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Recognition and cleavage of double-stranded DNA by yeast VMA1-derived endonuclease

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ABSTRACT

DNA endonuclease derived from the yeast VMA1-gene product recognizes and cleaves 31 base-pairs of double-stranded DNA (dsDNA). Mixtures of the endonuclease (VDE) with a full DNA substrate consisting of 34 base-pairs, with nicked substrates each having a nick in either DNA chain, and with cleaved substrates each having a cleaved-off chain are prepared. Molecular weights (MWs) of eluted peaks from gel filtration columns were estimated from elution profiles in the presence of Mg^{2+} ions. Each mixture exhibited an elute peak at about 63k MW, larger than the MW of VDE unbound to dsDNA. This indicates that VDE and dsDNA substrates form stable complexes. The mixture of VDE either with the full substrate or with the nicked substrate having a nick in the anti-sense chain eluted an additional 25k-MW peak, which presumably corresponds to a cleaved product. The complex of VDE with the full substrate was eluted at 62k-MW location in the absence of Mg^{2+} ions and yielded a single crystal. Stable complexes of VDE either with the dsDNA substrates or with the cleaved products are obtainable.

INTRODUCTION

The yeast *Saccharomices cerevisiae* gene encoding vacuolar membrane ATPase subunit 1 (VMA1) produces a 119 kDa polypeptide which autocatalytically excises out the 50 kDa DNA endonuclease (VDE) and religates its both ends into mature 69 kDa VMA1.[1,2] During meiosis, VDE cleaves the specific segment in the VMA1 gene that lacks the intervening VDE coding region.[3] The gene segment encoding VDE is then inserted into the cleaved location. Through this gene homing process, VDE propagates itself efficiently into yeast genes.

The segment recognized by VDE consists of asymmetrical 31-base double-stranded DNA (dsDNA)[4] and is referred into 2 regions; the minimal binding region of 17 base pairs and the cleavage region of 14 base pairs.[4] The cleavage at the latter region produces a 3'-hydroxyl overhangs of 4 bases. The comparison with the crystal structures of the endonucleases bearing the same active-site motifs suggested that VDE has 2 active sites facing to respective strands of the dsDNA.[5] The results obtained from the site-directed mutagenesis studies on putative active-site residues indicated that neither strands are cleaved and that the dsDNA with a nick only at one of the strands is not obtainable[6], suggesting that cleavage reactions at the sense and anti-sense strands proceeds cooperatively. One hypothesis states that the dsDNA alignment on the 2 active-sites are interlinked by a single Mg^{2+} ion[7] which is essential for cleavage reaction.[3] Another hypothesis states that one of the active sites facilitates the cleavage of the strand concertedly.[5]

In order to reveal the mechanism of the recognition and cleavage by VDE, analyses of crystal structures of complexes of VDE with dsDNA substrates are essential. Here, we have examined the VDE recognition of 5 different dsDNA substrates using gel filtration chromatography so as to obtain stable complexes.

MATERIALS AND METHODS

VDE was overexpressed in *E.coli* strain BLR(DE3)pLysS.[8] The soluble fraction of the cell lysate was subjected to ammonium sulfate precipitation. Precipitated proteins were purified on cation-exchange, anion-exchange, and gel filtration columns to homogeneity on SDS-PAGE.

DNAs purchased from Greiner Japan were annealed so as to form dsDNA substrates (Fig.1) by heating 95°C for 10 min and then being allowed to cool down to a room temperature. Annealed dsDNAs were purified either on a Superdex75 gel filtration column (Pharmacia) or on a HA-1000 hydroxyapatite column (Tosoh). The purified VDE and dsDNA solutions were kept free of Mg^{2+} ions.

Each dsDNA was mixed with VDE in a cleavage buffer containing 2.5 mM $MgCl_2$, 100 mM KCl, 2 mM 2-mercaptoethanol, 100 mM TrisHCl (pH 8.5) and kept at a room temperature overnight for the reaction. In addition, the full substrate consisiting of 34 base-pairs as well as the anti-sense-nicked substrate with a nick in the anti-sense chain was mixed with VDE in the buffer without Mg^{2+} ions.

Estimation of molecular weight (MW) was performed by analyzing elution profiles of the mixtures on the gel filtration column. The eluted peaks were pooled, and existence of VDE and dsDNA was assayed with Bradford method and with detection of fluorenscence of 4',6-diamidino-2-phenylindole, respectively.

