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Flexibility of DNA in complex with proteins deduced from the distribution of bending angles observed by scanning force microscopy

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Abstract

Flexibility and dynamics of DNA are important for DNA-binding and recognition by proteins. Here the flexibility of DNA is calculated from the distribution of DNA-bending angles of single DNA molecules as observed by scanning force microscopy by applying an equation that links the force constant of DNA-bending (f) to the variance of the distribution of bending angles (σ): $f = RT/\sigma^2$. Using published data, f is calculated to be 3–5 J/degree^2 for free DNA. Thus, bending DNA by 20° requires approx. 0.5–1 kJ/mol. This result shows that DNA is very flexible and readily can be bent by thermal motion. DNA-flexibility is not altered in some protein–DNA complexes (*Hha*I methyltransferase, *Eco*RV restriction endonuclease). In contrast, DNA-binding by *Eco*RI endonuclease increases DNA-flexibility and binding by *Eco*RI methyltransferase restricts the flexibility of DNA. During the transition of the RNA polymerase-σ^{54}–DNA complex from the closed to the open form and of cro repressor from a non-specific to a specific binding mode the flexibility of the DNA is strongly reduced.

Keywords: DNA-bending; DNA-binding; DNA recognition; DNA structure; Dynamics of DNA conformation; Protein–DNA interaction

1. Introduction

DNA is a flexible molecule [1] that can be stretched [2–4] and bent [5–7]. Many proteins that bind to DNA bend their DNA target upon binding [8,9]. In these cases the conformational changes of the DNA like bending or twisting are intimately coupled to complex formation [10,11]. The ability of the DNA to be distorted in a characteristic way is dependent on the sequence of the DNA [12–14]. Thus, DNA distortion can contribute to DNA recognition ('indirect readout')

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[10,11]. In addition, the accessibility of the DNA to enzymatic modification, like hydrolysis catalyzed by nucleases, is dependent on the flexibility of the DNA [15–18]. However, dynamic features of protein–DNA complexes are difficult to measure, so far.

Recently, many protein–DNA complexes have been investigated by scanning force microscopy, which allows to detect bending angles of DNA molecules [19–23] (data are summarized in Table 1). In these experiments protein–DNA complexes were loosely bound on a surface and equilibrated their conformation on the surface. It has been shown in many cases, that the observed mean bending angles closely correspond to bending angles determined by other techniques, lending credit to the reliability of the method [21,22]. However, as in these experiments the shapes of single DNA molecules are analyzed, not only the mean DNA-bending angle of all molecules can be analyzed, but also the variance of the distribution of bending angles. Gaussian distributions of bending angles are reported [19–23], excellent fits of the observed distributions to Gaussian curves are shown by Garcia et al. [22] and Rippe et al. [23]. The shape of this distribution reflects the dynamic properties of the system, i.e. the flexibility of the DNA. According to the laws of statistical thermodynamics the observed width of the distribution of bending angles is governed by the energy difference between DNA molecules having different bending angles as defined by the Boltzmann distribution (the wider the distribution the lower the energy difference between more or less bent molecules). By comparing the equations describing the experimentally observed Gaussian distributions of bending angles and the Boltzmann distribution, a quantitative relationship

Table 1
Distribution of bending angles of DNA molecules in complex with different proteins as observed by scanning force microscopy

DNA-protein complex[a]	Complex type	μ(degree)[b]	σ(degree)[b]	f[b] (J/degree^2)	Buffer conditions[c]	Ref.
Free DNA (used for *Hha*I methyltransferase study)	–	4	23	4.7	A	[22]
*Hha*I methyltransferase	Specific	2	28	3.1	A	[22]
*Eco*RI methyltransferase	Specific	51	17	8.5	A	[22]
*Eco*RI endonuclease	Specific	10	52	0.9	B	[21]
*Eco*RV endonuclease	Specific	43	29	3.0	B	[21]
Free DNA (used for RNA polymerase-σ^{54} study)	–	0	31	2.6	C	[23]
RNA polymerase-σ^{54}	Closed complex	49	24	4.3	C	[23]
RNA polymerase-σ^{54}	Open complex	114	18	7.6	C	[23]
λ-Cro	Non-specific complex	62	23	4.7	D	[20]
λ-Cro	Specific complex	69	11	20.3	D	[20]

