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Inserting foreign peptides into the major coat protein of bacteriophage M13

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Foreign DNA fragments were inserted into filamentous phage gene VIII to create hybrid B-proteins with foreign sequences in the amino terminus. The hybrid proteins are incorporated into the virions which retain viability and infectivity. Virions with hybrid B-proteins have the same contour length and the same number of B-protein molecules as virions with natural B-proteins. It was shown that for one of hybrid B-proteins the position of the processing site had changed.

B-protein; M13 bacteriophage; Protein engineering

1. INTRODUCTION

Gene VIII of the filamentous phage M13 encodes a major coat protein, B-protein, which covers the viral DNA. The idea to produce recombinant immunogens on the basis of the filamentous bacteriophage M13 with peptide epotodes inserted into the major coat protein seems rather promising. However, even individual amino acid substitutions disturb the processing of B-protein and the phage assembly [1,2]. Hence, it is very important to study the viability and structure of recombinant phages with hybrid B-protein.

In this paper we examined the effects of model peptide inserts into B-protein on viability, infectivity and structure of the phages.

2. MATERIALS AND METHODS

2.1. *Construction of mutant bacteriophage strains*

Mutagenesis of phages, insertions of oligonucleotide duplexes into the gene VIII and nucleotide sequencing were performed as described previously [9].

2.2. *Identification of the B-protein amino-terminal amino acid sequence*

The amino-terminal sequences were identified by the methods of Shively [5] and Elzinga [6]. Identification of PTH amino acids was carried out using HPLC with the RP-8 sorbent (2.3 mm × 44 mm).

2.3. *Electron microscopy*

Electron micrographs of M13 virions were obtained using a 'JEM-100C' electron microscope after the spreading of the virions on Formvar support in 10 mM citrate buffer, pH 7.0 and negative contrasting in 2% uranium acetate. Virion contour lengths were measured by the semi-automatic analyzer MOP/AM-03 (Reichert-Jung).

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3. RESULTS AND DISCUSSION

The carboxyl terminus of the B-protein is buried in the capsid, while the amino terminus is exposed on the surface of the virion [7]. Therefore, the amino-terminal region seems appropriate for peptide insertion because in this case the inserted peptides are exposed on the virion surface. The natural amino-terminus variability of the major coat proteins of the related phages Ff(M13, f1, fd), IF1, IKe [8] confirms the acceptability of amino terminus modification.

In a recent publication we reported the production of a M13B recombinant phage (Fig. 1) with the gene of ampicillin resistance [9]. This phage can be maintained in bacteria as a plasmid if insertions of the foreign peptide into the major coat protein result in loss of phage infectivity. The phages with chimeric variants of the B-protein were constructed on the basis of phage M13B1 (Fig. 1). The synthetic DNA fragment encoding model peptides was cloned into the RF of phage M13B1. The choice of the amino acid sequence of the model peptide was random in character. However, the general principles were the following: to retain the structure and function of the protein carrier, the physicochemical properties of the inserted peptide must be similar to the properties of the neighbouring locus. We chose the nucleotide sequence with the following features: in direct orientation it encodes the peptide with the amino acids D, Q, A, S and G that often occur in the amino-terminal regions of the coat proteins of the

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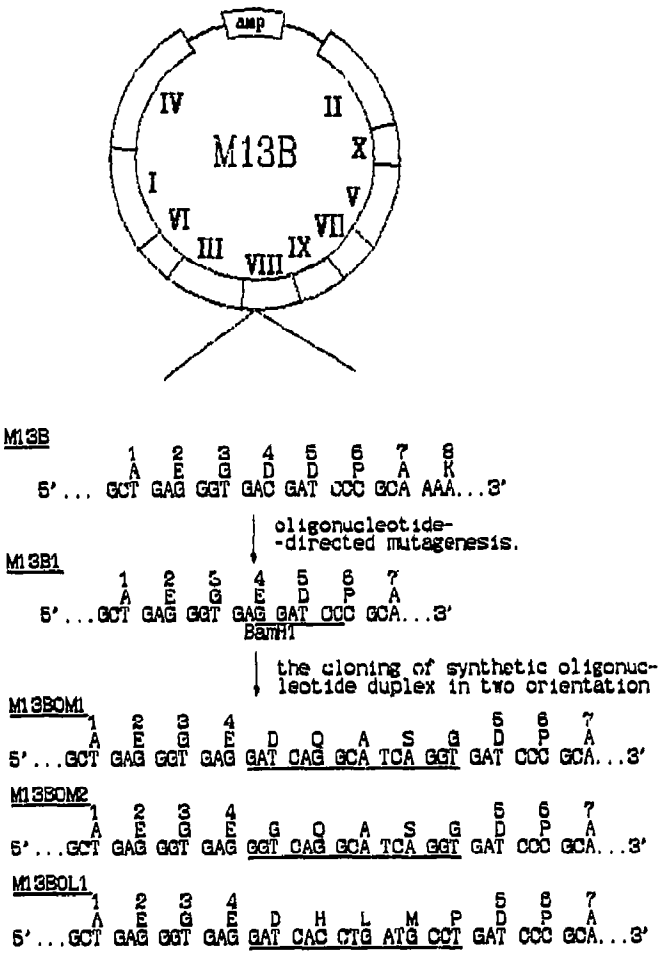

