LETTERS TO THE EDITOR

Recognition of *B* and *Z* Forms of DNA by *Escherichia coli* DNA Polymerase I

Since the substrate binding domain of the large proteolytic fragment of *Escherichia coli* DNA polymerase I has been shown to interact with the *B* forms of DNA, we have studied the ability of this enzyme to recognize structures other than the *B* form. The polymerase activity has been used to evaluate the degree of recognition of the *B* and *Z* forms of DNA. The *Z* form was found to promote less activity, indicating the probable inability of the polymerase to move along the conformationally rigid form of the template. The present study indicates that the *Z*-DNA found *in vivo* may have a role in the control of replication.

Recent observations on the crystal structure of the large fragment of *Escherichia coli* DNA polymerase I (Ollis *et al.*, 1985*a*) have raised the question of whether DNA conformations other than the *B* form can act as suitable templates. Error-free copying of genomic DNA is necessary to maintain transmission of genetic information from one generation to another. The structural polymorphism that has been observed in DNA (Wang *et al.*, 1979; Gupta *et al.*, 1980; Sasisekharan *et al.*, 1981; Dickerson *et al.*, 1982) suggests the possibility of a role for these different structures in the control of various biological processes, and the discovery of a novel left-handed zig-zag or "*Z*" conformation in synthetic and natural DNA sequences, has stimulated renewed interest in this subject (for a review, see Rich *et al.*, 1984). However, the possible biological function of *Z*-DNA structures remains largely speculative.

DNA replication and repair require the combined action of many polymerases that perform very accurately. These enzymes must therefore recognize specific DNA structures and then interact precisely with them to execute an error-free process. The first step in understanding the molecular mechanism of the precise interaction of polymerases with DNA templates comes from the crystal structure of the large fragment of *E. coli* DNA polymerase I. The large fragment has been shown to have a unique shape that suggests some aspects of its function (Ollis *et al.*, 1985*a*). It has been found to have two domains, the smaller of which is thought to be the active site for the 3′ → 5′ exonuclease activity. The larger domain is the binding site for DNA in the *B* form, with the flexible arm surrounding the DNA to facilitate a smooth procession of nucleotide incorporation.

This observation raises the question of whether DNA structures other than *B*-form will be recognized by the polymerases, and, if they are, then what is the nature of the interaction. To answer such questions we have used model systems such as the polymerase activity of *E. coli* DNA polymerase I as an index of its ability to recognize different forms of DNA.

Synthetic polynucleotides like poly(dG-dC) have been shown to undergo structural transition from the right-handed *B* form to the left-handed *Z* form, under the influence of various agents such as ethanol, or under conditions of high ionic strength or chemical modification (Rich *et al.*, 1984; Latha & Brahmachari, 1985*a*). Methylated bases (especially 5-methyl-cytosine) are common features of the eukaryotic genome (Bird, 1984). Methylation of dC residues in repeated dG-dC sequences facilitates the transition of such sequences to the *Z* form at physiological ionic strength (Behe & Felsenfeld, 1981; Latha & Brahmachari, 1985*b*). The presence of such structures in natural sequences and *in vitro* has been well documented by using various biochemical techniques (Rich *et al.*, 1984).

E. coli DNA polymerase I was obtained from Boehringer-Mannheim. Poly(dG-dC) and poly-(dG-dm^{5}C) were purchased from PL Biochemicals. Since poly(dG-dm^{5}C) is normally synthesized using poly(dI-dC) as template, the template was freed of any dI-dC by digestion with *Hha*I restriction endonuclease because any residual dI-dC may interfere with the characteristics of the template. Sedimentation analysis in alkaline medium (0·1 M-NaOH, 0·9 M-NaCl) was performed for both the polymers, and the $s_{20,w}$ data were analysed using the equation of Studier (1965), which indicated the presence of one nick per 200 to 250 bases. From the incorporation study at low temperature and 0·3 mM-Mg^{2+}, with *E. coli* DNA polymerase I in the presence of only one nucleotide [α-^{32}P]dCTP (Wu, 1970), the number of primer terminals was found to be identical for both polymers.

