RESTRICTION ENDONUCLEASE *Bam*H1 INTERACTION WITH A SYNTHETIC DUPLEX CONTAINING HALF-SIZE RECOGNITION SEQUENCES

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Received 6 July 1981

1. Introduction

Restriction endonuclease *Bam*H1 is a site-specific deoxyribonuclease which cleaves the phosphodiester bonds between the guanine residues within the duplex DNA sequence $5'\text{-}\overset{GGATCC}{CCTAGG}$ [1]. It is well known that restriction endonucleases recognise and cleave relatively short synthetic duplexes containing appropriate recognition sequence, therefore oligodeoxyribonucleotides called 'linkers' are widely used for genetic manipulations [2,3]. On the other hand, the synthetic oligonucleotides with defined sequences are useful tools to study mechanisms of interaction of the enzymes with DNA.

The minimal lengths of substrate duplexes have been investigated for a number of restrictases [4] and kinetic comparisons have been made between synthetic and natural substrates [5] in the hydrolysis reactions.

We have investigated *Bam*H1 restriction patterns of the self-complementary oligodeoxyribonucleotides. One of them, d(5′-CCAGGATCCTGG), contains a full-length recognition site. The second one, d(5′-TCCAGATCTGGA), contains a part of the recognition site, 5′-GATC flanked with the half-size recognition sequences 5′-TCC from its 5′-side and 5′-GGA from its 3′-side. Cleavage of the formed oligomer with *Bam*H1 shows an usual type of restriction pattern [1]. In contrast, the second one shows a restriction pattern which could be explained as being cleaved within the recognition sequence d(5′-GGA . . . TCC) formed by the ends of two substrate molecules. At the same time cleavage within the sequence d(5′-GATC) does not take place. These results support a symmetric binding model of a restriction nuclease with its recognition site [6] via interaction with one half of the recognition sequence.

2. Materials and methods

Endonuclease *Bam*H1 from *Bacillus amyloliquefaciens* was prepared as in [7]. Oligodeoxyribonucleotides were synthesised by the phosphotriester method [8]. Oligonucleotides were ^{32}P-labeled at the 5′-termini with polynucleotide kinase [9]. 5′-^{32}P-Labeled oligonucleotides (15–30 pmol/μl) were digested with *Bam*H1 (1–2 units/μl) at 0.5 for 4 h in 6 mM Tris–HCl (pH 7.4), 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol and 50 mM NaCl. Reaction was stopped by the addition of EDTA and urea up to 6 mM and 7 M, respectively. After digestion the samples were separated in 23% polyacrylamide gels as in [1]. Sequence analyses of the oligodeoxyribonucleotides and *Bam*H1 digestion products were performed as in [11].

3. Results and discussion

*Bam*H1 digestion of the oligonucleotide, d(5′-^{32}P-CCAGGATCCTGG) containing a full-length radioactive recognition site gives a product with an electrophoretic mobility equal to that of the expected digestion product, the tetranucleotide d(5′-^{32}P-CCAG) (not shown). Cleavage of the oligonucleotide, d(5′-^{32}P-TCCAGATCTGGA) gives three 5′-^{32}P-labeled products of a smaller length than a starting one (fig.1) and proceeds ~10-times slower, than the cleavage of the normal site. The length of the digestion products were determined by gel electrophoresis

