Nonspecific DNA Bending and the Specificity of Protein-DNA Interactions

D. A. Erie *et al.* (*1*) used scanning force microscopy to image the conformations of DNA molecules within specific and nonspecific complexes of the λ Cro protein and a 1-kb DNA fragment. These images revealed bent DNA within both types of complexes. They also revealed that Cro bent specific and nonspecific DNA by roughly equivalent amounts; the angles induced at specific DNA sites averaged 69° ± 11°, whereas the angles induced at nonspecific DNA sites averaged 62° ± 23°. The observation that Cro induced significant bends at nonspecific DNA sites led the authors to conclude that bending of nonspecific DNA by those proteins that bend specific DNA is advantageous because it increases binding specificity, the difference in free energy between specific and nonspecific complexes.

I present the argument of Erie *et al.* (*1*) in terms of an energy diagram (Fig. 1). Two limiting cases are shown, in which bending of a specific DNA site is accompanied by bending of a nonspecific DNA site (case 1) or not (case 2). Here, ΔG_{bend} represents the energy required to bend DNA; ΔG_{sp} represents the energy gained through specific DNA-protein contacts; ΔG_{nsp} represents the energy gained through nonspecific DNA-protein contacts; and $\Delta\Delta G$ represents the difference in energy between the specific and nonspecific complexes. Consider first case 1, in which Cro bends specific and nonspecific DNA equally. I assume for simplicity that the value of ΔG_{bend} depends only on the bend angle. In this case, both complexes suffer the same cost of bending DNA, and the value of $\Delta\Delta G^1$ represents the difference in the energy gained when Cro interacts with specific and nonspecific DNA: $\Delta\Delta G^1 = \Delta G_{sp} - \Delta G^1{}_{nsp}$. In case 2, in which Cro does not bend nonspecific DNA, ΔG_{bend} is larger for formation of the specific complex than for formation of the nonspecific complex. All else being equal, the absence of an unfavorable ΔG_{bend} term for nonspecific binding in case 2 lowers the free energy of the nonspecific complex relative to that of the specific complex: $\Delta\Delta G^2 = \Delta G_{sp} + \Delta G_{bend} - \Delta G^2{}_{nsp}$. The apparent result is an increase in binding specificity when Cro bends nonspecific DNA: $\Delta\Delta G^1$ is more favorable than $\Delta\Delta G^2$.

The argument described above cannot be correct because it does not predict the experimental result of Erie *et al.* (*1*); it predicts that Cro should not bend nonspecific DNA. The argument predicts that the complex between Cro and straight, nonspecific DNA ($P\text{-}D^2{}_{nsp}$ in case 2) will be lower in energy than that between Cro and bent, nonspecific DNA ($P\text{-}D^1{}_{nsp}$ in case 1); therefore the straight, nonspecific complex should be observed.

The argument of Erie *et al.* (*1*) requires that the amount of energy gained through protein-DNA interactions is largely independent of whether the DNA distorts upon binding, that is, $\Delta G^2{}_{nsp}$ equals $\Delta G^1{}_{nsp}$. However, it is more likely that the amount of energy gained through protein-DNA interactions is more favorable when the DNA distorts upon binding. Consider the binding reaction according to the pathway by which it likely occurs: first Cro binds linear DNA, then the DNA bends to make additional protein-DNA contacts. The DNA will bend only when it is energetically favorable to do so, when the incremental DNA-protein interaction energy gained when the nonspecific DNA bends (ΔG_{inc} in case 1) is equal to or greater than the energy required to distort the DNA (ΔG_{bend}). This incremental binding energy stabilizes the nonspecific complex ($P\text{-}D^{act}{}_{nsp}$ in case 1); relative to the specific complex ($P\text{-}D_{sp}$), and specificity ($\Delta\Delta G^{act}$) decreases. In other words, Cro bends nonspecific DNA to increase affinity. Bending DNA costs energy, but the act of bending must increase the stability of the protein-DNA complex, otherwise the DNA would not bend. This increase in stability leads to a reduction in binding specificity, not an increase. Although the partitioning of the free energy shown in case 1 may not be unique, if Cro is observed to bend nonspecific DNA, then the complex with bent DNA must necessarily be more stable than a complex with linear DNA. The energetic cost of DNA bending can contribute unfavorably to binding a correct DNA site and even more unfavorably to binding an incorrect DNA site (*2*–*6*). However, the bent complex will be observed only when the alternative—not bending—would lead to a less stable complex (*7*).

Alanna Schepartz
Department of Chemistry,
Yale University,
New Haven, CT 06520–8107, USA

REFERENCES AND NOTES

1. D. A. Erie, G. Yang, H. C. Schultz, C. Bustamante, *Science* **266**, 1562 (1994).
2. M. R. Gartenberg and D. M. Crothers, *Nature* **333**, 824 (1988).
3. T. A. Steitz, *Q. Rev. Biophys.* **23**, 205 (1990).
4. D. R. Lesser, M. R. Kurpiewski, L. Jen-Jacobsen, *Science* **250**, 776 (1990).
5. D. R. Lesser, M. R. Kurpiewski, T. Waters, B. A. Connolly, L. Jen-Jacobsen, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7548 (1993).
6. D. N. Paolella, C. R. Palmer, A. Schepartz, *Science* **264**, 1130 (1994).
7. I thank my co-workers N. Zondlo, A. Baranger, S. Metallo, and A. Moses and my colleagues T. Shrader, D. Crothers, P. Moore, P. Sigler, and T. Steitz for discussions and comments on the manuscript. Supported by grants from the National Institutes of Health and the National Foundation for Cancer Research.

