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Binding of meso-tetra (4-N-methylpyridyl) porphine to DNA

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ABSTRACT

The porphyrin photosensitizer, meso-Tetra (4-N-methylpyridyl) porphine tetraperchlorate is shown to unwind supercoiled ColEI DNA at a somewhat lower concentration than ethidium bromide. In contrast to this, the Fe(III) chelate of T4MPyP cannot unwind supercoiled DNA. It is concluded that these results corroborate our previous findings that, despite its large bulk, T4MPyP is fully capable of intercalating in DNA.

INTRODUCTION

Recently, it was reported from this laboratory that the photosensitizer, meso-Tetra (4-N-methylpyridyl) porphine tetraperchlorate (T4MPyP), Figure 1, could bind to DNA by intercalation (1).

Identification of T4MPyP as an intercalator was unexpected, since molecular model studies indicate that the hydrogen bonding between base pairs must be disrupted for insertion of the porphyrin in this manner. This suggests that the intercalation may depend upon the existence of "breathing modes" in DNA (2-4), or that non-B-form segments occur within the double helix (2,5) that favor intercalative binding. Here we demonstrate the ability of T4MPyP to unwind closed circular supercoiled DNA, a feature diagnostic for intercalation of this compound into DNA (6-8).

MATERIALS AND METHODS

Meso-Tetra (4-N-methylpyridyl) porphine tetraperchlorate

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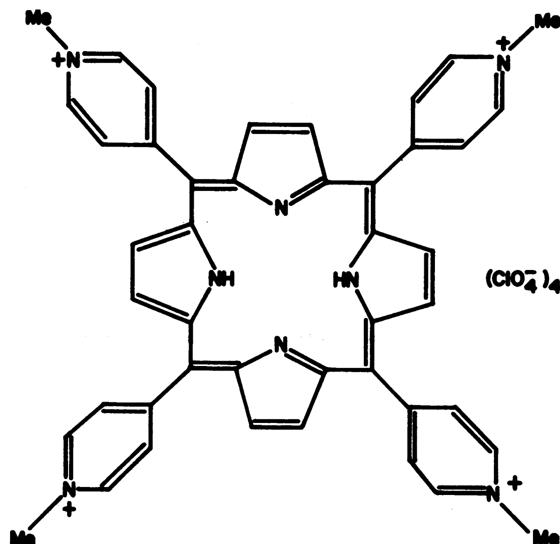

Figure 1. meso-Tetra(4-N-methylpyridyl)porphine tetraperchlorate.

(Figure 1) and its Fe(III)chelate were obtained from Dr. N. Datta Gupta, South Carolina State College.

Closed circular-superhelical DNA (Form I) from plasmid ColEI was isolated from JC411 cells using a Triton X-100 modification of the procedure of Clewell and Helinski (7). The DNA was purified using a hydroxyapatite column in place of a cesium chloride gradient.

The tube gel electrophoresis (8,9) of closed circular supercoiled DNA (Form I) and relaxed circular DNA (Form II) from ColEI, was carried out in a Tris-acetate buffer (pH 8.05), 50 mM Tris, 20 mM sodium acetate, 2 mM disodium EDTA and 90 mM NaCl, in either 0.7 or 1.0 percent agarose gels. The gels were prepared with a specified quantity of T4MPyP added to each tube. Each gel contained 0.40 μg of ColEI DNA. The electrophoresis was carried out for 6 hours at 24 ma and 20 volts. The gels were stained with ethidium bromide (0.5 μg/ml) in Tris-acetate buffer. Pictures of the gels were taken under ultra-violet light with Polaroid type 665 black and white positive/negative film.