Fig. 1. The scheme of hybrid phages construction. Phage M13B was produced by inserting a fragment of plasmid pBR327 containing the β-lactamase gene into the polylinker region of phage M13mp10. Phages with chimera variants of the B-protein were obtained in two stages. At the first stage, using oligonucleotide-directed mutagenesis, we introduced a unique recognition site of *Bam*HI restrictase into gene VIII encoding the B-protein. At the second stage, we cloned a synthetic DNA fragment 5'-GATCAGGCATCAGGT
TCCGTAGTCCACTGA-5'
in two orientations.

related phages (M13, IF1, IKe) [10] and the surface regions of other proteins [11]. The reverse DNA chain encodes the amino acids L, M and H. The physicochemical properties of L, M and H residues (size, hydrophobicity, charge) differ from those of the amino-terminal region of the coat protein, and insertion of these residues can disturb any stage of the phage assembly.

The phages with the inserts in direct and reverse orientation were designated M13BOM1 and M13BOL1, respectively. Their biological titers as compared to M13B decreased by one order, whereas there was only a 2-fold decrease in the amounts of purified single-

M13B	A	E	G	D	D	P	A	K	A...	
M13B1	A	E	G	E	D	P	A	K	A...	
M13BOM1	A	E	G	E	D	Q	A	S	G	D P...
M13BOM2	A	E	G	E	G	Q	A	S	G	D P...
M13BOL1					D	H	L	M	P	D P A...

Fig. 2. Amino-terminal sequences of the mature B-proteins for the recombinant phages, as determined by Edman degradation.

stranded DNA. These data demonstrate that insertion of the foreign peptides in the B-protein slightly affected the yield of the phage particles, but substantially affected the phage infectivity. Two subclones (M13BOM1 and M13BOM2) were selected by DNA sequencing. M13BOM2 had the transition (A→G) which caused the substitution of the aspartic acid residue by glycine at the (+)5 position of the chimeric B-protein (Fig. 1).

The B-protein of phage M13 is known to be synthesized in the cells as a precursor. The precursor has a signal peptide which is cleaved off during the assembly of the phage on the bacterial membrane. The influence of the foreign amino acid residues inserted into B-protein on the specificity of processing was a matter of interest. Therefore we determined the amino-terminal sequences of mature B-proteins of all phages. The sequences are given in Fig. 2. One can see that the natural cleavage site in the M13BOM1 and M13BOM2 phages remains unchanged. But the maturation of the hybrid M13BOL1 B-protein differs from maturation in other phages. In fact, insertion of the DNA sequence of reverse orientation disturbed the correct processing of the coat protein, whereas insertion of the sequence of direct orientation saved the cleavage point of the signal sequence. Therefore, the sequence remote from the recognition site of the *E. coli* signal peptidase affects the processing specificity of B-protein.

We measured the contour lengths of the filamentous virions for all the strains obtained. All virions have the same contour length within the accuracy of 3%. Using the method of spectrophotometric determination of the protein concentration in 0.1 N NaOH [12] we found that the DNA/protein ratio in all strains was also identical within a 1.5% accuracy. These data showed that the N-terminal insertion of the foreign peptide did not affect the structure-forming properties of B-protein.

Thus the present paper describes the possibility of generating the viable phages with foreign peptides inserted into the amino-terminal region of a major coat protein. Besides, we obtained some data concerning the influence of the amino acid sequence of the inserted peptide on the position of the processing points.

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