Electrophoretic behavior of d(GGAAAAAAGG)$_n$, d(CCAAAAAACC)$_n$, and (CCAAAAAAGG)$_n$ and implications for a DNA bending model

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ABSTRACT

Double stranded multimers $(C_2A_6C_2)_n$,$(C_2A_6G_2)_n$ and $(G_2A_6G_2)_n$ were prepared from chemically synthesized oligonucleotides to study the influence of sequences flanking the A_n tract on the curvature of DNA. All these duplexes, including polypurine·polypyrimidine one, exhibit strong retardation in polyacrylamide gel which is indicative of pronounced DNA curvature. It has been proposed previously that among the bends at the boundary with the oligo(A) tract two types should be distinguished: 5′-bends and 3′-bends (Koo et al., 1986) This distinction was deduced from different relative mobilities of two specially designed sequences having phased 5′-bends and 3′-bends, respectively. Our data indicate that the substitutions of nucleotides at both 5′ and 3′ boundaries of A_6 tract result in comparable changes in relative mobility. Therefore, for B-B′ bends it is important to take into account not only whether they are at the 5′ or 3′ end of an oligo(dA) tract, but also the particular sequences at the boundaries of this tract.

INTRODUCTION

Since the phenomenon of stable DNA bending (1) was discovered and its biological significance revealed (2), the classification and description of sequence-dependent DNA bends has become a matter of interest.

Several years ago models of sequence-dependent DNA curvature were rather contradicting but now they have the tendency to converge. One family of models was based on the general idea that the net curvature can be described in terms of different 'wedges' for 16 dinucleotide steps (or 10 unique steps) (3,4). A detailed model within this framework would comprise attributing the particular wedge parameters to dinucleotide steps (or groups of them). The last model of this sort (5) uses the available electrophoretic data and X-ray information (6,7) to deduce the parameters.

Another idea was that stable curvature is due to presence of neighboring B′ and B (or two B′) structures (8−10), the first one being typical of rather long A_nT_m tracts (11−16). Both ideas are valuable and may be reconciled by admitting the extended set of dinucleotide steps including (in addition to 10 common steps) those within the B′ tracts and in the vicinity of B-B′ or B1′-B2′ boundaries, the latter steps carrying bigger local curvature. Unfortunately, the available data are not sufficient for deduction of a complete and accurate model, thus only somehow restricted models can be used at present.

Within the framework of the simplest 'junction' concept where the bends are attributed only to junction dinucleotide steps there are two models (16,10) providing good qualitative agreement with experimental data ((2,16) and cited therein). They are close, though one of them was worked out from electrophoretic data (16) and the other one from conformational analysis (10). One particular question concerns the sequence-dependence of bends at B-B′ (or B′-B) boundaries. In the classical work of Koo et al. (9) two junctions B-A_n and A_n-B (equivalent to T_n-B and B-T_n, respectively) were distinguished. It was demonstrated that a larger bending angle should be attributed to the 3′-end of the A_n tract. However, in these experiments the only steps flanking the A_n tract were CA and AC at the 5′ and 3′ ends of the tract, respectively. Meanwhile, it cannot be excluded that changing CA and AC steps for other ones (e.g. GA and AG) would result in quite opposite relations between the bends at 3′ and 5′ ends. Moreover, there are some hints from our previous work (10) that different alternations of purines and pyrimidines can modulate the value and the direction of bending. Therefore, it is important to work out a more precise classification of bends and investigate the influence of a particular step sequence on the value and direction of bending.

In the present work we report the results of our measurements of electrophoretic anomalies for three kinds of duplexes (complementary chains are not shown): $(C_2A_6C_2)_n$, $(C_2A_6G_2)_n$ and $(G_2A_6G_2)_n$, which differ only by types of steps flanking the A_6 tract. A comparison of the first and the last multimers is of particular interest because they differ only by their steps flanking A_6, thus being identical in terms of the model distinguishing bends only according to the 5′ or 3′ end of the A_6 tract (9,16). The experiments demonstrate that the difference in relative mobilities between these sequences is comparable to that detected between $(C_2A_5CG_3CA_8)_n$ and $(C_2A_8CG_3CA_5)_n$ (called A_{8-5} and

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A_{5-8}, respectively) sequences (9) which were synthesized to reveal 3′ and 5′ end effects. Besides, it is demonstrated that the polypurine·polypyrimidine DNA duplex $(G_2A_6G_2)_n$ exhibits strong anomaly at 27°C reaching the value of 2 at 200 bp.

