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RECOMBINANT PROTEINS DERIVED FROM IMMUNODOMINANT REGIONS OF THE *GAG* AND *ENV* GENES OF HIV-1 AND THEIR POTENTIAL FOR USE IN SEROLOGIC DIAGNOSIS

J. KOVÁČ

Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Czecho-Slovakia

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Summary. - Based on the published sequences of human immunodeficiency virus (HIV-1) isolates highly conserved regions of the *gag* and *env* genes containing immunodominant epitopes were selected and expressed in *E. coli*. The expression vectors pKK24 and pEX41 produced viral proteins recognized by sera obtained from HIV-1 seropositive individuals. A testing system was designed to determine the practical value of bacterially synthesized proteins of HIV-1 *gag* and *env* genes for serodiagnosis of HIV-1 infection.

Key words: AIDS; HIV-1; immunodominant epitopes; recombinant proteins; immunoblot

It is well known that the primary aetiological agent of acquired immunodeficiency syndrome (AIDS) is a retrovirus referred to as human immunodeficiency virus (Barré-Sinoussi *et al.*, 1983; Gallo *et al.*, 1984). At present, antibody tests combining a screening assay (ELISA) and confirmatory testing are the most widely used techniques for establishing the presence of HIV infection. In practice, immunoblots (Western) of viral proteins obtained from infectious virus and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (PAGE) are most frequently used to confirm a positive ELISA result. However, the laboratory preparation of antigens for these tests is rather expansive, requires handling of live virus or virus-infected cells. The sensitivity of testing of the important anti-gp41 antibodies is relatively low - the glycosylated gp41 produces a diffuse band in SDS-PAGE and is present only in low concentrations on nitrocellulose strips after blotting (Brede, 1987).

A possible alternative is the use of suitable recombinant peptides, derived from parts of the HIV genome, as diagnostic antigens. In addition to three basic genes: *gag, pol* and *env* the HIV-1 genomes contain several other genes whose products play an important role in the regulation of viral gene expresssion (Dayton *et al.*, 1986; Fisher *et al.*, 1986; Sodroski *et al.*, 1987). Individuals infected with HIV-1 produce antibodies especially to structural proteins such as viral envelope and core proteins (Sarngadharan *et al.*, 1984). The HIV-1

envelope precursor protein of 160 kD is processed into two glycoproteins. Gp120 is heavily glycosylated and is known to be responsible for adsorption and gp41, the transmembrane protein which anchors the gp120/gp41 complex to the viral envelope (Kowalski *et al.*, 1987). In selecting an appropriate segment of the envelope transmembrane protein for bacterial expression, we sought for a region which exhibits a relatively high conservation of amino acid sequences between the various isolates of the AIDS virus. Amino acid sequences of the envelope protein complexes derived from the nucleotide sequences of seven AIDS virus isolates were compared. By computer analysis the secondary structure of gp120 and gp41 was predicted and 11 potential antigenic sites, 9 of which were located in the exterior part of the envelope protein and 2 in the membrane bound portion, were identified. These contain amino acids 613 to 635 and 722 to 745 (Modrow *et al.*, 1987). It has been shown that segments of gp41 including the first epitope when produced as recombinant gene products in the bacteria are recognized by HIV-1 positive sera in enzyme-linked immunosorbent assays and Western blots (Cabradilla *et al.*, 1988; Chang *et al.*, 1985); preliminary studies with synthetic peptides representing this region give similar results (Modrow *et al.*, 1987).

Virtually all individuals infected with HIV-1 produce antibodies to this region which is also highly conserved among different HIV-1 isolates (Modrow *et al.*, 1987). This antigenic site is located outside the lipid bilayer and is accesible to antibody recognition and reactivity. We have selected two *env* gene fragments of HIV-1, both cording for this immunodominant epitope. The larger one covers 45 amino acids from the carboxy-terminus of the 120 kD envelope protein and 130 amino acids from the N-terminal part of the gp41. The smaller fragment codes for amino acids 547 to 735 of the *env* gene. The protein coded by this fragment is devoid of a hydrophobic stretch of amino acids situated in the N-terminal part of gp41, for it is known that hydrophobic sequences may have a deleterious effect on recombinant protein synthesis in bacteria (Windheuser *et al.*, 1988).

