Thermodynamic Studies of Recombinant Dengue Virus NS1 Protein

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Dengue virus belongs to *Flaviviridae* family and represents one of the most important arbovirus. It infects more than 100 million people annually, representing a serious health problem worldwide. Its genome is composed of three structural proteins (C, prM and E), which form the viral envelope and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5), which are involved in the replication cycle. NS1 function is still not well understood, but several reports have shown that it is highly immunogenic and may induce protective antibodies with complement fixing activity, killing infected cells, being a potential target for dengue vaccine strategy. Our aim is to characterize biochemically NS1 protein and to investigate its function during dengue infection. First, the NS1 gene of dengue virus type 2 was cloned into pET plasmid and expressed into BL21pLyS bacterial cells. The histidine tag-containing protein was purified by Ni^{+2} column affinity chromatography. In order to investigate its folding, urea and guanidine curves was carried out using fluorescence spectroscopy. We observed that concentrations of 5,28M of urea and 2,36M of guanidine were able to promote 50% protein denaturation and the calculated ΔG_{unfold} was 1.9±0,2 kcal/mol. In parallel, NS1 recombinant protein was injected into mice in order to produce polyclonal antibodies against it. Currently, we are investigating alterations in the secondary structure by circular dichroism, the effects of temperature and pH in the protein stability using fluorescence spectroscopy and we are also optimizing the expression of NS1 protein into *Pichia pastoris* yeast cells to further comparison with bacterial-recombinant protein. To conclude we observed that NS1 recombinant protein is sensitive to guanidine and urea effect.

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