MOBILIZATION AND FUNCTION OF EXTRACELLULAR PHOSPHOLIPASE A₂ IN INFLAMMATION¹

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A common characteristic of inflammatory exudates is the accumulation of relatively high concentrations of phospholipase A₂ (PLA-2) in the cell-free fluid (1). Because many (by-)products of this enzyme's action can exert pro-inflammatory effects (e.g. eicosanoid derivatives of arachidonic acid), these observations have prompted considerable speculation about the possible (patho-) physiological role of extracellular PLA-2 in inflammation. These ideas, in turn, have triggered many investigators and pharmaceutical companies alike to search for "specific" inhibitors of PLA-2 that could be applied therapeutically in acute or chronic inflammatory diseases.

Despite these efforts, many fundamental questions remain that need to be addressed in order to obtain a clearer understanding of the significance of extracellular PLA-2 in inflammation and of the possible means to specifically modulate its action in vivo. These questions include the source(s) of extracellular PLA-2 in inflammation, the multiplicity and diversity of these enzymes, their molecular properties and biological target(s). All extracellular PLA-2 characterized to date (from insect to man) exhibit a common set of structural and functional attributes indicative of a family of highly conserved enzymes(2). These closely similar proteins can, nevertheless, differ considerably in their action on the phospholipids of natural membranes, possibly reflecting differences in "(hyper-)variable" regions that may determine ancillary functions needed for enzyme action on specific biological targets (3,4). A single organism can elaborate multiple extracellular PLA-2, presumably to perform different biological functions (2-4). The

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mapping of variable regions may ultimately facilitate decoding of PLA-2 function and also provide a target for truly specific inhibitors of PLA-2 function.

Our studies on the mobilization and function of extracellular PLA-2 in inflammation have evolved from our interest in the biochemical mechanisms of host defense against infection. The defense of the host from invading microorganisms is generally assumed to require ultimate digestion of microbial constituents but experimental evidence of the participants and determinants of this process is scanty. Our focus has been on the determinants of bacterial phospholipid degradation which accompanies the antibacterial action of polymorphonuclear leukocytes (PMN), first-line cells in host defense, since phospholipids are essential structural elements of cells and thus their breakdown might reflect and help determine the overall destruction of the bacterium. Using Escherichia coli as a test microorganism, we have found that the antibacterial action of PMN is accompanied by bacterial phospholipid degradation in which the bacterial outer membrane phospholipase A and both a PMN and an inflammatory fluid PLA-2 participate (5,6). It has been possible to establish the participation of each of these PLA by making use of mutant strains of E. coli that vary in their expression of the pldA gene encoding the bacterial outer membrane PLA (7,8). Because the membrane phospholipids of E. coli, like that of other cells, is normally resistant to the action of either endogenous or exogenous phospholipases (5,9), PLA action on bacteria ingested by PMN implies the participation of additional factor(s) which create conditions permissive for PLA activity. We have isolated a membrane-active bactericidal protein (bactericidal/permeability-increasing protein (BPI)) from PMN which activates bacterial phospholipid degradation by both the bacterial PLA and the host PLA-2 in a manner remarkably similar to that produced when E. coli are ingested by intact PMN (5,9).

PLA-2 ACTION ON BPI-TREATED E. COLI IS HIGHLY SELECTIVE

BPI treatment of E. coli triggers bacterial phospholipid degradation by only certain PLA-2 suggesting that this is a functional attribute dependent on some variable "domain" of PLA-2 (5). Chemical modification and primary structure analyses of a so-called "BPI-responsive" enzyme that was available in sufficient quantities for these structural studies (the basic isozyme of agkistrodon halys blomhoffii venom) revealed a cluster of basic residues in the NH₂-terminal 15 residues that appeared to be important for enzyme activity toward BPI-treated E. coli but not other substrates including autoclaved E. coli (10,11). In all PLA-2 sequenced to date, residues 1-12(13) are predicted (12) (or have been observed;13) to form an alpha-helix. An axial