The Low Dielectric Interior of Proteins is Sufficient To Cause Major Structural Changes in DNA on Association

Adrian H. Elcock* and J. Andrew McCammon

Department of Chemistry and Biochemistry
Department of Pharmacology
University of California at San Diego
La Jolla, California 92093-0365

Received December 4, 1995

It is well-known that many proteins induce large structural changes in DNA upon association. In particular, a common observation is bending of the DNA either toward[1] or away[2−4] from the approaching protein surface. The contribution of electrostatic effects to the former type of interaction has recently been investigated;[5,6] here we report on the role played by electrostatics in interactions of the latter type. In the cases where DNA bends away from the protein, as exemplified by the TBP−DNA and SRY−DNA crystal structures,[2−4] protein binding occurs in the minor groove of the DNA and is accompanied by a significant increase in the groove width. It has been suggested[7] that a significant contribution to this effect may come from the loss of solvent screening of phosphate charges which accompanies protein binding: the replacement of high-dielectric solvent by low-dielectric protein is expected to increase cross-strand phosphate repulsions.

To investigate this idea by computer simulation, a model protein, designed to fit snugly in the DNA minor groove, was constructed and placed at a distance of 30 Å from a DNA 16-mer. The model protein was uncharged and did not *directly* interact with the DNA in any way (i.e., there were no Coulombic or van der Waals interactions); the protein is therefore simply defined as a region of low dielectric immersed in a high-dielectric solvent. The response of the DNA to the approach of this model protein into the minor groove was then investigated by molecular dynamics simulations in which forces due to changes in the solvent and ionic environment were obtained from solutions to the Poisson−Boltzmann (PB) equation. The PB equation provides an approximate but extremely useful description of electrostatic effects in biological molecules,[8] its main advantage here being that it *implicitly* accounts for solvation and ion atmosphere effects, allowing the omission of the thousands of ions and water molecules that would otherwise be required in the simulations.

Simulations were carried out with a combination of the CHARMM[9] and UHBD[10] programs; the simulation protocol adopted here will be described in more detail elsewhere.[11] The CHARMM22 molecular mechanics force field[12] was used to represent all interactions between DNA atoms; additional weak harmonic restraints (k = 10 kcal mol^{-1} Å^{-1}) were applied to maintain base pairing. The forces on atoms due to this "gas-phase" force field were supplemented by PB electrostatic forces[13] used to model solvation effects. These forces were obtained by solving the PB equation on a 75^3 grid of 1.0 Å spacing, using an ionic strength of 150 mM. The response of the DNA to the approach of the protein was modeled with the technique of stochastic dynamics[14] using a low friction coefficient of 6.5 ps^{-1}; this method has worked well in similar simulations performed on small molecules.[15] The protein was moved closer to the DNA in 2.5 Å steps (to a final separation of 7.5 Å) with 1 ps of dynamics being performed at each step. PB solvation forces were updated every 0.1 ps. In total, therefore, the protein−DNA association reaction is completed in 10 ps. We note that this is several orders of magnitude faster than the real process; however, the purpose of the present simulations is to investigate only the likely structural consequences of protein approach. In this case then, MD has been used more as a means of searching configurational space for favorable protein−DNA geometries than as a realistic model for the time-dependent process of protein−DNA association. It should be noted that due to the absence of much solvent damping, the response of the DNA in these simulations is extremely rapid. A control simulation of the DNA alone, omitting the protein, was also performed in an exactly analogous fashion.

Approach of the low-dielectric protein causes significant structural changes in the DNA. This is best illustrated by comparing the protein−DNA complex obtained at the end of the simulations with what would have been obtained had the DNA structure been held fixed (Figure 1). For the latter, it can be seen that very considerable structural overlap occurs at the position of closest approach of the protein to the DNA: few major groove atoms remain exposed, and many phosphate groups are completely hidden. In contrast, the structure obtained at the end of the simulation shows the DNA phosphates no longer embedded within the protein but, instead, fully exposed to the high-dielectric solvent. This structural change is effected (or accompanied) by a dramatic opening up of the minor groove. These changes result from the presence of the protein: the central interstrand phosphate−phosphate distances in the protein−DNA system are on average 5.1 Å longer than those in the control simulation of the DNA alone. Approach of the protein and the accompanying exclusion of high-dielectric solvent cause a large unfavorable change in the solvation of the DNA. The structural response of the DNA in the simulations is such as to limit this loss of solvation, as can be seen in Figure 2, which plots the change in the solvation energy of the system as a function of the protein−DNA distance. The tendency of the phosphate groups to move toward high-dielectric regions effectively causes the DNA to adapt its shape to that of the approaching protein. In this sense, the DNA structural changes mark a clear case of "induced fit". It is interesting that although these changes are entirely electrostatically driven, they also result in a large increase in the buried surface area of the protein.

