

# EXPRESSION AND PURIFICATION OF RECOMBINANT HIV ENVELOPE POLYPEPTIDES

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## Introduction

The best strategy to control the AIDS epidemic is the development of an effective vaccine which should induce an immune response consisting of neutralizing antibodies targeted to conserved epitopes. Most of the neutralizing antibodies produced in HIV-1 infected individuals are directed against epitopes located within the C2V3C3 domains of the envelope glycoprotein gp120. Based on the evidence that linear peptides from the HIV envelope are capable of eliciting neutralising antibodies and, moreover, that the carbohydrate moiety of the envelope glycoproteins is implicated in the production of anti-HIV human antibodies with broad-spectrum neutralising properties against primary HIV isolates, it is anticipated that conjugation of oligosaccharides to envelope polypeptides derived from primary HIV isolates will result in neoglycopeptide vaccine antigens.

Therefore, the purpose of this work was the production, purification and characterisation of recombinant polypeptides representing the C2-V3-C3 Env region of primary isolates.

## Results

In order to produce new HIV peptide based immunogens, the *env* gene region coding for the C2V3C3 domains was amplified by PCR from proviral DNA of three HIV-1 and two HIV-2 infected patients. Patients infected with HIV-1 subtype B, C and G or HIV-2 group A were selected for amplification. Amplification products were cloned into the expression plasmid pTrcHis A and sequenced. Nucleotide sequences were analysed for contamination and genetic subtype by phylogenetic analysis. The corresponding amino acid sequences were inspected for relevant signature sequences, in particular, for charge, number and position of potential N-linked glycosylation sites and lysine residues. The recombinant polypeptides were expressed in *Escherichia coli* by induction with isopropyl- $\beta$ -D-thiogalactopyranoside. Kinetic expression assays were performed to determine the best time to collect the polypeptides for subsequent purification by immobilized metal affinity chromatography. Six new polypeptides were produced and purified in preparative scale. All polypeptides reacted in Western blot with HIV specific antibodies from HIV infected patients confirming their natural antigenicity.

## Future work

This involves the scale-up production of the native peptides and artificial glycosylation with oligosaccharides. The immunogenicity and antigenicity of the neoglycopeptides will be compared to the corresponding non-glycosylated peptides in an animal model.