when the pH differs from the isoelectric point and their net charge increases (e.g., Klug, 1979; Blank and Soo, 1987), while the entropy increase due to release of water molecules is the same at every pH. The effects of charge can usually be neglected at constant pH, but they must be considered when the protein ionizes due to a conformational change, as during hemoglobin oxygenation, where an analysis shows that both electrostatic (due to ionization of a histidine) and hydration energies (due to changes in the surface area in contact with water) are needed to account for the observations (Blank, 1975, 1994). The relation between molecular surface area in contact with water and the surface charge density has proven useful in understanding a number of biopolymer properties, for example, the dissociation of hemoglobin tetramers into dimers (Blank and Soo, 1987), cooperative interactions and the Hill coefficient (Blank, 1989, 1994), the high viscosities of concentrated hemoglobin solutions (Blank, 1984) and the relation between gating current and opening of voltage-gated channel proteins in excitable membranes (Blank, 1987). The idea can account for the different effects of electric and magnetic fields on the Na,K-ATPase reaction (Blank, 2005). The ability of changes in molecular charge to explain complex physiological effects, suggests that the same forces apply to DNA, and that local charging should favor local disaggregation.

Charging of DNA Segments and Chain Separation

EM fields activate DNA by affecting the competition between forces minimizing charge density and those minimizing molecular contact with water. Charge tends to increase the area occupied, since this decreases electrostatic repulsion.

Hydration energy and H-bond energy oppose an increase in area which means greater contact of DNA bases with water. The charge density on the bases is a measure of the electrostatic repulsion, and the surface area of the bases exposed to water is a measure of the hydration energy, so we can use these two measures to estimate the relative effects of electrostatic and hydration energies on the disaggregation of the two DNA chains. The displacement of an electron also affects H-bonds, but H-bond energy is small compared to hydration energies.

The DNA surface exposed to water can be estimated from model structures. The effect of charge can be estimated from measurements of hemoglobin disaggregation as a function of pH (Blank and Soo, 1987). In the hemoglobin experiments, the osmotic pressure increase enabled calculation of the surface area increase when tetramers split into dimers, while the added charge was determined by the titrated acid. It is important to note that the disaggregation equilibrium constant varied with the pH, but the surface charge density was the same at every pH. In hemoglobin, despite the increase in total positive or negative charge, the charge density remained at $\sim 0.1 \text{ nm}^{-2}$. Apparently, the surface charge density determines the balance between areas exposed to water and unexposed (aggregated) areas. One expects quantitative differences between hydration of proteins and nucleic acids, but we can assume that the energy associated with breaking of water-water bonds is the same, and that the hydration energies are probably comparable. What is different in DNA is the association with histones and other charged chemicals that would alter the equilibrium. In any case, we would expect DNA to maintain a particular charge density and start to disaggregate when the charge density exceeded that value.

The structure of DNA is quite complicated at the molecular level, but we can approximate the energetics with a simple geometric model that estimates the area exposed to solution and the surface charge density for small DNA segments before and after they disaggregate/separate. Figure 3A shows the model used to estimate surface charge density of a 4 bp DNA segment in two DNA chains, DNA I and DNA II, before disaggregation. Each segment is approximated as four cubes, and each cube of length a and facial area a^2 represents a base (B) connected to a ribose (R) phosphate (P) polymer chain that forms the backbone of DNA. The end segments are joined to other segments on the same chain, and do not contribute to the exposed area of the segment. A total of 24 faces of area a^2 are in contact with the aqueous solvent. In Figure 3B, the 4 bp segments on the two chains have disaggregated. The bases on segment II, shown as CTCT, are exposed to the aqueous solvent, as are their (hidden) conjugates GAGA on segment I. As in Figure 3A, the end segments joined to the rest of the same chain do not contribute to the exposed area of the segment, but the newly exposed bases make a total of 32 faces of area a^2 in contact with the aqueous solvent. When aggregated,

$$\text{total exposed area} = 24 \times 0.64 \text{ nm}^2 = 15.36 \text{ nm}^2. \quad (2)$$

When the 4 bp in contact split apart, they generate an additional $8 \times 0.64 \text{ nm}^2$ or 5.12 nm^2 for a total solvated area of 20.48 nm^2 .

Initially, the two segments (I and II) are attached and the surface charge, Q , which can be as high as 1 per PO_4 group, is spread over 15.36 nm^2 . If an EM field stimulus adds a single charge to the block, a charge of $Q + 1$ will now be spread over 20.48 nm^2 . If we assume that the surface charge density has the same value as a result of a DNA split and an increase in bases exposed to solution,

