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RUNGRUTAI UDOMSINPRASERT : MOLECULAR CLONING, EXPRESSION IN *E. COLI* AND PURIFICATION OF A NATIVE DENGUE TYPE 2 CLEAVAGE SITE PROBE FOR THE DETECTION OF NS3 PROTEASE ACTIVITY. THESIS ADVISORS: GERD KATZENMEIER, Ph.D., CHANAN ANGSUTHANASOMBAT, Ph.D., ALBERT KETTERMAN, Ph.D., 124 p. ISBN 974-663-261-2

Dengue virus encoded NS3 serine protease is considered important for viral replication due to its responsibility for specific cleavage of polyprotein precursor. Development of high throughput assay and design of inhibitors are urgently required for future progress in Dengue research. The aim of this thesis project was to produce a recombinant protein substrate containing the dengue virus type 2 polyprotein cleavage sites, NS2A-2B and NS2B-3, which would be useful as a probe for the detection of NS3 protease activity.

A DNA sequence encompassing the NS2A-2B and NS2B-3 junctions was amplified using full-length cDNA genome of dengue virus serotype 2 strain 16681 as a template. The PCR product was inserted into pUC18 for sequencing analysis and subcloned into pTrcHisB expression vector. It was fused N-terminally in-frame to the polyhistidine affinity tag. The cleavage site probe (CSP) fusion protein was expressed in *Escherichia coli* as inclusion bodies. A 28 kDa band of the target protein analyzed by SDS-PAGE reacted with Ni-NTA conjugated to alkaline phosphatase in Western blot analysis. Under denaturing conditions, the CSP fusion protein was purified by using metal affinity chromatography to greater than 80% purity and the purified protein was renatured 'on-column' by using urea-free buffer. The yield of purified CSP was approximately 1 mg/l. of *E. coli* culture as determined by Bradford's assay. However, the histidine tag could not be removed from the fusion protein when cleaved with enterokinase. The purified CSP labeled with FITC was incubated with clarified dengue 2-infected cell lysate to assay for NS3 protease activity.

The results show that the method of CSP-FITC labeling could be used for production of a sensitive probe for the detection of NS3 protease activity. This method may be modified for study of other proteins.