MATERIALS AND METHODS

DNA synthesis and purification: Three pairs of complementary oligonucleotides (CCAAAAAACC, GGGTTTTTTG, CCAAAAAAGG, GCCTTTTTTG, GGAAAAAAGG, CCCTTTTTTC) with a 5′ one-nucleotide extension were synthesized by the phosphoamidate solid-phase method and purified by HPLC.

Pairs of complementary (except for single-stranded residues at the 5′ ends) oligonucleotides were mixed, and mixtures containing 1 μg of oligonucleotides were kinased in total volume of 5 μl with 10 μCi of [γ^{32}P]ATP (sp. act. > 5000Ci/mmol) and 6 units of T4 polynucleotide kinase at 37°C during 15 minutes, and another 15 minutes with 5 nM of cold ATP and additional 4 units of T4 polynucleotide kinase; then heated to 60°C and cooled slowly to room temperature to form hybrids. Two μl of kinased mixtures were used in ligation reaction with 2 units of T4 DNA ligase in total volume of 10 μl. The reaction was allowed to proceed at room temperature during a time length optimized for a particular pair of oligonucleotides. Ligated products were run on non-denaturing 8% polyacrylamide gels (mono:bis acrylamide ratio= 29:1,90 mM Tris-borate, 10 mM EDTA, pH 8.3). The applied voltage was 7 v/cm and electrophoresis was carried out at 7 or 27°C. Multimers of PstI linkers (GCTGCAGC) were electrophoresed adjacent to a pBR322-SouIIIA digest to confirm their normal gel mobility.

RESULTS AND DISCUSSION

The magnitudes of anomalous electrophoretic mobilities of $(C_2A_6C_2)_n$, $(C_2A_6G_2)_n$ and $(G_2A_6G_2)_n$ multimers were determined in 8% polyacrylamide gel at 7°C and 27°C. The results shown in Fig. 2 clearly demonstrate that all three multimers have pronounced anomalies. However, there is a detectable difference between them. $(C_2A_6C_2)_n$ multimers exhibit the most anomalous migration in polyacrylamide gels while $(G_2A_6G_2)_n$ multimers have the smallest anomaly. The curves are rather similar in their shape and they tend to reach a plateau at lengths more than 200 bp (T=27°C). As expected, the anomalies increase at low temperature (T=7°C) and reach rather large values (R_L more than 3.). At low temperature the mutual positions of the curves remain unchanged. Bearing in mind these data let us dwell upon two points concerning sequence-dependent curvature.

Curvature of poly(Pu)·poly(Py)

The presence of phased oligo(dA) tracts rather than a particular sequence of purines and pyrimidines is the true reason of DNA curvaturre as has been clearly established (9,17) for a number of sequences with different alterations of purines and pyrimidines. For instance, Diekmann (17) has demonstrated that four multimers with the same purine/pyrimidine sequence exhibit strikingly different anomalies. However, the simplest test with the DNA fragment having poly(Pu)·poly(Py) has not been done until recently. According to a preliminary report (18) $(G_5A_5)_n$ multimers have anomalous mobility, the value of relative mobility at 200 bp being only 1.9 at 4°C in 8% gel. In our case the value

