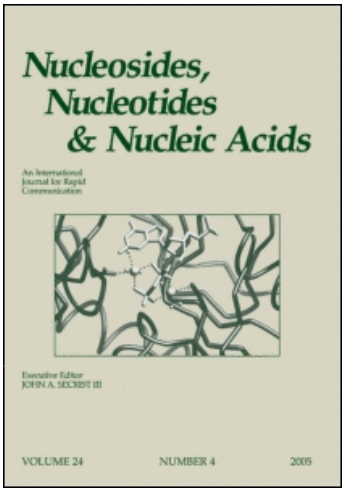

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Interaction of Restriction Endonucleases with Phosphorothioate-Containing DNA

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INTERACTION OF RESTRICTION ENDONUCLEASES WITH PHOSPHOROTHIOATE-CONTAINING DNA

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Abstract. The requirements for inhibition of cleavage of phosporothioate-containing DNA by the restriction enzymes *Ban*II and *Eco*RV with respect to number and position of these groups was determined.

Phosphorothioate-containing DNA and RNA have found wide application in molecular biology. One of the properties which makes this class of compounds attractive is its slow rate of nuclease - catalyzed hydrolysis.[1] Results obtained with approximately 30 restriction enzymes and phosphorothioate DNA have shown that these enzymes can be divided into three categories. Class I restriction enzymes are unable to catalyze the linearization of DNA which had been obtained by polymerisation with one particular nucleoside phosphorothioate triphosphate as substrate. In other cases, similar substitutions were only able to decrease the rate of cleavage of the phosphorothioate linkage (class II) or had little effect at all (class III).[2] The inability of the class I restriction enzymes to cleave phosphorothioate - containing DNA is the basis for efficient oligonucleotide - directed mutagenesis methods for single and double stranded DNA.[3,4] Despite these successful applications, the protective ability of the phosphorothioate residues has never been investigated in detail except for the restriction endonuclease *Nci*I.[3]

In every case studied to date, a phosphorothioate substitution of the phosphate which undergoes hydrolysis is a minimum requirement for complete inhibition of cleavage. Closer inspection of

the class I enzymes reveals that the nucleotide used to incorporate a phosphorothioate at the cleavage site is present more than once in the recognition sequence. In order to determine what a sufficient requirement for inhibition of cleavage is we have undertaken a more detailed study with two enzymes, *Ban*II and *Eco*RV by using DNA containing phosphorothioates placed at several specific positions within the recognition sequence of each enzyme. *Ban*II is a particularly suitable object for such a study as the *Ban*II site in M13mp2, 5′-GGGCTC-3′, is inhibited when dCMPS is incorporated into the complementary (-)strand whereas the sequence 5′-GAGCTC-3′ in M13mp18 is not.

It was determined for both enzymes that incorporation of a single phosphorothioate group at the potential site of cleavage reduces the rate of enzyme-catalyzed hydrolysis between 5- and 10-fold. An additional phosphorothioate group positioned immediately 3′- to the cleavage site and located outside the recognition sequence prevents cleavage by the enzyme *Ban*II.[5] No combination of phosphorothioate group substitution was found to confer complete protection of DNA against the hydrolytic activity of *Eco*RV. However, inhibition studies with phosphorothioate-containing DNA fragments showed that this enzyme binds strongly to such DNA in an unspecific manner. Fully dAMPS-substituted DNA containing a phosphorothioate at the site of cleavage was found to be completely resistant to hydrolysis.[6] It had been determined earlier for the restriction enzyme NciI that an additional phosphorothioate group 5′- to the cleavage site is required for complete protection.[2]

These results suggest that there is no simple predictable pattern for phosphorothioate inhibition of restriction enzymes.

REFERENCES

1. Eckstein, F. and Gish, G. (1989) TIBS 14, 97 - 100.
2. Taylor, J. W., Schmidt, W., Cosstick, R., Okruszek, A. and Eckstein, F. (1985) *Nucleic Acids Res.* 13, 8749-8764.
3. Nakamaye, K. L. and Eckstein, F. (1986) *Nucleic Acids Res.* 14, 9679 - 9698.

4. Olsen, D. B. and Eckstein, F. (1990) *Proc. Natl. Acad. Sci. USA* 87, 1451 - 1455.
5. Olsen, D. B. Kotzorek, G., Sayers, J. and Eckstein, F. (1990) *J. Biol. Chem.* 265, in press.
6. Olsen, D. B., Kotzorek, G. and Eckstein, F. (1990) *Biochemistry* 29, in press.