$$\frac{Q}{15.36} = \frac{Q + 1}{20.48}. \quad (3)$$

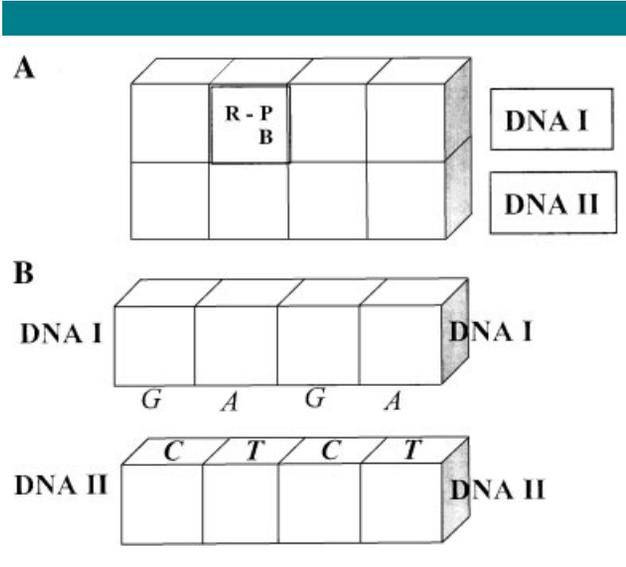


Fig. 3. A: Geometric model used to estimate surface charge density of a 4 bp DNA segment of two DNA chains, DNA I and DNA II, before separation. Each segment is approximated as four cubes, and each cube of length a and facial area a^2 represents a base (B) connected to a ribose (R) phosphate (P) polymer chain that forms the backbone of DNA. The end segments are joined to other segments, and do not contribute to the exposed area. A total of 24 faces of area a^2 are in contact with the aqueous solvent. B: When the 4 bp DNA segments on chains DNA I and DNA II separate, the bases on segment II, shown as CTCT, are exposed to the aqueous solvent, as are their (hidden) conjugates GAGA on segment I. As in subpart (A), the end segments joined to the rest of the same chain do not contribute to the exposed area, but the newly exposed bases make a total of 32 faces of area a^2 in contact with the aqueous solvent.

This leads to $Q = 3.0$, or three charges on the original 4 bp.

Repeating the calculation using different numbers of base pairs in a segment, the value $Q = 3$ appears to be a consequence of the idealized geometry we have chosen and the assumption of a constant surface charge density. The calculated values are based on approximations of molecular dimensions and neglect of interactions with histones, etc, but the value of Q is not unreasonable. Orthophosphate, an approximation for the ribose phosphate groups in DNA is about half ionized at pH 7.2. What has been demonstrated is that a polymer having the geometry of DNA can undergo aggregation-disaggregation transitions at various segment lengths with equal ease. DNA appears ready to be disaggregated and expose its code for transcription when there is a small change in the charge at a particular site. This may explain the specificity of transcription factors at particular sites and the ability of the same RNA polymerase mechanism to operate all along the chain.

Although disaggregation of DNA appears equally likely at all segment lengths, the strain of distorting the ribose-phosphate chain to pull out one base is probably too great. Also, the opening of 1 bp may not be enough to allow entry of RNA polymerase for transcription to proceed. With longer segments, there is less distortion to the DNA backbone, but more energy is needed to move the larger molecular mass after it has been hydrated. The balance between these two factors may coincide with the 4 bp unit CTCT associated with the response to EM fields. The perturbations of DNA structure due to interaction with proteins, as when bases flip out of a DNA double helix (Roberts and Cheng, 1998), can involve only a small number of base pairs.

The above mechanism offers a general rationale for disaggregation of DNA at small groups of bases, and the simple example made it appear that DNA is equally likely to disaggregate at all segment lengths and compositions. It is obvious that small differences in the actual dimensions of the individual bases and the groups that have interacted with them must affect the equilibria. The 4 bp unit CTCT associated with the response to EM fields and used in the example may be particularly effective. In addition to the low electron affinities, which enable electrons to be displaced relatively easily, the CTCT surface is 'molecularly smooth.' CTCT bases are pyrimidines and smaller than their complementary purines A and G, so a split forming CTCT and GAGA surfaces has a smaller total area than the usual mixture of pyrimidines and purines. A displaced electron at this site would have a greater effect on the charge density and create a greater driving force for separation. The smoother fit on the molecular level also leads to a lower tendency to form multiple H-bonds that increase the strength of adhesion between chains (Suehnel, 2002). Fewer multiple H-bonds would make it easier for base pairs to separate.

It is hard to make quantitative predictions, since both a 900 bp segment of the *c-myc* promoter with eight CTCT sequences, and a 70 bp region of the HSP70 promoter with only three CTCT sequences, respond to EM fields. However, in the experiments with the artificial construct, the EM field response appeared to be proportional to the number of CTCT groups present in the promoter (Lin et al., 1998). The nCTCTn sequences exist in a 3D configuration and are therefore also in contact with other DNA sequences that could be involved in the interaction. It is becoming clear from the discovery that genes could be affected by their position on the chromosomes, that overlap of genetic functional units is a widespread phenomenon. Given the fact that the DNA chain is contorted in space, and can be methylated, acetylated and phosphorylated, the positioning of the CTCT groups along the chain is probably also significant. CTCT groups separated by many base pairs may actually be quite close together in space, and some separations may allow two groups to act synergistically in helping the two chains to disaggregate.

Conclusion

Charge is a major factor controlling disaggregation of biopolymers at molecular cleavage planes. For this reason, transfer of charge in EM fields could contribute to separation of base pairs in DNA. A simple model of DNA geometry shows that an increase in local charge can cause separation of small groups of base pairs, and the low electronegativities of CTCT bases associated with the response to EM fields increase the likelihood of electron displacement. EM field initiated DNA separation can set in motion the inter-connected biochemical signaling pathways that are activated in the stress response.

Some clear implications of these ideas can be tested. The response of DNA to EM fields should vary with the charge and electron affinity of the DNA bases. Predictions about responses of DNA to charging should be testable through variations of pH by the selective binding of metal ions, histones and known transcription factors, or changes in the charge due to phosphorylation, acetylation, etc. It is also possible to test if the H-bond 'flicker' frequency in water is an upper limit for DNA response.

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