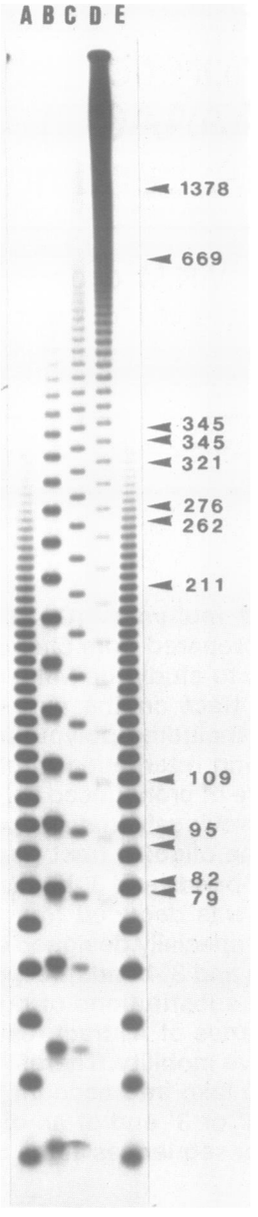

Figure 1. Autoradiogram of the multimers $(C_2A_6C_2)$, $(C_2A_6G_2)$ and $(G_2A_6G_2)$ electrophoresed on a non-denaturing 8% polyacrylamide gel at 27°. Lanes a and e: multimers of PstI linkers (GCTGCAGC); lane b: multimers of $(C_2A_6C_2)$; lane c: multimers of $(C_2A_6G_2)$; lane d: multimers of $(G_2A_6G_2)$. Size markers from pBR322-SouIIIA digest are shown by arrows.

is much greater even at higher temperature (R ≈ 2.8, T=7°C, 8% gel). This difference remains also at room temperature (R_L reaches the value of ≈2 for $(G_4A_6)_n$). A greater anomaly in the case of $(G_4A_6)_n$ (Fig. 2) may partly be a consequence of a longer oligo(A) tract.

Another aim of the present work was to compare anomalies of poly(Pu)·poly(Py) and other oligo(A) containing multimers with minimal differences in their sequence. $C_2A_6C_2$ and $G_2A_6G_2$ repeats have similar phases and the same length of oligo(A) tracts. Fig. 2 shows that the anomalies for these two sequences are rather large while the difference between them is much less. Poly(Pu)·Poly(Py) DNA has smaller anomalies. The difference in R_L between these two curves at 200 bp (T=27°C) makes up only ≈ 24% of the value for $(G_2A_6G_2)_n$. So, the main determinant of electrophoretic anomaly, as it has been previously

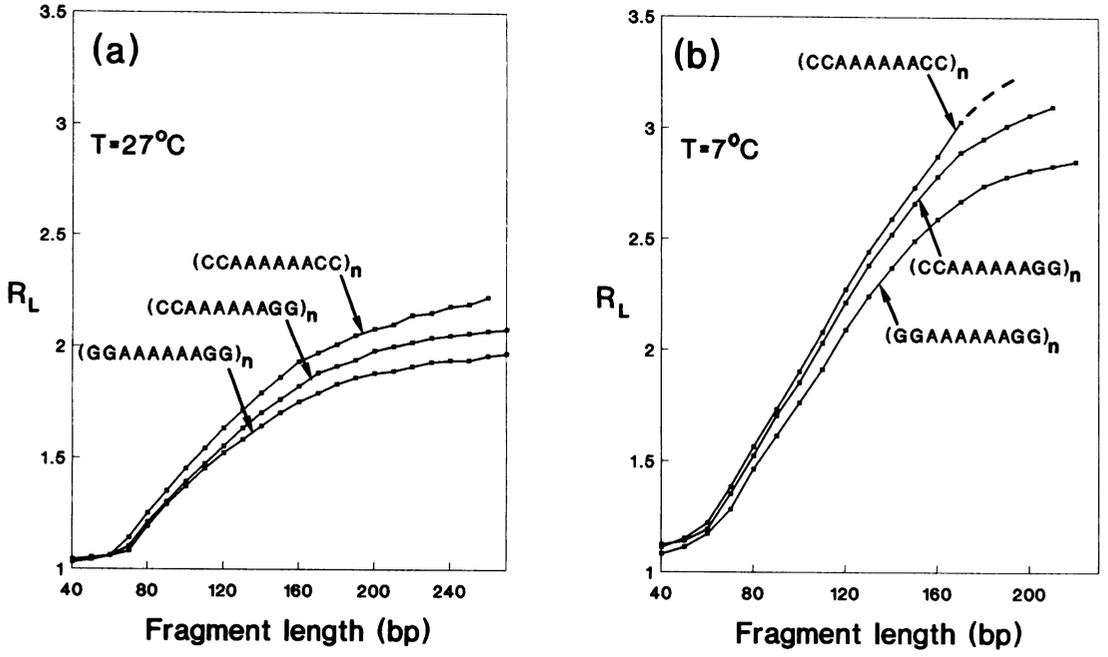

Figure 2. The plot of relative electrophoretic mobility of three multimers $(C_2A_6C_2)_n$, $(C_2A_6G_2)_n$ and $(G_2A_6G_2)_n$ at 27°C (a) and 7°C (b). These multimers differ only by types of steps flanking the A_6 tract. One of them, namely $(G_2A_6G_2)_n$, is polypurine·polypyrimidine duplex.