The *gag* gene codes for a primary translation product of 55 kD which is post-translationally processed to produce the internal core protein p24, and p15 and p17, which are present in lesser amounts (Marcus-Schura *et al.*, 1988). The availability of the HIV-1 core protein p24 is also of importance to identify a possible HIV infection by specific antibody detection. Comparison of the published HIV-1 sequences shows that the genetic information between *Hind* III sites of the *gag* open reading is also highly conserved and covers 80 % of the p24 coding region (Mous *et al.*, 1987). So we have isolated this gene segment and inserted it into an expression vector (Fig. 1). For construction of expression plasmids we have used the commercially available *E. coli* plasmid vectors pKK233-2 (Amann *et al.*, 1983) and pEX-1 (Stanley *et al.*, 1984).

The expression vector pKK233-2 contains the *trc* promoter - a hybrid promoter with the consensus 17 bp spacing between the -35 region of the tryp-

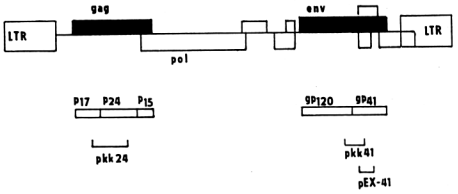

Fig. 1
Schematic representation of the genome structure of HIV-1 and the localization of DNA segments used to construct the pKK 41, pKK 24 and pEX-41 expression vector

tophan gene promoter and the -10 region of the *lac* UV5 promoter. Further it contains the *lac* Z ribosome binding site followed by an ATG initiation codon which is contained within an unique *Nco* I site. The vector enables expression of foreign proteins in an unfused fashion. In a *lac* I^Q host such as JM 105 the *trc* promoter is repressed but may be depressed at the appropriate time by the addition of isopropyl β-D-thiogalactoside (IPTG).

The 627 bp fragment of the *gag* gene and the 523 bp fragment of the *env* gene were inserted (Kováč *et al.,* in preparation) into the expression vector pKK233-2 creating recombinant plasmids pKK24 and pKK41 (Fig. 2). Upon transformation of *E. coli* cell strain JM 105, colonies were screened for expression of virus-specific proteins. Positive colonies were grown in bulk cultures at 37 ^{0}C, induced with IPTG and the proteins were analysed by SDS-PAGE and Western blot with a pool of HIV-1 positive sera. Two proteins, with apparent molecular weights of 25, 000 and 21, 000 reacted with anti-HIV-1 sera but not with normal sera. The 25 kD protein corresponds to the product of pKK24 and the 21 kD protein represents the *env* gene fragment synthesized by the pKK41 positive clones. Although the immunoreactivity study of the 21 kD *env* gene recombinant peptide provided us with promising results (Zachar *et al.,* 1990) the expresssion level of the pKK41 product did not give optimistic prospects for a satisfactory purification. Therefore, we decided to express the gp41 immunodominant epitope as a fusion protein in pEX-1.

This bacterial expression vector contains the PR promoter of bacteriophage lambda which controls expression of a *cro-lac* Z fusion gene. The 566 bp *Bsp* RI gene fragment of gp41 was ligated into the *SmaI* site of pEX-1. After transformation of *E. coli* pop 2136 containing the cI857 gene, colonies were screened for expression of HIV-1 proteins. Positive clones produced a 138 kD protein which consists of the HIV-1 *env* fragment fused to a 117 kD cro-β-gal fusion protein derived from pEX-1 (Fig. 3). These were grown in bulk cultures at 30 ^{0}C and induced to produce HIV-1 fusion protein by growth at 42 ^{0}C. Subsequently, bacteria were pelleted and suspended in 1/10 of the original volume of

