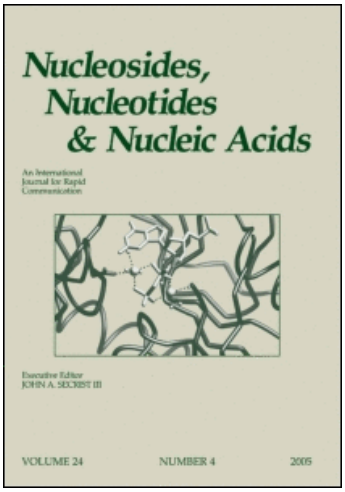

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THE INFLUENCE OF MODIFICATIONS ON THE CLEAVAGE OF OLIGONUCLEOTIDE DUPLEXES BY Eco R II AND Mva I ENDONUCLEASES

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ABSTRACT. To study the interaction of the restriction endonucleases MvaI and Eco RII with DNA we have synthesized some modified oligonucleotides. The results of hydrolysis demonstrate that both enzymes cleave their substrate by different mechanism.

Eco RII and MvaI endonucleases are isochizomer and recognize in DNA the (↓CCA/TGG) and (CC↓A/TGG) sequences. We have previously studied the cleavage specivity of Mva I and Eco RII endonucleases[1,2,3]. These data enable us to draw certain conclusions about the mechanism of substrate cleavage and about the structure of the enzyme-substrate complex. Here interaction of both enzymes with a set of special synthesized DNA-duplexes containing different modifications within the recognition site or different sequences in the flanking site has been studied.

RESULTS AND DISCUSSION

The oligonucleotides of Ia and the modified oligonucleotides were synthesized by the phosphotriester method in solution as described earlier[4]. The synthesis of oligonucleotides of III an IV were performed on the soviet

I 5′ ACCTACCXGGTGGT a X=T, b X=dFU
3′ TGGATGGACCACCA c X=riboU, d X=xyloT

II 5′ GATGCTGCCAACCTGGCTCTAGCTTCATAC
3′ CTACGACGGTTGGACCGAGATCGAAGTATG

III 5′ GCCAACCTGGCTCT IV 5′ AATGCCTGGCATT
3′ CGGTTGGACCGAGA 3′ TTACGGACCGTAA

V 5′ ACCTACm^{5}CTGGTGGT
3′ TGGATG GACCACCA

VI 5′ ACCTACCT GGTGGT
3′ TGGATGGAm^{5}CCACCA

VII 5′ ACCTACm^{5}CT GGTGGT
3′ TGGATG GAm^{5}CCACCA

VIII 5′ ACCTACCppTGGTGGT
3′ TGGATGG ACCACCA

synthesizer "Victoria 4" according to the phosphoramidite method. Id and VIII were synthesized according to[5]. Duplexes were labeled either in the upper or in the lower strand and the kinetic parameters were determined for both strands.

The replacement of dT by dFU (Ib) as well as by riboU (Ic) and xyloT (Id) in the central base pair leads to a significant altering either of the electronic and conformational structure of the duplex Ia. The initial rate (v_o) of digestion by MvaI for the modified strand is not changed, and even the hydrolysis of the unmodified strand is not remarkable inhibited. Contrary, the digestion of these duplexes by Eco RII is strong inhibited. These findings are in agreement with the results of hydrolysis of the pyrophosphate containing duplex VIII. As expected the modified strand is not digested by MvaI, whereas the unmodified strand underlies a normal digestion. A very different result was obtained for Eco RII. Replacement of Eco RII endonuclease scissile phosphodiester bond with pyrophosphate converts the substrate into its non-hydrolyzable analog, which cannot be cleaved[6].

