3736276 SCBC/M : MAJOR : BIOCHEMISTRY ; M.Sc. (BIOCHEMISTRY)
KEY WORD : DNP-11-dUTP/POLYMERASE CHAIN REACTION/DOT BLOT HYBRIDIZATION/*IN SITU* HYBRIDIZATION

KANCHANA DOKLADDA : DEVELOPMENT OF A NON RADIOACTIVE DNA PROBE METHOD FOR THE DIAGNOSIS OF BACULOVIRUS IN THE BLACK TIGER PRAWN. THESIS ADVISOR : PICHIT TOSUKHOWONG, Ph.D., PRAYAD KOMARATAT, Ph.D. 79 p. ISBN 974-589-100-2

DNP-11-dUTP (6(2,4 dinitrophenyl)-ε-aminocaproyl[5-(3-amino)allyl] dUTP was synthesized from dUTP by known reactions. The side chain was introduced by converting dUTP to aminoallyl dUTP with the yield of 16 percent. The final product was obtained by linking the amino end of aminoallyl dUTP with DNP-aminocaproic acid -N-hydroxy succinimide ester, the latter compound was synthesized from caproic acid, FDNB and N-hydroxy succinimide. The product was purified by Sephadex-G15 gel filtration and DEAE-cellulose column chromatography. It was characterized by UV-visible absorption spectrum which has maximum wavelengths at 365 nm and 238 nm and about 2 moles of acid labile phosphate per mole of nucleotide (calculated from reported molar extinction of 16000 at 360 nm).

DNP-11-dUTP was incorporated into target sequence of DNA of SEMBV virus in Bluescribe M-13 plasmid by PCR. The ratio of DNP-11-dUTP to dTTP 1:10 or 1:5 gave the same incorporation by PCR as the control judging from agarose gel electrophoresis. The incorporation result was also confirmed by enzyme immuno assaywith rabbit anti DNP antibody, followed by goat anti rabbit IgG conjugated with alkaline phosphatase.

By using DNP-11-dUTP labeled PCR product 5 pg of Bluescribe M-13 plasmid containing SEMBV DNA can be detected in dot blot hybridization.

Tissue from black tiger prawn infected with SEMBV virus was tested for the virus by *in situ* hybridization technique. Probe labeled with 1:5 of DNP-11-dUTP to dTTP or 1:10 ratio gave positive test.

In conclusion DNP-11dUTP can be conveniently synthesized and DNA probe with this nucleotide analogue can be used to detect target DNA by *in situ* hybridization technique.