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HIV-transmembrane glycoprotein GP41 ENV fragments with high antigenicity and their application to determine HIV-positive sera

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Abstract

Using a computer program, developed by Jameson and Wolf, potential antigenic sites of the transmembrane glycoprotein gp41 env fragment 583-623 are predicted. Two regions with a high antigenic index are indicated: The first one, already reported and located in the segment 603 to 609, and a second one at the C-terminus of the peptide fragment. Therefore, the peptide ARILAVERYLKDQQLLGI WGCSGKLICTTAVPWNASWSNKS, corresponding to the immunodominant region 583-623 on the env reading frame of the HIV-1 isolate BRU and involving these two antigenic sites, was synthesized by the solid phase method and tested in ELISA system.

INTRODUCTION

The humoral immune response to the human immunodeficiency virus (HIV-1), the causative agent of the acquired immune deficiency syndrome (AIDS), has been extensively studied. Synthetic peptides are used increasingly to probe antigenic or immunogenic regions on the surface of the virus proteins (ref. 1). As could be shown, a major immunodominant region, which is highly conserved among the HIV-1 strains, sequenced so far, is located on the transmembrane glycoprtein gp41 env. Furthermore, it is well established that several synthetic peptides, varying in length and/or position, but all corresponding to this region can be used as antigens in an ELISA (ref. 2,3). Though the main binding site was established as a 7 amino acid residue peptide reaching from amino acid 603 to 609 on the env reading frame (ref. 4-6), it could be shown that at least one more epitope is located near this site (ref. 7). For diagnostic purposes it is essential to use a peptide antigen which covers the whole immunodominant region. This region is likely to span from amino acid 583-623 on the env reading frame of the HIV-1 isolate BRU (ref. 8)

RESULTS AND DISCUSSION

A computer program, developed by Jameson and Wolf (ref. 9), integrating hydrophilicity (H), surface probability (S), backbone flexibility (F) and secondary structure (CF: Chou-Fasman, GR: Garnier-Robson) parameters and based on the following equation:

$$< Ai = \sum_{i=1}^{N} 0.3\,(H) + 0.15\,(S) + 0.15\,(F) + 0.2\,(CF) + 0.2\,(GR)$$

was used to predict potential antigenic sites of the transmembrane glycoprotein gp41 env fragment 583-623 (Fig. 1).

As seen from Fig. 1, two regions with a high antigenic index are indicated: The first one, already reported and located in the segment 603 to 609 and a second one at the C-terminus of the peptide fragment.

Therefore, the peptide with the sequence ARILAVERYLKDQQLLGIWGCSGKLICTTAVPWN ASWSNKS, corresponding to the immunodominant region 583-623 on the env reading frame of the HIV-1 isolate BRU and involving the two predicted antigenic sites, has been synthesized by the solid phase method using the Fmoc strategy and an ECOSYN P instrument (Eppendorf/Biotronik, Maintal, Germany, Fig. 2).

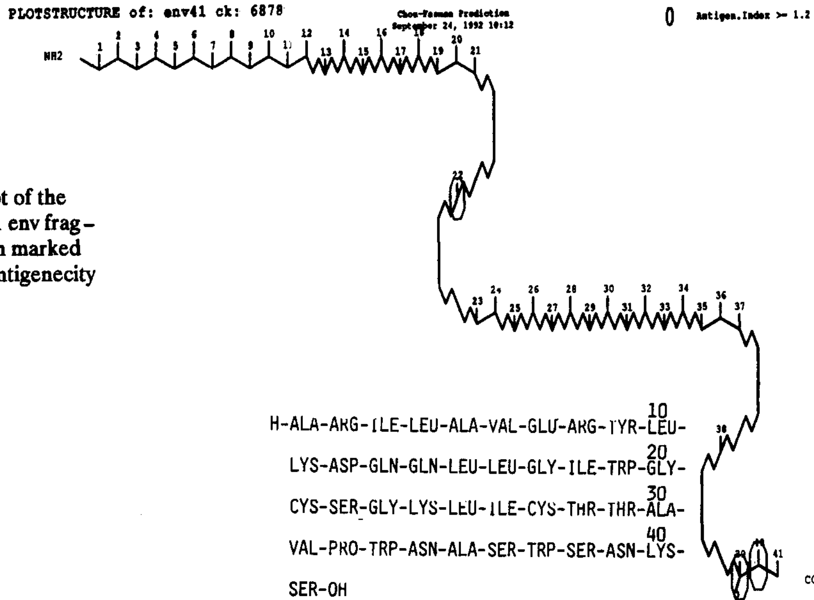

Fig. 1.
Chou Fasman plot of the glycoprotein gp41 env fragment 583-623 with marked regions of high antigenecity index.

