Release of a lysosomal phospholipase A from rabbit neutrophil leucocytes

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The appearance of prostaglandins in some inflammatory exudates is paralleled by infiltration of the inflamed area with polymorphonuclear leucocytes (PMN). A close correlation between the release of lysosomal enzymes from these cells, and the appearance of prostaglandins led to the suggestion that lysosomal phospholipases A cause the release of precursor fatty acids for prostaglandin production (Anderson, Brocklehurst & Willis, 1971). The importance of phospholipase A_2 in prostaglandin biosynthesis has been confirmed (Blackwell, Flower, Nijkamp & Vane, 1978). This communication describes the release and properties of a phospholipase A liberated from PMN leucocytes during phagocytosis of zymosan-complement (ZC) (Henson, 1971), and by calcium (Northover, 1977).

PMN's were obtained from female White New Zealand rabbits (2.5 kg) 18 h after intraperitoneal injection of sterile thioglycollate medium, U.S.P. (100 ml) as described by Northover (1977). Neutrophils (1×10^7) were incubated at 37° in medium containing (mM) NaCl 150, KCl 3, glucose 10, Hepes 5, pH 7.4, with varying amounts of ZC or calcium. Aliquots of the medium were incubated with [1-^{14}C]-oleate labelled *E. coli* for 60 mins to assay phospholipase A activity (Authi & Traynor, 1979). β-Glucuronidase, lysosyme and lactate dehydrogenase activities were also determined (Northover, 1977). Results of enzyme assays are expressed as percentages of the activities released after sonication (6 μm, 3 min) of the cells (for phospholipase activity), or treatment with Triton X-100 (0.2%).

ZC and calcium caused a time and concentration dependant increase in the release of phospholipase A activity. Maximal release of approximately 30% of the total activity was obtained at 2.5 mM calcium, or 2 mg/ml ZC. The release of phospholipase A followed that of β-glucuronidase and lysosyme, confirming a lysosomal location for the enzyme. No such release of the cytoplasmic marker lactate dehydrogenase was observed.

The released phospholipase A has two pH optima at pH 6 (broad) and pH 9 and a requirement for calcium. The enzyme appears to be of A_2 specificity since 95% of the label incorporated into phospholipid was in the 2-position and no radioactivity was recovered in the lyso product. A phospholipase A with similar properties has been found in rabbit peritoneal exudates after injection of glycogen (Fransen, *et al.*, 1978).

The lysosomal phospholipase A described may be of importance in the observed production of prostaglandins by PMN leucocytes during phagocytosis (Higgs, McCall & Youlten, 1975) and in the pathogenesis of inflammatory diseases generally.

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