RESULTS

In order to suppress the charge effect of the porphyrin, electrophoresis was carried out in 90 mM NaCl. This was determined to be the highest concentration that could be used and still demonstrate the end-point at which form I DNA completely unwinds and comigrates with form II DNA. At a higher concetration of NaCl, the electrophoretic migration is greatly inhibited, while, at lower concentrations the results cannot be interpreted unequivocally. Even at 90 mM NaCl, the charge effect is apparent as a reduction in the migration rate of chromosomal DNA with increasing concentration of porphyrin, as shown in Figure 2. This effect also accounts for the reduced migration rate of form II DNA, and it also responsible for some of the reduction noted for form I DNA and II comigrate, indicating that the supercoiling of form I has been relaxed. This end-point precedes a slight increase in the migration rate,

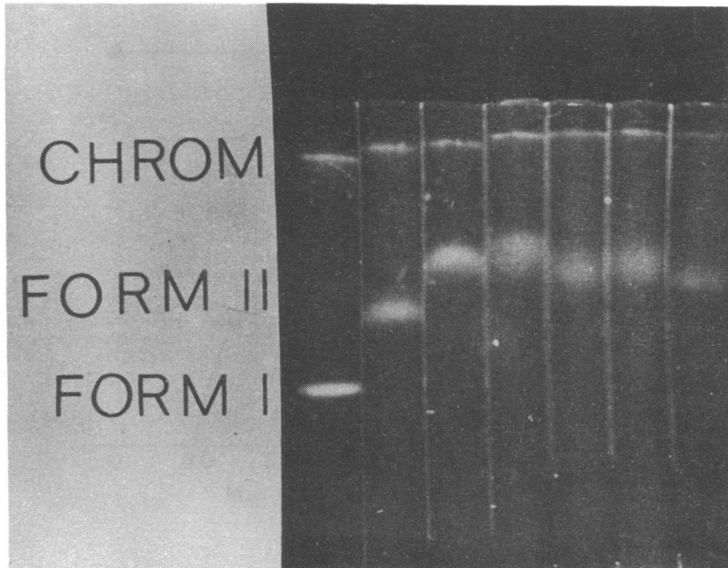

Figure 2. Tube gel electrophoresis of form I and form II ColEI DNA in 0.7 percent agarose gels. Chromosomal DNA is also apparent. Each gel contained (left to right) 0, 0.4, 1.2, 1.6, 2.0, 2.5 or 3.0 µg/ml of T4MPyP.

indicating that the direction of the supercoiling has been reversed (6,8,9).

The electrophoresis titration was repeated using smaller increments of T4MPyP so that the end-point could be determined with a greater precision. The data, plotted in Figure 3, shows a comparison of T4MPyP with its Fe(III)Cl chelate and ethidium bromide, a well-known intercalator (6). Fe(III)ClT4MPyP has the capacity to bind to DNA by electrostatic forces; however, it is not expected to intercalate because the tetrapyrrole ring is puckered to accommodate Fe(III) bound to a chlorine in the axial position. The resulting nonplanarity should prevent an intercalative interaction.

In accordance, with this, the results indicate that Fe(III)ClT4MPyP cannot unwind form I DNA, and does not affect its migration rate up to a concentration of 2.6 μM (shown only to 1.0 μM in Figure 3). Conversely, T4MPyP is even somewhat

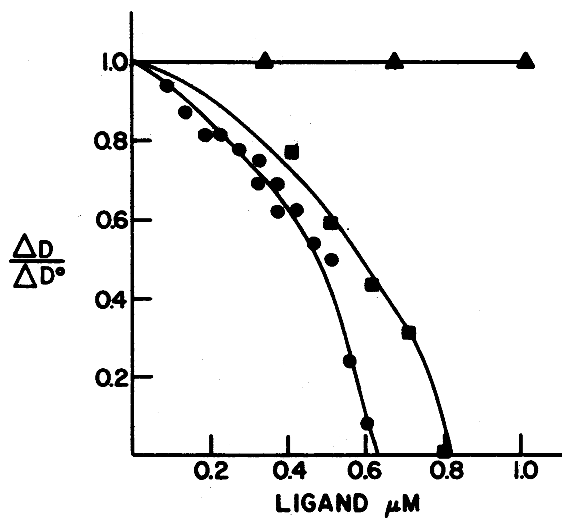

Figure 3. Migration of closed circular supercoiled DNA in 1% agarose gels containing varying concentrations of T4MPyP -●-, Fe(III)ClT4MPyP -▲-, and ethidium bromide -■-. ΔD is the distance between form I and form II in each gel, and ΔDo is the distance in the corresponding control gel. The titration end-point (i.e., comigration of forms I and II) is obtained from the intercept of the curve on the abscissa.

more effective than ethidium bromide in relaxing form I DNA. Its end-point was found to be approximately 0.6 µM compared to 0.8 µM for ethidium bromide.

