X-Ray Diffraction Studies on *Mycobacterium Smegmatis* DNA

MALINI RAJAGOPALAN, SAMIR K. BRAHMACHARI, and V. SASISEKHARAN, *Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560 012, India;* and RAKESH SRIVASTAVA,* *Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore-560 012, India*

A and B are the two interconvertible forms observed for different natural DNAs. Generally, the A-pattern for a given DNA fiber was obtained at 75% relative humidity while the same fiber gave the B-pattern at higher r.h.[1,2] (92%). Since there have been improvements in preparation techniques and hence the availability of salt-free DNA, the classical A-to-B transition is now seldom observed. Hamilton et al.[3] investigated in detail the fiber-diffraction patterns of A-form DNA from various sources. Except for *Mycobacterium tuberculosis*, DNA fibers from all other sources gave the typical A-pattern characteristic of calf thymus DNA.

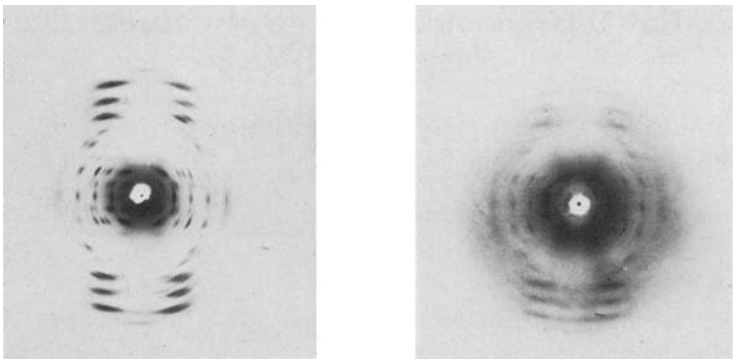
In this report, we present fiber-diffraction studies on *Mycobacterium smegmatis* DNA with high GC content (70%). This was done as part of the investigation of mycobacteria as the group which includes some of the dreadful pathogens that cause tuberculosis, leprosy, and many other diseases. Recently, it has been shown that the *Mycobacterium smegmatis* genomic DNA has two distinct stretches, with different GC contents.[4] Such an arrangement of sequences may be of some physical significance for the structure of DNA and, thereby, of physiological significance. Hence, *Mycobacterium smegmatis* DNA was subjected to x-ray diffraction studies. We observed that at a 1:1 Na^+:phosphate concentration, this DNA exhibits a characteristic A-pattern (unlike *Mycobacterium tuberculosis*[3]) throughout the humidity range 30–98%.

The *Mycobacterium smegmatis* DNA was extracted and purified[5] (free of RNA, protein, and polysaccharide). This DNA was dialyzed extensively against 3 m*M* NaCl and 0.1 m*M* EDTA, maintaining the DNA concentration at 1 mg/mL. This dialyzed solution was then centrifuged at 45,000 rpm for 24 h at 10°C, using a SW-50 rotor. The gel obtained after centrifugation was used in drawing the fibers. To remove the polysaccharide, *Mycobacterium smegmatis* (free of RNA and protein) DNA was treated with cetyl trimethyl ammonium bromide (CTAB) and precipitated with alcohol.[5] Diffraction patterns of the fiber were taken at different r.h.'s, using appropriate saturated solutions. The relative humidity change was brought about by introducing the saturated solution into a Lindemann capillary (1-mm diameter). The Lindemann capillary helps in equilibrating the fiber under the particular r.h. A flat-plate camera was used to record the photographs.

We examined the diffraction patterns of *Mycobacterium smegmatis* (1:1 Na^+: phosphate) DNA fibers at 30, 40, 78, and 98% r.h. Figure 1(a) shows the diffraction pattern observed at 40% r.h. It is interesting to note that a characteristic A-DNA

* Present address: Human Genetics Laboratory, Yale University, New Haven, Connecticut.

