E113 Comparative Analysis of Two Insecticyanins from Larval Hemolymph of *Artogeia rapae*.

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Two blue-pigment binding proteins designated as BP-1 and BP-2 are present in larval hemolymph of *Artogeia rapae* L. and fluctuate in expression during development. BP-1 exists in large amounts during larval stage and disappears around 1 day after pupation in hemolymph. However, BP-2 begins to increase after prepupal stage. BP-1 and BP-2 are easily separated by 75% ammonium sulfate, and then purified effectively by ion exchange chromatography and Prep-Cell Unit. Both BP-1 and BP-2 each consist of 4 subunits, which has molecular masses of 20,244 Da and 19,878 Da, respectively. Native molecular masses of BP-1 and BP-2 were determined to be approximately 80 kDa and isoelectric points are 7.0 and 6.8, respectively. BP-1 and BP-2 are almost similar in amino acid composition except methionine which content is 0.05% in BP-1 and 0.42% in BP-2. Also, they are almost identical in amino acid sequence on N-terminal end except the first amino acid which is asparagine in BP-1 and aspartic acid in BP-2.

E114 Gonadotropin-Releasing Hormone (GnRH) Neuron-Specific Splicing of GnRH Transcript

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The rat GnRH gene consists of four short exons and three introns (A, B, and C). Northern blot and RT-PCR analyses showed a strong expression of the GnRH mRNA in the preoptic area (POA), olfactory bulb, and piriform cortex. Interestingly, several putative GnRH splicing intermediates longer than mature GnRH mRNA were seen in several extrahypothalamic tissues including cortex, brain stem, cerebellum, pituitary, and ovary. To examine the splicing activity of each GnRH intron, in vitro splicing reactions using HeLa nuclear extract (NE) were performed. While intron B, and intronC could be efficiently spliced by the HeLa NE, intron A could not be or marginally spliced. Point mutation study revealed that acceptor site of intron A is very weak. There exist two putative exonic splicing enhancers (ESEs) in exon 3 and exon 4. The ESE on exon 4 (ESE4) is much stronger than that on exon 3. In the presence of the nuclear extract from GnRH-producing GT1-1 neural cells, there was an enhancement in the splicing activity of intron A. These results strongly suggest that the enhanced splicing activity of intron A is a prerequisite for mature GnRH mRNA production.