A model of the intercalation of T4MPyP into the B form of DNA is shown in Figure 4. It must be emphasized that to intercalate T4MPyP, the hydrogen bonds between base-pairs must be ruptured. Once intercalated, however, the hydrogen bonds can be reformed with only a minimal distortion of the helix. Two important features of this model should be noted. First, it is the tetrapyrrole ring that interacts with the bases; the pyridine rings do not participate. Second, the pyridine rings tend to project away from the helix axis into the grooves, such that three of the four positively charged N-methyl groups are in close contact with phosphate groups, suggesting that a strong

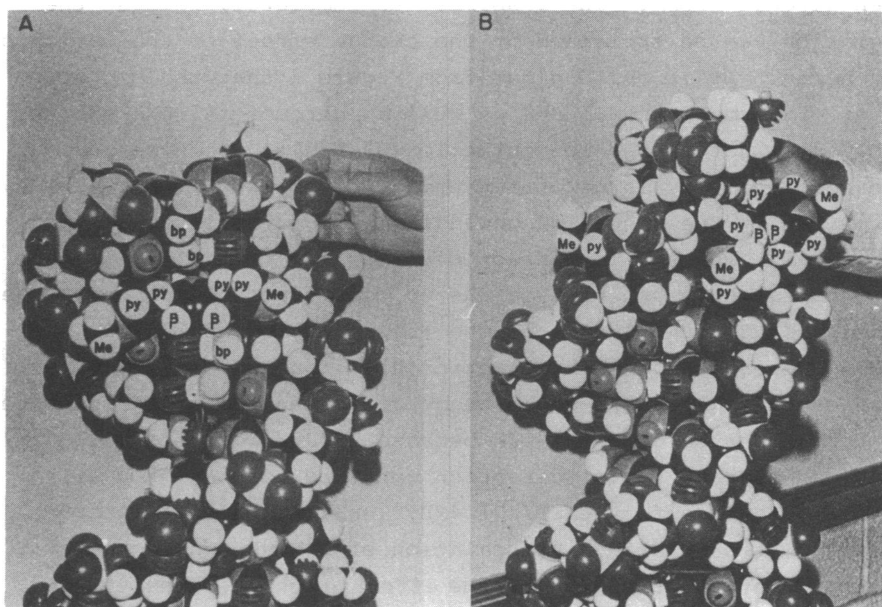

Figure 4. CPK model of T4MPyP intercalation in B-form DNA. A, view of the major groove. B, rotation of view A to the right to show one of the protrusion of one of the pyridyl groups into the minor groove. Me = methyl group of T4MPyP, py = pyridyl hydrogens of T4MPyP, β = pyrrol hydrogens of T4MPyP, and bp = base pair.

electrostatic component is a factor in this intercalation.

DISCUSSION

The electrophoresis assay alone does not provide sufficient evidence to conclude that a particular substance is an intercalator; only that it can unwind supercoiled DNA, a property shared by all intercalators (6). The sum of the evidence, however, from Tm, CD, viscosity, and binding analysis, together with corroboration from the electrophoresis assay, present a very strong case for the identification of T4MPyP as an intercalator.

The electrophoresis assay was characterized by a complete titration to an end-point, preceding at least a partial reversal of the direction of migration. This demonstrates that form I DNA has not been nicked, but relaxed from a superhelix to an open circular form. The inability to completely reverse the migration can be explained by the charge effect of the cationic porphyrin. It is quite clear from Figure 2 that the migration rates of forms I and II DNA as well as chromosomal DNA are reduced somewhat as the concentration of T4MPyP is increased. The comparatively large effect noted in the case of form I DNA, is primarily the result of unwinding induced by intercalation of T4MPyP. The smaller effect ovserved for form II DNA and chromosomal DNA, however, is due to the reduction of the charge density of these polymers by the intercalation of T4MPyP. If T4MPyP were bound only by electrostatic forces to the external surfaces of DNA, there would be no effect on the migration rate of form II or chromosomal DNA because T4MPyP would be pulled away and migrate in the direction opposite to that of DNA. This is the case for Fe(III)ClT4MPyP, which does not intercalate. At saturation,intercalation of T4MPyP can account for an estimated 30% decrease in the effective charge density of DNA. The subsequent reduction in migration rate could be even greater since a further reduction in charge density may result from extension of the DNA along the helix axis that occurs upon intercalation (10).