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.50

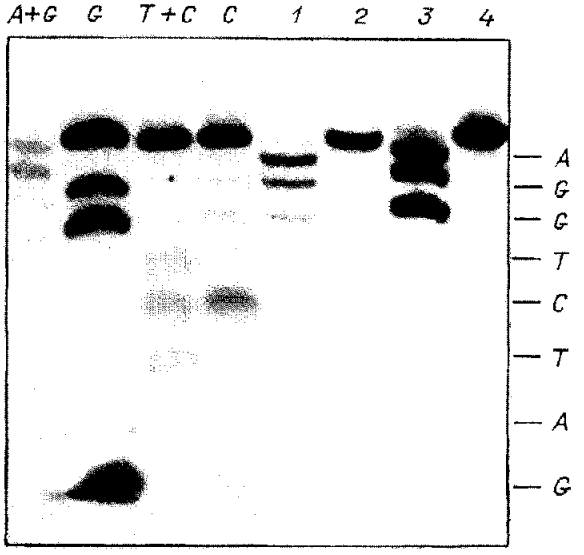

Fig.1. Autoradiography of the gel slab containing oligonucleotide TCCAGATCTGGA fragments after its cleavage by Maxam–Gilbert method [11] and by *Bam*H1 restriction endonuclease: (1,3) *Bam*H1-hydrolysate of the oligonucleotide; (2,4) starting oligonucleotide; (1,2) samples were precipitated by ethanol and dissolved in loading buffer before electrophoresis; (3,4) samples were loaded on the gel without ethanol precipitation.

using length markers obtained from the starting oligomer by the Maxam–Gilbert chemical method. It is seen that the digestion products are oligonucleotides containing 11, 10, and 9 residues with 3′-terminal bases, G, G and T, respectively. Relative amounts of these products are 20%, 30% and 50% as revealed by counting the radioactivity of bands after gel electrophoresis. The *Bam*H1 restriction of the oligonucleotide, d(5′-TCCAGATCTGGA), seems to be surprising for two reasons:

(i) There are 3 digestion products in spite of the general idea about high specificity of the class II restriction endonucleases action [12].

(ii) None of the cleavage sites within the oligonucleotide sequence are in agreement with the known *Bam*H1 restriction pattern [1], i.e., the restriction does not occur via recognition of the hexanucleotide sequence and its subsequent cleavage at the left side from the symmetry centre [1].

We have confirmed that the enzyme preparation used in this study shows usual properties. Restriction of self-complementary oligonucleotide d(5′-CCAGGATCCTGG), containing the recognition site inside the chain, gives expected products. It has been shown also that the enzyme does not cut the oligonucleotide d(ACGGAATTCCAT) lacking the recognition sequence.

These results could be explained by the supposition that in this case the enzyme binds with the composite recognition site formed by the ends of two different duplex molecules. One can support this conclusion by taking into account the rigidity of the oligodeoxyribonucleotide duplexes.

T-4 DNA-ligase is known to link DNA duplexes with 'blunt' ends [12]. Endonuclease *Bam*H1 molecule seems to have a similar mechanism of interaction with the duplexes. The enzyme probably joins the DNA molecules together creating a complex where two duplexes form one double helix with the same parameters as in covalently linked polynucleotide. Endonuclease *Bam*H1 molecule consists of two subunits, both apparently containing independent binding and hydrolytic centers [13,14]. The binding center of each subunit seems to interact with the recognition site d(5′-TCC) of the oligonucleotide. Two bound oligonucleotide molecules form a double helix similar to the non-cleaved substrate recognition site d(5′-GGA . . . TCC), then the hydrolytic center acts. The hydrolytic step is less specific than the binding one since the phosphodiester bond cleavage occurs both in canonical and in neighbouring positions. This indefiniteness of the cleavage position could be due to the relative mobility of the two duplex molecules which are not connected one to another by covalent bonds. The ends of the oligonucleotides are kept together due to interaction with the enzyme molecule which must be flexible as all the proteins are, hence the intersubunit region could be slightly deformed.

This suggestion is in a good agreement with the symmetric binding model proposed in [6]. According to this model a subunit protein has a second-order rotation symmetry axis so that each subunit interacts with one half of the recognition-site nucleotides.

It is of sufficient interest to check the substrate properties of synthetic oligonucleotides which are able to form the *Bam*H1 recognition site cut in other positions within the hexanucleotide sequence. It seems to be probable that other restriction endonucleases can interact with the substrates containing similar 'cleaved' recognition sites. Both possibilities are now under investigation in our laboratory.

Acknowledgements

The authors would like to acknowledge Dr V. A. Karginov, for carrying out the sequence analysis on the oligodeoxyribonucleotides and Dr G. F. Mishenina for providing the *Eco*RI linker dodecanucleotide.

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