Poly(dG-dm^{5}C) has been shown to undergo transition from *B* to *Z* form in the presence of Mg^{2+} at millimolar concentrations (Behe & Felsenfeld, 1981). Under identical conditions, however, the unmethylated polymer does not undergo this transition. The change from *B* to *Z* form has been well characterized by techniques such as circular dichroism, ultraviolet and nuclear magnetic resonance spectroscopy (Rich *et al.*, 1984). We have followed this transition by ultraviolet spectroscopy (Fig. 1). The activity with poly(dG-dm^{5}C) as
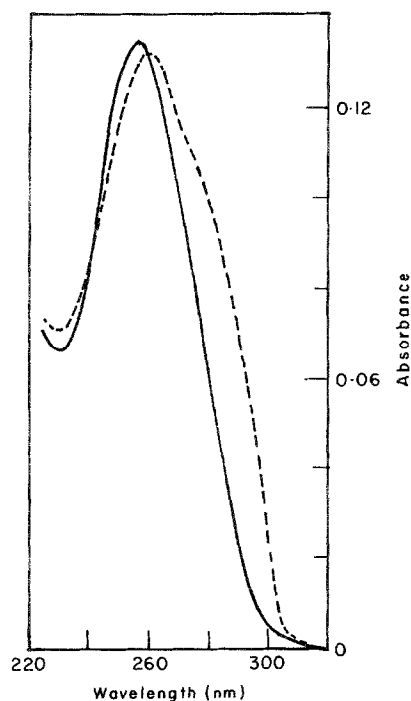
Figure 1. Representative ultraviolet spectra of poly(dG-dm⁵C) in the enzyme assay buffer (see the legend to Fig. 2). In the presence of: 0·3 mM-Mg²⁺ (———), B form; 3 mM-Mg2⁺ (— — —), Z form. Characteristic B and Z-form circular dichroism spectra were also obtained under these conditions and were similar to those reported by Behe & Felsenfeld (1981).

template was monitored under different concentrations of Mg²⁺, with poly(dG-dC) as a control. The poly(dG-dm⁵C) template showed nearly identical activity with respect to poly(dG-dC) at 0·3 mM-

Mg²⁺, where both templates exist in the B form (Fig. 2).

In the case of poly(dG-dC), an expected increase in the incorporation of labelled nucleotide was observed with increasing Mg²⁺ concentration, but this was not the case with the methylated polymer. At 3 mM-Mg²⁺, a sharp fall in activity was observed with the methylated polymer (Fig. 2). The fall in activity cannot be explained as an inhibitory effect of either Mg²⁺ concentration or cytosine methylation, as Mg²⁺ is essential for enzyme activity and the methylated polymer does not inhibit the enzyme at 0·3 mM-Mg²⁺. The only difference between the use of the methylated polymer and that of the unmethylated polymer is a structural transition brought about by Mg²⁺, as shown, by the Mg²⁺ titration value (Fig. 3). Titration of poly-(dG-dm⁵C) with Mg²⁺ under assay-buffer conditions (see the legend to Fig. 2) showed a $B \rightarrow Z$ structural transition with a mid-point around 1 mM-Mg²⁺ (Fig. 3). This transition reached a plateau value of around 4 mM-Mg²⁺. The $B \rightarrow Z$ transition with the methylated polymer correlated well with the decrease in template activity with poly(dG-dm⁵C) with respect to poly(dG-dC) (Fig. 3). It may be argued further that, since there is no m⁵C in $E.\ coli$ DNA, the organism may not have evolved a system of recognizing m⁵C. But the observation that at 0·3 mM the enzyme is equally active with the methylated and the unmethylated template, indicates clearly that methylation $per\ se$ of the template does not inhibit enzymic interaction. We have observed that brominated poly-(dG-dC) in the Z form does not serve as a template for $E.\ coli$ DNA polymerase I and avian myeloblastosis virus DNA polymerase (Brahmachari $et\ al.$, 1985). Similarly, there is low polymerase
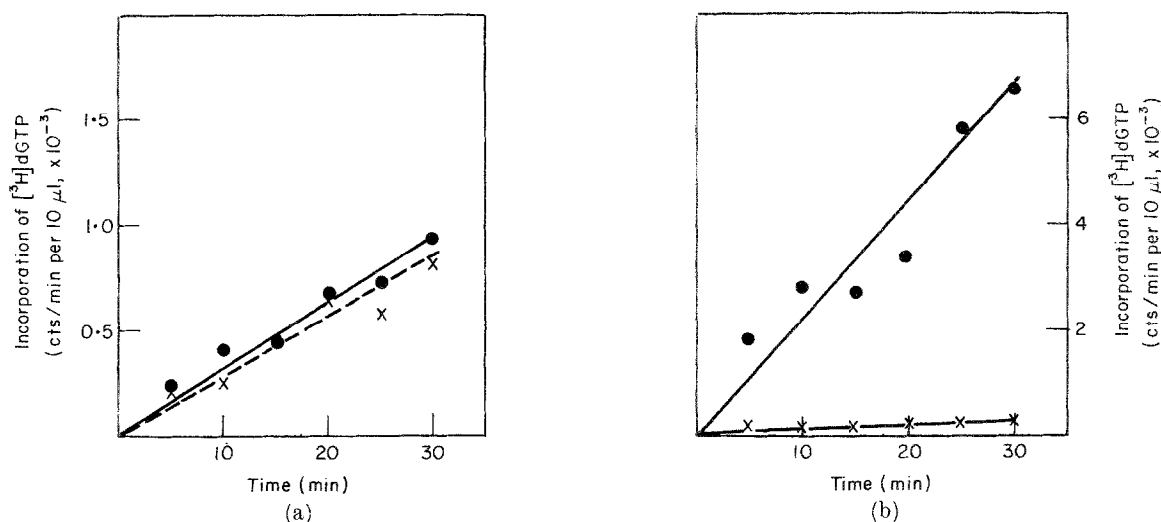