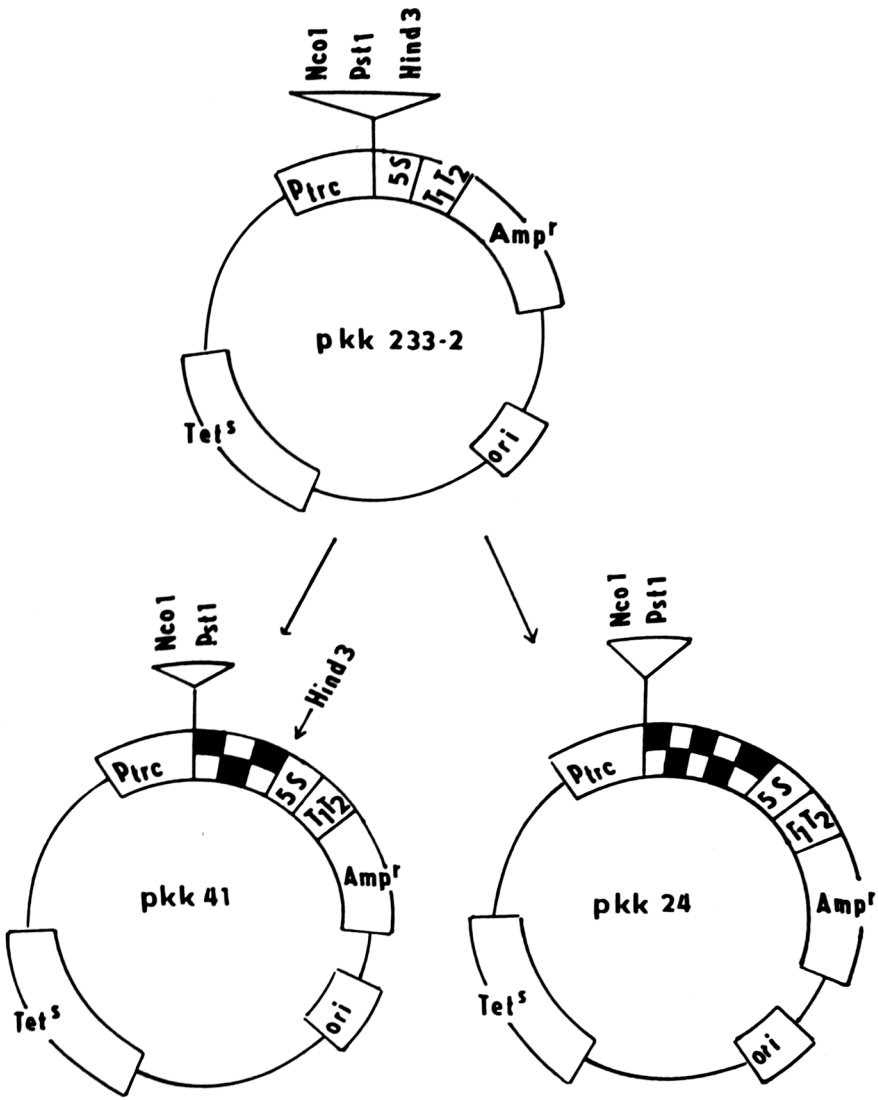

Fig. 2
Construction of recombinant plasmids pKK 41 and PKK 24 which express a portion of the *env* and *gag* genes, respectively. For construction of the expression plasmid pKK 41, the 1.429 kb *Bgl* II fragment (nucleotides 7179–8608) of the HIV-1 *env* gene was subcloned into pUC 18. After amplification a 523 bp *Pst* I-*Hind* III fragment was excised and ligated into pKK 233-2