[a]All experiments were carried out on mica in air using the tapping mode under similar buffer conditions. The corresponding force constants of DNA-bending are calculated using Eq. (5).
[b]μ, mean bending angle; σ, variance of distribution of bending angles observed; f, force constant of bending (derived using Eq. (5)).
[c]Buffer conditions: A, 18 mM HEPES (pH 7.6), 0.9 mM DTT, 9 mM $MgCl_2$; B, not specified; C, 22 mM HEPES/KOH (pH 8.0), 10 mM magnesium acetate, 40 mM potassium acetate, 0.01 mM DTT; and D, 20 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 7.5 mM potassium acetate.

between the variance of the distribution of DNA-bending angles and the flexibility of DNA can be derived.

2. Theoretical approach

If DNA is regarded as a spring, its flexibility is quantitatively described by the force constant f of the spring towards bending. Thus, a relation between the variance of the distribution of DNA-bending angles and f has to be derived. According to the Gaussian distribution the fraction (X_φ) of DNA molecules having a bending angle of φ is proportional to the mean bending angle (μ) and the variance of the distribution (σ):

$$X_\varphi \sim \exp\left[-1/2((\varphi-\mu)/\sigma)^2\right] = \exp\left[-(\varphi-\mu)^2/2\sigma^2\right] \quad (1)$$

(with φ, bending angle; X_φ, fraction of molecules having a bending angle of φ; μ, mean bending angle; σ, variance of distribution).

In statistical thermodynamics the occupation of a state is correlated to its energy as given by the Boltzmann distribution. In this distribution the fraction of DNA molecules having a bending angle that differs by φ from the mean conformation is proportional to their energy (ε_φ):

$$X_\varphi \sim \exp(-\varepsilon_\varphi/RT) \quad (2)$$

(with ε_φ, energy required to a bend the DNA by φ).

If one regards DNA as an elastic spring that responds linearly to deviations of the bending angle from its equilibrium value, the energy required for bending depends on the bending angle as follows:

$$\varepsilon_\varphi = -1/2\, f\, (\varphi-\mu)^2 \quad (3)$$

(with f, force constant of bending).

Combining Eq. (2) and Eq. (3) yields:

$$X_\varphi \sim \exp(-\varepsilon_\varphi/RT) = \exp\left[-f(\varphi-\mu)^2/2RT\right] \quad (4)$$

By comparing Eq. (4) and Eq. (1) one obtains:

$$f = RT/\sigma^2 \quad (5)$$

Eq. (5) describes the desired quantitative relationship between the standard deviation σ of observed bending angles and the flexibility of DNA. Although this relationship has been reported previously [21] it so far has not been applied on scanning force microscopy data, which image DNA under near native conditions. It is especially useful to analyze changes of the flexibility of DNA caused by protein-binding.

3. Results and discussion

Changes in the conformation and dynamics of DNA can be expected to influence the thermodynamics of protein–DNA interactions [10]. In particular, the sequence-dependent structural polymorphism of DNA contributes to DNA recognition by proteins ('indirect readout') [10,11,14,15]. Indirect readout is a complicated phenomenon that includes not only static sequence-dependent structures but dynamic effects as well. However, very few studies so far have addressed this question. For example, by ^{31}P NMR changes of the dynamics of the DNA were observed with lac repressor headpiece–operator complexes. It has been shown that high-affinity protein–operator complexes retain the inherent high-flexibility of the free operator, whereas the DNA is conformationally restricted in lower-affinity complexes [25].

Recently, many protein–DNA complexes as well as free DNA have been observed by scanning force microscopy. So far, these results were only interpreted with respect to the mean bending angle of the DNA. Published results are summarized in Table 1. However, as single DNA molecules are investigated the distribution of bending angles can also be derived from the data, that allows straightforward conclusions as to the flexibility of the DNA (Eq. (5)).

