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Chimeric Synthetic Peptide as Antigen for Immunodiagnosis of HIV-1 Infection

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One chimeric peptide incorporating antigenic sequences from the gp41 transmembrane region (peptide H-18) and the gp120 envelope region (peptide H-15) corresponding to amino acids (587–617) on gp41 and (495–516) on gp120 of human immunodeficiency virus (HIV 1) was synthesized. Both sequences were separated by two glycine residues. This peptide was evaluated as antigen in an ultramicro-enzyme-linked immunosorbent assay (UMELISA) with samples derived from HIV-1 (*n* = 30) with different titers of antibodies and healthy blood donors (*n* = 30). The results were compared to plates coated with monomeric peptides and to plates coated with two monomeric peptides together. Results demonstrated that monomeric peptides gp41 (H-18) and gp120 (H-15) were good as antigens with samples that present antibodies to these regions. The chimeric peptide was the most antigenic. Those results may be related to the peptide structure, adsorption to the solid surface, and epitope accessibility to the antibodies. This chimeric peptide would be very useful for HIV-1 diagnostics. © 2000 Academic Press

Key Words: **chimeric synthetic peptide; UMELISA; HIV-1.**

The diagnosis of HIV-1 (1) infection depends on detecting the presence of virus or a serologic response to the virus. In the routine screening of blood and byproducts for transfusion, HIV-1 infection is recognized by detection of specific antibodies. The recommended procedure is enzyme-linked immunosorbent assay (ELISA) (2–4) screening followed by Western blot test (WB) confirmation of suspected positive specimens (5). Earlier versions used viral lysates antigens in solid phase, so these tests became less sensitive and specific. Later, the most important proteins for diagnostic of the virus were obtained by recombinant DNA technology (6) These assays are more sensitive and specific because these antigens are not contaminated with human cellular proteins. However, the assays present unspecific reactions because the contaminants from the host are recognized unspecifically by the sample's antibodies. Unspecificity does not occur when synthetic peptides are used. HIV virus conserved domains have been used showing a high specificity and sensitivity in detecting antibodies in sera from infected patients (7).

Although two or more antigens can be used for diagnostic assays, this could affect the sensitivity and specificity of the test because equivalent binding of these molecules to the solid surface, competition for the binding and spatial distribution of antigenic determinants in peptides on the solid phase. There is a tendency toward using synthetic chimeric peptides to avoid those problems and improve assay's sensitivity and specificity (8). The assessment of a chimeric peptide of one immunodominant region of the gp41 transmembrane protein and gp120 envelope protein of HIV-1. Besides, comparative results of a chimeric peptide, monomeric peptides and monomeric peptides mixed are presented in this report.

MATERIALS AND METHODS

Peptide synthesis. Chimeric and monomeric peptides as shown in Table 1, were synthesized manually by the standard solid-phase method (9–11). The first two represented immunodominant sequences from gp41 and gp120. The last chimeric peptide included sequences from gp41 (H-18) and peptide gp120 (H-15). Two glycine residues were added to chimeric peptide to separate two immunodominant sequences.

The peptides were assembled on *tert*-butoxycarbonyl(t-Boc)-βAla-OCH_2-PAM resin (0.47 mmol/g; 100–200 mesh) purchased from Bachem (Switzerland). Derivated of amino acids were obtained from Bachem (Switzerland). All the solvents used (dichloromethane (DCM), 2-propanol (iPrOH), *N,N'*-dimethylformamide (DMF) and the reagents (trifluoroacetic acid (TFA), *N*-ethyldiisopropylamine (DIPEA), *N*-ethyldiisopropylcarbodiimide (DIPCDI)) were pure for synthesis (Merck, Germany). The couplings with DIPCDI 0,2 mol/L in DCM or 1-hydroxybenzotriazole (HOBT) esters (Sigma) in DMF, were monitored by the qualitative ninhydrin test (12) and usually completed within 1 h. The α-amino groups were protected with t-Boc.

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TABLE 1

Synthetic Peptides Used in This Study

Code	Sequence
H-18	ERYLKDQQLLGIWGCSGKLICTTAVPWNA
H-15	KIEPLGVAPTKAKRRVVQREKR
Qm	(H-18)-GG-(H-15)

The t-Boc group was removed at each cycle by 37.5% TFA in DCM, followed by neutralization with 5% DIPEA in DCM. The peptides were cleaved from the resin using "Low–High" method (13, 14) with hydrogen fluoride (HF) purity for analysis (Fluka, Switzerland) in the presence of scavengers (anisole, dimethyl sulfide, 1,2-ethanedithiol and *p*-cresole) and then extracted with 30% acetic acid and the solutions were lyophilized.

