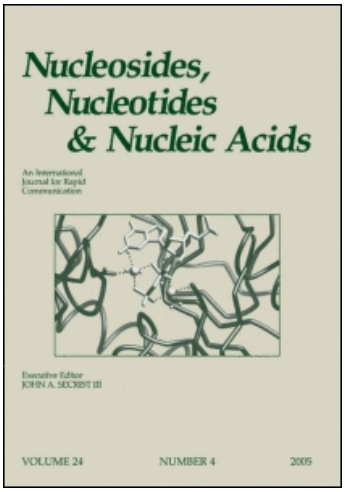

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Stabilisation of DNA by Incorporation of Phosphothioate Groups

F. Eckstein[a]
[a] Max-Planck-Institut für experimentelle Medizin, Göttingen, West-Germany

To cite this Article Eckstein, F.(1985) 'Stabilisation of DNA by Incorporation of Phosphothioate Groups', Nucleosides, Nucleotides and Nucleic Acids, 4: 1, 77 — 79
To link to this Article: DOI: 10.1080/07328318508077831
URL: http://dx.doi.org/10.1080/07328318508077831

NUCLEOSIDES & NUCLEOTIDES, 4(1&2), 77-79 (1985)

STABILISATION OF DNA BY INCORPORATION OF PHOSPHOTHIOATE GROUPS

F. Eckstein

Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie,

D-3400 Göttingen, West-Germany

Summary: The synthesis of Rp- and Sp-d(GGsAATTCC), an octanucleotide containing the recognition sequence for the restriction endonuclease Eco R1 as well as a phosphorothioate group at the cleavage site is described. Only the Rp-diastereomer is a substrate for Eco R1. Viral DNA, containing exclusively phosphate groups in the (+)strand, and phosphorothioate groups 5'- to deoxyadenosine in the (-)strand yields nicked DNA on cleavage by Eco R1 as an isolatable intermediate. Other restriction enzymes show a similar pattern of hydrolysis. - The influence of phosphorothioate groups on the B Z conformational change is demonstrated on phosphorothioate analogues of $d(C-G)_4$ and $d(G-C)_4$.

Phosphorothioate analogues of nucleotides have proven to be extremely useful for the elucidation of certain stereochemical aspects of the mechanism of enzyme catalysed phosphoryl and nucleotidyl transfer reactions[1,2,3]. In particular they can be used to determine the stereochemical course of such reactions.

2'-Deoxynucleoside 5'-O-(1-thiotriphosphates) are good substrates for various DNA polymerases. All of these accept only the diastereomers of the Sp-configuration as substrates and catalyse the polymerisation reaction to proceed with inversion of configuration at phosphorus, thus introducing a phosphorothioate internucleotidic linkage of the Rp-configuration. The introduction of phosphorothioate groups into DNA has certain consequences with respect to degradation by nucleases. In general phosphorothioate esters and diesters are hydrolysed more slowly than the corresponding phosphate esters and this is also reflected in nucleases. Thus, the 5'→3' exonuclease of DNA polymerase I degrades the phosphorothioate internucleotidic linkage only extremely slowly. This is also true for the 3'→5' exonuclease of this enzyme[4,5]. Since this exonuclease has a proofreading function, the incorporation of deoxynucleoside phosphorothioates into DNA causes a higher frequency of base mismatches and thus mutations[4,5,6,7].

As preliminary data suggest that also restriction endonucleases cleave phosphorothioate internucleotidic linkages only slowly we decided to investigate this cleavage reaction more closely. In order to do this d(GGsAATTCC), an

 0732-8311/85/0401-0077$3.50/0

octamer which contains the recognition sequence for the restriction endonuclease Eco R1 as well as a phosphorothioate group at the cleavage site, was synthesized by the phosphoramidite approach on silica gel support[8]. Two procedures were employed to obtain the octamers with the phosphorothioate group in either the Rp or the Sp-configuration. In one the phosphite between dG and dA was oxidized by the addition of sulfur on the polymer bound growing oligonucleotide chain resulting in a mixture of phosphorothioate groups of the Rp and Sp-configuraiton. They became separable by HPLC after enzymatic phosphorylation of the octamer. In the other the two diastereomers of d Gp(S)A were synthesized, separated and then introduced as a bloc after phosphitylation to the polymer bound oligonucleotide. The phosphorothioate octamers were identified by desulfurisation with iodine to the parent compound as well as by FAB mass spectrometry. Only the octamer with the phosphorothioate group in the Rp-configuration was cleaved by Eco R1, with a rate approximately 20 times more slowly than the all phosphate-containing octamer. The stereochemical course of the enzymatic reaction was determined by carrying out the hydrolysis in the presence of $H_2{}^{18}O$ and ^{31}P-NMR analysis of the [^{18}O]dATPαS obtained by phosphorylation of [^{18}O]dAMPS obtained from complete digestion of the first reaction product with nuclease P1. The Eco R1 catalysed reaction proceeds with inversion of configuration[9].

