The Absence of Histone in the Bacterium *Escherichia coli*

II. X-ray Diffraction of Nucleoprotein Extract

By M. H. F. WILKINS, Ph.D., and G. ZUBAY*, Ph.D.

(From the Medical Research Council, Biophysics Research Unit, King's College, London)

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ABSTRACT

X-ray diffraction photographs of a nucleoprotein preparation from *Escherichia coli* show the *A*-type pattern of crystalline deoxyribonucleic acid (DNA). This suggests that a large part of the DNA is free from protein. In higher organisms DNA does not exist in this form but is closely bound to protein.

The chemical analyses in Part I suggest there may be a considerable difference between bacterial deoxyribonucleoprotein (DNP) and that of higher organisms. X-ray diffraction study of the DNP provides information about molecular configuration and thus complements the chemical data. The diffraction pattern of DNP (1) in widely different types of cells, *e.g.* calf thymus, sea urchin sperm, fowl erythrocytes, is very similar and shows that protein of histone type and DNA are combined in a characteristic way. A different structure, nucleoprotamine, also exists but it is known only in the sperm of some species. It might be thought that the nucleohistone structure is essential to the genetic functioning of chromosomes. However, the present results indicate that this is unlikely because, although genetic processes in bacteria are probably fundamentally similar to those in higher organisms, and the structure of DNA is the same, the nucleohistone configuration is not found in the bacterium studied.

EXPERIMENTAL

The x-ray technique was similar to that described by Langridge *et al.* (2). Fibres of approximately 100 μ diameter were pulled from a concentrated gel of DNP in water; they were mounted in slight tension. Diffraction photographs were taken with (*a*) a Philips type micro camera with 100 μ glass collimator and 25 mm. distance from specimen to film and (*b*) a larger camera having 100 μ lead pinholes and 50 mm. distance from specimen to film. Cu K α-radiation from a Hilger semi-microfocus x-ray tube was used. The humidity around the specimen was controlled by hydrogen passed through a suitable salt solution.

RESULTS

At high relative humidity, 92 per cent RH, the diffraction pattern (Fig. 1) strongly resembles the *B* type given by the sodium salt of DNA (3). It is distinguished from the nucleohistone pattern mainly by the arrangement of spots on the 2nd layer line, the presence of a well defined first layer-line, and the absence of a meridional 34A reflection. At 75 per cent RH the diffraction pattern (Figs. 2 *a* and 2 *b*) of DNA is very clearly visible and well oriented, the characteristic crystalline *A*-type pattern (4) being superimposed on a B_2 type (5, 6). Diffuse unoriented rings corresponding to spacings of about 4.5 and 9.5A are also present.

INTERPRETATION OF RESULTS

The diffuse rings in the diffraction photographs resemble those produced by free protein and do not appear when histone or protamine are combined with DNA. The presence of the rings indicates that a large part of the protein in the specimen is not bound closely to DNA.

The sharp spots given by the DNA show that the molecules are largely intact and have not suffered appreciable denaturation or enzymatic degradation during extraction. The intensity of the DNA pattern shows that a fraction (probably more than 30 per cent) of the DNA molecules are packed together in a regular way to form microcrystalline regions in the fibre. The sharpness of

* National Science Foundation Postdoctoral Fellow of the United States.

the *A* pattern (*e.g.*, the approximate 6 A spot on the 2nd layer line defines the angle of diffraction to within about 2 per cent) shows that these regions are about 400 A in dimension and hence include portions of at least 20 parallel DNA molecules. This crystalline arrangement will be determined mainly by interactions between the phosphate groups on neighbouring molecules and is unlikely to exist if the great majority of these groups are not free from protein. The presence of impurity bound to a macromolecule does not necessarily affect its crystallization for it is known (7) that the attachment of certain small groups to globular proteins does not alter the arrangement of the molecules in the crystal. It is rather surprising, however, to find the DNP preparation, which may be regarded as DNA with a large amount of attached impurity, forming microcrystals of DNA. A similar effect, however, has been found before with DNA preparations containing protein. For example, a partly purified DNA preparation made by Dr. K. S. Kirby (8) from calf liver contained about 20 per cent non-histone protein and gave an *A*-type x-ray diffraction pattern. The probable explanation in the present case is that the protein occurs in fragments at-