The charge effect also explains the smaller-than-expected increase in viscosity obtained for T4MPyP-bound DNA (1). As

already noted, intercalation tends to extend DNA in the direction of the helix axis. This is expected to result in an increase in its viscosity. Reduction of the charge density of DNA, however, relaxes its rigid structure and thereby decreases its viscosity. The net result of these two effects is a small increase in viscosity.

It is instructive to compare the properties of T4MPyP with those of nonintercalating ligands that unwind supercoiled DNA. Cis and trans dichlorodiammine platinum (II) compounds unwind supercoiled DNA by an unknown non-intercalating mechanism (11). The unwinding has been shown to be time-dependent and apparently involves covalent binding. These characteristics are not shared by T4MPyP and cannot explain the mechanism of its unwinding ability.

Another important comparison is with the steroidal diamines that unwind and reverse supercoiled DNA, and stabilize the DNA double helix (6). These are the properties associated with intercalators. The balance of the evidence suggests that the binding of these molecules to DNA is by a nonintercalative mechanism (12), although, they may mimic intercalators by binding to DNA kinks (2). Firstly, they are nonplanar molecules without aromatic character, whereas, all known intercalators including T4MPyP are planar aromatic molecules. Secondly, they do not increase the viscosity of DNA. Thirdly, they must be used at significantly higher concentrations than for known intercalators to completely unwind supercoiled DNA. For example, approximately an order of magnitude higher concentration of irediamine A is required to relax ϕX174RF DNA than is required for ethidium bromide (6). T4MPyP, as reported here, is equivalent or somewhat more efficient than ethidium bromide. A direct comparison of the binding characteristics of irediamine A and T4MPyP to DNA show the former to have a binding constant of the order of 10^3 in 0.1 M NaCl (13), while the latter has been measured at 10^7 in approximately 0.2 M NaCl (1). Moreover, in the case of T4MPyP, there is evidence for binding at concentrations of NaCl as high as 1.0 M (1).

Perhaps the most compelling evidence in favor of the intercalation of the T4MPyP is the demonstration that Fe(III)Cl-

T4MPyP cannot unwind supercoiled DNA. Since it is as fully capable as T4MPyP of interacting with DNA through electrostatic forces,the observed differences in the activity of these two compounds is compatible with intercalative binding for the latter. This, in turn, adds to the evidence presented previously and fully supports our earlier conclusion (1).

ACKNOWLEDGEMENTS

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REFERENCES

1. Fiel, R.J., Howard, J.C., Mark, E.H. and Datta-Gupta, N. (1979) Nucleic Acids Res. 6, 3093-3118.
2. Sobell, H.M., Reddy, B.S., Bhandary, K.K., Jain, S.C., Sakore, T.D. and Seshadri, T.P. (1977) Cold Springs Harbor Symp. 42, 87-102.
3. Li, H.J. and Crothers, D.M. (1969) J. Mol. Biøl. 39, 461-477.
4. McGhee, J.D. and von Hippel, P.H. (1975) Biochemistry 14, 1281-1296.
5. Wang, A.H-J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., van Boom, J.H., van der Marel, G. and Rich A. (1979) Nature 282, 680-686.
6. Waring, M. (1970) J. Mol. Biol. 54, 247-279.
7. Clewell, D.B. and Helinski, D.R. (1969) Proc. Natl. Acad. Sci. U.S.A. 62, 1159-1166.
8. DeLeys, R.J. and Jackson, D.A. (1976) Biochem. Biophys. Res. Commun. 69, 446-454.
9. Espejo, R.T. and Lebowitz, J. (1976) Anal. Biochem. 72, 95-103.
10. Lerman, L.S. (1964) J. Cell and Comp. Physiol. 64: Sup. 1, 1-18.
11. Cohen, G.L., Bauer, W.R., Barton, J.K. and Lippard, S.J. (1979) Science 203, 1014-1016.
12. Waring, M. and Henley, S.M. (1975) Nucleic Acids Res. 2, 567-586.
13. Saucier, J-M. (1977) Biochemistry 16, 5879-5889.