The purity of individual peptides was examined by reverse-phase high-performance liquid chromatography (RP-HPLC) (15) with on octadecyl (C18) silicated column (Pharmacia Biotech) with a gradient of eluent A (0.1% (V/V)TFA in H_2O; eluent B, 0.05% (V/V) TFA in acetonitrile; gradient, 0–60% B over 60 min; flow rate 0.5 ml/min. The peptides were detected by UV at $\lambda = 226$ nm.

Mass spectrometry. Peptide molecular weights were confirmed by mass spectrometry MALDI-TOF (Matrix-Assisted Laser Desorption of ions time-of-flight) (16–18) using a Protein TOF mass spectrometer (Bruker Analytical Systems, Inc.).

Coated ultramicro-ELISA plates (UMELISA). Polystyrene plates (Greiner Labortechnik, Germany) were incubated with 15 μL/well of either monomeric synthetic peptides (2 μg/mL) or monomeric peptides together (2 μg/mL each one mixed), and a chimeric peptide (4 μg/mL). All peptides were dissolved in carbonate-bicarbonate buffer (0.05 mol/L, pH 9.6). Later the plates were incubated at 37°C for 4 h. The unbound peptides were aspirated and washed with one wash of 0.14 mol/L phosphate-buffered saline (PBS) with 0.05% Tween 20. Wells were blocked by addition of 30 μL/well of buffer (0.1% of bovine serum albumin in PBS, with 0.5% Tween 20) and incubated overnight at room temperature. Plates were sealed with plastic sealing tape and stored at 4°C until used.

Ultramicro-enzyme-linked immunosorbent assay (UMELISA). Specimens were diluted 1:20 in buffer Tris–HCl (0.015 mol/L, pH 7.8) and 0.05% Tris with 0.05% Tween 20) in 20% of sheep serum and added to coated plates at 10 μL/well. Plates were incubated at 37°C for 30 min. After washing three times with 30 μL/well of Tris/Tween, sheep anti-human IgG phosphatase alkaline conjugate (Boehringer Mannheim GmbH, Germany) was added to the plates at 10 μL/well and incubated for 30 min at 37°C. Followed by three washes with Tris/Tween, substrate 4-methylumbelliferylphosphate (4-MUP) (Koch Light Ltd., Haverhill, Suffolk, England) was dissolved in diethanolamine buffer and added 10 μL/well and incubated at room temperature for 30 min. The fluorescence intensity was measured on a SUMA PR-521 plate reader (Immunoassay Center, Havana, Cuba) (excitation at 365 nm and emission at 450 nm). All assays included positive and negative controls. The samples were analyzed in duplicate.

Commercially available panels containing HIV-1 infected samples were tested. Boston Biomedical Inc. (BBI; Rockville, MD) supplied three HIV panels: Anti-HIV-1 Low Titer Performance Panel PRB106 ($n = 15$), Anti-HIV-1 Seroconversion Panel Q (Modified) PRB917 ($n = 5$), Anti-HIV-1 Seroconversion Panel PRB-952 ($n = 6$) and Anti-HIV-1 Panel from Cuba ($n = 19$) was supplied by the Research AIDS Center (Havana, Cuba).

Cutoff value. To determine the cutoff value, 500 healthy blood donors' and 58 HIV 1 seropositives' confirmed sera were studied. The cutoff value (CO) was 0.30.