Taken together these results suggest that MvaI and Eco RII cleave their substrates by different mechanism. It has to be assumed that MvaI endonuclease with its both subunits[7] cuts the two phosphodiester bonds in two separate single strand scissions. Contrary, Shabarova et al.[6] suppose a simultaneous cleavage of both strands for Eco RII digestion. In comparision with our previous studies on the Eco RII recognition[1] it has to be noted that the protein-DNA contact by MvaI endonuclease is realized independently on a wide range of modifications within the recognition site whereas any interruption of the structure of the recognition site leads to a significant inhibition of digestion by Eco RII. The results of digestion of duplexes V-VII confirm this assumption . The substitution of the internal C in one or both strands by m^{5}C does not remarkable change the rate of hydrolysis.

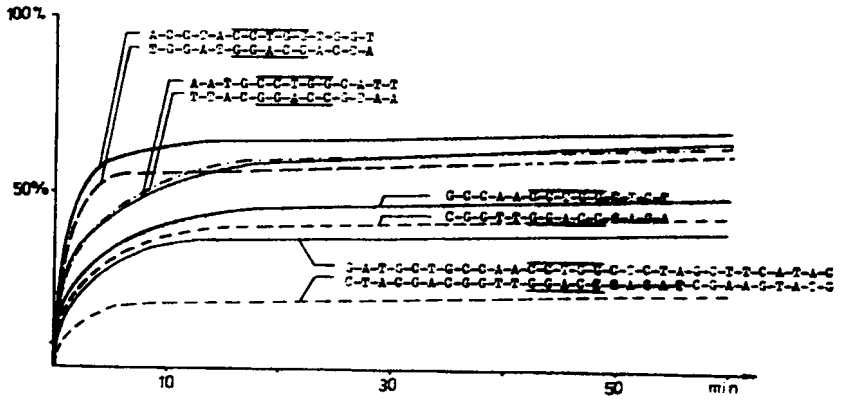

Figure 1: Eco RII digestion

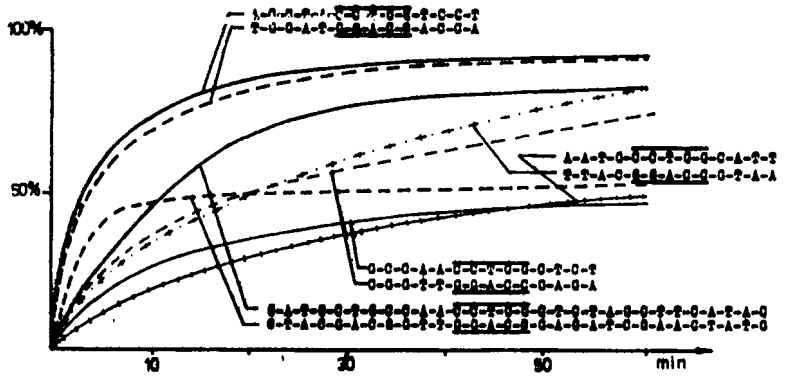

Figure 2: MvaI digestion

For further studies on the mechanism we have examined the hydrolysis of duplexes II-IV which are natural substrates existing in different helical geometries. Duplex Ia exists in a A-form, whereas II and III exist in the classical B-form. FIG.1 and FIG.2 show the results of digestion by both enzymes. It is clearly shown that cleavage of the A-form duplex Ia proceeds appreciably faster than the cleavage of II both by MvaI -and Eco RII endonucleases. Similar results were obtained for digestion of III (as part of II) which indicates that the effectivity of cleavage is independently on the chain length of the duplexes. The data obtained demonstrate that hydrolysis by MvaI and Eco RII endonucleases depends on the geometry of DNA. It is interesting to note that in the case of MvaI both stands

were hydrolyzed with different cleavage rates. Proceeding from these results we have studied digestion of the quasi palindromic duplex IV. Only in the case of hydrolysis by MvaI the rate of cleavage of dA-containing strand is noticeably faster than that of dT-containing strand. One possible reason is that MvaI unlike Eco RII remarkably differs in hydrolysis of its two scissile bonds C-A and T-C.

Taken together all results suggest that Eco RII essentiell needs contact positions to the DNA, but does not differs in hydrolysis of its scissile bonds. Otherwise, MvaI cuts its scissile bonds with different cleavage rates independently on superpositions within the recognition site.

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