19 January 1995; accepted 18 April 1995

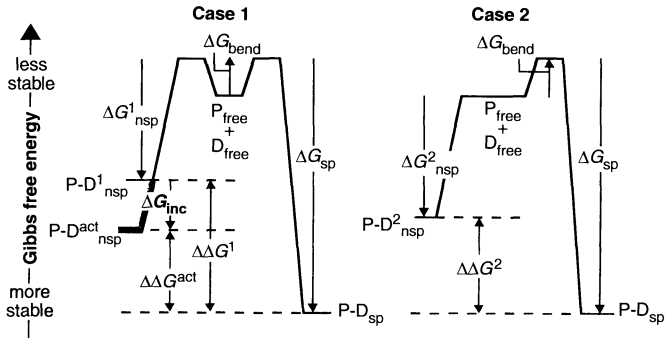

Fig. 1. Free energy diagrams illustrating the effect of nonspecific DNA bending on the stabilities of specific ($P\text{-}D_{sp}$) and nonspecific ($P\text{-}D_{nsp}$) complexes of protein (P_{free}) and DNA (D_{free}). ΔG_{bend}, energy required to bend DNA; ΔG_{sp}, energy gained through specific DNA-protein contacts; ΔG_{nsp}, energy gained through nonspecific DNA-protein contacts; and $\Delta\Delta G$, difference in energy between the specific and nonspecific complexes. Shown are the relative energies of $P\text{-}D_{nsp}$ and $P\text{-}D_{sp}$ expected if the energy gained through nonspecific DNA-protein contacts is independent of whether the protein binds bent DNA (case 1) or linear DNA (case 2). Here, $\Delta G^1{}_{nsp}$ equals $\Delta G^2{}_{nsp}$, and as a result $\Delta\Delta G^1$ is larger than $\Delta\Delta G^2$. Also shown are the relative energies expected if the energy gained through nonspecific DNA-protein contacts is larger (by the amount ΔG_{inc}) in the case where the nonspecific DNA bends. Here, ΔG_{inc} lowers the energy of $P\text{-}D^1{}_{nsp}$ to $P\text{-}D^{act}{}_{nsp}$ and as a result $\Delta\Delta G^{act}$ is smaller than $\Delta\Delta G^2$.

Response: We thank Schepartz for pointing out a potential ambiguity in our discussion of the role of DNA bending in protein binding specificity. In our report (*1*), we showed that Cro induces DNA bending when bound specifically and nonspecifically to DNA. This study provided evidence that large DNA conformational changes can occur in an ensemble of nonspecific protein-DNA complexes. We suggested that bending of the nonspecific DNA may

be an important component of the mechanism of specific site recognition by Cro. Moreover, we argued that inducing a bend at all locations along the DNA is not incompatible with the mechanism of facilitated target recognition ("sliding") that has been proposed for Cro. Finally, we suggested that the energy cost associated with bending the DNA both at specific and nonspecific sites may contribute to the binding specificity of the protein.

We would like to clarify the interpretation of this last point. Our intention was to isolate the energy cost of bending the DNA upon protein binding and to determine its contribution to binding specificity. In our analysis, we referred to an ideal, hypothetical reference state in which all protein-DNA contacts are present but in which there is no energy cost of bending the DNA, that is, the ideal state is one in which the bending rigidity of the DNA has been "turned off."

This ideal, hypothetical reference state is defined thermodynamically by $\Delta G_{i,NR} \equiv \Delta G_i - \Delta G_{i,B}$, where ΔG_i is the total free energy of the specific (i = S) or nonspecific (i = NS) complex, $\Delta G_{i,B}$ is the energy required to bend the DNA in the respective complex, and NR stands for no rigidity of the DNA. In general, the binding specificity, $\Delta\Delta G_{Sp}$, of a protein is defined as

$$\Delta\Delta G_{Sp} \equiv \Delta G_S - \Delta G_{NS} = \Delta G_{S,NR} + \Delta G_{S,B} - (\Delta G_{NS,NR} + \Delta G_{NS,B}) = \Delta\Delta G_{Sp,NR} + \Delta\Delta G_{Sp,B}$$

where $\Delta\Delta G_{Sp,NR} \equiv \Delta G_{S,NR} - \Delta G_{NS,NR}$, and $\Delta\Delta G_{Sp,B} \equiv \Delta G_{S,B} - \Delta G_{NS,B}$. $\Delta\Delta G_{Sp,B}$ is the contribution of the differential energy of bending of the DNA between the specific and nonspecific complexes to the binding specificity of the protein.

