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Studies on the Mechanism of Eicosanoid Biosynthesis in the Primitive Arthropod, *Limulus polyphemus*

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ABSTRACT

Studies on the Mechanism of Eicosanoid Biosynthesis

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The studies presented are designed to increase our understanding of the biochemical pathways involved in inflammation using a primordial model. *Limulus polyphmus* is an ancient marine arthropod which has retained practically the same bodily form for 300 million years. *Limulus* has a single circulating blood cell, the granular amebocyte. This hemocyte was studied to determine if it could produce eicosnaoids involved in the inflammatory process, like those seen in mammalian cells, when stimulated with calcium ionophore and the fatty acid precursor, arachidonic acid. The studies revealed that the cell is capable of producing eicosanoids, and the major metabolite was 8-hydroxyeicosatetraenoic acid (8-HETE). HETEs are known to be involved in both inflammatory and reproductive processes of invertebrates and mammals.

The lipid composition of the amebocyte was then examined to determine the possible pools of eicosanoid precursors in the hemocytes. This analysis revealed large levels of twenty carbon polyunsaturated fatty acids, especially arachidonic and eicosapentaenoic acids, are present in the amebocyte membranes. The phospholipid class analysis revealed that phosphatidylethanolamine levels (42.2%) were followed by phosphatidylcholine (36.3%), phosphatidylserine (9.0%), phosphatidylinositol (6.2%) and sphingomyelin (4.6%). Cardiolipin (1.6%) was also present, as well as

trace amounts of lysophosphatidylcholine. The phosphatidylethanolamines contained plasmalogens (62%) and alkylacyl phospholipids (27%), but little diacyl phospholipid (11%). The phosphatidylcholines were diacyl (39%), alkylacyl (35%) and plasmalogen (26%). PI and PS were predominantly diacyl. Especially interesting was the presence of 16:0e/20:4 phosphatidylcholine, a precursor to platelet activating factor (PAF), and this is the first documentation of a specific PAF precursor in an invertebrate hemocyte.

A putative phospholipase A_2 (PLA_2) was partially purified by three chromatographic steps and characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot and ESI-MS. The band reactive to polyclonal recombinant human PLA_2 in the Western blot was 18.5 kDa protein in the ESI-MS analysis. A partial sequence revealed that the protein was previously described, though with alternative activity (Fujii *et al.* 1992). The protein reacted in a well-established *E. coli* PLA_2 assay and demonstrated specificity with no general lipase activity. The protein's activity was inhibited in a dose-dependent fashion by the irreversible inhibitor, manoalide, as well as by BPB which binds to histidine at the active site of PLA_2s. The protein did not lose activity after heating alone, but activity was abolished after heating with ßME. The characterization of this putative PLA_2 from *Limulus* will contribute to our understanding of the evolution of the pathways involved in inflammation, as well as possible alternate functions for this protein in invertebrates.

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