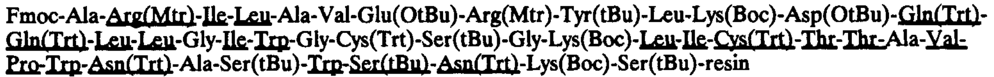

Fig. 2 Coupling strategy for the synthesis of fragment 583-623 of the glycoprotein gp41 env of HIV-1. Side chain protecting groups used in the synthesis are given in parentheses. Double coupling steps are underlined.

After removal of the protecting groups and cleavage from the Wang resin, the peptide mixture was precipitated with diethyl ether, collected by centrifugation and lyophilized from water. Four peptide fractions with molecular masses of 4581 (I), 4452 (II), 4802 (III) and 4670 Da (IV) were obtained when the crude synthetic product was separated by RPHPLC (Fig. 3).

As shown in Fig. 4, the molecular masses were determined relative to a reference peak of bovine insulin as an internal standard using a matrix-assisted laser desorption mass spectrometer (Lasermat, Finnigan MAT, Bremen, Germany). The molecular mass of fraction I is only 6 Da higher than the calculated value of 4576.3 Da for the desired sequence. Fraction II has a lower molecular mass as compared with the value of 4576.3 Da, and fractions III and IV have higher ones. This suggests that

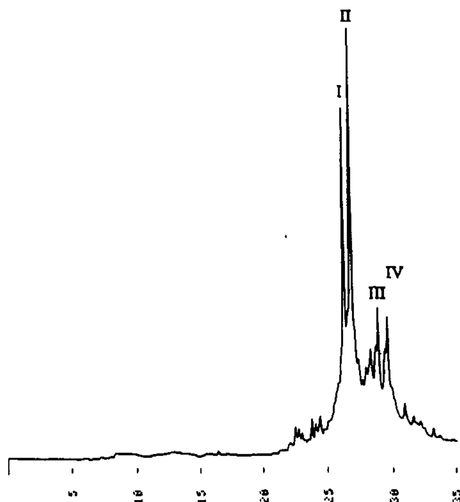

Fig. 3.
Preparative HPLC separation of the crude synthetic mixture of fragment 583-623 gp41 env of HIV: Column, Nucleosil (Macherey-Nagel, Düren, Germany) $7C_{18}$, 250x10mm; Solvent A 0.1% TFA/H_2O, B 60% CH_3CN/0.085% TFA/H_2O, gradient 15-100%B in 35 min., flow rate 2.5 ml/min., detection 214 nm, sample 5.1 mg/ml, injection 0.1 ml, equipment Eppendorf/Biotronik; absorption versus time.

