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The inhibition of restriction endonucleases due to Z-DNA in negatively supercoiled plasmid

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Plasmid pGC20 containing the $(dGC)_9$ insert in *Sma*I recognition site has been used to study the inhibition of cleavage by different restriction endonucleases due to Z-DNA formation in $(dCG)_{10}$ sequence of the negatively supercoiled plasmid. Data obtained indicate the different sensitivity of restriction endonucleases to DNA conformational perturbations resulted from the Z-DNA formation. Therefore, the inhibition of DNA cleavage by a particular restriction endonuclease cannot serve as a criterion for the estimation of the length of B–Z junctions in circular supercoiled DNAs.

Z-DNA; Negatively supercoiled plasmid; Restriction endonuclease; Inhibition

1. INTRODUCTION

Sequences (dGC) integrated into plasmids have been shown to be capable of forming left-handed Z-DNAs on negative supercoiling while the remaining plasmid DNA still held the B conformation [1,2]. Data obtained from the cleavage of B–Z junctions by SI and *Bal*31 nucleases [3,4] as well as most results of chemical modification in these regions by various modifying agents (see for example [5–8]) showed that several base pairs with either broken or strongly distorted Watson-Crick hydrogen bonds existed at B–Z junctions.

In addition to such a type of perturbations in B–Z junction regions, other changes in the conformation and conformational flexibility of the DNA douplex near Z-DNA are possible. These changes are not revealed by the techniques of chemical modification, but can be detected by restriction endonucleases very sensitive to changes in the conformation of both recognisable and flanking DNA sequences [9,10]. The results of the *Bam*HI digestion experiments showed that the degree of enzyme inhibition decreases as the distance between the recognition sequence and Z-DNA insert increases. In terms of this criterion, the length of the B–Z junction was determined to be less than 8 bp long [10]. However, the nucleotide composition and structure of flanking sequences are known to exert a different effect on various restriction endonucleases [11]. That is why we decided to study the effect of a $(dCG)_{10}$ sequence in the Z form on the activity of different restriction endonucleases and, if possible, to use the inhibition data for determining the length of the B–Z junction in plasmid pGC20.

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2. MATERIALS AND METHODS

Plasmid pGC20 prepared by integrating a $(dGC)_9$ sequence into the *Sma*I recognition site in the polylinker of plasmid pUC19 [12], was isolated [24] from an *E. coli* IM83 cell. The plasmids pGC20 and pUC19 (kindly supplied by Dr S. Mirkin) were cleaved by *Eco*RI, *Sac*I, *Bam*HI, *Xba*I, *Sal*I, *Kpn*I purchased from Amersham. All digestions were carried out at 37°C for 1 h at the enzyme/DNA ratio of 5 units/1 μg of DNA in appropriate buffer mixtures. Plasmid pGC20 was relaxed by adding ethidium bromide (Sigma) to a DNA solution up to a concentration calculated upon the known value of the superhelical density of pGC20 DNA and its concentration in solution. The value $\sigma_0 = -0.067$ for the isolated plasmid pGC20 was determined by titrating the plasmid DNA with an ethidium bromide solution and recording the amplitude of linear dichroism in the flow at 260 nm. DNA digestions were electrophoresed on 0.8% agarose gel using a buffer solution containing 40 mM Tris-acetate and 2 mM EDTA, pH 8.0.

3. RESULTS AND DISCUSSION

Fig. 1 presents the nucleotide sequence of the pGC20 polylinker adjacent to the $(dGC)_9$ insert, and shows the recognition sites of six restriction endonucleases located on both sides of this insert and at different distances from it. Based on the results of our preliminary *Bam*HI inhibition experiments (not shown) we decided to use the inhibition of cleavage by several restriction endonucleases as a criterion for estimating the length of the B–Z junction in the plasmid pGC20. For this purpose, plasmid pGC20 was digested by six endonucleases selected in such a way that the recognition sites for two different enzymes were almost equally located in relation to $(dCG)_{10}$ sequence on both sides of it (see Fig. 1). Fig. 2 presents an electrophoregram of supercoiled pGC20 DNAs digested by *Bam*HI and *Kpn*I, *Xba*I, and *Sac*I, *Sal*I and *Eco*RI. One can see that 5 out of the 6 restriction endonucleases were either fully or partly inhibited. Under these conditions, only *Eco*RI completely

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...GTCGACTCTAGAGGATCCCC(GC)$_6$GGGTACCGAGCTCGAATTC...
SalI XbaI BamHI Z-DNA KpnI SacI EcoRI

Fig. 1. The nucleotide sequence of a pGC20 polylinker adjacent to Z-DNA formed in a $(dCG)_{10}$ sequence. The recognition sites of six restriction endonucleases are shown in the figure.

linearized the scDNA. To reinforce the assumption that these enzymes were really inhibited due to a Z-DNA, the digestions of the DNA relaxed by ethidium bromide pGC20 in which $(dCG)_{10}$ is in the B form were carried out with them. In that case, all 6 restriction endonucleases almost completely linearized the DNA (Fig. 3). It is possible that the relaxed form of pGC20 DNA is cleaved by all the six enzymes not (or not solely) because the conformation of $(dCG)_{10}$ sequence changes from Z to B form, but due to altering of the tertiary structure of circular plasmid DNA. In order to check this possibility, we used the same enzymes to cleave the plasmid pUC19 with nearly the same superhelical density but, in contrast to plasmid pGC20, without $(dGC)_9$ insert. As one can see in Fig. 4, all 6 restriction endonucleases also linearized the supercoiled pUC19 almost completely. This control experiment confirms that the cleavage of supercoiled plasmid pGC20 is inhibited by Z-DNA formation in C–G sequence plasmid pGC20 is inhibited by Z-DNA formation in C-G sequence rather than by changing in the DNA tertiary structure.