To determine the effect of phosphorothioate substitution at restriction endonuclease cleavage sites, M13 viral, circularly closed single (+)strand was used as a template for the synthesis of the (-)strand in the presence of various dNTPαS as substrates for DNA polymerase. The resulting double stranded viral DNA is a hybrid where only the (-)strand contains phosphorothioate groups. On incubation of such a hybrid synthesized with dATPαS instead of dATP with Eco R1, a nicked intermediate in the first phase of the reaction can be seen as the sole product by gel analysis. Upon longer incubation times linear DNA is produced. This result is to be expected from the observations made with the phosphorothioate containing octamer. A similar result is seen in the reaction with Bam H1. Reactions with Ava I on DNA where the phosphorothioate group is 5'- to dC result in the production of the nicked material as the sole product indicating that the phosphorothioate of the Rp-configuration is extremely stable towards hydrolysis by this enzyme. However, in the reaction with Sal I no nicked intermediate is detectable, the first product seen being molecules with cleavage in both strands presumably indicating that a nicked intermediate cannot be released from the enzyme-DNA complex. These observations suggest that incorporation of phosphorothioate groups at particular restriction cleavage sites might be of use for directing strand cleavage, and thus in mutagenesis experiments.

Experiments with the phosphorothioate analogues of poly[d(G-C)] indicate that the phosphorothioate group has a profound influence on the B→Z

transition[10,11]. Thus, a phosphorothioate group 5' to deoxycytidine facilitates it whereas such a group 5' to deoxyguanosine prevents it. These data point to a possible involvement of the phosphate groups in this process. Studies with phosphorothioate analogues of $d(C-G)_4$ and $d(G-C)_4$ where a phosphorothioate group of either the Sp- or the Rp-configuration is incorporated 5'- to dG in the former and 5'- to dC in the latter, show that the configuration of the phosphorothioate plays an important role in this process[12]. Thus only Sp-phosphorothioate 5'- to dG and Rp 5'- to dC allow this conformational transition to occur. The kind of influence these phosphorothioates exert on an oligonucleotide to facilitate or prevent this transition is not clear. Inspection of models suggests that a direct interaction of sulfur with other atoms is not involved. We thus conclude that possibly hydrophobic interactions play a role in this process.

REFERENCES

1. Eckstein, F. Ang. Chem. 1983, 95, 431-447; Ang. Chem. Int. Ed. Engl. 1983, 22, 423-439
2. Gerlt, J. A., Coderre, J. A., Mehdi, S. Adv. in Enzymol. 1983, 55, 291-380
3. Frey, P. A. New Comprehensive Biochem. (ed. Ch. Tamm) 1983, Vol. 3, 201-248
4. Kunkel, T. A., Eckstein, F., Mildvan, A. S. Koplitz, R. M., Loeb, L. L. Proc. Natl. Acad. Sci. USA 1983, 78, 6734-6738
5. Gupta, A. P., Benkovic, P. A., Benkovic, S. J. Nucleic Acids Res. 1984, in press
6. Shortle, D., Grisafi, P., Benkovic, S. J., Botstein, D. Proc. Natl. Acad. Sci. USA 1982, 79, 1588-1592
7. Abarzna, P., Marians, K. J. Proc. Natl. Acad. Sci. USA 1984, 81, 2030-2034
8. Connolly, B. A., Potter, B.V.L., Eckstein, F., Pingoud, A. and Grotjahn, L. Biochemistry 1983, 23, 3443-3453
9. Connolly, B. A., Eckstein, F. and Pingoud, A. J. Biol. Chem. 1984, 259, 10760-10763
10. Jovin, T. M., van de Sande, J. H., Zarling, P. A., Arndt-Jovin, D. J., Eckstein, F., Füldner, H. H., Greider, C., Grieger, I., Hamori, R., Kalisch, B., McIntosh, L. P., Robert-Nicoud, M. Cold Spring Harbor Symp. Quant. Biol. 1983, 47, 143-154
11. Jovin, T. M., McIntosh, L. P., Arndt-Jovin, D. J., Zarling, D. A., Robert-Nicoud, M., van de Sande, J. H., Jorgenson, K. F., Eckstein, F. J. Biomol. Structure and Dynamics 1983, 1, 21-57
12. Cosstick, R. and Eckstein, F. Biochemistry, submitted