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Arylphorin from Larval Hemolymph of the Chinese Oak Silkworm, *Antheraea pernyi*

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Arylphorin (APA;*Antheraea pernyi* Arylphorin) has been purified from the 5th instar larval hemolymph of the Chinese oak silkworm, *Antheraea pernyi*. The hemolymph contained a lot of proteins including a major storage protein. APA was purified by a simple preparative PAGE and diffusion. The preparation was shown to be homogeneous more than 95% by SDS-PAGE. The native molecualr weight of APA was 460 kDa with a 80 kDa single subunit, suggesting hexamer. The protein had high amounts (17.7%, w/w) of phenylalanine and tyrosine. Rabbit antibody prepared against the purified protein cross-reacted with the 5th instar larval hemolymph proteins of *A. pernyi* and *A. yamamai*, but not with those of *Bombyx mori* and *B. mandarina*. With these results, it was concluded that APA was fall into as an arylphorin family having close relationship with *A. yamamai*. Arylphorins were known to be glycosylated. APA was stained by Schiff's reagent, suggesting a glycoprotein. Arylphorin had 4.8% (w/w) sugar, and mannose and *N*-acetylglucosamine were major components. PNGase-F digestion or hydrazinolysis produced the same high-performance liquid chromatographic profile of 2-aminobenzamide labeled glycans, suggesting APA had only *N*-linked oligosaccharides. Matrix-assisted laser desorption ionization-mass spectrometry analysis confirmed that the major glycans consisted of high-mannose type structures. Sequencing of glycan pool for further characterization showed that arylphorin had a glycan family of high-mannose type structures with and without core fucose residue. Interestingly, the most abundant form was $Man_{10}GlcNAc_2$, which was not described for *N*-glycan in insects. This work presents the characteristics of a purified arylphorin from *A.* pernyi and provided the first and most complete view of glycan structures of any arylphorins studied to date.