A restriction endonuclease *Sua*I from the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*

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A type II restriction endonuclease (*Sua*I) has been isolated from the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*. The enzyme is an isoschizomer of *Bsp*RI. It does not cut *S. acidocaldarius* DNA, as the recognition sequence GGCC in this DNA contains modified nucleotide(s). The enzyme is most active at 60–70° C and is highly thermostable.

Restriction endonuclease Bsp*RI isoschizomer* Sua*I* *Archaebacteria*

1. INTRODUCTION

The definition of archaebacteria as a third kingdom of life besides those of eubacteria and eukaryotes, based upon 16 S rRNA sequence analysis, was supported by the finding of certain unique features of archaebacterial molecular phenotype [1]. Along with these there exists a number of specific resemblances between archaebacteria and eukaryotes. These include the properties of archaebacterial elongation factor EF-2 [2], DNA-dependent RNA polymerases [3,4] and DNA polymerases [5], and the finding of introns in tRNA genes of archaebacteria [6]. On the other hand, archaebacteria have clearly prokaryotic organization and some other properties of eubacteria, e.g. anti Shine-Dalgarno sequences in the terminus of 16 S rRNA [7], and arrangement of rRNA genes in the order 16 S–23 S–5 S [8].

Archaebacteria might have analogues to the eubacterial system of controlling lateral gene flow. Recently, evidence of in vivo restriction and modification of halophage S45 and the presence of strain-specific endonucleolytic activities in cell lysates of *Halobacterium* was reported [9]. In addition, a type II restriction endonuclease (*Tha*I) occurs in *Thermoplasma acidophilum* [10]. We report here the purification of one more archaebacterial type II restriction endonuclease: *Sua*I from *Sulfolobus acidocaldarius*.

2. MATERIALS AND METHODS

S. acidocaldarius DSM 639 was grown at 82°C as described in [11].

Assays for *S. acidocaldarius* restriction enzyme activity were carried out in 20 μl of 0.05 M Tris-HCl, pH 7.8, 0.005 M $MgCl_2$, 0.025 M KCl, 0.001 M dithiothreitol containing 2 μg pBR322 DNA. Either 0.5 or 1.0 μl of enzyme sample was added and the reactants incubated at 65°C for 60 min. The reactions were stopped by adding 3 μl of 0.2 M EDTA, 50% glycerol and 0.1% bromphenol blue. Aliquots of 18 μl were electrophoresed in 2% agarose or 6% polyacrylamide slab gels as described in [12]. DNA was detected by staining in ethidium bromide.

High-M_r DNA was prepared from *S. acidocaldarius* according to [13], pBR322 and pUC19 DNAs as in [12].

Restriction endonucleases *Bsp*RI, *Hin*cII, *Hin*dIII, *Eco*RI and T_4 DNA ligase were purchased from Ferment (Glavmikrobioprom, USSR). Digestion and ligation of DNAs with these enzymes were carried out according to the conditions recommended by the supplier.

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Double digestion of pBR322 DNA by *Bsp*RI and *Sua*I was performed as follows. DNA was digested by *Bsp*RI, the digestion products extracted twice with phenol-chloroform (1:1) and once with chloroform, precipitated with ethanol (2.5 vols), recovered by centrifugation, dissolved in the assay mixture for *Sua*I and further digested by *Sua*I.

For estimation of the lengths of restriction fragments *Sau*3A-digested pBR322 DNA and *Bsp*RI-digested SV40 DNA fragments were used as markers.

3. RESULTS AND DISCUSSION

3.1. *Isolation of restriction enzyme* SuaI

Frozen cells (6 g) of *S. acidocaldarius* were thawed and suspended in 30 ml buffer A (0.05 M Tris-HCl, pH 8, 0.04 M KCl, 0.005 M 2-mercaptoethanol, 5% glycerol) containing 0.4 M KCl and 300 μg/μl phenylmethylsulfonyl fluoride. The cells were lysed by addition of Triton X-100 and EDTA to the suspension, to final concentrations of 0.1% and 0.005 M, respectively. After sonication (6 × 30 s) the cell debris was removed by centrifugation (20000 × *g*, 1 h). The supernatant was collected, dialysed against buffer A, and applied to a 30 ml phosphocellulose (Whatman P11) column equilibrated with the same buffer. A 200 ml gradient (0–1 M KCl in buffer A) was applied to the column. In fractions eluted at 0.35–0.5 M KCl the presence of a restriction endonuclease was detected. These fractions were combined, dialysed against buffer A, and applied to a 10 ml column of BioRex 70 (BioRad) equilibrated with buffer A. The column was developed with a 100 ml gradient (0–1 M KCl in buffer A). The enzyme activity eluted as a single peak at about 0.4 M KCl. The peak fractions were combined. 1 μl of this enzyme preparation cleaved 3 μg pBR322 DNA in 1 h under the standard assay conditions.

With a slight deviation from convention, the enzyme was called *Sua*I, to distinguish it from *Sac*I.

*Sua*I purified by Bio-Rex chromatography seems to be free of contaminating non-specific nucleases. Increasing the digestion time from 1 to 12 h neither reduced the length of pBR322 DNA fragments nor affected the sharpness of DNA bands in the gel.

3.2. *Properties of restriction enzyme* SuaI

The enzyme cuts pBR322 DNA, producing more than 10 fragments (fig.1A). The lengths of the 11 largest fragments are 587, 540, 504, 458, 434, 267, 234, 213, 192, 184 and 124 base pairs. These fragment sizes are exactly those of *Bsp*RI-digested pBR322 DNA [12].