So far, only two data sets are reported for free

DNA molecules (Table 1). Having a mean bending angle of 4° and 0° the images display a very broad distribution of bending angles ($\sigma = 23°$ and 31°, Table 1). Using the variance of bending angles observed in these experiments, one obtains a force constant for bending DNA of 4.6 and 2.6 J/degree^2. Therefore bending DNA by 20° from its equilibrium conformation requires only approx. 0.5–1 kJ/mol if one assumes Eq. (3) to be valid over this range. It should be noticed that the equilibrium conformation can be bent. The bending energy can be compared to values calculated from the persistence length of the DNA using Eq. (6) [30,20]:

$$\varepsilon_\theta = PRT\theta^2/2l \quad (6)$$

(with ε_θ, energy required to bend a DNA fragment of length l by an angle of θ; P, persistence length) [30,20].

Applying Eq. (6) and using a persistence length of the DNA of 130 bp [31,32] one can estimate that approx. 1 kJ/mol is required to bend a DNA piece comprising 20 bp by 20°. Thus, the bending energy derived on the basis of Eq. (5) and Eq. (6) are in excellent agreement to each other. However, it should be noticed, that the persistence length is an averaged property, whereas the method applied here, allows to calculate local, sequence-specific bending energies. The persistence length of DNA is dependent on the concentration and type of counterions [33], covalent structure of the DNA [34], chemical modifications [35] and helix type [36].

In addition, changes of the flexibility of DNA can occur upon protein binding. These changes can be analyzed in quantitative terms using the method of data analysis presented here. Like in free DNA, in protein–DNA complexes the force constants of DNA-bending reflect the energy required to bend the DNA out of its equilibrium conformation, which is strongly bent in some complexes. As shown in Table 1, a broad range of force constants of bending is observed in protein–DNA complexes. In some cases the force constant of DNA-bending is similar to that in free DNA, meaning that the conformation of the DNA is not restricted in complex with these proteins. When bound to the *Eco*RI restriction endonuclease the DNA even gains flexibility. In other cases, distortion of the DNA is more difficult in complex with a protein as illustrated by the values of f calculated for the *Eco*RI methyltransferase and the λ-cro protein in complex with specific DNA. In these cases strong bending is observed with a narrow distribution of bending angles, which indicates that the protein significantly restricts the conformational freedom of the DNA. For example, bending DNA that is specifically bound to λ-cro by 20° out of its equilibrium conformation requires 4 kJ/mol, thus, the energy barrier for DNA-bending is 5–10-fold higher than in free DNA. Moreover, the data given in Table 1 demonstrate that the increase in bending observed during formation of an open complex of the RNA polymerase-σ^{54}–DNA complex goes in parallel with a more constrained conformation of the DNA. Interestingly, the transition of the cro protein from a non-specific to a specific complex also strongly restricts the flexibility of the DNA, but does almost not alter the mean bending angle.

These results show, that DNA is very flexible and can be bent considerably even by thermal motion. Perhaps this low energetic barrier between differently bent DNA conformations may explain the long dispute on how much and where certain DNA sequences are bent [24]. In addition, the high-flexibility of DNA even in complex with many proteins may be responsible for the observation, that in some cases largely different DNA-bending angles of protein–DNA complexes were measured, when different techniques are used, because under the experimental conditions of different techniques, that inevitably are not identical, dissimilar average structures might be preferred. For example, DNA-bending angles of the basic region-leucine zipper protein complex Jun–Fos vary between 94° and $< 5°$ [26] and the DNA-bending angle of DNA bound by the *Eco*RI restriction enzyme is approx. 10° when observed by scanning force microscopy [21] or X-ray crystallography [27], but a value of 55° has been deduced from gel shift experiments [28]. It should be noticed that DNA bound by *Eco*RI has the highest flexibility of all samples that are investigated so far (Table 1), supporting the interpreta-

tion, that DNA-flexibility may obscure these results at least in part. In the light of these findings, the values of DNA-bending angles determined by averaging techniques have to be interpreted with some caution and DNA-flexibility has to be taken into consideration. In addition, these few examples illustrate that the method of data analysis presented here potentially provides important information about the contribution of DNA-flexibility to the mechanism of DNA-binding and recognition by proteins, because it allows a site-specific analysis of changes in the flexibility of DNA during formation of protein–DNA complexes.