Table I. List of different dinucleotide steps at the B-B′ boundaries [a, b].

Type number	Junction dinucleotide	Complementary dinucleotide	5′ or 3′ end of adenine chain [3,10]
1.	5′-Ca-3′	5′-tG-3′	5′-bend
2.	5′-Ta-3′	5′-tA-3′	5′-bend
3.	5′-Ga-3′	5′-tC-3′	5′-bend
4.	5′-aG-3′	5′-Ct-3′	3′-bend
5.	5′-aC-3′	5′-Gt-3′	3′-bend

[a] A and T nucleotides within A_nT_m tracts are designated by lower case letters.
[b] In each case complementary dinucleotide (column 3 of the Table) has identical bend. Formally, the binding parameters for complementary step can be obtained by changing sign of tilt angle.

revealed (9), is due to the presence of phased boundaries of B and B′ DNA fragments irrespective of the particular sequence in the B-tract.

Classification of bends at the B−B′ (or B′−B) junction

To consider different steps at the B-B′ boundaries and to avoid misunderstanding in terminology let us first focus on some structural features and formal approximation of the junction model as we treat it.

1). The anomalous B′ structure can be formed at A_nT_m tracts (n=0,1,...; m=0,1,..; n+m ≥4) and the internal part of these tracts is approximately straight. (We will refer to nucleotides consistuting such a tract as 'a' or 't' instead of A or T to distinguish them from A and T nucleotides in B DNA).

2). DNA tracts other than long A_nT_m tracts can be attributed to B-fragments if their length is greater than several base pairs. The internal part of random sequence B-tracts is also considered as having a straight helical axis.

3). The bends appear at the boundaries of B-B′. If in the B′-B′ junction there are one or two bp disrupting the anomalous DNA structure the bend of another type appears because B-region is not yet formed (this case will not be considered in the present paper, see e.g.(10)).

4). The bend at the B-B′ junction can be spread over several base pairs in the vicinity of the junction step but it can be formally attributed to this step. As the first approximation it can be proposed that the amplitude and phase of the bend depends only on the type of junction dinucleotide.

Within the framework of these definitions there can be 5 different junction dinucleotides (see Table I). In papers (9,16) the only distinction was made between 5′- and 3′-bends with respect to the A_n tract. It means that the $\alpha(1) \approx \alpha(2) \approx \alpha(3)$ and differs from $\alpha(4) \approx (5)$, where $\alpha(i)$ is the vector of value and direction of bending and i=1, 2..,5 is the type of boundary dinucleotide step (Table I). As a matter of fact, the background experimental data (9) demonstrate only but $\alpha(1) \neq \alpha(5)$, because the sequences analyzed contain no steps of type 2,3 and 4.

Generally speaking, there are no reasons to state that the differences of $\alpha(i)$ within the 5′-group (types 1,2,3) or within the 3′-group (types 4,5) are much less than the differences of $\alpha(i)$ between the members of these two groups. As to the differences between $\alpha(1)$ and $\alpha(5)$, apart from electrophoretic

data on A_{5-8} and A_{8-5} multimers, there are additional hints from structural data (19,4,10) pointing to possible modulation of bending parameters due to purine-pyrimidine alterations (1−YR, 5−RY, see Table I). So far, there have not been an experimental data to allow a direct comparison with the other types of bends.

