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Chimeric Synthetic Peptides from the Nucleocapsid p24 Protein of Human Immunodeficiency Virus Type-1

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Two chimeric synthetic peptides incorporating antigenic sequences from N-terminal (peptide C14) (134–163) and C-terminal (peptide C15) (335–364) of the p24 protein of human immunodefiency virus (HIV- 1), were synthesized. Peptides C14-GG-C15 and C15-GG-C14 represented sequences from the p24 protein in both possible orders, separated by two glycine residues as arm spacers. These peptides were evaluated as antigen in an Ultramicroenzyme-linked immunosorbent assay (UMELISA) using sera of HIV-1-infected individuals (n = 16) with different titers of antibodies and the specificity was evaluated with healthy blood donors (n = 20). The results were compared to plates coated with monomeric peptides C14 and C15. The chimeric peptide C14-GG-C15 was the most antigenic. Those results may be related to the peptide structure, the sequence order in the chimeric peptide, and epitope accessibility to the antibodies. This chimeric peptide would be very useful for HIV-1 diagnostics. © 2001 Academic Press

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The human immunodeficiency virus type 1 (HIV-1) is the cause of the acquired immunodeficiency syndrome (1). The viral genes encode core structural proteins (gag), enzymes (pol), envelope proteins (env), and complex set of regulatory proteins. The gag gene of human immunodeficiency virus type 1 (HIV-1) encodes a polyprotein precursor, p55, that is cleaved by the virus-encoded protease into three proteins: p24, p17, and p15 (2–4).

Antibodies against the capsid proteins appear early in infection and are useful serological markers for HIV-1 infection (5, 6).

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Synthetic peptides are being used to map sequential antibody binding sites on proteins either immobilized on solid phase or solubilized and bound to wells of microtiter plates.

Linear B cell epitopes have already been identified within p24 (7). Researchers have obtained different results about the recognition in HIV-1-infected individuals human sera antibodies, that reflect slight differences in the aminoacids composition of the peptides or the method of peptide presentation.

The region N-terminal and C-terminal of p24 protein were considered immunodominant domains of this molecule (8, 9), which is well recognized by sera from HIV-1 infected patients.

Currently, synthetic peptides derived from conserved regions are being used, showing high sensitivity and specificity in detecting serum antibodies from infected patients. Although two more antigens can be used for diagnostic assay, sensitivity, and specificity of the tests could be affected by equivalent binding of the molecules to the solid surface, competition for the binding, and spatial distribution of antigenic determinants in peptides bound to the solid phase.

There is a trend toward using synthetic chimeric peptides to avoid those problems and improve assay's sensitivity and specificity (10, 11)

In this study, antigenic sequences derived from N-terminal (C14) and C-terminal (C15) from the p24 protein of HIV-1 virus were assembled in chimeric peptides C14-GG-C15 (PQ1) and C15-GG-C14 (PQ2). T

he objective of the present report was to examine the effect of the epitope orientation on C14 and C15 antigenicity to diepitope chimeric peptides in which the C14 and C15 sequences occupied different positions.

MATERIALS AND METHODS

Peptide synthesis. Chimeric and monomeric peptides as shown in Table 1, were synthesized manually by the standard solid-phase method (12–13). The first two represented immunodominant sequences of the N-terminal of p24 (C14) and the C-terminal of p24 (C15). The last chimeric peptides (C14-GG-C15) (PQ1) and (C15-GG-

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TABLE 1

Synthetic Peptides Used in This Study

Code	Sequence
C14	PIVQNLQGQMVHQAISPRTLNAWVKVVEEK
C15	LKALGPAATLEEMMTACQGVGGPGHKARVL
PQ1	C14-GG-C15
PQ2	C15-GG-C14

C14) (PQ2) included sequences from C14 and C15 peptides. Two glycine residues were added to chimeric peptides to separate two immunodominant sequences.

Fmoc-based chemistry was utilized for the preparation of peptides using 0.20 g (0.47 mmol/g; 200–400 mesh) of 4-(2′, 4′-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxymethyl-polystyrene resin purchased from Bachem (Switzerland). Derivated of aminoacids were obtained from Bachem (Switzerland). All the solvents used (dichloromethane (DCM), *N*,*N*′-dimethylformamide (DMF) and the reagents (trifluoracetic acid (TFA), *N*-ethyldiisopropylamine (DIPEA) (Merck, Germany), 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate) (TBTU) Bachem (Switzerland), Piperidine (Sigma) were pure for synthesis. Coupling reactions were carried out using the respective Fmoc-aminoacids and a mixture of TBTU, HOBt, and DIEA in 1 h with DMF as solvent. Fmoc-cleavage and extent of coupling in each step were monitored using a qualitative ninhydrin test (14). Fmoc groups were removed in 50% piperidine/DMF mixture. Peptides were cleaved from the resin with a mixture of TFA, anisole, dimethyl sulfide, 1,2-ethanditiol, and p-cresole at room temperature. The peptides were precipitated with ice-cold ether. The mixture was centrifuged and the ether decanted. Fresh ether was added to resuspend the peptide. Washing was carried out three times. Finally the peptides were liofilized with water.