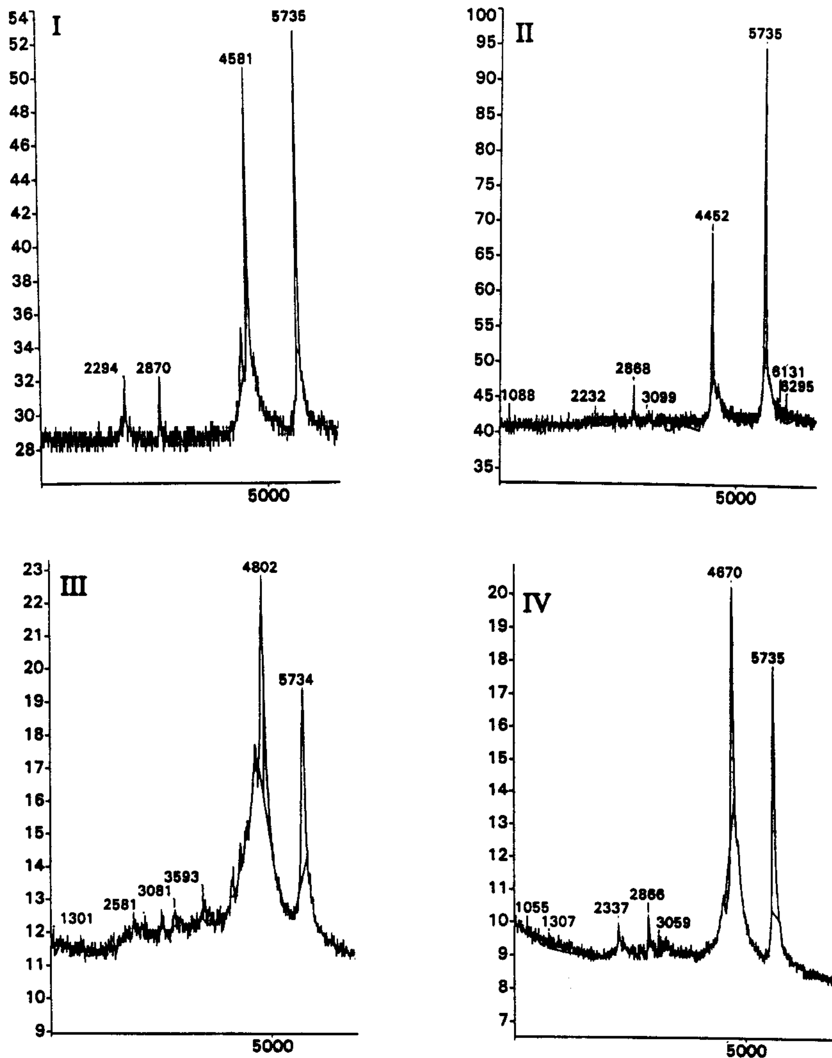

Fig. 4. LDMS spectra of the HPLC fractions I, II, III and IV of the synthetic fragment 583-623 of the HIV-1 glycoprotein gp41 env. Number of shots, 6; laser power, 24; matrix, α-cyano-4-hydrocinnamic acid; peak at m/z 5735, bovine insulin as internal standard; intensity versus m/z.

fraction II is produced by a synthetic deletion step and that fraction III and IV still have covalently bound protecting groups. The spectra in Fig. 4 illustrates also the homogeneity of the fraction I and II and the heterogeneity of fractions III and IV.

For elucidation of the primary structures of fractions I and II, portions of these were reduced, alkylated with 4-vinyl pyridine and digested with proteinase Asp-N. The molecular masses of fragments 1-11 (1335 Da) for both products are identical, corresponding to the theoretical value for this sequence. The molecular masses of the fragments 12-41 are 3272 Da and 3146 Da respectively for the HPLC- isolated Asp-N products of fractions I and II. The mass deficit of 126 Da suggests that fraction II is produced by deletion during the stepwise synthesis. Sequence analysis of HPLC-isolated chymotryptic fragments of fractions 12-41 showed that fraction I contains the desired peptide sequence, while in the peptide of fraction II Lys^{40} is deleted.

ELISA measurements, employing these peptide as an antigen adsorbent, have shown to reproducibly detect antibodies in sera of patients with HIV-1 infection. They were also efficiently used in a combined ELISA system for simultaneous detection of anti HIV-1/2 antibodies and HBsAg. Furthermore, this assay with peptide fraction I and II showed significant higher sensitivity with all sera in comparison with the activity of the commercially used 26 amino acid peptide 593-618 (Figs. 5,6).

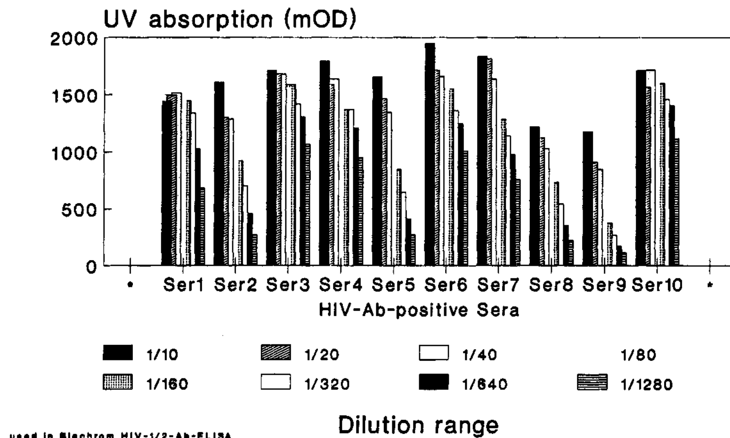

Fig. 5. ELISA activity of a commercial HIV-1/2 peptide mixture with diluted (dilution ratio see under the plot) HIV-positive sera.

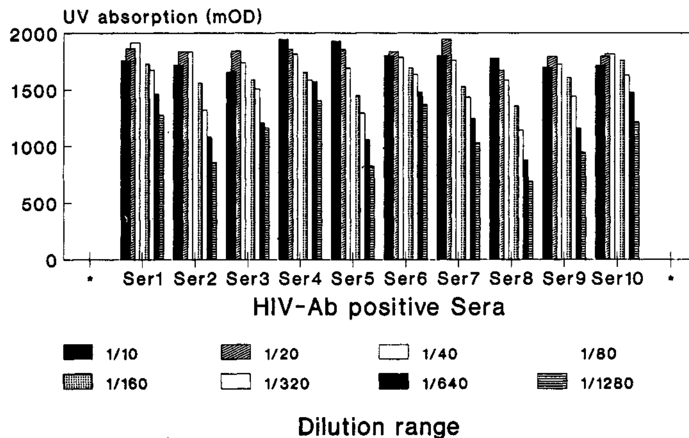

Fig. 6. ELISA activity of the synthetic glycoprotein gp41 env 583-623 fragment with diluted (dilution ratio see under the plot) HIV-positive sera.

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