Optical Rotatory Dispersion and Circular Dichroism of Superhelical Deoxyribonucleic Acid

By A. M. CAMPBELL and D. S. LOCHHEAD

Institute of Biochemistry, University of Glasgow, Glasgow W.2, U.K.

(Received 28 April 1971)

The difference in tertiary structure between covalently closed circular supertwisted DNA and the relaxed circular form has frequently been demonstrated. It is generally assumed that the twisting of the covalently closed molecules reflects the difference between the conditions prevailing in the cell and those under which the same DNA is usually investigated *in vitro*. Wang (1969b) has shown that the range of superhelix densities for such DNA is from -0.026 to -0.039 superhelical turns per ten base-pairs under normal growth conditions.

Superhelix density is sensitive to changes in temperature and ionic strength (Wang, 1969a; Wang, Baumgarten & Olivera, 1967). Such variations are presumed to reflect changes in the secondary structure of the DNA molecules, leading to alterations in the tertiary structure. In the work reported here the reverse hypothesis was tested. In attempts to determine whether the tertiary restraints imposed on the molecule lead to any measurable distortion in the secondary structure of the DNA, we have found, under conditions of fairly low ionic strength, no detectable difference between the O.R.D. (optical rotatory dispersion) and C.D. (circular dichroism) spectra of covalently closed circular supertwisted DNA and those of the relaxed form. However, under conditions of high ionic strength, where the secondary structure of DNA is believed to tend towards the C structure (Tunis-Schneider & Maestre, 1970), there is a considerable difference in the spectra of the two forms of DNA, presumably resulting from the tertiary restraint on the supercoiled form.

The replicative double-stranded form of bacteriophage ϕ X174 DNA was prepared by the method of Rush, Kleinschmidt, Hellman & Warner (1967) from infected cells of *Escherichia coli* C. The final separation of the two forms of the DNA was achieved on gradients of caesium chloride-propidium iodide (3,8-diamino-5-diethylamino-propyl - 6 - phenylphenanthridinium di - iodide) (Hudson, Upholt, Devinny & Vinograd, 1969) ($500\mu\text{g/ml}$) run in a Spinco model L ultracentrifuge for 48h at 43000 rev./min in the Ti 50 angle rotor. The propidium iodide was separated from the DNA on columns of Dowex 50. All experiments were performed under sterile (i.e. nuclease-free) condi-

tions. The sedimentation coefficients of the two types of DNA were determined with a Spinco model E analytical ultracentrifuge by band sedimentation and were corrected for the solvent (Bruner & Vinograd, 1965). The $s_{20,w}^0$ of the superhelical DNA was 21S, and that of the circular form 17S. These values were determined both before and after measurement of the spectra.

The O.R.D. spectra were determined with a Bendix-Bellingham and Stanley recording spectropolarimeter at 20°C and the C.D. spectra with a Cary 60 recording spectropolarimeter at 27°C. U.v.-absorption measurements were made with a Cary 15 spectrophotometer, by using a value of $E_{1\text{cm}}^{1\%}$ of 200 at 260nm (Samejima & Yang, 1965). This value may be altered slightly in solutions of high ionic strength (Emanuel, 1960), but the effect is negligible under the conditions employed.

Figs. 1(a) and 1(b) show the C.D. and O.R.D. spectra of both forms of ϕ X174 DNA in our standard buffer (0.3M-NaCl-0.03M-sodium citrate, pH 7.0). No attempt was made to obtain information in either instrument below 230nm since the transmission of light in our buffers was judged to be too low for accurate determinations. Fig. 1 shows little difference between the two forms of DNA, and the spectra correspond well to those expected for a DNA with a G+C content of 42% (Samejima & Yang, 1965). Under these conditions the DNA is presumably in the B structure (Tunis-Schneider & Maestre, 1970) and the superhelical turns, which are presumably between 12 and 18 in number (Wang, 1969b), do not lead to any detectable distortion in secondary structure.

