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 bends 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\textsubscript{bend} represents the energy required to bend DNA; ΔG\textsubscript{sp} represents the energy gained through specific DNA-protein contacts; ΔG\textsubscript{rms} represents the energy gained through nonspecific DNA-protein contacts; and ΔG 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\textsubscript{bend} depends only on the bend angle. In this case, both complexes suffer the same cost of bending DNA, and the value of ΔG\textsuperscript{1} represents the difference in the energy gained when Cro interacts with specific and nonspecific DNA: ΔG\textsuperscript{1} = ΔG\textsubscript{sp} - ΔG\textsubscript{rms}. In case 2, in which Cro does not bend nonspecific DNA, ΔG\textsubscript{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\textsubscript{bend} term for nonspecific binding in case 2 lowers the free energy of the nonspecific complex relative to that of the specific complex: ΔG\textsuperscript{2} = ΔG\textsubscript{sp} + ΔG\textsubscript{bend} - ΔG\textsubscript{rms}. The apparent result is an increase in binding specificity when Cro bends nonspecific DNA: ΔG\textsuperscript{1} is more favorable than ΔG\textsuperscript{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-D\textsuperscript{2} in case 2) will be lower in energy than that between Cro and bent, nonspecific DNA (P-D\textsuperscript{2} 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, ΔG\textsuperscript{2} equals ΔG\textsuperscript{rms}. 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 bends 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\textsubscript{inc}) in case 1 is equal to or greater than the energy required to distort the DNA (ΔG\textsubscript{bend}). This increment bending energy stabilizes the nonspecific complex (P-D\textsuperscript{act} in case 1); relative to the specific complex (P-D\textsubscript{sp}), and specificity (∆G\textsuperscript{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—non-bending—would lead to a less stable complex (7).

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REFERENCES AND NOTES

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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
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