PURIFICATION AND CHARACTERIZATION OF A PHOSPHOLIPASE A2 FROM HUMAN OSTEOARTHRITIC SYNOVIAL FLUID

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SUMMARY

Phospholipase A2 (PLA2) from human osteoarthritic synovial fluid was purified to homogeneity in three steps. The NH2-terminal amino acid sequence and biochemical characteristics of the enzyme were identical to the Peak A PLA2 activity of rheumatoid synovial fluid (1). The enzyme exhibited an apparent mass of 14,000, an absolute Ca++-dependence, an alkaline pH optimum, and was inhibited by sodium deoxycholate (DOC), NaCl and 0.5 M Tris-HCl. The enzyme strongly preferred phosphatidylethanolamine (PE) as substrate over phosphatidylcholine (PC) or phosphatidylinositol (PI), and hydrolyzed PE containing arachidonic acid or linoleic acid in the sn-2 position at similar rates. Heparin bound to the enzyme but did not inhibit catalytic activity. In addition, the human enzyme was not inhibited by the acidic 'chaperone' subunit of crotoxin despite considerable sequence similarity with the basic PLA2 subunit of the neurotoxin. The enzyme was capable of hydrolyzing E. coli membrane phospholipids in the presence of the neutrophil bactericidal/permeability increasing protein (BPI). This finding, coupled to the reported pro-inflammatory activity and presence of the enzyme in inflammatory cells, supports the hypothesis that it may be a component of the host defense mechanism which can, under certain conditions, contribute to the pathogenesis of inflammatory disease.
INTRODUCTION

Phospholipases A2 are a group of generally small, Ca++-dependent enzymes which catalyze the hydrolysis of the sn-2 acyl ester bond of phosphoglycerides to produce lysophospholipids and free fatty acids (2). The enzymes are widespread in nature and occur in both cell-associated and extracellular forms. The cellular enzymes serve important physiological roles in phospholipid metabolism, and in membrane remodeling and repair (3-5). PLA2 also plays a key role in regulating the biosynthesis of inflammatory lipid mediators, including eicosanoids and platelet activating factor (PAF), by liberating their rate-limiting precursors, arachidonic acid and lyso-PAF, respectively (6-8).

Extracellular PLA2s are particularly abundant in mammalian pancreatic tissue and in the venom of snakes and arthropods, where they serve a digestive function. The venom enzymes additionally exhibit a wide range of toxic effects which may or may not depend on their enzymatic activity (9,10). Increasing evidence indicates that mammalian extracellular PLA2s may contribute to the pathogenesis of inflammatory disease (11). Circulating PLA2 levels are greatly elevated during endotoxin shock in rabbits (12) and septic shock in man (13) and correlate directly with the magnitude and duration of hypotension. The administration of purified extracellular PLA2 produces hypotension in experimental shock models (11,12). PLA2 has been found in the cell-free inflammatory peritoneal exudates of rabbits (14) and rats (15) and in the synovial fluid of patients with inflammatory arthritides (16). The circulating levels of PLA2 are also elevated in patients with rheumatoid arthritis, reflecting the systemic nature of the disease, and correlate significantly with the PLA2 activity in synovial fluid and with clinical and laboratory indices of disease activity (17). Intraplantar injection of partially purified human synovial fluid PLA2 induces edema in mice (18), and intra-articular injection of the enzyme in rats causes inflammatory and proliferative changes resembling those seen in experimental models of inflammatory arthritis and in rheumatoid arthritis (19).

Despite the growing interest in mammalian non-pancreatic extracellular PLA2s, relatively little is known about the structural and functional properties of these enzymes due to their scarcity. Recently, the extracellular PLA2s present in rabbit (14) and rat (15) inflammatory exudates, and secreted from rat (20) and rabbit (21) platelets have been isolated and partially sequenced. They were found to share considerable sequence homology with the cellular PLA2s purified from pig ileum (22) and rat spleen membranes (23). These enzymes all lacked a half cystine at residue 11, making them more closely related structurally to the Group II crotalid/viperid venom PLA2s than to the Group I enzymes of mammalian pancreas and venoms of elapid and hydrophid snakes (24).