

## Immunogenicity of Dengue E-protein Peptides Modified with Para-nitro Phenylalanine

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### Abstract

Few conserved epitopes from the Envelope (E) protein of the dengue virus have previously been identified to show a broader immunogenicity and neutralization towards all the four dengue virus serotypes. Inclusion of an unnatural amino acid such as para-nitro phenylalanine in the place of a phenylalanine in a protein, has previously been reported to enhance the immunogenicity. The aim of this research was to explore the effect of having para-nitro phenylalanine in the place of phenylalanine of those conserved E protein epitopes in enhancing their immunogenicities. Therefore peptides representing these epitopes, namely P1/E, P2/E, P3/E and P4/E, were commercially synthesized with and without the inclusion of a para-nitro group on phenylalanine. Three mice (Balb/c) batches were immunized with each peptide. The immunogenicity of these peptides was studied, by assessing the antibody levels generated against the corresponding peptide in collected antisera. The whole E protein was used as a positive control and the adjuvant alone was used as the negative control. All the peptides, with or without the modification, induced antibodies in mice as measured using ELISA assays. Mean OD values of unmodified and modified peptides are as follows; P1/E:  $0.390 \pm 0.02a$ , P2/E:  $0.305 \pm 0.01a$ , P3/E:  $0.244 \pm 0.02b$ , P4/E:  $0.238 \pm 0.01a$ , Mod-P1/E:  $0.311 \pm 0.01b$ , Mod-P2/E:  $0.166 \pm 0.02b$ , Mod-P3/E:  $0.371 \pm 0.03a$ , Mod-P4/E:  $0.253 \pm 0.01a$ . Out of the four peptides studied, P3/E with the para-nitro phenylalanine modification (Mod-P3/E), produced significantly high antibody levels as compared to its unmodified version. Interestingly for rest of the peptides, there were no significant differences in the levels of antibodies between the ones with and without para-nitro phenylalanine. Accordingly, this study does not provide sufficient enough evidence to support the claim that inclusion of an unnatural amino acid in the place of a phenylalanine in a protein enhances the immunogenicity in the tested E protein epitopes of the dengue virus.

**Keywords:** *Dengue, Conserved E-protein Epitopes, Unnatural amino acid, Immunogenicity*