Substrate specificity of new restriction endonuclease FaiI

Details
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A few site-specific endonucleases are currently known which recognize and cleave short (3- and 4-letter) degenerate DNA sequence. These are restriction endonucleases CviJI and different isoshizomers from eukaryotic Chlorella virus, which recognizes and cleaves the nucleotide sequence 5'-RGCY-3' [1]. In the present work we describe the substrate specificity of a new site specific endonuclease FaiI, which is capable to cleave degenerate 5'-YATR-3' (four main sequences: CA^TG, CA^TA, TA^TG and TA^TA) and with the less effectiveness the majority other variants of three-letter sequence 5'-YAT-3' . This enzyme was isolated from bacterial strain Flavobacterium aquatile by the chromatographic methods

MATERIALS AND METHODS

Oligodeoxyribonucleotide duplexes of the following composition, which served as a substrate for endonuclease FaiI, were used in the experiments:

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lly     3z P-5′-CGAGTTCATGGCTGGGCCCAAC -3′
            3′-GCTCAAGTACCGACCCGGGTTG-5′
llz     32p-5′-CGAGTTCATAGCTGGGCCCAAC-3′
            3′-GCTCAAGTATCGACCCGGGTTG-5′
llg     3zp-5′-CGAGTTTATTACTGGGCCCAAC-3′
            3′-GCTCAAATAATGACCCGGGTTG-5′
15g     32p-5′-CGAGTTGATGGCGCGGCCCAAC-3′
            3′-GCTCAACTACCGCGCCGGGTTC-5′
l 5e    3z P-5′-CGAGTTGATCGCGCGGCCCAAC -3′
            3′-GCTCAACTAGCGCGCCGGGTTC-5′

Fai3    32 P-5′-CGAGTTTGTAGCTGGGCCCAAC-3′
            3′-GCTCAAACATCGACCCGGGTTG-5′
Fai4    32p-5′-CGAGTTTACAGCTGGGCCCAAC-3′
            3′-GCTCAAATGTCGACCCGGGTTG-5′
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Recognition sequence for FaiI in each duplex is marked in bold. Recognition sequence for restriction endonuclease FaeI CATG^ is underlined

One of the chains of oligonucleotide duplex was labeled at 5’-end using T4-polynucleotide kinase and γ-[32P]ATP. After oligonucleotide purification, complementary unlabeled oligonucleotide was added, the tube was heated at 95°C for 5 minutes followed by cooling to room temperature on the bench. Hydrolysis of oligonucleotide duplexes with 2 units of endonuclease FaiI was conducted in 10 µl of the reaction mixture containing SE-buffer “B” (10 mM Tris HCl pH 7.9 (at 25°C), 10 mM MgCl2, 1mM DTT) and oligonucleotide duplex at the concentration of 62.5 nM at the temperature of 50°C for 25 min. Electrophoresis of the hydrolysis products was carried out in denaturing 20% PAAG with 7 M urea in tris-borate buffer. Gel autoradiography was performed using the Personal Molecular Imager (BioRad, USA).

RESULTS AND DISCUSSION

DETERMINATION OF SITE-SPECIFIC ENDONUCLEASE FAM DNA CLEAVAGE POSITIONS

Comparison of lengths of DNA fragment, produced during cleavage of oligonucleotide duplex 11y with FaiI and restriction endonuclease FaeI (recognition site 5'-CATG^-3') was carried out. Products of partial hydrolysis of the same duplexes with exonuclease ExoIII were used as a fragments length marker. Results of oligonucleotide duplexes digestion are presented on **Fig. 1.**

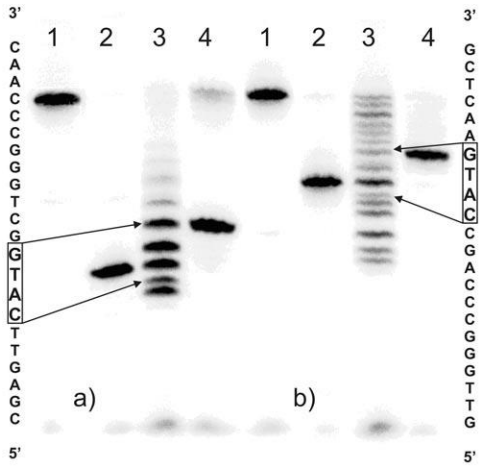

Figure 1. Determination of FaiI cleavage position on duplexes 11y (a) and 11yk (b) in comparison with FaeI.

As shown in **Fig. 1**, length of DNA fragments, which are produced in course of the oligonucleotide duplexes 11y cleavage with FaiI, are two nucleotides shorter than products of FaeI digestion. Thus, the endonuclease FaiI cleaves the recognition sequence 5'-CATG-3' after adenine, producing blunt ends.

SUBSTRATE SPECIFICITY OF FAIL

On the figures below data on synthetic oligonucleotide duplexes cleavage with FaiI and corresponding sites for FaiI are presented.

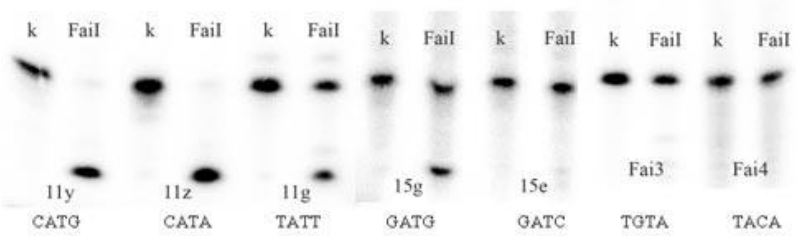

Figure 2. Intact (k) and hydrolyzed (FaiI) duplexes with ^{32}P- labeled one strand was separated in 20% PAAG with 7M urea.

Fig. 2 shows the data on synthetic oligonucleotide duplexes cleavage with FaiI. According to data of Fig.2 FaiI displays maximal activity with substrates containing recognition sequence YATR (5'-CATG-3' - duplex 11y, 5'-CATA-3' - duplex 11z) and we observe a full DNA digestion. In the case of substitution of purine or pyrimidine (5'-TATT-3' duplex 11g, 5'-GATG-3' – duplex 15g) we observe a partial DNA hydrolysis with a weak activity.
In the case of simultaneous substitution of purine and pyrimidine DNA cleavage doesn't occur (5'-GATC-3' - duplex 15e). Replacement of central AT dinucleotide results in the absence of enzyme's activity as well (5'-TGTA-3' - duplex Fai3, 5'-TACA-3' - duplex Fai4).
Thus, FaiI displays maximal activity in hydrolysis of DNA substrates containing YATR site.

FaiI may be used in the preparation of a quasi-random shotgun library [1]. Besides, FaiI-generated oligodeoxyribonucleotides may be used in some molecular biology applications, i.e., DNA labeling, detection, high-resolution restriction mapping [1].

REFERENCES

1. Swaminathan, N., Mead, D.A., McMaster, K., George, D., Van Etten, J.L., Skowron, P.M. Molecular cloning of the three base restriction endonuclease R.CviJI from eukaryotic Chlorella virus IL-3A. – 1996 - Nucleic Acids Res. 24: 2463-2469.