obtained with the green fluorescent protein. These data are sufficient to suggest conformational rigidity of both proteins, but other evidence is adduced from: (*a*) the relative insensitivity of aequorin blue fluorescence to oxygen quenching (bimolecular quenching constant [k_{bm}] 0.09 M^{-1} s^{-1}) which is comparable to the lower values observed by Lakowicz and Weber (3) in the study of oxygen quenching of proteins. The green protein fluorescence is essentially insensitive to the quenching effects of oxygen ($k_{bm} < 0.004$ M^{-1} s^{-1}). (*b*) Fluorescence lifetimes and anisotropies are essentially invariant across the emission band and hence reveal no evidence of dipolar relaxation around the chromophore in the green fluorescent protein; and there is only marginal dipolar relaxation in aequorin. (*c*) The green fluorescent protein is denatured by sodium dodeceylsulfate and guanidinium chloride only under harsh conditions and is markedly resistant to proteolytic cleavage by most commonly proteases.

These data, taken together show that the chromophore in Ca^{++} discharged aequorin is rigidly bound; in the green fluorescent protein not only is the chromophore very tightly bound, but the entire protein appears to be markedly conformationally inflexible.

Supported in part by U. S. Public Health Service grant HL 12186, The Mayo Foundation, and a grant from the Research Corporation. The NMR experiments were performed at the Purdue Biochemical Magnetic Resonance Laboratory, supported by National Institutes of Health grant RR-01077.

Received for publication 15 December 1979.

REFERENCES

1. Blinks, J. R., F. G. Prendergast, and D. G. Allen. 1976. Photoproteins as biological calcium indicators. *Pharmacol. Rev.* **28**:1–93.
2. Prendergast, F. G. and K. G. Mann. 1978. Chemical and physical properties of aequorin and the green fluorescent protein isolated from *aequorea forskalea*. *Biochemistry.* **17**:3448.
3. Lakowicz, J. R., and G. Weber. 1973. *Biochemistry.* **12**:4171.

THE EFFECT OF ETHIDIUM BROMIDE ON DNA INTERNAL MOTIONS

Michael Hogan and Oleg Jardetzky, *Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, California 94305 U.S.A.*

Recent nuclear magnetic resonance studies of long DNA fragments have shown that the internal structure of B-form DNA is not rigid, but instead experiences large fluctuations in nucleotide conformation which occur with a time constant near 10^{-9} s. (Hogan and Jardetzky, 1979; Bolton and James, 1979; Klevan, et al., 1979).

Based upon ^{31}P NMR relaxation data, Klevan, et al. (1979) and Bolton and James (1979) have proposed that the backbone phosphates of DNA experience a large, fast internal motion in the helix, but that the fast phosphate motion is limited to rotations about phosphate bonds ω and ω'. As has been stated (Klevan, et al., 1979), such rotation can produce only small displacements of the two nucleosides adjoining the phosphate in the helix. Therefore, the models in Bolton and James (1979) and in Klevan, et al. (1979) do not predict fast motions which may occur at other positions in the helix.

However, as we have shown (Hogan and Jardetzky, 1979; unpublished data), the B-DNA helix must experience large fluctuations in conformation of the base and sugar as well as the deoxyribose-phosphate backbone of the helix. The lines of the ^{1}H, ^{31}P, and ^{13}C spectra of the bases and sugars are too narrow to be due to overall motion alone.

The best fit of ^{1}H, ^{13}C, and ^{31}P NMR relaxation times to a two-state model predicts substantial fluctuations about a geometry near that of a B-DNA helix: Position C_2 of the deoxyribose sugar, ±35°; the base planes, ±20°; the phosphate-proton vectors in the backbone, ±25°. Within the accuracy of the model, the time constants for these internal motions are the same: $2 \pm 1 \times 10^{-9}$ s. (Hogan and Jardetzky, 1979).

On the basis of these findings, we have proposed a working hypothesis which predicts that the base plane motion which we monitor must arise from the motion of the C_2 axis (the short axis of the base planes in the helix). We propose that at the same time, deoxyribose sugar-pucker geometry fluctuates inside the helix with a time constant near 10^{-9} s. The rapid change in sugar conformation necessarily changes the geometry of the sugar-phosphate backbone. Because they are coupled in this way, the time constants measured at phosphate and in the sugar are the same.

To appreciate fully the effect of such motions on the NMR properties of DNA, it is necessary to consider exactly what would happen if these motions were stopped. During times shorter than 10^{-4} s, the long axis of a 300-base-pair-long DNA helix ($L = 1,200$ Å) moves in solution as if it were a rod with a time constant for that motion near 10^{-5}s. (Hogan, et al., 1978). Using standard relationships (Woessner, 1962), we calculate that in a rigid 300-base-pair-long helix, the linewidths of backbone phosphates and of most nucleotide protons would be $>>1,000$ Hz; T_1 values would be $>>10$ s.

If T_1 values or linewidths were of this order of magnitude, resonances could not be detected by ordinary NMR pulse sequences. Therefore, ^{13}C, ^{1}H, and ^{31}P NMR spectra can be measured in long DNA helices only because nucleotides in the helix experience conformational fluctuations near 10^{-9} s. with amplitudes $> \pm 20°$.

The geometric constraints placed upon DNA when ligands bind to the helix make it likely that large DNA internal motions will be severely hindered in a tight DNA-ligand complex. If, due to ligand binding, the amplitude of DNA motions were reduced or if the rate of the motions were made significantly slower, then DNA regions at the binding site would behave as if rigid, i.e., ^{31}P or ^{1}H NMR spectra of bound DNA regions would then become unmeasurable in solution.

In the present study using ^{31}P and ^{1}H NMR, we have measured DNA area and relaxation time changes which occur when the intercalating drug ethidium bromide (EB) binds to a 300-base-pair-long DNA fragment. We find that when EB binds to DNA, ^{1}H, and ^{31}P NMR resonance area becomes unmeasurable within a two-base-pair-long region. We also find that the relaxation properties of DNA outside the binding site are nearly unaffected by bound EB. From these observations, we conclude that base plane, deoxyribose, and phosphate backbone internal motions are "frozen out" within the two base-pair long EB-DNA complex, but that DNA regions immediately adjacent to the complex are nearly unaffected.

Received for publication 17 December 1979.

REFERENCES

Bolton, P. H., and T. L. James. 1979. *J. Am. Chem. Soc.* In press.
Hogan, M. E., and O. Jardetzky. 1979. *Proc. Natl. Acad. Sci. U.S.A.* In press.
Klevan, L., I. M. Armitage, D. M. Crothers. 1979. *Nucleic Acids Res.* 6:1607–1616.
Woessner, D. E. 1962. *J. Chem. Phys.* 27:697–655.