Crystallization of the complex of VDE with the full substrate

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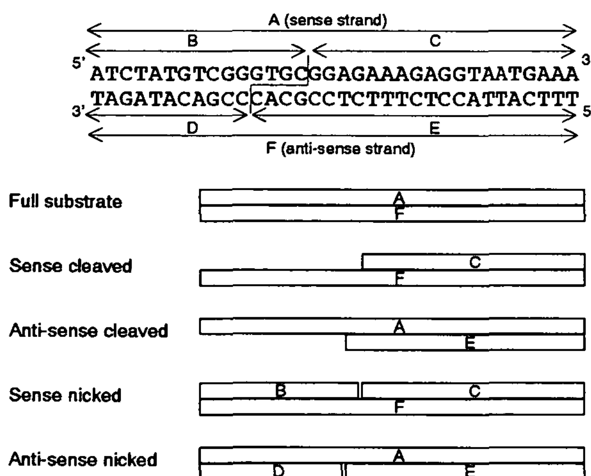

Fig 1. Constructions of dsDNAs used in the experiment.

was attempted by use of the hanging-drop vapor-diffusion method.

RESULTS AND DISCUSSION

Elution profiles and estimated MWs of the eluted peaks are shown in Fig 2. From all the mixtures consisting of VDE and dsDNA, common peaks located at 63k-MW were eluted. This MW value is larger than that of VDE or 34-base dsDNA when it is solely eluted. Through the assays of protein and dsDNA constituents, formations of stable complexes of VDE with resultant substrates are confirmed. These complex formations clearly demonstrate that the minimal binding region mainly consisting of C and E chains (Fig.1) is enough for recognition by VDE.[4] The peak obtained from the mixture with the full substrate dsDNA was eluted from the hydroxyapatite column at a different location in the elution profile, as compared with that of the sole VDE.

The mixture of VDE either with the full substrate or with the anti-sense-nicked substrate yielded an additional peak at 25k-MW location. In the absence of Mg^{2+} ion, this 25k-MW peak is not eluted from the mixture. The peak, therefore, corresponds to the shorter cleaved product consisting of B and D chains, which is eluted at a 21k-MW location when solely applied on the column, although the longer cleaved product consisting of D and E chains is eluted at a 37k-MW location when solely applied. The longer cleaved product seems to remain complexed with VDE, since elution peaks of the longer cleaved product was not obtainable from the prepared mixtures. This observation is consistent with the finding through the gel-shift experiments.[9]

From the denaturing gel electrophoresis experiment, it is reported that VDE readily cleaves both the sense- and anti-sense-nicked substrates. The VDE mixture with the sense-nicked substrate was eluted as a single peak, as in the mixtures with the sense- and anti-sense-cleaved substrates. This is possibly due to the slow or incomplete cleavage in the present experiment at a room temperature, which is examined under non-denaturing conditions.

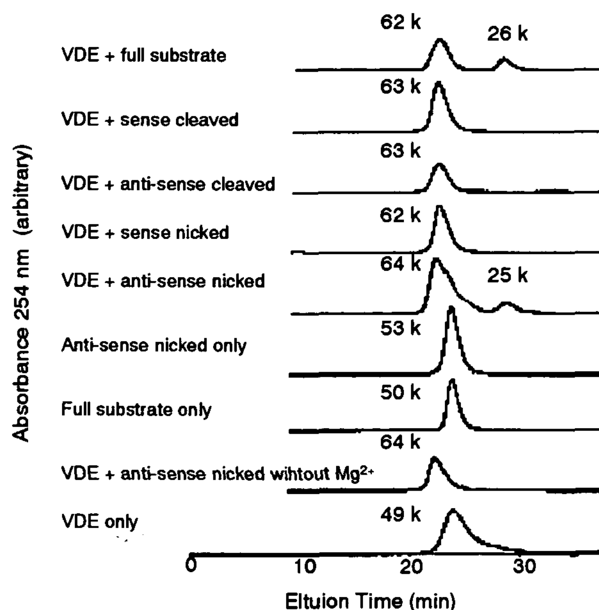

Fig. 2 Elution patterns of reaction mixtures of VDE and sDNAs from the Superdex75 gel filtration column. The number indicates the molecular weight of each peak, which is estimated from the elution time.

The complex either with the full substrate or with the anti-sense-nicked substrate is also obtainable in absence of Mg^{2+} ions, as shown in Fig. 2. The complexes of VDE with partially cleaved substrates as well as with the longer cleaved product are obtainable even in the presence of Mg^{2+} ion. as well as with the longer cleaved product.

The crystallization trial from the eluted complex have been attempted. Single crystals with the largest dimension of 0.1 mm were obtained by mixing the complex solution of VDE and the full substrate with a PEG 4000 solution in the absense of Mg^{2+} ions.

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