Figure 2. Enzyme activity of $E.\ coli$ DNA polymerase I with poly(dG-dC) and poly(dG-dm⁵C) as templates. The assay buffer was 67 mM-potassium phosphate (pH 7·4), 1 mM-2-mercaptoethanol, 16 μg template/ml, 0·04 unit enzyme/100 μl, [³H]dGTP with a final spec. act. of 12 μCi/nmol. The reaction volume was 100 μl. Samples (10 μl) were spotted onto Whatman no. 3 paper. The reaction was stopped with acetic acid, and followed by chromatography in 0·5 M-ammonium acetate, 50% (v/v) ethanol to remove the free nucleotides. After chromatography, the paper was dried and counted in a liquid scintillation counter. (a) 0·3 mM-Mg²⁺: —●——●—, poly(dG-dC); −×−−×−, poly(dG-dm⁵C). (b) 3·0 mM-Mg²⁺: —●——●—, poly(dG-dC); −×——×−, poly(dG-dm⁵C).
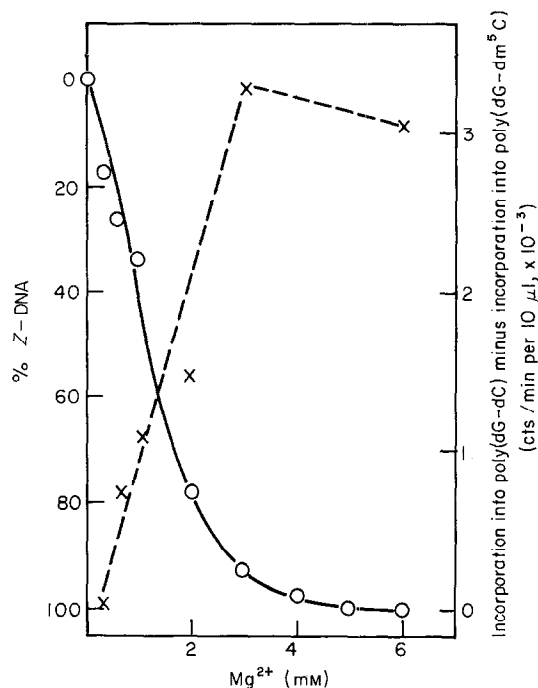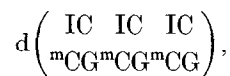
Figure 3. The difference in suitability as a template between poly(dG-dm⁵C) and poly(dG-dC), as a function of Z-DNA content. Z-DNA content was determined by ultraviolet spectroscopy. Percentage Z form for poly-(dG-dm⁵C) (-○—○-); incorporation into poly(dG-dC) minus incorporation into poly(dG-dm⁵C) (-×--×-). Assay buffer as in the legend to Fig. 2. Template concentration, 16 μg/ml.

activity with the Z^* form (van de Sande & Jovin, 1982) of poly(dG-dC) as template in 12% (v/v) ethanol and 8 mM-Mg^{2+} (Brahmachari *et al.*, 1985). The present study, which has been carried out under physiological conditions, suggests that the formation of Z-DNA structure inhibits the activity of *E. coli* DNA polymerase I. It is known that many other templates behave in an autocatalytic manner with respect to *E. coli* DNA polymerase I, whereas poly(dG-dC) shows linear kinetics (Wells *et al.*, 1977). Hence, the template activity of poly(dG-dC) is independent of the number of 3'-OH termini but is dependent upon the concentration of the template. Since at 0·3 mM-Mg^{2+}, poly(dG-dC) and poly(dG-dm⁵C) have identical polymerase activity, it may be assumed that poly(dG-dm⁵C) also shows linear kinetics and not autocatalytic activity. Since the concentrations of both the templates were the same in all the experiments, and because the kinetics of incorporation are linear the total incorporation should give a true indication of their suitability as templates, even if the number of 3'-OH termini were different in each case. However, in this case the number of 3'-OH termini were indeed the same.