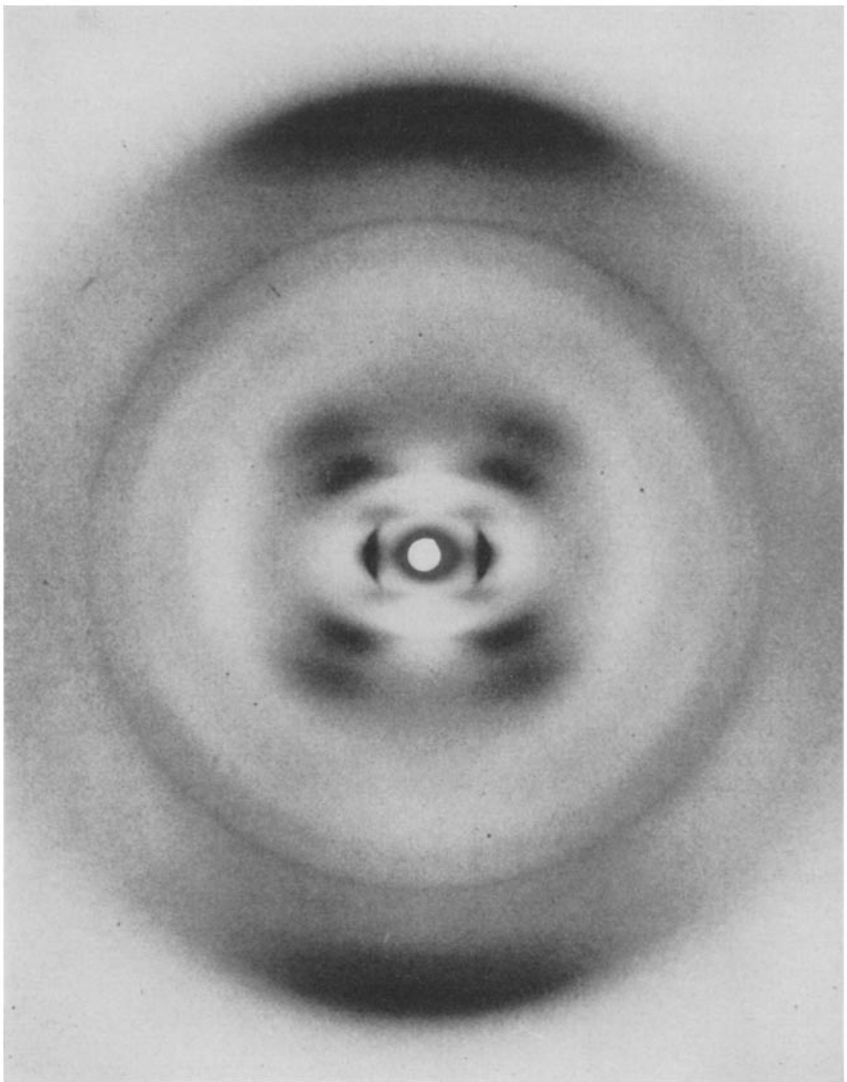

FIG. 1. X-ray diffraction photograph of bacterial DNP preparation at 92 per cent relative humidity.

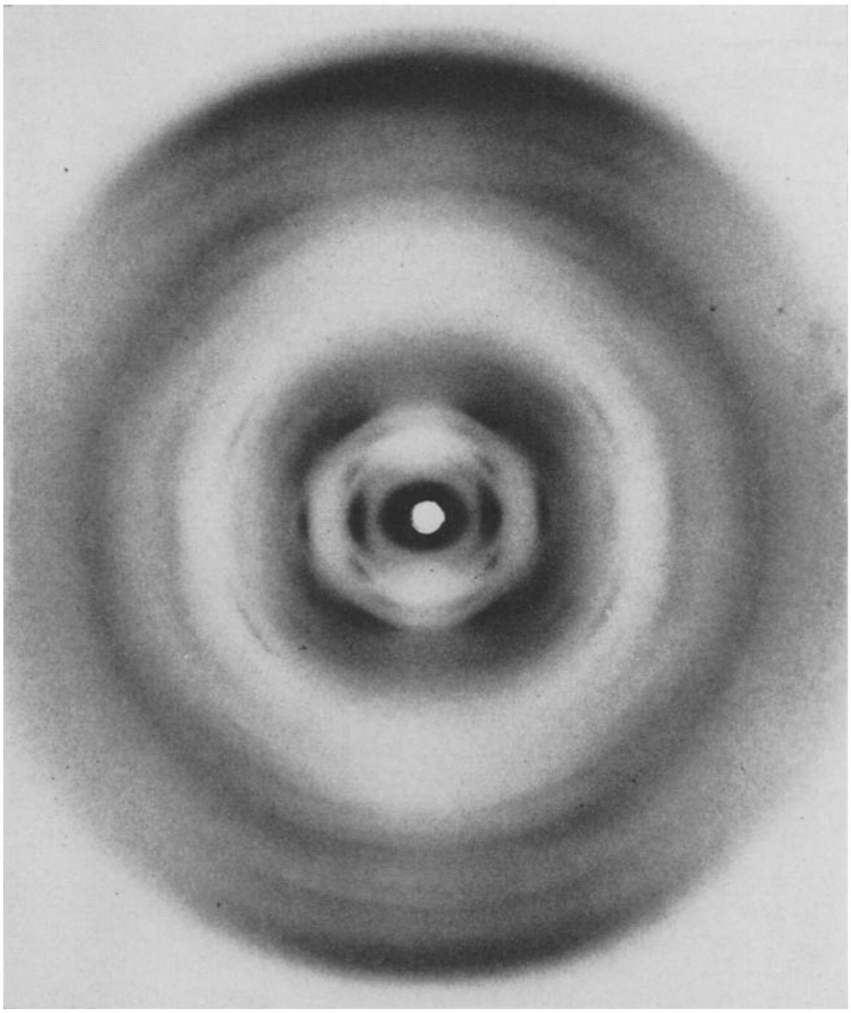

FIG. 2 *a*. X-ray diffraction photograph of bacterial DNP preparation at 75 per cent relative humidity.