Sequences of analyzed multimers contain the same A_6 tracts and different boundary dinucleotides, namely types 1 and 5 in $(C_2A_6C_2)_n$, 1 and 4 in $(C_2A_6G_2)_n$ and 3 and 4 in $(G_2A_6G_2)_n$. According to the original (9) and the refined (16) junction models, $(C_2A_6C_2)_n$ and $(G_2A_6G_2)_n$ are expected to have identical bending parameters and hence identical curves of electrophoretic anomalies. However, our experiments reveal differences in electrophoretic mobility of these two multimers (Fig. 2). It can be seen from the experimental curves (Fig. 2) that the difference in anomalous mobility reaches 24% for $(C_2A_6C_2)_n$ and $(G_2A_6G_2)_n$ at 200 bp. The observed difference (24% of the lower curve level) is comparable with that of $(C_2A_5CG_3CA_8)_n$ and $(C_2A_8CG_3CA_5)_n$ (26% of the lower curve level) sequences (9) that were studied in order to distinguish between the bends at 3′ and 5′ of oligo(A) tract. It should be mentioned that when the substitution is made only at one boundary the decrease of relative mobility is approximately 12% (Fig. 2). Nevertheless the difference is too large to attribute bends of type 1 and 3 to one group (the same statement is valid for types 4 and 5).

A direct interpretation of these results in terms of bend angles and phases is rather difficult. Even in the most simple case of the $(C_4A_6)_n$ and $(G_4A_6)_n$ the difference may be attributed either to diminished bend angles or to less fitted phases (or both) of two junction bends. In the case of $(G_2C_2A_6)_n$ a possible small bend at GC step (4) provides an additional uncertainty.

Anyway, it can be concluded that for B-B′ bends it is important to take into account not only whether they are at the 5′ or 3′ end of an oligo(dA) tract, but also the sequences at the boundaries of this tract. In particular, it seems that GA (and/or AG) steps have smaller bend angles and/or more roll-like direction of bend compared to CA (and/or AC) steps.

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Abbreviations: A,C,G,T − dA,dC,dG,dT, respectively; all sequences were written from 5′ end to 3′ end; B − B-form of DNA typical of random sequence; B′ − anomalous DNA structure at long A/T tracts; R_L − the value of electrophoretic anomaly, i.e. the ratio of the actual fragment length to the apparent one, which is determined from comparison with size marker.

REFERENCES

1. Marini,J.C., Levene,S.D., Crothers,D.M. and Englund,P.T. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7664−7668.
2. Structure and Expression, Vol. 3: DNA Bending and Curvature (Olson,W.K., Sarma,M.H., Sarma,R.H., Sandaralingam,M. eds).
3. Trifonov,E.N. (1980) *Nucl. Acids Res.* **8**, 4041−4053.
4. Calladine,C.R. (1982) *J. Mol. Biol.* **161**, 343−352.
5. Calladine,C.R., Drew,H.R. and McCall,M.J. (1988) *J. Mol. Biol.* **201**, 127−137.
6. Nelson,H.C.M., Finch,J.T., Luisi,B.F. and Klug,A. (1987) *Nature (London)* **330**, 221−226.
7. Coll,M., Frederick,C.A.,Wang,A.H.-J. and Rich,A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8385−8389.
8. Wu,H.-M. and Crothers,D.M. (1984) *Nature (London)* **308**, 509−513.
9. Koo,H.-S., Wu,H.-M. and Crothers,D.M. (1986) *Nature (London)* **320**, 501−506.
10. Chuprina,V.P. and Abagyan,R.A. (1988) *J. Biomol. Struct. Dyn.* **6**, 121−138.
11. Diekmann,S. (1987) *EMBO J.* **6**, 4213−4217.
12. Leroy,J.-L., Charretier,E., Kochoyan,M. and Gueron,M. (1988) *Biochemistry* **27**, 8894−8898.
13. Burkhoff,A.M. and Tullius,T.D. (1988) *Nature* **331**, 455−457.
14. Hagerman,P.J. (1985) *Biochemistry* **24**, 7033−7037.
15. Chuprina,V.P. (1987) *Nucl. Acids Res.* **14**, 293−311.
16. Koo,H.-S. and Crothers,D.M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1763−1767.
17. Diekmann,S. (1986) *FEBS Letters* **195**, 53−56.
18. Shlyakhtenko,L., Lubchenko,Yu.L., Chernov,B. and Zhurkin,V. (1989) In: Book of Abstracts of Sixth Conversation in Biomolecular Stereodynamics (R.H.Sarma, ed.), Albany, N.Y., pp. 311−312.
19. Dickerson,R.E., Kopka,M.L. and Pjura,P. (1985) In: Biological Macromolecules and Assemblies, (Jurnac,F. and McPherson,A., eds.) Wiley, N.Y., Vol. 2, 37−126.