Biopolymers, Vol. 22, 1633–1635 (1983)
© 1983 John Wiley & Sons, Inc.
CCC 0006-3525/83/071633-03$01.30



(a) (b)

Figure 1. (a) A-type diffraction pattern obtained for *Mycobacterium smegmatis* DNA fiber at 1:1 Na^+:phosphate concentration at r.h. 40% (using saturated calcium chloride solution). The pattern obtained is similar to that of the A-form of calf thymus DNA. (b) Diffraction pattern of the same fiber at 98% r.h. Note that this pattern is identical to (a).

pattern was obtained where the central spots occur in small groups rather than singly, as in the case of *Mycobacterium tuberculosis* DNA.[3] Our finding raises some doubt that the difference in diffraction pattern between that reported earlier[3] and ours may not be due only to the difference in the molecular arrangement in the lattice. This difference from similar organisms might be due to the difference in the preparation of samples—in particular, polysaccharide contamination in the DNA sample. Partial retention of polysaccharide in the preparation by CTAB treatment of the fiber of *Mycobacterium smegmatis* DNA showed an A-pattern; however it is semicrystalline in nature. At a slightly higher concentration of salts, the familiar A-to-B transition was obtained with this DNA fiber.

Fibers drawn using *Mycobacterium smegmatis* DNA containing the critical salt concentration gave the A-DNA pattern at all relative humidities up to 98%. Figure 1(b) shows the diffraction pattern obtained at 98% r.h. At lower r.h. (9%), the structure collapsed, suggesting that a minimum water content is necessary to maintain the ordered structure, which corresponds to 35% r.h. This finding is similar to the earlier report of Cooper and Hamilton[6] that up to a salt content of 6% (w/w), the A-conformation was retained even at 98% r.h. In our case, not only could we specify the sample of DNA used, but also indicate the cation concentration at which the DNA structure would be locked into the A-conformation (1:1 Na^+: phosphate), as compared to their calculation of salt content (w/w).

It was earlier suggested that DNA may adopt the A-conformation *in vivo* and can be implicated as a structural element during transcription.[7-9] Shakked et al.[10] studied the crystal form of d(GGTATACC) and showed that it forms a duplex similar to A-form DNA. The fact that the octamer sequence is present in many promoters, and that it adopts the A-form as one of the stable structures, suggests that we cannot rule out the possibility of the involvement of the A-form during transcription. Conner et al.[11] worked on the crystal of d(CCGG), the most potential sequence for methylation, and found that in the A-form, the interstrand base-stacking of the guanines exists and that the cytosine bases are exposed for possible modifications. This stretch d(CCGG) has the highest frequency of modification. This modification is necessary for protection against DNA cleavage by the restriction enzymes.

In light of our observations and recent crystallographic studies, it is not unlikely to expect the A-conformation to be stabilized under critical salt concentration *in vivo*. Further work in this direction is required to substantiate this.

This work was partially supported by the DST-SERC Scheme, Government of India. R. S. thanks the Indian Council of Medical Research for a predoctoral fellowship.

References

1. Langridge, R. D., Marvin, D. A., Seeds, W. E., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F. & Hamilton, L. D. (1960) *J. Mol. Biol.* **2,** 38–64.
2. Fuller, W., Wilkins, M. H. F., Wilson, H. R., Hamilton, L. D. & Arnott, S. (1965) *J. Mol. Biol.* **12,** 60–80.
3. Hamilton, L. D., Barclay, R. K., Wilkins, M. H. F., Brown, G. L., Wilson, H. R., Marvin, D. A., Ephrussi-Taylor, H. & Simmons, N. S. (1959) *J. Biophys. Biochem. Cytol.* **5,** 397–403.
4. Norgard, M. V. & Imaeda, T. (1980) *J. Bacteriol.* **144,** 766–771.
5. Srivastava, R. (1980) Thesis submitted to the Indian Institute of Science, Bangalore.
6. Cooper, P. J. & Hamilton, L. D. (1966) *J. Mol. Biol.* **16,** 562–563.
7. Arnott, S., Fuller, W., Holgson, A. & Prulton, J. (1968) *Nature* **220,** 561–564.
8. Florentiev, V. L. & Ivanov, V. I. (1970) *Nature* **228,** 519–522.
9. Wachsman, W. & Antony, D. D. (1980) *Biochemistry* **19,** 5981–5986.
10. Shakked, Z., Rabinovich, D., Cruse, W. B. T., Egert, E., Kennard, O., Sala, G., Salisbury, S. A. & Viswamitra, M. A. (1981) *Proc. Roy. Soc. London* **B213,** 479–487.
11. Conner, B. N., Takano, T., Tanaka, S., Itakura, K. & Dickerson, R. E. (1982) *Nature* **295,** 294–299.

Received October 20, 1982
Accepted March 3, 1983