Proc. Nat. Acad. Sci. USA
Vol. 70, No. 7, pp. 2167–2170, July 1973

Variation of Type-B DNA X-Ray Fiber Diagrams with Base Composition

(DNA structure/x-ray fiber diffraction/dependence on base composition)

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Communicated by André Lwoff, April 30, 1973

ABSTRACT **Eight natural DNAs of widely differing base composition have been studied by x-ray diffraction in fibers at high relative humidity. The resulting type B diffraction diagrams showed that all of the DNAs had a 34-Å pitch and 3.4-Å interbase pair separation. However, the intensity distribution on the inner three layer lines was a strong function of the base content. In diffraction diagrams of very AT-rich DNA, the intensity of the first and third layer line was 2- or 3-times stronger than in the patterns of GC-rich DNA. These high humidity diffraction patterns agree with x-ray scattering from solutions of DNA. The results are interpreted to imply that each AT base pair may have a different cross section than a GC pair. If this is so, it would appreciably alter the currently held ideas concerning DNA recognition.**

Until recently, it was generally accepted that DNA could exist only in one of three forms, and also that the structure of DNA was independent of the nature of the DNA. The latter conclusion was based on an x-ray fiber diffraction study of only the A forms of various natural DNAs (1). Nevertheless, x-ray fiber diffraction experiments on AT-rich DNAs at intermediate and low relative humidities (r.h.) have shown that the secondary structure under these conditions does depend on the base composition (2, 3) and that there exists at least several new forms for DNA.

At high water contents, in fibers and in solution, calf-thymus DNA exists in a conformation of the B type (4, 5). However, no extensive x-ray fiber diffraction study of the high-humidity configuration of various DNAs has been reported. [A short preliminary report of three high-humidity patterns has recently been published (6)]. The high-humidity experiments presented here were prompted by the finding that the wide-angle x-ray scattering patterns of natural and synthetic DNAs in solution were, indeed, functions of the nature of the DNA (7). The wide-angle x-ray scattering patterns from all natural DNAs in solution show two intensity maxima near 13 and 10 Å. (Other maxima are observed at smaller equivalent Bragg spacings.) To a first approximation, these maxima correspond to the maximum of the molecular transform of the fiber diffraction pattern on the second and third layer lines, respectively. The x-ray intensity corresponding to the first layer line is mostly obscured by the small-angle scattering of the whole molecule in solution. Nevertheless, the contribution from the first order bessel function term does raise the overall intensity scattered in the neighborhood of 20–30 Å, and the relative intensity in this region was observed to be significantly larger for DNA very rich in AT (7).

Abbreviation: r.h., relative humidity.

It was also noticed that the sharpness of the 13- and 10-Å maxima were functions of the base composition, but recen- experiments show that the width of these maxima is also aft fected by the salt content. However, the ratio of the intensity of the 10-Å maxima to the one at 13 Å does not seem to vary, at least over the range 0.05–1 M monovalent salt. In the previous report (7), I stressed only the difference between DNA very rich in AT and typical or GC-rich DNA and declined to comment on the apparent differences between DNA very rich in GC and DNA moderately rich in AT because a sufficient number of experiments had not been done. After subsequent experiments with DNA of *Micrococus luteus* and *Escherichia coli*, it became evident that the ratio of the 13-Å to the 10-Å maximum, and thus the ratio of the second to the third "layer line," was a semi-continuous function of the AT content (Table 1). The x-ray fiber diffraction studies reported here on DNA at high humidity confirm and extend these findings.

MATERIAL AND METHODS

DNA from yeast mitochondria, *Sarcina lutea*, and both *E. coli* and *Cytophaga johnsonii* were gifts from G. Bernardi, M. Leng, and W. Guschebauer, respectively. *Clostridium perfringens* and *M. luteus* DNA were purchased from Sigma Biochemical Co. *Staphylococcus aureus* and calf-thymus DNA were isolated in this laboratory (2, 5). These DNAs gave the expected melting behavior and UV spectrums.

Fibers were obtained from a wet gel either by stretching while drying or by drying on a fixed support under controlled conditions of relative humidity and temperature. Photographs were taken with nickel-filtered radiation from a copper anode with an average exposure time of about 3 hr at a film-to-fiber distance of 30 mm. For this study, it was desirable to obtain noncrystalline diffraction patterns so as to minimize lattice sampling of the molecular transforms. Still, the maximum values of the layer-line intensity determined from crystalline and noncrystalline patterns were in general agreement.

