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Insertion of short hepatitis virus A amino acid sequences into poliovirus antigenic determinants results in viable progeny

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In an infectious poliovirus cDNA construct, the determinant encoding antigenic epitope N-Ag1 (in a loop located between two β-strands in polypeptide VP1) was altered by site-directed mutagenesis, to be partially similar with the determinants for presumptive epitopes in polypeptides VP1 or VP3 of hepatitis A virus (HAV). The modified constructs proved to be infectious. However, another construct, in which the same locus encoded a 'nonsense' and a relatively hydrophobic amino acid sequence, exhibited no infectivity. These data showed the feasibility of the insertion of foreign sequences in a specific antigenically active locus of the poliovirus icosahedron, and suggest some limitations with respect to the sequences to be 'transplanted'.

Poliovirus; Hepatits A virus; Chimeric virus; cDNA, infectious

1. INTRODUCTION

Hepatitis A virus (HAV) belongs to the Picornaviridae family, which also includes polioviruses and numerous other small naked (i.e. lacking a lipoprotein membrane) icosahedral animal viruses, with single-stranded RNA genome of positive polarity. Since hepatitis A is a major medical problem and since no efficient anti-HAV vaccine is yet available, several approaches to design such vaccines are currently being explored. One such approach may consist of introducing HAV antigenic determinants into structurally related attenuated poliovirus strains (the Sabin strains), which have an excellent record as efficient and safe oral vaccines. The feasibility of such an approach could be demonstrated by the successful construction of chimeric polioviruses exhibiting antigenic properties of two different serotypes [1–3]. The site of choice for manipulating with antigenic determinants in the poliovirus particle, is the amino acid sequence from positions 93–103 corresponding to a loop between two β-strands in the capsid polypeptide VP1 (antigenic site N-Ag1). Although this site appears to tolerate different kinds of substitutions and insertions and the newly introduced amino acid sequence could be antigenically active [1–5], the extent to which the site could be modified without an adverse effect on the self-assembly and stability of the viral icosahedron remains unknown. Neither is the exact structure of the antigenic site of HAV known.

On the basis of these considerations, we began construction of a variety of derivatives of the Sabin poliovirus strains with antigenic determinants made more or less similar with presumptive antigenic determinants of HAV. Two such derivatives are described here. In addition, a cDNA construct, in which the N-Ag1-coding region was replaced by a biologically irrelevant sequence of the same length, turned out to be non-infectious, suggesting that some limitations on the structure to be 'transplanted' should exist.

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2. MATERIALS AND METHODS

A full-length cDNA copy of the Sabin type 1 genome was prepared by using standard procedures [6]. The first strand was synthesized by reverse transcription using oligo(dT)$_{12-18}$ as primers as well as oligodeoxyribonucleotides corresponding to positions 5031–5050 and 2421–2440 of the viral genome. The second strand was synthesized with the help of *E. coli* DNA polymerase I in the presence of RNase H. *Eco*RI linkers were ligated to virus-specific double-stranded DNA fragments followed by the insertion of these fragments into the *Eco*RI site of pBR322. The search for clones with specific inserts was accomplished with the help of ^{32}P-labeled oligonucleotides. Since the 5′-terminal sequence was missing from the isolated clones, the appropriate segment was chemically synthesized (positions 1–33 and 34–70 of the (+) and 1–66 of the (−) strand, respectively); the synthesized segment had an *Eco*RI site adjacent to its 5′ terminus and a *Kpn* site at the 3′ terminus.

For the mutagenesis, a *Pst*I fragment of the Sabin type 1-specific cDNA, encompassing nucleotides 2245–3419, was cloned into M13mp10. The U-containing DNA template was produced by grow-

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ing the phage in *E. coli* RZ1032 cells. The mutagenesis was accomplished with the help of synthetic oligodeoxyribonucleotides as described [7]. Mutant clones were selected by the hybridization with labeled probes.

The infectivity of plasmids or their transcripts was assayed using 7-day-old primary cultures of monkey kidney cells. After removal of the media, the cells were incubated for 7 min at room temperature with Earle's saline containing 100 μg/ml of DEAE-dextran. The saline was removed and an appropriate dilution of the RNA (or DNA) in 0.1 ml of 0.87% NaCl, 0.01 M phosphate, pH 7.1, containing 500 μg/ml of DEAE-dextran was added. The bottles were incubated for 15 min at room temperature with permanent gentle shaking. The agar overlay was finally added.