tached to the DNA at relatively few points and hence portions of the DNA molecule are free from protein and can crystallize. An alternative explanation might be that protein is bound to the bases of DNA and lies at the bottom of the large helical groove on the molecule where it would not interfere with the packing of the molecules. Since the bases in the *A* structure are displaced from the helix axis, this protein would form roughly a cylinder coaxial with the helix. If there were protein in such a position, the relative intensities of the spots on the equator of the *A* pattern would be different from those obtained with pure DNA. The spots in the x-ray photograph of the bacterial preparation are not well defined and are superimposed on an uneven background and as a result accurate measurement of their intensities is not possible. Rough values obtained with a densitometer were not significantly different from those for DNA. Calculation, with these results, shows that the weight of protein that might be attached to the bases of DNA is not likely to be more than about 20 per cent that of DNA.

The presence of the B_2 pattern shows that some of the molecules are in the *B* configuration. All DNA specimens give a *B* pattern above 85 per cent RH. The best preparations of DNA give the *A* pattern at 75 per cent. A mixture of B_2 and *A* patterns is observed with some specimens of DNA, but the factors that determine the propor-

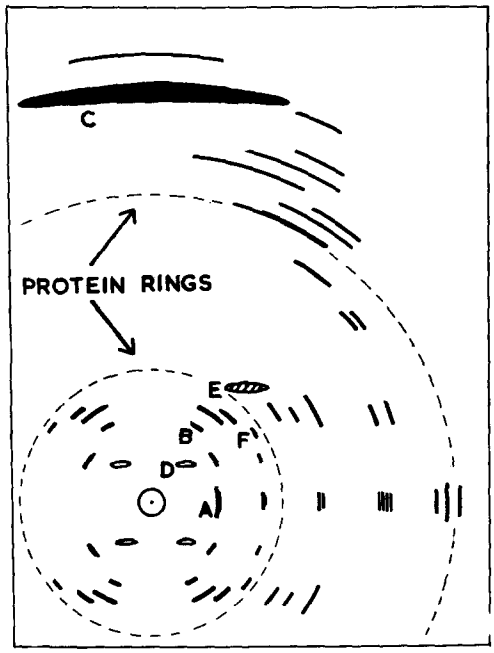

FIG. 2 *b*. Diagram showing the main features of the x-ray diffraction photograph at 75 per cent relative humidity. *A*, *B*, *C*, *D*, *E*, *F* are from the B_2 pattern of DNA; *A*, *B*, and *C* show clearly, *D* is also visible, but *E* and *F* are obscured by the protein ring. The other arcs on the diagram represent the more prominent parts of the *A* pattern of DNA.

tion of molecules in the *A* and *B* configurations at 75 per cent RH are not fully understood. The B_2 pattern is readily obtained at 75 per cent RH by keeping fibres of DNA in tension (5, 6). This is accounted for by the fact that transition from *B* to *A* configuration involves shortening of the molecules.

CONCLUSION

The DNP material prepared from *E. coli* without the use of deproteinizing agents contains a considerable proportion of DNA free of protein. Products which give similar x-ray diffraction patterns may also be obtained from higher organisms if deproteinizing agents are used. It seems likely that in intact bacteria most of the length of the DNA molecules does not have any protein firmly attached to it. However, the possibility cannot be entirely excluded that protein has been removed by enzymes from the DNA during the preparation of the DNP.

BIBLIOGRAPHY

1. Wilkins, M. H. F., *Cold Spring Harbor Symp. Quant. Biol., 1956*, **21**, 75.
2. Langridge, R., Wilkins, M. H. F., Wilson, H. R., Hooper, C. W., and Hamilton, L. D., data in preparation.
3. Feughelman, M., Langridge, R., Seeds, W. E., Stokes, A. R., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F., Barclay, R. K., and Hamilton, L. D., *Nature*, 1955, **175**, 834.
4. Wilkins, M. H. F., Seeds, W. E., Stokes, A. R., and Wilson, H. R., *Nature*, 1953, **172**, 759.
5. Wilson, H. R., and Wilkins, M. H. F., data to be published.
6. Wyckoff, H. W., Ph.D. Thesis, Cambridge, Massachusetts Institute of Technology, 1955.
7. Kendrew, J. C., and Perutz, M. F., *Ann. Rev. Biochem.*, 1957, **26**, 353.
8. Kirby, K. S., *Biochem. J.*, 1958, **70**, 260.