Densitometer scans were made with a Joyce–Loebel recording densitometer. Traces along a layer line and also parallel to the meridian through the intensity maximum on each layer line were used to determine the relative intensity and the background. The results given in Table 2 are measured intensity values minus background. The experimental error in the intensity determination is estimated at 10–20%. This error is quite large due to difficulties in working with film and fibers at very high r.h. and problems involving densitometer measurements on noncrystalline patterns. It must be stressed that the aim of this paper is to present a

trend, not to give absolute values. These will come from future studies using a counter diffractometer.

RESULTS AND DISCUSSION

The intensity distributions in the high-humidity patterns of three DNAs with 69, 50, and 31% AT at the same salt content–about 2% NaCl–were shown in a preliminary report to be a function of AT content (6). One of the results of the present study is that the type-B patterns are not appreciably influenced by salt content. This can be seen from a comparison of the high humidity type-B pattern of *C. perfringens* DNA in Fig. 1, which contained 4% Na_2SO plus 2% NaCl, with that shown in ref. 6. To be certain that no type-A DNA was contributing to the diffraction, lithium salt fibers were photographed. In Fig. 1, the high-humidity type-B patterns from sodium and lithium *M. luteus* DNA are presented for comparison. The change from sodium to lithium had a negligible effect on the type-B patterns. Both conclusions are in accord with the original studies on calf-thymus DNA (8, 9).

Fig. 1 shows typical high-humidity x-ray fiber diagrams from DNA of very high, intermediate, and very low AT. Although all of the natural DNA patterns have the same meridional spacing (3.4 Å) and layer line spacing (34 Å), the distribution of intensity on the first three layer lines shows a striking variation with base composition. For DNA richest in AT, the magnitude of the intensity of the innermost spot