3. RESULTS AND DISCUSSION

As a first step to our goal, a full length cDNA copy of the Sabin type 1 genome was prepared. The plasmid (pOLS1) was combined from 5 fragments as outlined in fig.1. The virus-specific sequence was recloned into plasmid pGEM3 under the control of an SP6 promoter; the resulting plasmid was named pPVS. Neither pPVS, nor its RNA transcripts were found to be infectious when assayed for plaque formation in monkey kidney cells. The reason for non-infectivity of pPVS was not further investigated, but a new construct was made by combining the sequences of pPVS encoding the appropriate segment of the Sabin type 1 polypeptide VP1 as well as the SP6 promoter (and adjacent sequences) with the remainder virus-specific sequences from an infectious plasmid pSVp harboring cDNA of a Sabin type 1-related strain Mahoney (pSVp was kindly provided by D. Baltimore). The way by which the new plasmid, pPVS-3M, was constructed, is shown in fig.1. Both pPVS-3M and its transcript proved to be infectious.

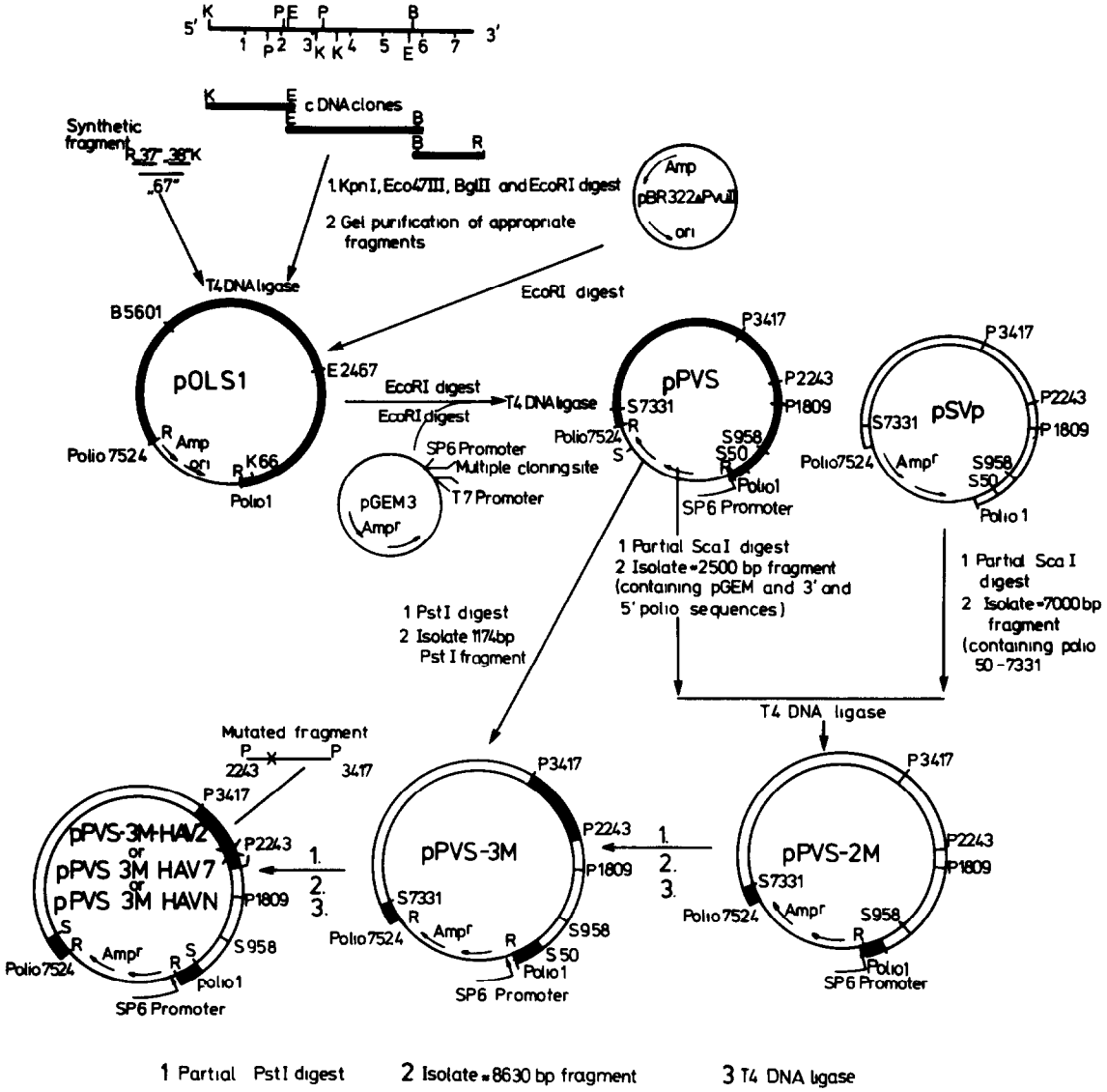

Fig.1. Strategies for plasmid construction. Sites for restriction endonucleases *Kpn*I (K), *Eco*47III (E), *Bgl*II (B), *Eco*RI (R), *Sca*I (S) and *Pst*I (P) are shown. pBR322Δ*Pvu*II was derived from pBR322 by the deletion of a short *Pvu*II-*Eco*RI segment, addition of an *Eco*RI linker to the blunt end and ligation.