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References

[1] J.M. Withka, S. Swaminathan, J. Srinivasan, D.L. Beveridge, P.H. Bolton, Science 255 (1992) 597–599.
[2] T.T. Perkins, S.R. Quake, D.E. Smith, S. Chu, Science 264 (1994) 822–826.
[3] P. Cluzel, A. Lebrun, C. Heller, R. Lavery, J.-L. Viovy, D. Chatenay, F. Charon, Science 271 (1996) 792–794.
[4] S.B. Smith, Y. Cui, C. Bustamante, Science 271 (1996) 795–799.
[5] D.M. Crothers, T.E. Haran, J.G. Nadeau, J. Biol. Chem. 265 (1990) 7093–7096.
[6] D.M. Crothers, Science 266 (1994) 1819–1820.
[7] J.K. Strauss, L.J. Maher III, Science 266 (1994) 1829–1834.
[8] R. Tjian, T. Maniatis, Cell 77 (1994) 5–8.
[9] M.H. Werner, A.M. Gronenborn, G.M. Clore, Science 271 (1996) 778–784.
[10] T. Härd, T. Lundbäck, Biophys. Chem. 62 (1996) 121–139.
[11] T.A. Steitz, Q. Rev. Biophys. 23 (1990) 205–280.
[12] O.Y. Fedoroff, B.R. Reid, V.P. Chuprina, J. Mol. Biol. 235 (1994) 325–330.
[13] V.B. Zhurkin, N.B. Ulyanov, A.A. Gorin, R.L. Jernigan, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 7046–7050.
[14] P.J. Hagerman, Biochem. Biophys. Acta 1131 (1989) 125–132.
[15] A.A. Travers, Ann. Rev. Biochem. 58 (1990) 427–452.
[16] I. Brukner, M. Dlakic, A. Savic, S. Susic, S. Pongor, D. Suck, Nucleic Acids Res. 21 (1993) 1025–1029.
[17] G. Meiss, P. Friedhoff, M. Hahn, O. Gimadutinow, A. Pingoud, Biochemistry 34 (1995) 11979–11988.
[18] S. Windolph, A. Fritz, T. Oelgeschläger, H. Wolfes, J. Alves, Biochemistry 36 (1997) 9478–9485.
[19] W.A. Rees, R.W. Keller, J.P. Vesenka, G. Yang, C. Bustamante, Science 260 (1993) 1646–1649.
[20] D.A. Erie, G. Yang, H.C. Schultz, C. Bustamante, Science 266 (1994) 1562–1566.
[21] C. Bustamante, C. Rivetti, Ann. Rev. Biomol. Struct. 25 (1996) 395–429.
[22] R.A. Garcia, C. Bustamante, N.O. Reich, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 7618–7622.
[23] K. Rippe, M. Guthold, P.H. von Hippel, C. Bustamante, J. Mol. Biol. 270 (1997) 125–138.
[24] M.C. Wahl, M. Sundaralingam, Curr. Opin. Struct. Biol. 5 (1995) 282–295.
[25] C. Karslake, M.V. Botuyan, D.G. Gorenstein, Biochemistry 31 (1992) 1849–1858.
[26] P.J. Hagerman, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 9993–9996.
[27] Y. Kim, J.C. Grable, R. Love, P.J. Greene, J.M. Rosenberg, Science 249 (1990) 1307–1309.
[28] J.F. Thompson, A. Landy, Nucleic Acids Res. 16 (1988) 9687–9705.
[30] J.A. Schellman, Biopolymers 13 (1974) 217–226.
[31] C.R. Cantor, P.R. Schimmel, Biophysical Chemistry, Part III, W.H. Freeman, New York, 1980.
[32] P.J. Hagerman, Ann. Rev. Biophys. Chem. 17 (1988) 265–286.
[33] C.G. Baumann, S.B. Smith, V.A. Bloomfield, C. Bustamante, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 6185–6190.
[34] P. Furrer, J. Bednar, A.Z. Stasiak, V. Katrich, D. Michoud, A. Stasiak, J. Dubochet, J. Mol. Biol. 266 (1997) 711–721.
[35] B. Revet, J.M. Malinge, E. Delain, M. Le Bret, M. Leng, Nucleic Acids Res. 12 (1984) 8349–8362.
[36] E. Charney, H.H. Chen, D.C. Rau, J. Biomol. Struct. Dyn. 9 (1991) 353–362.