Substrate specificity of new restriction endonuclease SetI

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Tomilova J.E., Degtyarev S.Kh

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 SetI, which is capable to cleave DNA sequence 5'- ASST^ -3' (four main sequences: ACGT^, AGCT^, ACCT^ and AGGT^). SetI was isolated from an E.coli strain that carries the cloned SetI gene from Streptomyces werraensis 37

MATERIALS AND METHODS

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

11a ^{32}P-5’-CGAGTTTAT**AACT**GGGCCCAAC-3’
3’-GCTCAAATA**TTGA**CCCGGGTTG-5’

11g ^{32}P-5’-CGAGTTTAT**TACT**GGGCCCAAC-3’
3’-GCTCAAATA**ATGA**CCCGGGTTG-5

11h ^{32}P-5’-CGAGTTTAT**CACT**GGGCCCAAC-3'
3’-GCTCAAATA**GTGA**CCCGGGTTG-5

11i ^{32}P-5’-CGAGTTTAT**GACT**GGGCCCAAC-3’
3’-GCTCAAATA**CTGA**CCCGGGTTG-5

11j ^{32}P-5’-CGAGTTTAT**AGCT**GGGCCCAAC-3’
3’-GCTCAAATA**TCGA**CCCGGGTTG-5’

11k ^{32}P-5’-CGAGTTTAT**ACCT**GGGCCCAAC-3’
3’-GCTCAAATA**TGGA**CCCGGGTTG-5’

Recognition sequence of SetI in each duplex is marked in bold. Recognition sequence of restriction endonuclease PspOMI (5’-G^GGCCC-3’) is underlined.

One chain of oligonucleotide duplex was labeled at 5'-end using T4-polynucleotide kinase and γ-[^{32}P]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 1 units of endonuclease SetI was conducted in 10 μl of the reaction mixture containing SE-buffer "Y" (33 mM Tris acetate pH 7.9 (at 25°C), 10 mM $Mg(CH_3COO)_2$, 66mM KCH_3COO, 1mM DTT) and oligonucleotide duplex at the concentration of 62.5 nM at the temperature of 55°C for 15 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

Determination of site-specific endonuclease SetI DNA cleavage positions. Comparison of lengths of DNA fragment, produced during cleavage of oligonucleotide duplex 11a with SetI and restriction endonuclease PspOMI (recognition site 5'-G^GGCCC-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**.

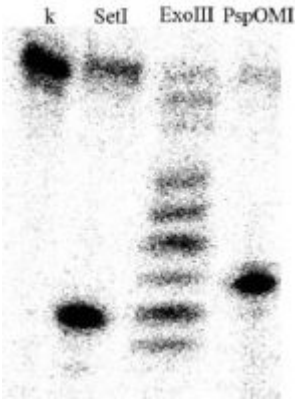

Figure 1. Determination of SetI cleavage position on duplex 11a in comparison with PspOMI.

As shown in **Fig. 1**, length of DNA fragments, which are produced in course of the oligonucleotide duplexe 11a cleavage with SetI, are one nucleotides shorter than products of PspOMI digestion. Thus, the endonuclease SetI cleaves DNA just after the recognition sequence, producing four nucleotides 3'-steaky ends.

On the figures below data on synthetic oligonucleotide duplexes cleavage with SetI are presented.

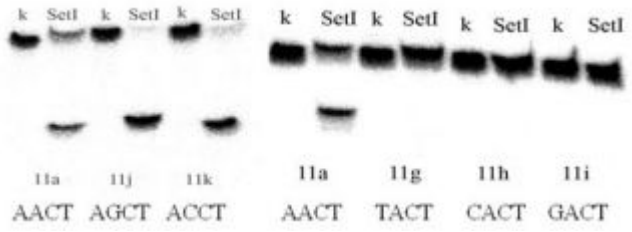

Figure 2. Intact (k) and hydrolyzed (SetI) ^{32}P labeled duplexes were separated in 20% PAAG with 7M urea.

According to data of Fig.2 SetI displays maximal activity with substrates containing recognition sequence ASST (5'-AGCT-3' - duplex 11j, 5'-ACCT-3' - duplex 11k) and we observe a full DNA digestion. In the case of substitution of cytosine or guanine (5'-AACT-3' duplex 11a) we observe a partial DNA hydrolysis with a weak activity.
Replacement of first adenine and second cytosine or guanine results in the absence of enzyme's activity (5'-TACT-3' - duplex 11g, 5'-CACT-3' - duplex 11h, 5'-GACT-3' - duplex 11i).
Thus, SetI displays maximal activity in hydrolysis of DNA sequence 5'-ASST-3'. SetI cleaves some other sites with a weak activity.

SetI may be used in the preparation of a quasi-random shotgun library [1]. Besides, SetI-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.