Nalumon Thadtapong 2013: Pyruvate Kinase from *Streptomyces antibioticus* and *S. coelicolor*: Characterization of the Nucleotide Sequence and Kinetic Property. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Somchai Pornbanlualap, Ph.D. 130 pages.

2'-Deoxynucleoside triphosphates (dNTP) are becoming indispensible reagents used for amplification of DNA in PCR techniques. This thesis describes an enzymatic method for the synthesis of dNTPs at 100 mM-scale and cloning of pyruvate kinase from *Streptomyces coelicolor* and *S. antibioticus*. The first step in synthesis of dNTP involves phosphorylation to 2'-deoxynucleoside monophosphates (dNMP) to their diphosphoric forms, using 2'-deoxynucleoside monophosphate (dNMP kinases). However, because no one single dNMP kinase was found to able to convert all four dNMPs to dNDPs, phosphorylation of 2'-dAMP and 2'-dCMP to their respective diphosphoric forms was accomplished using *Escherichia coli* adenylate kinase (ADK) and cytidine monophosphate kinase (CMK), respectively. The phosphorylation of 2'-dGMP and 2'-dTMP were accomplished using one enzyme, T_4 phage deoxynucleotides kinase (DNK). All of the enzymes described had been previously cloned and over-expressed in laboratory. The second step involves phosphorylation of 2'-dNDP to 2'-dNTP with pyruvate kinase (PYK). Although the type II *E. coli* PYK had been cloned and over-expressed in *E. coli*, this recombinant was unstable and aggregated upon prolonged incubation at 37°C. This enzyme was replaced by type I PYK from *E. coli* and from other organisms such as *Streptomyces antibioticus* and *S. coelicolor.* The *pyk* gene from *S. coelicolor* was cloned, sequenced and expressed in *E. coli* host. When induced with lactose, *S. coelicolor* PYK (ScPYK) was expressed as inclusion bodies. The purified protein contains two bands with a molecular weight of 51 and 60 kDa, respectively. The 51 kDa protein corresponds to the predicted molecular weight of ScPYK whereas the 60 kDa protein is proposed to be the phosphorylated form of PYK or protein resulted from an error during protein translation. In addition to ScPYK, the 1,437 bp *pyk* gene from *S. antibioticus* was isolated by PCR, completely sequenced and will be over-expressed and purified in the future. The putative *pyk* gene from *S. antibioticus* is predicted to encode for the type I PYK with the deduced amino acid sequence of 478 residues. To synthesized dNTP, the type I PYK from *E. coli* was chosen. Using ADK or CMK in combination with type I *E. coli* PYK, dATP or dCTP was successfully synthesized at 100 mM-scale with a yield of approximately 92%. Using DNK in combination with PYK, the synthesis of dGTP and dTTP were accomplished at 10 mM and 50 mM-scale, respectively. Synthesis of dGTP requires ATP as phosphoryl donor whereas synthesis of dTTP requires dTTP as phosphoryl donor.

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