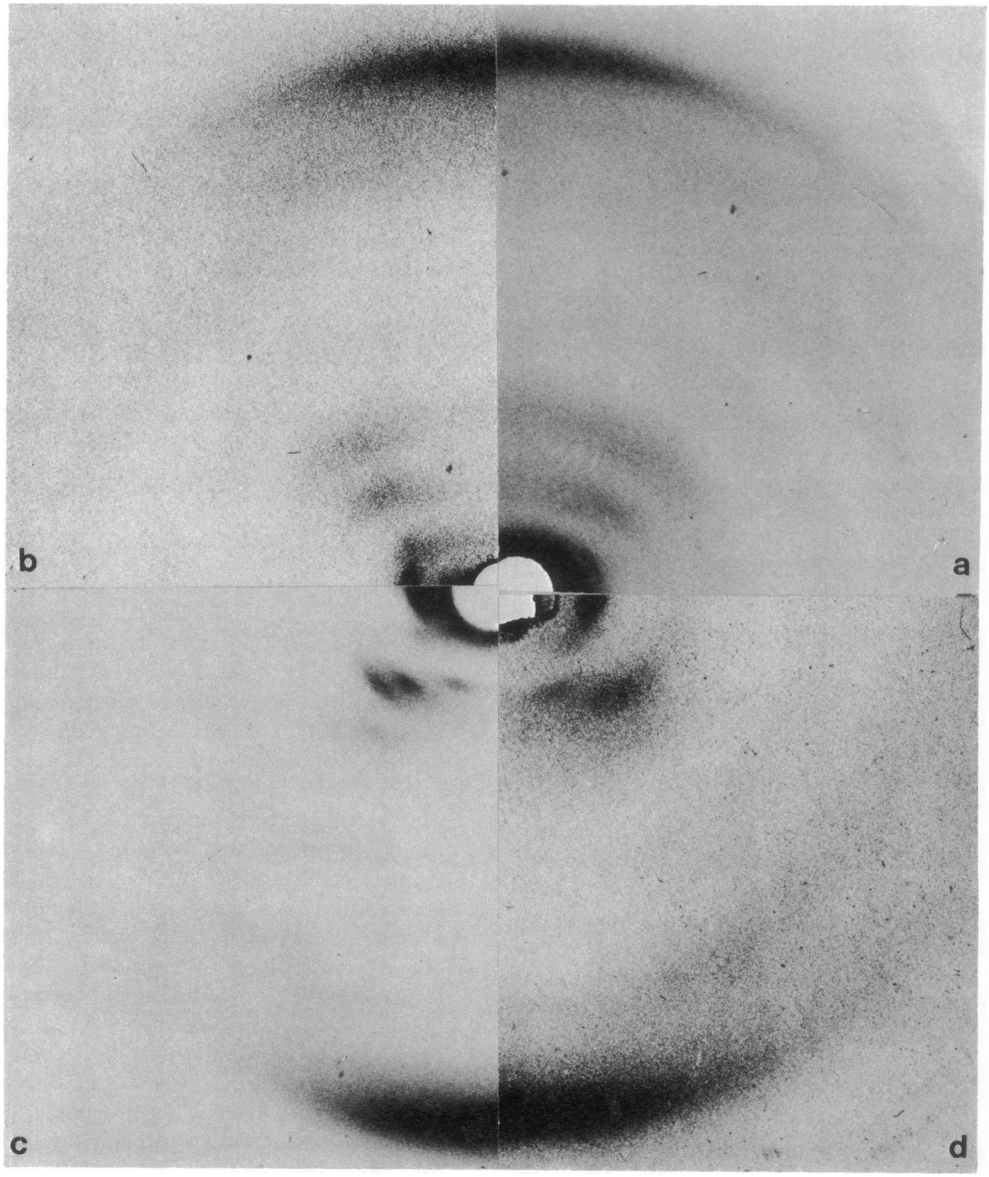

FIG. 1. A composite of type B DNA x-ray fiber patterns from three DNAs. (*a*) Yeast mitochondria DNA (82% AT) containing 4% by dry weight NaCl at 92% r.h. With this DNA it was not possible to obtain a well-oriented x-ray pattern, and the first layer-line spot is close to the central scattering. However, it is evident that the maximum value of the diffracted intensity is highest on the first layer line, followed by a weaker second layer line and a slightly less intense third layer-line spot. (*b*) *C. perfringens* DNA (69% AT) containing 4% Na_2SO_4 and 2% NaCl at 98% r.h. The first and second layer lines appear to be equally strong. (*c*) Sodium *M. luteus* DNA (31% AT) containing 5% NaCl at 98% r.h. Note the very strong intensity on the second layer line, which dominates the central diffraction. (*d*) Lithium *M. luteus* DNA containing 4% LiCl at 98% r.h. The distribution of intensity is the same as with sodium salt. This result shows that no type-A DNA is contributing to the diffraction.

TABLE 1. *Intensity ratios of the wide-angle x-ray scattering maxima of various DNA solutions*

DNA (Na salt)	AT (%)	I(10 Å)/ I(13 Å) × 100
Poly [d(A-T)]·poly [d(A-T)]	100	88
Clostridia perfringens	69	70
Bacillus cereus	65	69
Calf thymus	58	66
Escherichia coli	50	62
Micrococcus luteus	31	53

The data are from ref. 7. To a first approximation these ratios are related to the relative maximum intensity on the third layer line to that of the second layer line, but contributions from other layer lines are expected to increase the intensity scattered near 10 Å from DNA solutions.

TABLE 2. *Maximum relative intensities of DNA x-ray fiber patterns at high humidity*

DNA (Na salt)	A + T/ G + C of DNA	Maximum relative intensity*	
		First layer line	Third layer line
Yeast mitochondria	4.6	105	85
C. perfringens	2.3	85	80
S. aureus	2.0	80	75
C. johnsonii	1.9	75	75
Calf thymus	1.3	65	60
E. coli	1.0	55	55
M. luteus (Na)	.45	30	35
M. luteus (Li salt)	.45	30	35
S. lutea	.41	35	40

* Given as percent of the maximum value of the x-ray intensity on the second layer line, which is equal to 100 for each DNA.

on the first layer line is the highest, followed by a slightly weaker second layer line, and a weaker third layer-line intensity spot. But with increasing GC content the second layer-line spot becomes more intense, so that with DNA very rich in GC it completely dominates the pattern. Table 2 presents the values obtained from densitometer traces of various high-humidity patterns.

The type-B patterns from all eight of the DNAs studied here and those published for sodium *E. coli* (10), calf-thymus (4), and T2 DNA (1) follow this trend of increasing intensity on the first and third layer lines with increasing AT.

The variations in the ratio of the intensity maxima of the layer lines was not related to the fiber-pulling conditions or salt content; and, as comparison of Tables 1 and 2 shows, the ratio of the third to the second layer line parallels that of the 10-Å to the 13-Å maximum in solution. The results cannot be explained by a change in the molecular packing, but require a modification of the secondary structure dependent on base composition.