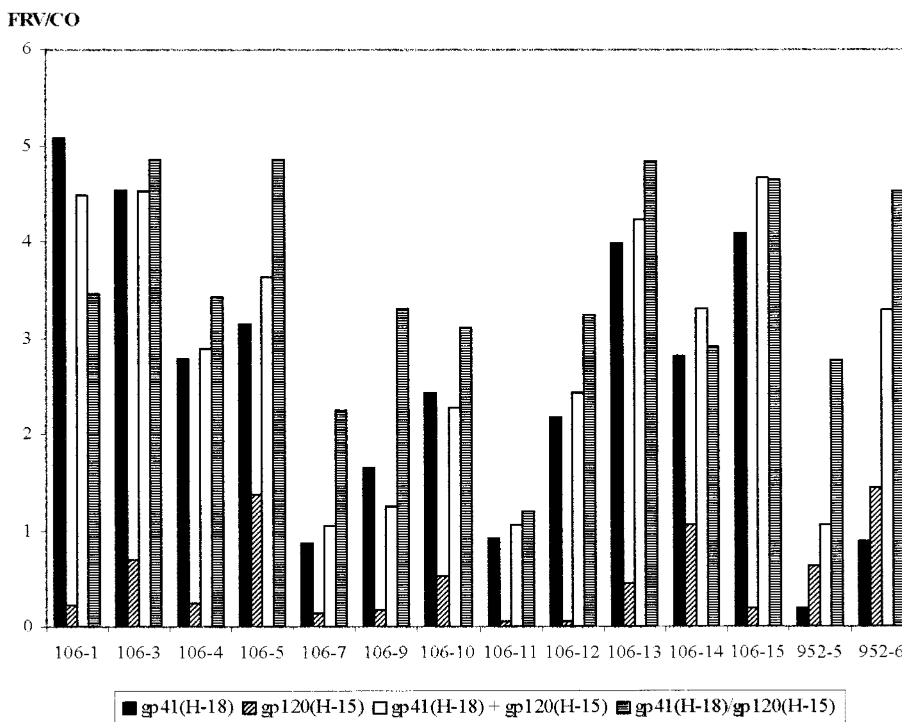

FIG. 1. UMELISA test of the synthetic peptides of HIV 1 according to what was stated under Materials and Methods. Reactivity of low titer and seroconversion HIV 1 ($n = 14$) on monomeric peptides gp41 (H-18) and gp41 (H-15), monomeric peptides together and the chimeric peptide Qm.

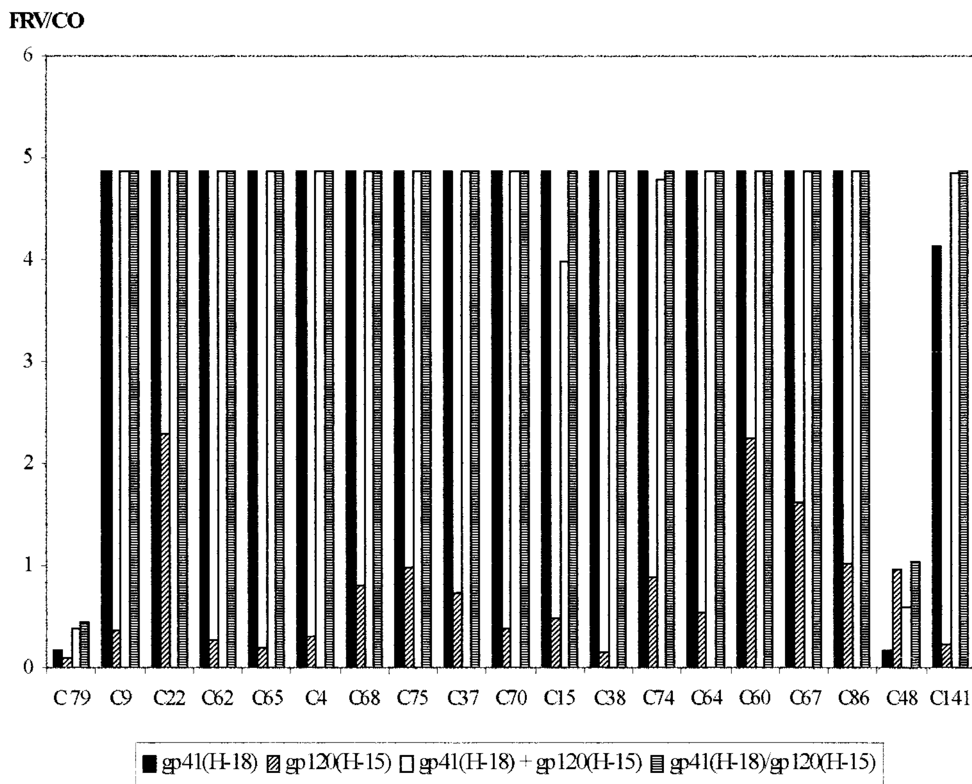

FIG. 2. UMELISA test of the synthetic peptides of HIV 1 according to what was stated under Materials and Methods. Reactivity of HIV 1 (n = 19) of Cuban seropositive specimens using monomeric peptides gp41 (H-18) and gp41 (H-15), monomeric peptides together and the chimeric peptide Qm.

All numeric results were means of duplicates, expressed as specimen fluorescence to cutoff ratios (FRV/CO) (FRV was Sample/positive fluorescence ratio value). Ratios ≥1 were considered reactive.

RESULTS AND DISCUSSION

All the peptides were synthesized by the solid phase method. Peptide purity was greater than 90%.

The sequence of monomeric peptides was deduced from (Bru Isolate) (HIV-1) reported by Wain-Hobson *et al.* (1985). Peptides were selected after analyzing the hydrophilicity profile of protein gp41 and gp120 according to Hopp and Woods (1981) (19).

After the monomeric peptides were analyzed in UMELISA with positive HIV-1 sera, positive by Western blot confirmatory test (WB) (H-18) and gp120 (3) were selected to be used because they were proved to be the most reactive. The obtained result concerning gp41 (H-18) coincides with the results reported from the study carried out by Stigler *et al.* (20) where it is said that the epitope sequence (CSGKLICTTAVPW), was identified as the best binding peptide ($K_D = 1 \times 10^{-8}$ mol/L) with Fab fragment of the human monoclonal antibody 3D6.