It is important to emphasize that in these simulations no direct forces acted between the protein and the DNA; groove opening does not result, for example, from steric clashes with the protein since no van der Waals interactions operate between the protein and the DNA. The structural changes observed therefore result

(1) Schultz, S. C.; Shields, G. C.; Steitz, T. A. *Science* **1991**, *253*, 1001.
(2) Kim, Y.; Geiger, J. H.; Hahn, S.; Sigler, P. B. *Nature* **1993**, *365*, 512.
(3) Kim, J. L.; Nikolov, D. B.; Burley, S. K. *Nature* **1993**, *365*, 520.
(4) Werner, M. H.; Huth, J. R.; Gronenborn, A. M.; Clore, G. M. *Cell* **1995**, *81*, 705.
(5) Strauss, J. K.; Maher, L. J. *Science* **1994**, *266*, 1829.
(6) Crothers, D. M. *Science* **1994**, *266*, 1819.
(7) Travers, A. A. *Nat. Struct. Biol.* **1995**, *2*, 615.
(8) Honig, B.; Nicholls, A. *Science* **1995**, *268*, 1144.
(9) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
(10) (a) Madura, J. D.; Davis, M. E.; Gilson, M. K.; Wade, R. C.; Luty, B. A.; McCammon, J. A. *Rev. Comput. Chem.* **1994**, *5*, 229. (b) Madura, J. D.; Briggs, J. M.; Wade, R. C.; Davis, M. E.; Luty, B. A.; Ilin, A.; Antosiewicz, J.; Gilson, M. K.; Bagheri, B.; Scott, L. R.; McCammon, J. A. *Comput. Phys. Commun.* **1995**, *91*, 57.
(11) Elcock, A. H.; Potter, M. J.; McCammon, J. A. In *Computer Simulation of Biomolecular Systems*; Wilkinson, A. J., Weiner, P., Eds.; ESCOM: Leiden, The Netherlands, Vol. 3, to be published.
(12) Mackerell, A. D., Jr.; Wiórkiewicz-Kuczera, J.; Karplus, M. *J. Am. Chem. Soc.* **1995**, *117*, 11946.
(13) Gilson, M. K.; Davis, M. E.; Luty, B. A.; McCammon, J. A. *J. Phys. Chem.* **1993**, *97*, 3591.
(14) Brooks, C. L., III; Karplus, M.; Pettitt, B. M. *Proteins: A Theoretical Perspective of Dynamics, Structure, and Thermodynamics*; Advances in Chemical Physics, Vol. LXXI; John Wiley: New York, 1988.
(15) Gilson, M. K.; McCammon, J. A.; Madura, J. D. *J. Comput. Chem.* **1995**, *16*, 1081.

