MODULATION OF PHOSPHOLIPASE A₂
ACTIVITY IN HUMAN SYNOVIAL FLUID BY
CATIONS

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Abstract—Cell-free, Ca²⁺-dependent phospholipase A₂ activity (PLA₂) was measured in human synovial fluid of patients with various kinds of arthritis using [1-¹⁴C] oleate-labelled autoclaved Escherichia coli as substrate. PLA₂ activity at pH 7.0 and with 5 mM added Ca²⁺ was stimulated and then inhibited in a dose-dependent fashion by NaCl; maximal stimulation of 8.8 fold was found at 150 mM Na⁺. Similar effects were obtained with K⁺, Li⁺ and Ru⁺. In the absence of added Na⁺, PLA₂ activity was maximal with 25 mM Ca²⁺ (145 nmols/hr/mg), but in the presence of 150 mM Na⁺, activity was maximal with 4 mM Ca²⁺ (415 nmols/hr/mg). PLA₂ activity was optimal between pH 6.5 - 8.0 in presence of 150 mM Na⁺ and 4 mM Ca²⁺. There was no significant difference between PLA₂ activity in synovial fluids from rheumatoid and other types of arthritis. Neutral active, Ca²⁺-dependent PLA₂ activity in acid extracts of human platelets, plasma, polymorphonuclear leukocytes and synovial fluid varied in response to added Na⁺. Neutral active, Ca²⁺-dependent PLA₂ activity in acid extracts of human platelets, plasma, polymorphonuclear leukocytes and synovial fluid varied in response to added Na⁺. In presence of 150 mM added Na⁺ and 5 mM Ca²⁺, PLA₂ activity in human synovial fluid was inhibited by all multivalent cations tested. In the absence of Na⁺, Cu²⁺ and Mg²⁺ stimulated PLA₂ activity in a dose dependent fashion; whereas, Fe²⁺, Fe³⁺ and Al³⁺ were inhibitory. The extent of stimulation by Mg²⁺ was inversely related to the concentration of added Ca²⁺.

INTRODUCTION

Mammalian cells and organs (1–3), in general, contain two major classes of phospholipases A (PLA). Membrane-associated phospholipases A that are Ca²⁺-dependent and active at neutral pH (1) and lysosomal phospholipases A that are active at acid pH are generally soluble and inhibited by mono and divalent cations (3). We (4, 5) and others (6, 7) have demonstrated the presence of a potent cell-free, neutral-active, and Ca²⁺-dependent PLA₂ in the synovial fluid.
of patients with various kinds of arthritis. Both local and systemic inflammatory processes in human and animals are associated with high levels of extracellular phospholipase A₂ activity (8–10), and stimulated inflammatory cells release phospholipases A₂ (11–13). Recently, attention has been focused on possible proinflammatory actions of extracellular PLA₂s and their products since this enzyme contributes to the arachidonate mobilization and eicosanoid production in normal cell function and in injury. In this paper, we describe the optimization of the in vitro assay for neutral active, Ca²⁺-dependent PLA₂ activity in human synovial fluid and the unusual modulation of enzymatic activity by mono- and multivalent cations.

MATERIALS AND METHODS

The chemicals and reagents used in this study were of analytical grade or better. Synovial fluids were obtained from patients by the Staff of Connective Tissue Diseases, Medical College of Virginia, Richmond. Fluids were aspirated from active joints of patients with diagnosed arthritis; using plastic syringes without anticoagulant, fluids were centrifuged at 400 g for 15 min to sediment cells. The resulting cell-free supernatant was diluted 1 : 49 (v/v) with ice-cold distilled water and was assayed for PLA₂ activity and protein content. An acid extract was prepared by mixing equal volumes of synovial fluid and ice cold 0.36 N sulfuric acid–1.6 M NaCl. After 3 h at 4°C, the mixture was centrifuged at 10,000 g for 30 min. The supernatant was dialyzed overnight against two changes of 10 mM sodium acetate buffer, pH 4.5, and the dialysate was centrifuged at 10,000 g for 30 min. The resulting supernatant is designated acid extract. Outdated human platelets were acid extracted as described by Jesse and Franson (14). Human plasma was obtained from the blood of healthy donors. An acid extract was prepared as described under preparation of synovial fluid acid extract. Isolation and acid extraction of human polymorphonuclear leukocytes were done as described by Marki and Franson (15). PLA₂ activity was measured by an established method using [1-¹⁴C]oleate-labeled autoclaved E. coli as substrate (16). Assay mixtures in total volume of 0.5 ml contained 100 mM BisTris, pH 7.0, 5.0 mM Ca²⁺, and 2.5 × 10⁵ cells of autoclaved E. coli phospholipid (5000 cpn and 5 nmols phospholipid) unless indicated otherwise. Time and protein content were adjusted to ensure linear kinetics. The reaction was stopped by the addition of 3.0 ml chloroform–methanol (1 : 2, v/v), and the lipid was extracted and separated as previously described (16). PLA₂ activity is expressed as nanomoles phospholipid hydrolyzed per hour per milligram protein. Protein was determined by the method of Bradford (17) using BSA as standard.

RESULTS

In preliminary experiments, we noted that PLA₂ activity in human synovial fluid was modulated by the concentration and chemical nature of the buffer used. Figure 1 shows the effects of several buffers on PLA₂ activity. Tris HCl, a primary amine, stimulated enzymatic activity in a concentration-dependent manner, whereas BisTris and HEPES, as tertiary amines, had no effect on activity regardless of the concentration used. Because the stimulatory effects of