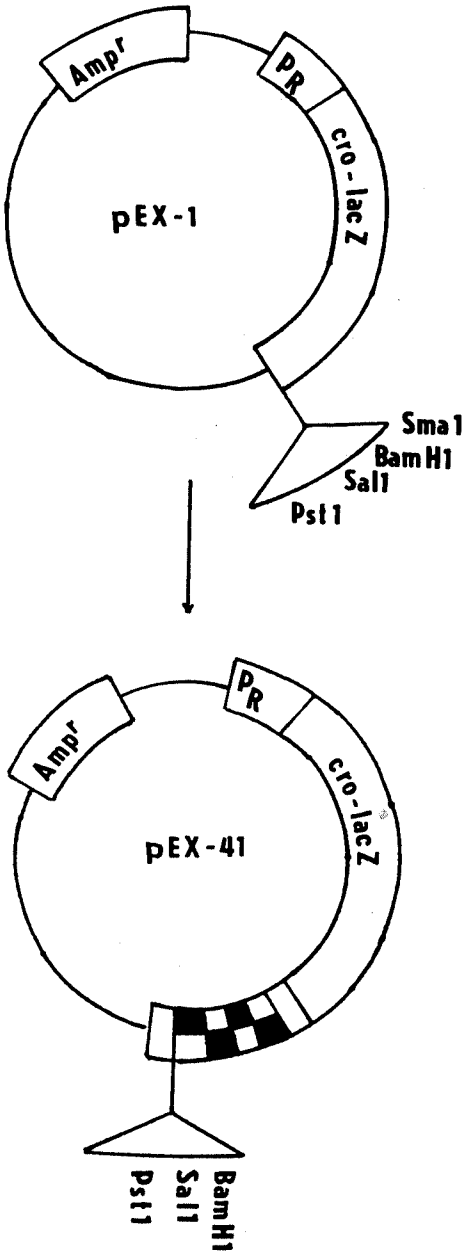

Fig. 3
Construction of the recombinant plasmid pEX-41 carrying a fragment 566 bp of HIV-1 *env* gene

Table 1. Results of recombinant imunoblots and HIV-1 Western blots (DuPont/BioTech) on 40 proficiency test specimens[a]

No. of sera	Recombinant immunoblot	Western blot	Recombinant immunoblot		Western blot	
			p24	gp41	p24	gp41
20	P	P	+	+	+	+
6	P	P[b]	+	−	+	+
2	P	P	±	+	+	+
1	I	I	+	−	±	±
2	N	I	+	−	−	−
2	N	N	−	−	±	−
7	N	N	−	−	−	−

[a] Proficiency test specimens were received from the Centers for Disease Control, Atlanta
[b] Specimens were characterized as HIV-1 antibody positive on the basis of reactivity with other *env* and *pol* gene proteins
P: HIV-1 positive; N: HIV-1 negative; I: Indeterminate
+: reactive; −: non reactive; ±: weak reactivity

0.01 mol/l Tris-HCl (pH 8.0) containing 0.001 mol/l EDTA, 1 % SDS, 5 % mercaptoethanol, and 10 % glycerol. The lysate was used to prepare nitrocellulose strips for immunoblot testing of previously characterized sera (National Reference Laboratory for AIDS, Bratislava).

In order to evaluate the immunological reactivity of pKK24 and pEX-41 a set of 3 strips was prepared. It contained one strip on the basis of a total cell lysate harbouring pEX-41, second one on the basis of pKK24 and a third one containing proteins of the *E. coli* host cell strain. Prior to testing of sera with specific anti-HIV-1 antibodies we examined this test system with 17 sera from patients with high anti-*E. coli* titres (kindly provided by G. Füst, Budapest). These sera did not contain antibodies to *E. coli* proteins of the same molecular weight as the recombinant proteins – products of pKK24 and pEX-41 and did not produce false-positive results. Further 40 sera were tested in our test system and the results were compared with those obtained with DuPont/BioTech Western blot assay (WB) licensed by the U.S. Food and Drug Administration (Table 1.). From these, 28 sera which gave positive results in the commercial tests were also classified positive by the recombinant immunoblot system. Discrepant results were observed in WB in the reactivity to p24 and gp41 when comparing the conventional antigen and the recombinant proteins; 6 sera reacted positively with pEX-41 product, but negatively with gp41 in the conventional WB. On the other hand 7 sera scored differently when comparing the pKK24 reactivity with the reaction against p24 in classical WB. No false positive results with the

pEX-41-pKK24 immunoblot system were observed. From 3 sera classified indeterminate by the conventional WB, 2 were negative and 1 was inconclusive in the recombinant test (exhibiting weak reaction against each one the recombinant peptides).

Our results show that a confirmatory test using recombinant derived p41 and p24 compares favourably with other tests using several viral antigens. Although limited in extent our immunoreactivity study suggests the superiority of the described recombinant p41 antigen over the viral antigen in detecting anti-gp41 antibodies by WB. It is noteworthy that antibodies to viral gp41 seem to be more specific to HIV-1 than do antibodies to the core antigens or the *pol* p31 protein (Hofbauer *et al.,* 1988). A more comprehensive study to evaluate the suitability of pEX-41 and pKK24 for the development of an alternative confirmatory test seems advisable.

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