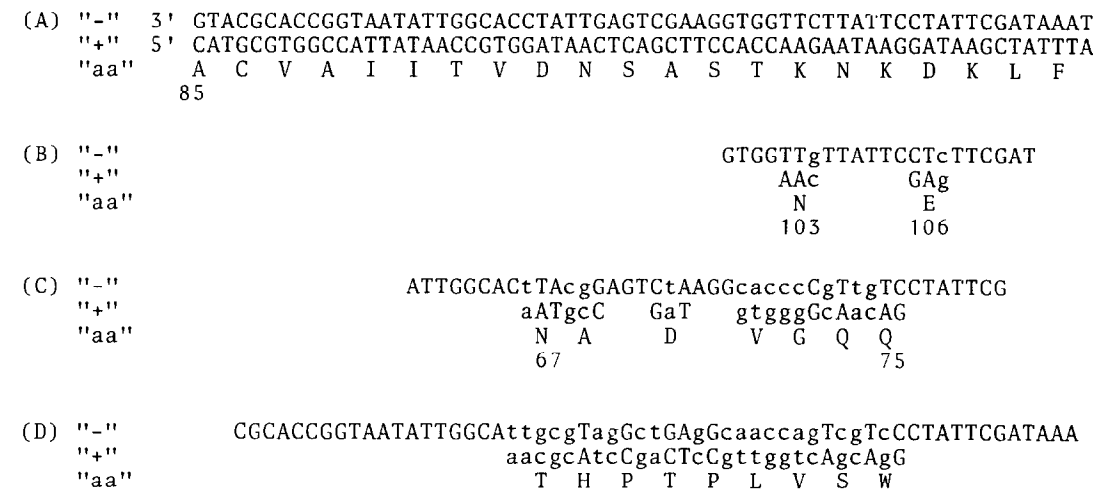

Fig.2. Oligonucleotides used for mutagenesis, and amino acid changes produced. The primary structure of a segment of the Sabin 1 gene for VP1 is shown in (A). Derivation of plasmids pPVS-3M-HAV2, pPVS-3M-HAV7 and pPVS-3M-HAVN are given in (B), (C) and (D), respectively. The primary structures of the appropriate segment of Sabin-1 (−)DNA strand (A) and of the oligonucleotides used for mutagenesis (B–D) are shown in lines '−'. The primary structures of the (+)DNA strands and of proteins are shown in lines '+' and 'aa', respectively. The mutated nucleotides are shown in lower case letters. Only the changed codons and amino acids are indicated in the mutated plasmids. The amino acid numbering in (B) and (C) is given for HAV VP1 and VP3, respectively.

Then, we attempted to introduce into the N-Ag1 coding segment of pPVS-3M some alterations which should make this antigenic site partially similar to presumptive antigenically active sites in the HAV VP1 or VP3 [6,8]. Plasmid pPVS-3M-HAV2 was constructed in such a way as to encode an HAV VP1-specific amino acid sequence (positions 103–106) instead of the poliovirus-specific sequence in positions 99–102. To do this, only 2 nucleotide changes should be made, with 2 codons being altered (fig.2). The second plasmid, pPVS-3M-HAV7, could code for amino acid positions 67–75 of HAV VP3 (instead of poliovirus VP1 positions 93–101). This required 7 codons to be mutated (fig.2). The mutagenesis was carried out first in a virus-specific DNA segment cloned in M13mp10 and then full-length plasmids were restored as shown in fig.1.

The infectivity assays with plasmids pPVS-3M-HAV2 (having substitutions resembling the appropriate site in HAV VP1) and pPVS-3M-HAV7 (substitutions resembling the site in HAV VP3) showed both of them as being infectious. Thus, our results support the feasibility of alterations of the N-Ag1 site in poliovirus VP1 as a tool for creating novel virus variants. The antigenic and serological properties of pPVS-3M-HAV2 and pPVS-3M-HAV7 are being investigated.

Although the genomic region we are manipulating could be changed in various ways, some alterations appeared to be prohibited. Thus, plasmid pPVS-3M-HAVN, which encoded a biologically irrelevant peptide in positions 99–107 (due to a local frame shift), exhibited no infectivity. It could be supposed that the lethal phenotype of this construct was due to relative hydrophobicity of the inserted sequence. Although it is yet to be rigorously proved that non-viability of pPVS-3M-HAVN could be explained solely by the intentionally introduced genomic alterations, possible limitations for the design of artificial viable viruses should be taken into account.

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