Analysis of the energy diagrams presented by Schepartz leads to the same conclusion regarding the contribution of the differential energy of bending ($\Delta\Delta G_{Sp,B}$) to binding specificity as that presented in our report, that is, that protein-induced bending of nonspecific DNA can increase binding specificity. Comparison of the minimum energy states (this point is not being argued because we agree that these would be the observed states) of case 1 and case 2 from figure 1 of the comment by Schepartz reveals that in case 1, the contribution of the differential energy of bending to specificity ($\Delta\Delta G_{Sp,B}$) is zero, whereas in case 2, it is positive (unfavorable). Because we have factored out all contributions other than that of the unfavorable cost of DNA bending, our analysis is not dependent on the values of ΔG^1_{nsp} and ΔG^2_{nsp}, as is suggested by Schepartz.

In contrast, Schepartz's analysis is valid only for a range of values of ΔG^1_{nsp} and ΔG^2_{nsp}, including the case where these two terms are equal, as is assumed in her diagrams. This choice amounts to imposing the condition that the nonspecific contacts made with straight DNA by a protein that bends the nonspecific DNA (case 1) are the same or similar to those made with straight DNA by a protein that does not bend the DNA upon nonspecific binding (case 2). There is no reason to believe that ΔG^1_{nsp} should be equal or very close to ΔG^2_{nsp}, because the conformation of the DNA in the two final states is markedly different. It is more likely that a protein that bends the DNA to form a nonspecific complex may lose or gain some of the nonspecific interactions that would have been available to it were it not to bend the DNA. The implication of Schepartz's diagrams is as follows. If the protein in case 2 can make the same contacts (which do not require bending of the DNA) as the protein in case 1, then there should in principle be no reason why, following Schepartz's argument, this protein would not "choose" to gain further stabilization by bending the DNA. The implication of this argument is that a protein that bends the specific site must always bend the nonspecific sites. However, Schepartz's argument assumes the separability of the ΔG^1_{nsp} and the ΔG_{inc}. In general, we do not have the information necessary to carry out this partitioning of the total favorable free energy of binding, and therefore the final energy of the complex cannot be predicted a priori. Our analysis nevertheless avoids this problem by isolating the one contribution to the total energy of binding for which we have independent experimental information, that is, the energy cost of bending the DNA.

Schepartz suggests that the likely pathway for binding of Cro is that it first binds linear DNA and then bends the DNA to make additional contacts. There is, however, no experimental evidence to support this hypothesis. Because the argument about energetics is made from this starting point, implicit in figure 1 of Schepartz's comment is that in case 1 (where the protein bends the DNA), the interactions that the protein could make with straight DNA are necessarily favorable ($\Delta G^1_{nsp} < 0$), that is the protein interacts favorably with straight DNA even though the final DNA conformation is bent. There is no reason to make this assumption; it is possible that the interaction of such a protein with straight DNA is unfavorable ($\Delta G^1_{nsp} > 0$). In this case, all of the favorable interaction energy would have to come from contacts resulting from the distortion of the DNA (ΔG_{inc} in Schepartz's notation).

Our data on Cro support the hypothesis that differences in bending energy might modulate specificity of protein-DNA interactions [(2–6) in the comment by Schepartz]. If we had observed that the nonspecific complexes were not bent (Schepartz's case 2), then $\Delta G_{NS,B} = 0$, and the differential energy of bending of DNA would make only unfavorable contributions to specificity ($\Delta\Delta G_{Sp,B} > 0$), because energy is required to induce DNA bending. Furthermore, the value of $\Delta\Delta G_{Sp,B}$ would not depend on the position on the template where the nonspecific binding occurs and would depend only on the bending rigidity of the specific site. Consequently, in such a case, the bending rigidity of the DNA as a function of DNA sequence could not modulate specificity.

Specificity is dominated by differences in energy between the final states of the specific and nonspecific complexes and is independent of the path by which the complexes are formed. To determine the contributions to specificity, it is necessary to compare the difference in energy between the final states of specific and nonspecific complexes for two different classes of proteins, namely, a protein that bends the DNA in the final state and a protein that can make all the same contacts with its site without bending the DNA. On the contrary, comparing the energies between different states (real or hypothetical) along the pathway to the formation of the final state provides no information about the contributions of bending to binding specificity. For Cro, the putative "straight state" must be of higher energy than the observed bent state, but as pointed out by Schepartz, this is not (nor can it be) a final state, so the comparison is not possible. Comparing final states of two different classes of protein, in contrast, can be used to elucidate the contributions to specificity associated with the differential energy of bending of the DNA, as was suggested in our report.

More data will be required to determine whether DNA bending in nonspecific complexes is a general property of proteins that bend their specific sites. Such data should indicate for which cases the suggestion that the sequence-dependent bendability of the DNA can modulate specificity is applicable.

Dorothy A. Erie
Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27514, USA
Carlos Bustamante
Institute of Molecular Biology, Howard Hughes Medical Institute, University of Oregon, Eugene, OR 97403, USA

REFERENCES AND NOTES

1. D. A. Erie, G. Yang, H. C. Schultz, C. Bustamante, *Science* **266**, 1562 (1994).
2. We thank S. Smith and J. Schellman for useful discussions.

2 March 1995; accepted 18 April 1995