The results of our digestion experiments indicate that the degree of enzyme inhibition does not always correlate with the distance between recognition sites and Z-DNA. A clearcut correlation can be followed up in the series *Kpn*I-*Sac*I-*Eco*RI (see Figs 1 and 2); the degree of enzyme inhibition drops down as the recognition sites become more removed from a Z-DNA, and *Eco*RI causes complete digestion of pGC20 under these conditions. Such a correlation is not found for the series *Bam*HI-*Xba*I-*Sal*I whose recognition sites are located symmetrically versus a Z-DNA. On the contrary, *Bam*HI linearizes about 50% of supercoiled DNA within 1 h; whereas *Xba*I and *Sal*I whose recognition sites are 7 and 13 bp from Z sequence, respectively, are

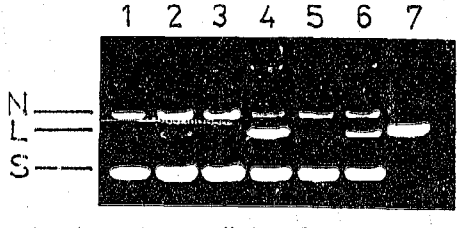

Fig. 2. The digestions of supercoiled pGC20 by six different restriction endonucleases: (1) nondigested supercoiled pGC20 (control); (2) pGC20 digested by *Sal*I; (3) by *Xba*I; (4) by *Bam*HI; (5) by *Kpn*I; (6) by *Sac*I; (7) by *Eco*RI. S = cupercoiled DNA; L = linear DNA; N = nicked DNA.

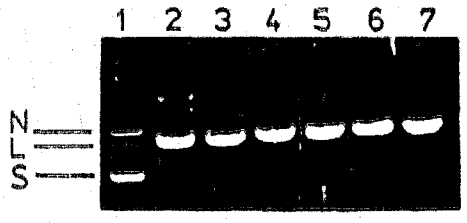

Fig. 3. The digestions of relaxed pGC20 by six restriction endonucleases: (1) nondigested supercoiled pGC20 (control); (2) relaxed pGC20 digested by *Sal*I; (3) by *Xba*I; (4) by *Bam*HI; (5) by *Kpn*I; (6) by *Sac*I; by *Eco*RI. S = supercoiled DNA; L = linear DNA; N = nicked DNA.

inhibited almost completely. Therefore, although the recognition sites of *Bam*HI and *Kpn*I as well as *Sal*I and *Eco*RI are located at virtually identical positions versus a Z-DNA, the activities of these enzymes are quite different.

Based upon the known mechanism of the site-specific recognition and interaction of EcoRI with DNA [13], we can suggest that the original conformation and conformational flexibility of recognisable and flanking sequences can exert a noticeable influence on the efficiency of recognition and the enzyme binding to DNA. It is quite likely that unique conformational features of these appear under the action of two factors: a perturbance caused by the presence of Z-DNA in the flanking sequence and an additional effect exerted by the negative superhelicity of circular DNA. On the other hand, as was found with restriction substrates and the binding of these [11], we may therefore speculate that the different effect of a Z-DNA on the enzyme activity of restriction endonucleases is related to the different manners by which these enzymes recognise appropriate sequences and bind to them. Thus, the results presented here permit one to conclude that the alteration of conformational properties of DNA spread over rather long distances from the $(dCG)_{10}$ sequence in Z form and have an influence on the efficiency of enzyme/DNA interaction. As for the possibility of studying B-Z junctions in supercoiled DNAs, our data indicate that the inhibition of cleavage by any particular restriction endonuclease cannot serve as a criterion for determining the length of these DNA regions.

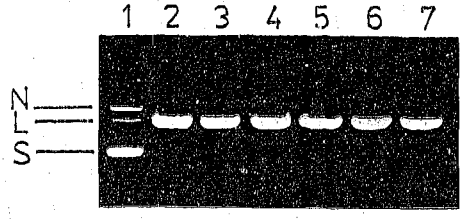

Fig. 4. The digestions of supercoiled pUC19 by six restriction endonucleases: (1) nondigested pUC19 (control); (2) pUC19 digested by *Sal*I; (3) by *Xba*I; (4) by *Bam*HI; (5) by *Kpn*I; (6) by *Sac*I; (7) by *Eco*RI. S = supercoiled DNA; L = linear DNA; N = nicked DNA.

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