It is interesting to note that earlier workers synthesized poly(dG-dm⁵C) enzymically, in an indirect way using poly(dI-dC), whereas for other polynucleotides, the same polynucleotide was used

as the initiating template (Wells *et al.*, 1970; Gill *et al.*, 1974; Behe & Felsenfeld, 1981). Our results demonstrate clearly that poly(dG-dm⁵C) in the Z form is not an efficient template at optimal Mg^{2+} concentration. An essential feature of the Z-DNA conformation is that every purine residue in an alternating purine-pyrimidine sequence adopts the *syn* conformation (Rich *et al.*, 1984). Conformational studies have shown that inosine does not prefer to adopt the *syn* conformation but guanosine does (V. Sasisekharan, personal communication). Thus, poly(dI-dC) and the hybrid

$$d\left(\begin{array}{ccc} IC & IC & IC \\ {}^mCG & {}^mCG & {}^mCG \end{array} \right),$$

after one round of synthesis cannot adopt the Z conformation in the presence of optimal Mg^{2+} concentration. It has been shown by two groups of workers (Vardimon & Rich, 1984; Zacharias *et al.*, 1984) that *Hha*I endonuclease does not cleave its natural substrate if the latter is in the Z conformation. Since the poly(dI-dC) chain of the hybrid can be selectively cleaved using *Hha*I endonuclease, the hybrid template could not have been in the Z form. Hence the enzymic synthesis of poly(dG-dm⁵C) using poly(dI-dC) as template is feasible. This result, which has not been considered previously in the light of structural observations, may be explained by the present study, which shows that the Z conformation must be responsible for the inhibition of the polymerase activity.

Induction of the $B \rightarrow Z$ transition under superhelical tension (Rich *et al.*, 1984), the presence of Z-DNA binding and stabilizing proteins in *Drosophila* cells (Nordheim *et al.*, 1982), wheat germ (Lafer *et al.*, 1985), and simian virus 40 minichromosomes (Azorin & Rich, 1985), and the presence of Z-DNA in the transcriptionally active but replicatively inactive macronucleus of *Stylonychia mytilus* (Lipps *et al.*, 1983), suggests the possibility of the control of replication by Z conformations. In addition, repair of the open imidazole ring form of 7-methylguanine by specific DNA glycosylase (Lagravère *et al.*, 1984) and that of O^6-methylguanine by O^6-methylguanine DNA methyl transferase (Bioteux *et al.*, 1985) have been shown to be very poor when the template is in the Z form. Also, RNA polymerases have been shown to be less active on a template that is in the Z form rather than in the B form (Butzow *et al.*, 1985; Durand *et al.*, 1983; van de Sande & Jovin, 1982). Our observation that the template in the Z form is less suitable than that in the B-form for *E. coli* DNA polymerase I supports our hypothesis that the Z conformation may inhibit normal replication.

Although the repair function of *E. coli* DNA polymerase I may seem of greater significance than the present study, a report on the high sequence homology of the DNA binding domain of phage T7 DNA polymerase (Ollis *et al.*, 1985b) with *E. coli* DNA polymerase I strengthens our argument. The preorientation of the incoming nucleotide in the B form in the enzyme–substrate complex of *E. coli*

DNA polymerase I, as suggested by nuclear magnetic resonance studies (Sloan *et al.*, 1975), and also the crystal structure studies of the DNA binding domain (Ollis *et al.*, 1985a), complement the present study by indicating that the *B* form is a better template for DNA polymerization and for repair. The rigid conformation of *Z*-DNA and the non-specific interaction involving N7 of the guanine residue exposed on the surface of the *Z* helix with the proton-donating side-chain of the protein, like arginine, in the binding site may reduce the processing activity of the enzyme. This inhibitory effect of the *Z* structure in DNA synthesis provides a possible mechanism by which small stretches of *Z*-DNA may act as modulators of DNA synthesis.