0002-7863/96/1518-3787$12.00/0 © 1996 American Chemical Society

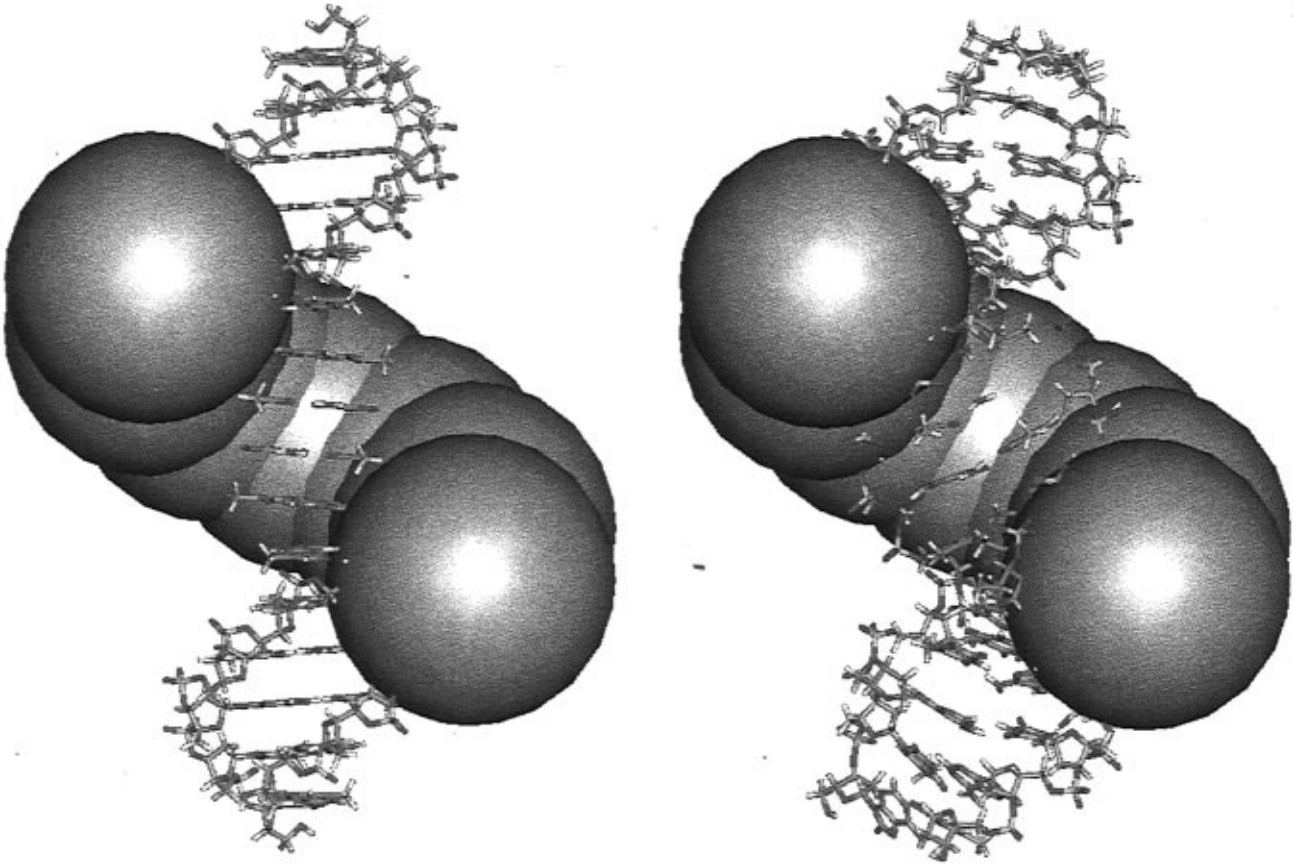

Figure 1. Structure of the protein−DNA system at the end of the simulation (right) compared with what would have been obtained if the DNA had been held rigid (left). The phosphate groups are represented by yellow atoms for phosphorus and red atoms for oxygen.

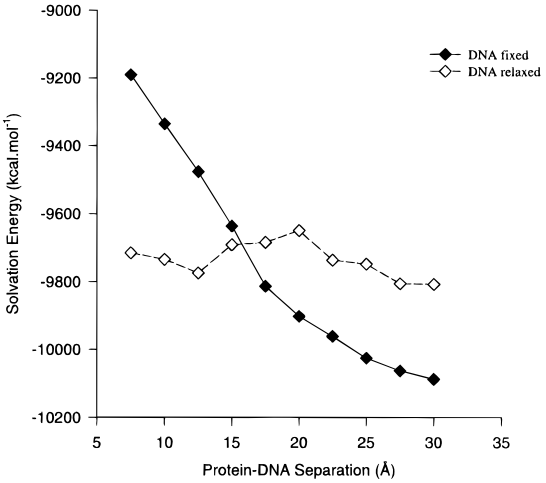

Figure 2. Electrostatic solvation energy of the protein−DNA system as a function of the separation. All calculations were performed with the UHBD program. Solutions to the nonlinear Poisson−Boltzmann equation were obtained on a 75^3 grid of spacing 1 Å at an ionic strength of 0.15 M. The dielectric of the protein and DNA regions was set to 1.0, and that of the solvent region was set to 78.5, appropriate for water at 25 °C.

only from changes in the solvent and ionic environment of the DNA, in particular, the decreased solvent shielding of phosphate repulsions. The simulations suggest, therefore, that large structural changes in DNA can be induced without introducing charge−charge interactions between the protein and the DNA, by modifying the charge−charge interactions already present within the DNA. Favorable electrostatic interactions between the protein and the DNA will typically contribute to bending of DNA *toward* a protein surface, and charge neutralization of the phosphates on one face of the DNA, effectively reducing phosphate repulsions, has been shown to make a significant contribution to such bending.[5,6] The structural changes observed here, in contrast, result from *increased* phosphate repulsions. In both cases, however, the DNA essentially "bends itself" on approach of the protein. In real protein−DNA systems, it is likely that both effects will contribute; certainly, it should not be thought that minor-groove binding will always result in dramatically increased groove width: positively charged residues in the minor-groove binding domain would be expected to compensate for the dielectric effect and favor a narrow groove, for example. The probable importance of dielectric effects suggested by the present results, however, is underlined by the fact that the DNA-binding domains of the proteins inducing the most dramatic changes in DNA structure are extremely hydrophobic.[7] It is interesting to speculate whether the dielectric-induced effects seen here might also contribute to conformational changes in other highly-charged systems such as lipids.

Acknowledgment. This work was supported in part by grants from the National Institutes of Health, the National Science Foundation, and the National Science Foundation MetaCenter Program. A.H.E. is very grateful to the Wellcome Trust for financial support under Grant 041157/Z/94/Z.

JA954061M