Figs. 1(c) and 1(d) show the effect on both the C.D. and the O.R.D. spectra of raising the Na^+ concentration to values above 4M. The spectra from the two forms of the DNA are markedly different. The height of the peak in the O.R.D. spectrum of the superhelical DNA at 290nm is double that of the non-superhelical but otherwise identical DNA, and the cross-over differs by a value of 6.5nm. In the C.D. spectra the height of the peak at 280nm in the superhelical form is also double that for the non-superhelical DNA and the cross-over is shifted by 5nm. By contrast, the difference in the troughs of the two forms, in both O.R.D. and C.D. spectra, is very slight, and the

Fig. 1. (a) C.D. and (b) O.R.D. of double-stranded ϕ X174 DNA in 0.3M-NaCl-0.03M-sodium citrate buffer, pH 7.0; (c) C.D. and (d) O.R.D. of double-stranded ϕ X174 DNA in 4M-NaCl-0.03M-sodium citrate buffer, pH 7.0. Experimental details are given in the text. —, Supercoiled DNA (form I); ----, relaxed circular DNA (form II).

values are similar to those obtained under conditions of low ionic strength. Although the effect of high salt concentration is much greater on the non-superhelical DNA, both forms are affected to some extent and even the supertwisted molecules show a decrease in height of the high-wavelength peak, to 70% of the low-salt value, on the transition to high salt concentration. The small difference in the temperature at which the two spectra were determined has clearly had little effect on the structural differences between the two forms. Wang (1969a) has shown that the total difference in superhelical structure between 20 and 27°C should be about 0.5 superhelical turns per molecule.

Studies on the C.D. of DNA in 92% relative-humidity films have shown that its structure in solution under normal conditions of low salt concentration corresponds closely to the B form. The high-salt structure has been tentatively identified with that of the lithium salt of DNA at 66% relative humidity, i.e. the crystallographic C form (Tunis-Schneider & Maestre, 1970). The most significant difference between these two forms of DNA is that the B structure is believed to have 10 residues per turn and the C structure 9.3. Thus by changing from the B to the C conformation the supertwisted DNA would be obliged to take on more than 30 extra superhelical turns for a molecular

weight of 3.4×10^6 . In a similar system with Cs^+ as a counterion, Wang (1969a) has shown that the actual value as judged by the ethidium bromide-binding technique is very much smaller. If there is a gradual transition between the B and C structures as the ionic strength is raised (Tunis & Hearst, 1968; Tunis-Schneider & Maestre, 1970), the requirement to take on extra superhelical turns that are thermodynamically unfavourable to the molecule may make the superhelical DNA lag behind the relaxed DNA as the salt concentration is raised.

The significance of the superhelix-induced variation in secondary structure is clear when one considers that most studies on such DNA species are carried out in the analytical ultracentrifuge in high concentrations of caesium chloride. Since Cs^+ is approximately twice as effective as Na^+ in effecting the transition into the high-salt structure (Tunis & Hearst, 1968), the secondary structures of the two forms of DNA cannot be the same in the presence of concentrations of caesium chloride above 3M. Thus, when supertwisted DNA is investigated under these conditions, the average rotation per base-pair, θ , may not be the same in the two forms, and consequently τ , the number of superhelical turns determined by the dye-binding technique, may not be the same as τ^0 , the true number of superhelical turns.

We suggest that the tertiary restraints imposed

by the covalent closure of the DNA molecule affect the secondary structure during the transition from the low-salt to the high-salt form of DNA.

We are grateful to Dr G. Wood of Strathclyde University for providing access to the Cary 60 recording spectropolarimeter for C.D. measurements and to Dr K. Sargent of the Microbiological Research Establishment, Porton, for his help in the provision of bacteriophage-infected cells. Thanks are due to Professor J. N. Davidson, F.R.S., and to Professor R. M. S. Smellie for their interest and for providing facilities with the aid of a grant from the Cancer Research Campaign.

- Bruner, R. & Vinograd, J. (1965). *Biochim. biophys. Acta*, **108**, 18.
- Emanuel, C. F. (1960). *Biochim. biophys. Acta*, **42**, 91.
- Hudson, B., Upholt, W. B., Devinny, J. & Vinograd, J. (1969). *Proc. natn. Acad. Sci. U.S.A.* **62**, 813.
- Rush, M. G., Kleinschmidt, A. K., Hellman, W. & Warner, R. C. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 1676.
- Samejima, T. & Yang, J. T. (1965). *J. biol. Chem.* **240**, 2094.
- Tunis, M. J. B. & Hearst, J. E. (1968). *Biopolymers*, **6**, 1218.
- Tunis-Schneider, M. J. B. & Maestre, M. F. (1970). *J. molec. Biol.* **52**, 521.
- Wang, J. C. (1969a). *J. molec. Biol.* **43**, 25.
- Wang, J. C. (1969b). *J. molec. Biol.* **43**, 263.
- Wang, J. C., Baumgarten, D. L. & Olivera, B. M. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 1852.