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BENCHAMAS SUBSIN : MOLECULAR CLONING, EXPRESSION IN *E. COLI* AND PARTIAL PURIFICATION OF THE DENGUE VIRUS TYPE 2 - PROTEASE COMPLEX NS2B-NS3. THESIS ADVISOR : GERD KATZENMEIER, Ph.D., SAKOL PAMYIM, Ph.D., CHANAN ANGSUTHANASOMBAT Ph.D. 145p. ISBN 974-661-523-8

Dengue virus is the etiologic agent of dengue fever (DF) and dengue hemorrhagic fever (DHF). There are four antigenetically related but distinct dengue virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4), which all cause DF/DHF. Currently, there are no drugs available for a causative treatment of these diseases. Dengue virus type 2 contains a positive sense RNA genome of 10.7 kb encoding a polyprotein of 3391 amino acids. The polyprotein is proteolytically processed by a combination of host- and virus-encoded enzymes into at least 10 distinct polypeptides, 3 structural proteins and 7 nonstructural (NS) proteins. The generation of mature nonstructural virus proteins is catalyzed by the NS2B-NS3 protease complex encoded by the virus genome. The 69 kDa NS3 protein is a multifunctional polypeptide required for virus replication and contains a serine-protease domain in the N-terminal part of the molecule. The NS3 serine protease exhibits remarkable specificity for dibasic amino acid residues at the protein junctions in the polyprotein precursor. NS2B, a 14 kDa protein has been shown to act as cofactor or effector for the NS3 protease enhancing cleavage efficiency at various sites. The cleavage events during polyprotein processing are likely to be essential for maturation of the virus. The dengue NS2B-NS3 protease complex represents a potential target for the design of novel antiviral agents which are equally effective against all 4 serotypes.

The sequence encoding NS2B-NS3 was amplified by PCR using a full-length cDNA of DEN-2 genome as a template. The 2.3 kb NS2B-NS3 PCR gene segment was cloned into pBluescript IIKS$^-$ and analyzed by DNA sequencing. The sequence obtained was identical to the sequence reported for DEN-2 strain 16681. The NS2B-NS3 fragment was subcloned into the pCAL-n vector. The recombinant plasmid pCAL/NS2B-3 was transformed into *E. coli* BL21(DE3)pLysS. After induction with IPTG, the 87 kDa tag-NS2B-NS3 fusion protein could not be detected in SDS-PAGE. The NS2B-NS3 fragment was subcloned into the expression vector pTrcHisA where it was fused N-terminally in frame to a poly-histidine peptide for purification by metal chelate affinity chromatography. The recombinant plasmid pTH/NS2B-3 was transformed into *E. coli* JM109. Upon induction, an expression peak of the 88 kDa tag-NS2B-NS3 fusion protein was observed in SDS-PAGE after 4 hrs induction. In Western blot analysis, the tag-NS2B-NS3 fusion protein cross-reacted with Ni-NTA conjugated alkaline phosphatase and human antiserum from dengue infected patients. The target protein appeared to be associated mostly with sedimentable inclusion bodies. Inclusion bodies were solubilized in 6M guanidine hydrochloride and partial purified by Ni-metal affinity columns. The solubilized sample was eluted with a denaturing buffer containing 8 M urea. Protein concentration was determined at OD 280 nm and the sample was analyzed by electrophoresis. Partially purified fusion protein was obtained from the affinity chromatography and will be used for subsequent biochemical characterization.