Before considering the possible nature of the structural modification responsible for the different x-ray patterns, the invariant features should be pointed out: the meridional spacing and layer-line separation of the fiber patterns and the cross-section radius of gyration in solution (7) do not significantly vary with AT. In other words, the inter-base pair separation, pitch, and radius seem to be independent of the chemical composition. Thus the only allowed alterations that might explain the diverse intensity distributions are changes in either the base-pair tilt or the angle between the bases in a pair. Detailed model calculations will be necessary to decide between these two possibilities. Either of these would result in important variations in the geometry of the large and small DNA groove. A variation in the angle between the bases is in better agreement with the x-ray scattering at intermediate angles and with recent electrophoresis experiments showing that the mobility and, therefore, the cross section is dependent on the base composition (11). A change in the relative position of the two strands would result in a corresponding distortion of the base-pair hydrogen bonds or it would result from another type of base pairing in AT-rich DNA. One must remember that the base pairing in cocrystals of nucleotides, where the only definitive studies have been made, is indeed related to the nature of the bases. A:T never has been observed in a Watson–Crick base pair; it is only found with Hoogsteen pairing (12).

The x-ray results can be explained by either a variation of the structure over relatively large regions in clusters of a dozen or more base pairs or a difference in the structure of each AT and GC pair or group of a few nucleotides. The apparently continuous character of the changes in the diffraction, which can perhaps be related to the monotonic increase in density on CsCl or NaI gradients (13, 14) with GC, would be somewhat more consistent with the second explanation. If the latter mechanism is correct, a part of the paradox of how double-stranded DNA is recognized has a straightforward solution; each base pair (or small group of pairs) has a characteristic and directly detectable structure. The presence of a given base would be manifested by a characteristic change in the local dimensions of the DNA grooves.

I should like to close with a brief discussion of the different classes of DNA structure. The word *form*, which has been used to connote a unique configuration, should be applied only to the type-A conformation. Only the type-A structure has been observed to be independent of both base composition (1) and salt and water content (9). The results presented here show that there are many B-type structures that depend upon the type of the DNA, and it has been observed that the structural parameters of the C, P, and T classes vary with the environment (15, 3). Furthermore, it has recently been found that sodium DNA can exist in a B-like structure at humidities as low as 44%, and it seems that the B conformation at low humidities may not be the same as at higher water contents (2).

The A form might provide a good substrate for RNA and DNA polymerase, since it would not vary with local changes in the base content along the DNA. Although this would suggest that the A form is the "active" configuration, its invariant property makes it very poor recognition DNA; this role is probably played by the other classes of DNA structure.

I thank Miss Rosette Lipiec for her assistance in this study. This work was supported by a grant from the Delegation Generale a la Recherche Scientifique et Technique.

1. Hamilton, L. D., Barclay, R. K., Wilkins, M. H. F., Brown, G. L., Wilson, H. K., Marvin, D. A., Ephrussi-Taylor, H. & Simmons, N. S. (1959) *Biophys. Biochem. Cytol.* **5,** 397–403.
2. Bram, S. (1972) *Biochem. Biophys. Res. Commun.* **48,** 1088–1092.
3. Bram, S. & Tougard, P. (1972) *Nature New Biol.* **239,** 128–131.
4. Franklin, R. E. & Gosling, R. G. (1953) *Nature,* **171,** 740–741.
5. Bram, S. & Beeman, W. W. (1971) *J. Mol. Biol.* **55,** 311–324.
6. Bram, S. (1973) *C.R. Acad. Sci. Ser. D* **276,** 657–659.
7. Bram, S. (1971) *Nature New Biol.* **232,** 174–176.
8. Langridge, R., Seeds, W. E., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F. & Hamilton, L. D. (1960) *J. Mol. Biol.* **2,** 19–37.
9. Cooper, P. J. & Hamilton, L. D. (1966) *J. Mol. Biol.* **16,** 562–563.
10. Wilkins, M. H. F., Stokes, A. R. & Wilson, H. R. (1953) *Nature* **171,** 738–740.
11. Zeiger, R. S., Salomon, R., Dingman, C. W. & Peacock, A. C. (1972) *Nature New Biol.* **238,** 65–69.
12. Hoogsteen, K. (1959) *Acta Crystallogr.* **12,** 822–828.
13. Schildkraut, C. L., Marmur, J. & Doty, P. (1962) *J. Mol. Biol.* **4,** 430–443.
14. Anet, R. & Strayer, D. R. (1969) *Biochem. Biophys. Res. Commun.* **37,** 52–58.
15. Marvin, D. A., Spencer, M., Wilkins, M. H. F. & Hamilton, L. D. (1961) *J. Mol. Biol.* **3,** 547–565.