Inhibition of restriction enzyme's DNA sequence recognition by PUVA treatment

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ABSTRACTS
Applying various restriction enzymes on a specially designed 1.5 kb DNA fragment revealed that the inhibitory effects of PUVA treatment on restriction endonuclease activities are caused by recognition inhibition. In this study, Restriction enzymes which have a 5'-TpA sequence at the cleaving site (Kpn I, Xba I, Pme I, and Dra I), and non-cleaving site (Pac I) in recognition sites, or have two 5'-TpA sequences at the recognition site and a non-specific sequence between recognition and cleaving site (BciV I) were inhibited by PUVA treatment. Most of the other restriction enzymes used in this study which do not have a 5'-TpA sequence at their recognition site were not inhibited by PUVA treatment, although a 5'-TpA sequence is located adjacent (Sma I) or very close (BamH I, Sac I and Pst I) to the recognition and cleaving site for them.

INTRODUCTION
Psoralens are used to treat psoriasis with a combination of UVA irradiation (known as PUVA therapy) (1). PUVA treatment is known to inhibit DNA synthesis at the S phase of a cell cycle (2), resulting in the inhibition of cell division. Psoralens are also known to bind to double strand DNA as either monoadducts or bis-adducts (cross-links) by [2+2] cycloaddition (3). The inhibition of DNA synthesis at the S phase of cell cycle by PUVA is therefore thought to be caused by these photoproducts. The binding mechanism of psoralens to a double strand DNA has been well investigated. Using supercoiled plasmid DNA and six restriction enzymes, Kittler et al. (4) reported that the activities of Kpn I, Ssp I, Dra I, and Rsa I, that have 5'-TpA sequence at their restriction sites, were inhibited by PUVA treatment, while the activities of EcoR I and BamH I, which have 5'-ApT sequence at the sites, were not inhibited. They associated this inhibitory effects with the preference of PUVA on 5'-TpA rather than on 5'-ApT. They also reported that the DNA that has 5'-TpA sequences in close vicinity to the restriction site for BamH I was cleaved and concluded that only the central bases within the recognition sequence are decisive for the inactivation of the restriction enzymes.

However, the activity of restriction enzymes involves two steps; the recognition step and the cleaving step. Some restriction enzymes have cleaving sites in its recognition sites as all of the restriction enzymes used in Kittler's experiment while the others such as BciV I have the cleaving site separately from recognition. Therefore, the use of the latter type of restriction enzymes makes it possible to distinguish if the activities of the restriction enzymes are inhibited at the recognition step or at a cleaving step. We used a specially designed DNA fragment with polyrestriction sites for a variety of restriction enzymes and compared the inhibitory effect of PUVA treatment on the activities of restriction enzymes which have 5'-TpA sequences at cleaving sites in restriction sites with the effect on restriction enzymes which have 5'-TpA sequences at the non-cleaving site of restriction sites (Pac I) or at an non-specific sequence between the recognition site and cleaving site in the restriction site (BciV I). In this report, we present evidence for the inhibition of restriction enzymes by PUVA treatment at the recognition step of the enzyme reaction.
Figure 2. Effects of PUVA treatment of the 1.5 kb DNA fragment on the activities of restriction enzymes which have poly-restriction sites on the fragment. 8-MOP (upper) and angelicin (lower) were incubated with 1.5 kb DNA fragments either with UVA irradiation (47.5 kJ/m²) for 1.5 hr on ice (U) or in the dark on ice for 1.5 hr (D). The restriction enzymes were indicated on the top of each two sets of lanes. UT: untreated 1.5 kb DNA fragment.

EXPERIMENTAL

A DNA fragment (1.5 kb) was synthesized by PCR using pNEB193 as a template. The mixtures of the DNA fragment (100 ng) and 200 pmole of 8-MOP (8-methoxypsoralen) or angelicin on ice were irradiated with UVA (47.5 kJ/m²) followed by restriction enzyme digestions and analysis by agarose gel electrophoresis.

RESULTS AND DISCUSSIONS

Fig. 1 shows the nucleic acid sequences of each restriction enzymes used in this study together with respective recognition sites, cleaving sites, and non-specific sequences in the restriction sites. Kpn I, Xba I, Pme I, and Dra I have respective 5'-TpA sequence at the cleaving sites in their respective recognition site of restriction sites. On the other hand, Pac I has a 5'-TpA sequence at the non-cleaving sequence in the recognition site of restriction sites. BciV I has two 5'-TpA sequences at the non-cleaving sequence in the recognition site and at the non-specific sequence of the restriction site. Other restriction enzymes do not have the sequence. As shown in Fig. 2, all of the restriction enzymes which have 5'-TpA at restriction site sequences were inhibited by 8-MOP and angelicin with UVA irradiation. In addition, little inhibitory activity of PUVA on BamH I, which has a 5'-TpA sequence at two nucleotides away from its "cleaving site" on the DNA fragment, suggested that cleaving activity is also not affected by psoralens bound to the neighboring 5'-TpA. Therefore it was suggested that the inhibitory effect of PUVA treatment on BciV I was provoked by only the binding of psoralens to the 5'-TpA sequence located at the "recognition site" of the restriction site, not by the binding of psoralens to the 5'-TpA at the non-specific sequence of the restriction site.

Based on those results, it is possible that the recognition step of the other DNA binding enzyme such as DnaA, which initiates the DNA replication process when it binds to the AT-rich 9 bp repetitive sequences (9-mers) in bacterial replication origin (5), could also be inhibited. Thus, the inhibition effect of PUVA on DNA synthesis at S phase of the cell cycle (2) could be in part explained by this inhibition of DnaA.

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REFERENCES