A new chimeric peptide with the two sequences together (gp41 and gp120) were decided to be synthesized because some samples were not detected with positive bands to gp41 and gp120 by WB test.

How synthetic peptides behave against positive samples to HIV 1 is showed in Fig. 1.

Peptides performance is showed in Fig. 1 where: peptide H-18 detected (10/14) samples, with 9 samples showing (FRV/CO) values of >2, while one sample each showing moderate to low (FRV/CO). Peptide H-15 detected (3/14) samples, with 3 samples showing (FRV/CO) moderate to low. Peptides H-18 and H-15 together detected (14/14) samples, with 10 samples showing (FRV/CO) values of >2. It detected 4 moderate to low samples. Chimeric peptide Qm detected (14/14) samples, with 13 samples showing (FRV/CO) values of >2, while one sample each showing moderate to low (FRV/CO).

Peptides performance is showed in Fig. 2 where: peptide H-18 detected (17/19) samples, with 17 samples showing (FRV/CO) values of >2. The two nondetected samples do not have bands to gp41 env protein in Western blot confirmatory test. Peptide H-15 detected (4/19) samples, with two samples showing (FRV/CO) values of >2, while two samples showing moderate to low (FRV/CO). Peptides H-18 and H-15 together detected (17/19) samples, with 17 samples showing (FRV/CO) values of >2. Chimeric peptide Qm detected (18/19) samples, with 17 samples showing (FRV/CO)

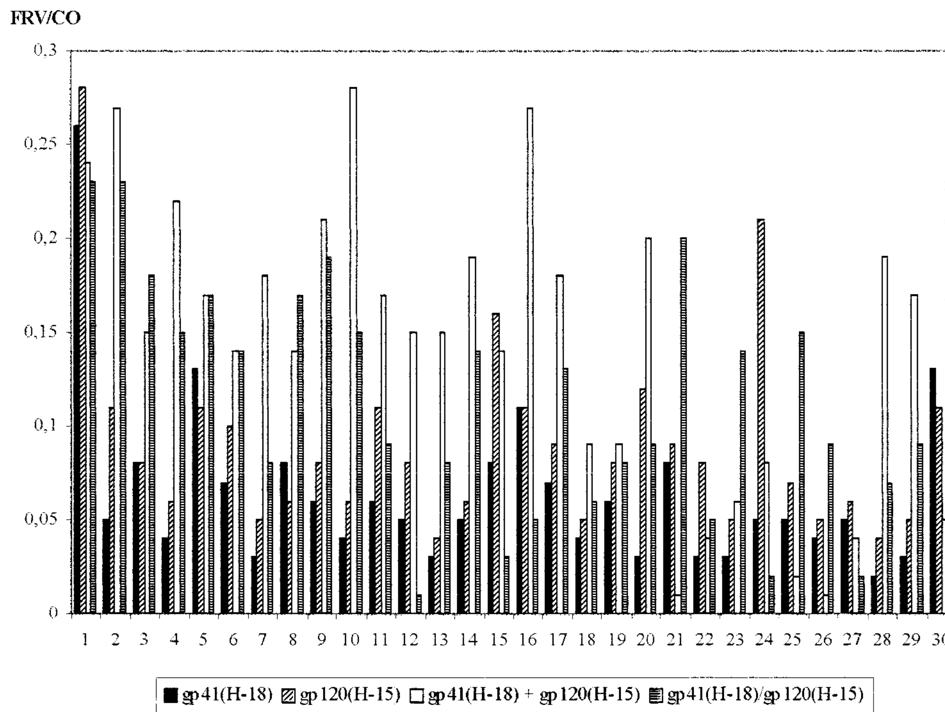

FIG. 3. UMELISA test of the synthetic peptides of healthy blood donors. Reactivity ($n = 30$) on monomeric peptides gp41 (H-18) and gp41 (H-15), monomeric peptides together and the chimeric peptide Qm.

values of >2, while one sample showing moderate to low (FRV/CO). The one nondetected sample do not have bands to gp41 and gp120 env proteins in WB test.

In order to assess peptide specificity, 30 samples from healthy blood donors were tested, as it is showed in Fig. 3 where all specimens were finally considered as negative.

Concluding, we have observed that the use of chimeric peptides as antigens certainly resolves the problem of competition and limited binding of one of the peptides in solid phase. Besides, chimeric peptides have the advantage of two epitopes in the same molecule and acquire a spatial structure that resembles the one that is going to be studied.

The chimeric peptide that was obtained through this work is very useful for detecting antibodies to HIV-1.

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