Attawan Sunantakarnkij 2008: Cloning, Expression and Purification of the Novel Adenosine Deaminase from *Streptomyces antibioticus*. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Assistant Professor Somchai Pornbanlualap, Ph.D. 79 pages.

2'-Deoxycoformyin (2'-dCF) is a naturally occurring nucleoside antibiotic that had been isolated from *Streptomyces antibioticus*. 2'-dCF has been demonstrated to be one of the most potent inhibitors of adenosine deaminase. Thus, the ability of 2'-dCF producing *Streptomyces* to tolerate the inhibitory effect of this antibiotic is a pre-requisite for antibiotic production. A novel ADA, designated as ADA-II, had been proposed to be the key enzyme that confers *S. antibioticus* resistant to the inhibitory effect of 2'-dCF during biosynthesis of this antibiotic. Because antibiotic resistant gene and antibiotic biosynthetic genes are often clustered, cloning of the *ada*-II gene can possibly allow one to obtain the entire 2'-dCF biosynthetic genes.

Three different PCR-base methods were used to clone and determine the complete nucleotide sequence of the *ada*-II gene. The central region, 3'-downstream region, and 5'- upstream region of the *ada* gene was determined by normal PCR, inverse PCR, and single primer PCR, respectively. The complete ORF of the putative *ada*-II is 1041 bp in length and encodes for a protein of 346 amino acid residues (37.8 KDa). The deduced amino acid sequence of this *ada* gene shows 88%, 87% and 84% homology to ADA from *S. coelicolor* A3(2), *S. avermitilis* MA-4680 and *S. verginia*, respectively. When aligned to murine ADA (MuADA), seven of the eight catalytically active residues were also conserved in *S. antibiotics* ADA (StADA).

The putative *ada*-II gene was expressed in *E. coli* BL21 (DE3) as non his-tagged, his-tagged and fusion protein, using pET-26b, pET-28b and pYTB12, respectively. However, the protein formed inclusion bodies (IBs) in all cases upon induction with IPTG. Therefore, the protein in the IBs was unfolded and refolded with 2 M urea. After refolding and purification of the his-tagged protein, no deaminase activity was detected. However, because the calculated molecular weight of this putative ADA (37.8 KDa) is similar to that of ADA-II (38 KDa) purified from cell-free extract of *S. antibioticus*, therefore it is likely that this ORF is encoded for ADA-II. Base on multiple alignment of ADA from various organisms, a concerted addition-elimination type (SN2) mechanism is proposed for StADA.

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