Alteration of the Specificity of *Pst* I Restriction Endonuclease

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Abstract: The influence of factors on the substrate-specificity of *Pst* I restriction endonuclease has been studied with the method of electrophoresis. The results show that, the specificity of *Pst* I almost can not be influenced by the single alteration of the concentration of Tris·HCl, Mg²⁺ or Na⁺ in the reaction system, but it can be altered by the reduction of any two of them. The specificity can not be altered by the single alteration of pH or the replacement of Mg²⁺ with Mn²⁺. The addition of glycerol or dimethylsulfoxide (DMSO) to the reaction system results in the relaxation of the substrate-specificity of *Pst* I, but dimethylmethylformide, glycol and ethyl alcohol can not bring about the alteration of *Pst* I specificity. Through the method of cloning and sequencing, the nucleotides of No. 1 and 6 in the recognition sequence of *Pst* I have changed (1C→A or 6G→T). Used with the enzyme analysis of an artificially synthetic DNA segment containing a special sequence, the nucleotides of No. 1 and 6 have both changed (1C→A and 6G→T). The recognition sequence of *Pst* I is speculated to be changed from CTGCA·G to TGCA·T.

Key words: restriction endonuclease; specificity; star activity
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0 Introduction

Type I restriction endonucleases are widely used in the analysis and manipulation of DNA molecules. Up to now, among 3 107 kinds of restriction endonucleases (the number is lately estimated.), the substrate-specificity has been examined for only a few endonucleases; *EcoR* I, *EcoRV*, *BamH* I, *Hind* II and so on. Their specificity were found to be altered under modified conditions; ( at higher pH, lower ionic strength, substitution of the metallic cofactor and the addition of organic solvents). Through analysis, the relaxation of recognition sequence has two types. One is that the recognition sequence is shortened. In 1975, Polisky *et al.* observed that the recognition sequence of the *EcoR* I enzyme was reduced from the six bases to the four internal bases in 25 mmol/L Tris·HCl, pH8.5, 2mmol/L MgCl₂[1]. The other is that some nucleotides of the canonical site are substituted. For instance, the recognition sequence G¹GATCC of the *BamH* I enzyme can turn into G¹GAACC , G¹GCTCC , G¹GGTCC with its specificity altered[2]. Recently, Clifford reported that the relaxed specificity of endonucleases was related to hydrostatic pressure and osmotic pressure[3].

Malyguine previously reported that the specificity of *Pst* I was relaxed in the presence of an organic solvent[4]. Our laboratory had examined the isoschizomers, *Bsp63* I, *Bsp78* I, of *Pst* I. We had found that under some same conditions, the specificity of *Bsp63* I was altered while that of *Bsp78* I wasn't[5]. So far nobody has systematically studied the influence of the single factor such as pH, ionic strength on the specificity of restriction endonucleases, and has reported the recognition sequence and the cutting site of *Pst* I as well (the altered specific activity, namely star activity, was referred to as *Pst* I *, as designated for *EcoR* I *). In this paper, we describe our studies.
1 Materials and Methods

1.1 Materials

*Pst* I, *Sac* I, *Nsi* I, agarose, X-GAL, IPTG were all the products of Promega. The plasmid pUC18DNA were purchased from SABC. All chemical reagents were analytical reagent grade. The oligodeoxynucleotides was synthesized by YUANPINGHAO BIOTECH CO. LTD. with the synthesis efficiency being 99%.

5'd (C--G--C--A--T--G--C--A--T--G--C--G) 3'
3'd (G--C--G--T--A--C--G--T--A--C--G--C) 5'

Buffer I : 90 mmol/L Tris*•*HCl, 10 mmol/L MgCl₂, 50 mmol/L NaCl, pH 7.5.

Buffer II : 20 mmol/L Tris*•*HCl, 5 mmol/L MgCl₂, 20% glycerol, pH 8.5.

1.2 Methods

1.2.1 Restriction enzyme digestion

These were done under the condition as the following figures describes. The reaction was terminated by adding the mixture (200 mmol/L EDTA and 0.05% bromophenol blue). The resulting mixture was then fractionated by electrophoresis in 1% agarose slab gels containing 0.5 mg/L ethidium bromide at 5 V/cm, and DNA was detected under ultraviolet light at 254 nm and photographed in the addition of a red light-filter lens.

1.2.2 Recombination of the digests

10 µg pUC18DNA was cleaved with *Pst* I of the right amount in buffer I for 14 h at 37°C (the maximum condition for *Pst* I *"* activity, Fig. 1) treated with klenow fragments for 40 min at 37°C. The extracted solution contained *Pst* I digestive fragments of varied lengths, and could be directly used for the ligation (Fig. 2). In the ligation, the vector pGEM-5Z was used this time, with a 1 : 3 molar ratio of vector:insert. The total volume of the reaction system was 10 µL.

1.2.3 Transforming and screening of the recons

The products of the ligation above were transformed into competent bacteria DH5α. The recombinant plasmids were extracted and screened as the reference (2).

1.2.4 Sequence analysis

This was done by SAIBAISHEN CO. LTD.

1.2.5 High-pressure liquid chromatography

The samples were prepared by incubating the synthetic DNA segments with *Pst* I in the presence of buffer I for 14 h at 37°C (3). Waters 515 high-pressure liquid chromatography was performed on a system consisting of a reverse phase C18 column, a gradient eluent (triethylammonium acetate and methanol, pH 7.0).

![Fig. 1 Degree of pUC18 DNA cleavage after incubation with different periods](image)

![Fig. 2 Cloning procedure of pUC18 DNA segments](image)

2 Results and Discussion

2.1 Effect of Ionic Strength, pH, Organic Solvent and Mn²⁺ on the Specificity

2.1.1 Effect of ionic strength

The optimum reaction medium for *Pst* I is buffer I : 90 mmol/L Tris•HCl (pH 7.5), 10 mmol/L MgCl₂ and 50 mmol/L NaCl. There is only one cutting site for *Pst* I in PUC18 DNA. And it was completely cleaved into linear DNA by the