The purity of monomeric 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 UltraMicroELISA plates (UMELISA). Polystyrene plates (Greiner labortechnik, Germany) were incubated with 15 μL/well of monomeric synthetic peptides (2 μg/mL) and a chimeric peptides (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 during 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 a volume of 30 μL/well of Tris/Tween, sheep anti-human IgG phosphatase alcaline 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.

Anti-HIV-1 panel from Cuba ($n = 16$) was supplied by the National Reference Center for Retroviruses and Santiago de las Vegas Sanatorium (Havana, Cuba).

Cut-off value. To determine the cut-off value, 500 healthy blood donors and 58 HIV 1 seropositives confirmed sera, were studied. The cut-off value (CO) was 0.30.

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 conventional solid-phase peptide methodology using Fmoc chemistry. Peptides purity was greater than 90%.

The sequence of monomeric peptides was deduced from (ARV2/SF-2 Isolate) (HIV-1) reported by Levy *et al.* (1985) (*Science* **227,** 484–492).

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

Peptides performance is showed in Fig. 1 where peptide C-14 detected (1/16) positive samples, with one sample showing (FRV/CO) values of >2. Peptide C-15 detected (4/16) samples, three samples showing (FRV/CO) values of >2, and one sample showing moderate to low (FRV/CO).

The chimeric peptides performance is showed in Fig. 1 where peptide PQ1 detected (14/16) samples, with

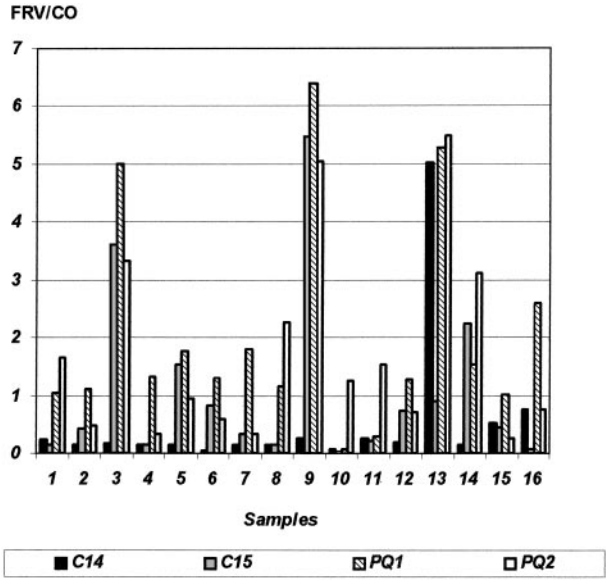

FIG. 1. UMELISA test of the synthetic peptides of HIV 1 according to what was stated under Materials and Methods. Reactivity of the monomeric peptides (C14 and C15) and chimeric peptides (PQ1 and PQ2) with sera of HIV 1-infected individuals ($n = 16$).

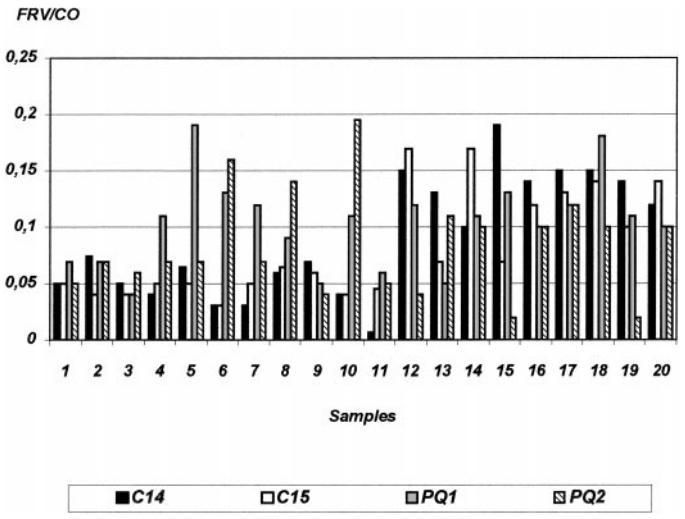

FIG. 2. UMELISA test of the synthetic peptides of the HIV-1. Reactivity of the monomeric peptides (C14 and C15) and the chimeric peptides (PQ1 and PQ2) with healthy blood donors ($n = 20$).

four samples showing (FRV/CO) values of >2, while 10 samples that showing moderate to low (FRV/CO). Peptide PQ2 detected (8/16) samples, with five samples showing (FRV/CO) values of >2, while three samples showing moderate to low (FRV/CO).

In order to assess peptide specificity, samples from healthy blood donors ($n = 20$) as it is showed in Fig. 2 were tested where all specimens were finally considered as negative.

Our results have demonstrated that chimeric peptides can be used to detect antibodies to regions at the N- terminal and C-terminal part of the p24 sequence, the epitope orientation from the chimeric peptides were very determinant in its antigenicity. This may be related to the peptide adsorption to the solid surface and epitope accessibility to the antibodies.

The chimeric peptide PQ1 (C14-GG-C15) obtained was the most antigenic peptide, and is very useful for detecting p24 antibodies to HIV-1 virus.

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