Fig.1B depicts the digestion of pBR322 and pUC19 DNAs by *Sua*I, *Bsp*RI and double digestion of these DNAs by both enzymes. In all 3 cases the fragment patterns are indistinguishable. This indicates that *Sua*I recognizes the same tetranucleotide GGCC as does *Bsp*RI [14].

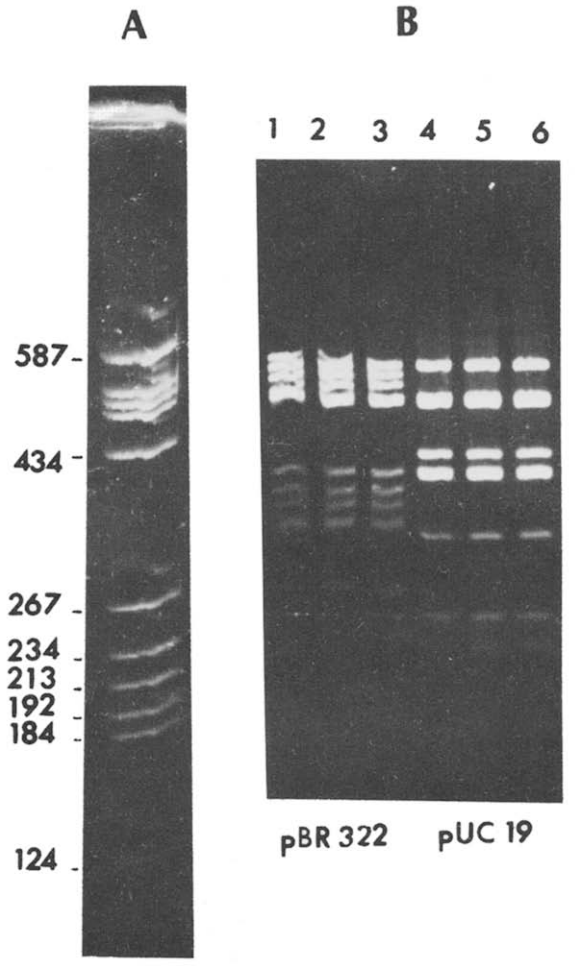

Fig.1. Digestion patterns of pBR322 DNA obtained in 6% polyacrylamide gel (A) and of pBR322 and pUC19 DNAs obtained in 2% agarose gel (B). (A) *Sua*I digest (lengths of fragments in base pairs indicated on the left). (B) Lanes: (1,4) *Bsp*RI digest, (2,5) *Sua*I digest, (3,6) *Bsp*RI-*Sua*I double digest.

The tetranucleotide GGCC can be cleaved by *Sua*I either symmetrically or nonsymmetrically, thus producing termini with a single-stranded end. To distinguish between these 2 possibilities an attempt was made to ligate blunt-ended *Hin*cII-digested pUC19 DNA with *Sua*I-digested DNA fragment. The latter was one of the DNA fragments produced by *Sua*I digestion of DNA from clone Dm I containing *D. melanogaster* rRNA genes [13]. After ligation and transformation, recombinant DNAs were double digested by *Hin*dIII and *Eco*RI. The fragments produced had lengths identical to those of the original fragments (not shown). The successful ligation indicates that *Sua*I produces blunt-ended fragments, thus cleaving the recognition sequence in the centre (GG↓CC).

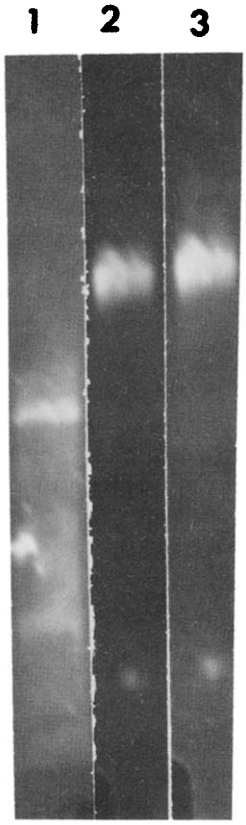

Fig.2. Digests of *S. acidocaldarius* DNA by *Bsp*RI (lane 1) and *Sua*I (lane 2); lane 3, undigested *S. acidocaldarius* DNA. The digestion patterns were obtained in 1% agarose gel.

On the basis of the data presented we conclude that *Sua*I is an isoschizomer of *Bsp*RI.

*Sua*I requires Mg^{2+}, the optimal concentration of $MgCl_2$ being 5 mM. KCl at concentrations above 100 mM significantly inhibits enzyme activity, but 10–30 mM KCl is necessary for maximal activity. The pH optimum is in the range 7.5–8.0.

*Sua*I is most active at 60–70°C. At 40°C the activity is about 5-times lower. The enzyme is highly thermostable. Preincubation of the enzyme for 30 min at 80°C in the assay mixture in the absence of DNA resulted in 50% loss of activity.

*Sua*I is the second restriction enzyme isolated from archaebacteria, the first having been isolated from *T. acidophilum* [10]. The occurrence of (at least) one more enzyme has been detected in strains of *Halobacterium* [9]. Here the enzyme is a component of the restriction-modification system. The same might be true of *Sua*I. This suggestion is based on the fact that *S. acidocaldarius* DNA is not cleaved by *Sua*I, while this DNA is effectively digested by *Bsp*RI which does not discriminate methylated cytosine (fig.2). It must also be noted that our preliminary data point to the existence of 5-methylcytosine and some minor bases, as yet unidentified, in *S. acidocaldarius* DNA.

The occurrence of a restriction enzyme in *S. acidocaldarius* was first detected by P. McWilliam (unpublished).

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