E347 Characterization of Sepiapterin Reductase cDNA clone (SSC801) from *Dictyostelium discoideum*

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Tetrahydrobiopterin (BH4) is an interesting compound having various functions in nature. Although it is a well-known cofactor for some enzymes, there still remains a function not completely understood in higher animals and less is known in micro-organisms. The cDNA project of *Dictyostelium* provided us a cDNA homolog (SSC801) of sepiapterin reductase (SR) catalyzing the last step of BH4 synthesis. An unique feature of *Dictyostelium* is that it produces mostly D-threo form (the oxidized form was named dictyopterin) of BH4 instead of more popular L-erythro form. However, when we incubated the recombinant SR enzyme with 6-pyruvoltetrahydropterin synthase, the second enzyme of the BH4 biosynthesis providing the substrate for SR, and its in vivo substrate dihydroneopterin triphosphate, the major product was biopterin. The result suggest that there may exist another enzyme producing D-threo form, which may be an isoform or different one. Another possibility is an epimerase, which converts BH4 into H4-dictyopterin.

E348 Expression and Characterization of Human Calicivirus 2C-like Protein in *Escherichia coli*

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Human calicivirus (HuCVs) has been reported as a cause of nonbacterial acute gastroenteritis in human. The HuCV genome is consisted of three open reading frames (ORFs). Deduced amino acid sequence from the cDNA indicates that ORF1 codes for nonstructural protein. Comparison of the amino acid sequence within the ORF1 suggestes that it possesses poliovirus 2C-like helicase, 3C-like protease and 3D-like RNA dependent RNA polymerase motif. The amino-terminal truncated 2C-like protein of HuCV obtained from Korean patient was expressed in *Escherichia coli* as an aggregated insoluble form. The inclusion body, solubilized by treating with 8 M urea, was purified by affinity column followed by ion-exchange chromatography. The denatured protein was renatured by dialysis against refolding buffer. The purified recombinant 2C-like protein showed polynucleotide-stimulated NTPase activity. Analysis of the recombinant 2C-like NTPase showed that substrate, pH, temperature and divalent ion concentration optima were ATP, pH 7.5, 37℃ and 3 mM Mg^{2+} respectively. These studies suggested that the 2C-like protein of HuCVs might be RNA helicase.