N. Ramesh
Yogesh S. Shouche
Samir K. Brahmachari†

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012, India

References

Azorin, P. & Rich, A. (1985). *Cell*, **41**, 365–374.

Behe, M. & Felsenfeld, G. (1981). *Proc. Nat. Acad. Sci., U.S.A.* **78**, 1619–1623.

Bird, A. P. (1984). *Nature (London)*, **307**, 503–504.

Boiteux, S., Costa de Oliveira, R. & Laval, J. (1985). *J. Biol. Chem.* **260**, 8711–8715.

Brahmachari, S. K., Ramesh, N., Das, M. R. & Parnaik, V. K. (1985). *Biochem. Int.* **11**, 281–290.

Butzow, J. J., Shin, A. Y. & Eichhorn, G. L. (1984). *Biochemistry*, **23**, 4837–4843.

Dickerson, R. E., Drew, H. R., Conner, B. N., Wing, R. M., Frantini, A. V. & Kopka, M. L. (1982). *Science*, **216**, 475–485.

Durand, R., Job, C., Zarling, A. D., Teissere, M., Jovin, T. M. & Job, D. (1983). *EMBO J.* **2**, 1707–1714.

Gill, J. E., Mazrimas, J. A. & Bishop C. C. Jr (1974). *Biochim. Biophys. Acta*, **335**, 331–348.

Gupta, G., Bansal, M. & Sasisekharan, V. (1980). *Proc. Nat. Acad. Sci., U.S.A.* **77**, 6486–6490.

Lafer, E. M., Sousa, R., Rosen, B., Hsu, A. & Rich, A. (1985). *Biochemistry*, **24**, 5070–5076.

Lagravère, C., Malfoy, B., Leng, B. & Laval, J. (1984). *Nature (London)*, **310**, 798–800.

Latha, P. K. & Brahmachari, S. K. (1985a). *Soc. Biol. Chem. (India) Biochem. Rev.* **55**, 1–16.

Latha, P. K. & Brahmachari, S. K. (1985b) *FEBS Letters*, **182**, 315–318.

Lipps, H.-J., Nordheim, A., Lafer, E. M., Ammermann, D., Stollar, B. D. & Rich, A. (1983). *Cell*, **32**, 435–441.

Nordheim, A., Tessen, P., Azorin, F., Kwon, A. Ha., Moller, A. & Rich, A. (1982). *Proc. Nat. Acad. Sci., U.S.A.* **79**, 7729–7733.

Ollis, D. L., Brick, O., Hamlin, R., Xuong, N. G. & Steitz, T. A. (1985a). *Nature (London)*, **313**, 762–766.

Ollis, D. L., Kline, C. & Steitz, T. A. (1985b). *Nature (London)*, **313**, 818–819.

Rich, A., Nordheim, A. & Wang, A. H.-J. (1984). *Annu. Rev. Biochem.* **53**, 791–846.

Sasisekharan, V., Gupta, G. & Bansal, M. (1981). *Int. J. Biol. Macromol.* **3**, 1–8.

Sloan, D. L., Loeb, A. L., Mildvan, A. S. & Feldman, R. J. (1975). *J. Biol. Chem.* **250**, 8913–8920.

Studier, F. W. (1965). *J. Mol. Biol.* **11**, 373–390.

van de Sande, J. H. & Jovin, T. M. (1982). *EMBO J.* **1**, 115–120.

Vardimon, L. & Rich, A. (1984). *Proc. Nat. Acad. Sci. U.S.A.* **81**, 3268–3272.

Wang, A. H.-J., Quingley, G. J., Kolpak, F. J., van der Marel, G., van Boom, J. H. & Rich, A. (1979). *Nature (London)*, **282**, 680–686.

Wells, R. D., Larson, J. E., Grant, R. C., Shortle, B. E. & Cantor, C. R. (1970). *J. Mol. Biol.* **54**, 465–497.

Wells, R. D., Blakesley, R. W., Burd, J. F., Chan, H. W., Dodgson, J. B., Hardies, S. C., Horn, G. T., Jensen, K. F., Larson J., Nes, I. F., Selsing, E. & Wartell, R. M. (1977). *C.R.C. Crit. Rev. Biochem.* **4**, 305–340.

Wu, R. (1970). *J. Mol. Biol.* **51**, 501–521.

Zacharias, W., Larson, J. W., Kilpatrick, M. W. & Wells, R. D. (1984). *Nucl. Acids Res.* **12**, 7677–7692.

† Author to whom all correspondence should be addressed.

Edited by A. Klug