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Phospholipase A_2 of human monocytes with molecular weight of 73kD induces the production of prostaglandin derivatives and leukotriene B_4 in the suspension of human neutrophil granulocytes

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In our laboratory the phospholipase A_2 (PLA_2) with molecular weight of 73 kD, i.p. of 8.2 (PLA_2-73) was described [6]. This enzyme derives from human mononuclear cells, supposedly from monocytes, and represents a new type of human phopholipases A_2, not known earlier [10, 11]. We observed previously that this enzyme can induce the production of platelet activating factor (PAF) in the suspension of human neutrophils [6] and can cause a suppression of the random migration of these cells [7]. The production of PAF requires the activation of PLA_2 in the leukocytes [8]. At the same time, PLA_2 produces arachidonic acid, as well [5]. The derivatives of arachidonic acid, especially prostaglandin $F_{1\alpha}$ and $F_{2\alpha}$ ($PGF_{1\alpha}$, $PG_{2\alpha}$) and leukotriene B_4 (LTB_4) are potent mediators of inflammations, beside PAF [4, 9]. In this study, using mass spectrometric analysis, we have found $PGF_{1\alpha}$, $PGF_{2\alpha}$ and LTB_4 in the suspension of human neutrophils treated with PLA_2-73. In a next series of experiments, we have demonstrated that the protein of 68 kD, often observable in the solution of PLA_2-73, is an enzymatically inactive degradation product of the active enzyme of 73 kD.

Materials and Methods

Preparations of cells

Heparinized venous blood was obtained from healthy volunteers. Mononuclear cells and neutrophils were separated by Ficoll (Pharmacia Fine Chemicals, Sweden) — Uromiro (Bracco Industria Chimica, Italy) density centrifugation followed by dextran sedimentation of the neutrophil-rich pellet. Residual erythrocytes were lysed with hypotonic saline. The neutrophil granulocytes were washed and suspended in TC-199 media [2]. Their purity was above 95%.

Preparation and identification of phospholipase A_2 was carried out according to our previous description [6].

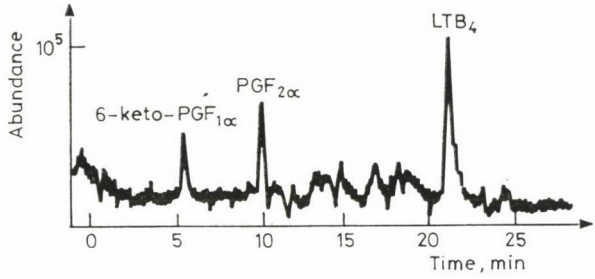

FIG. 1. TSP-HPLC-MS total ion current profile of a supernatant of human neutrophil granulocytes treated with PLA_2-73

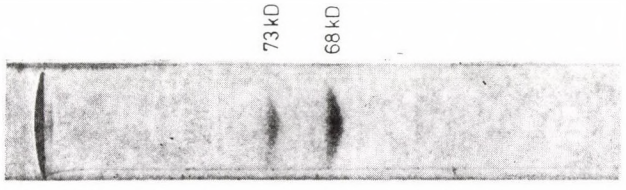

FIG. 2. SDS-PAGE picture of active phospholipase A_2 (73 kD) and of inactive fragment of enzyme (68 kD)

Identification of prostaglandin derivatives and leukotriene B_4

Thermospray-high-performance liquid chromatography-mass spectrometry (TSP--HPLC-MS) was carried out [1] for the detection of $PGF_{1\alpha}$, $PGF_{2\alpha}$ and LTB_4 in the supernatants of neutrophils, treated with PLA_2-73.

RESULTS

In order to measure the production of $PGF_{1\alpha}$, $PGF_{2\alpha}$ and LTB_4 by human neutrophils, we have analysed the TSP-HPLC-MS characteristic peaks of $PGF_{1\alpha}$, $PGF_{2\alpha}$ and LTB_4 in the culture supernatants of neutrophils treated with PLA_2-73 for 24 hours at 37 °C. Figure 1. illustrates that the characteristic peaks of $PGF_{1\alpha}$, $PGF_{2\alpha}$ and LTB_4 molecules could be found in the spectrum of the supernatant derived from the neutrophil cell culture.

Figure 2 illustrates the protein bands of the active enzyme (73 kD) and of the inactive fragment of enzyme (68 kD). The inactivation and degradation of purified active enzyme can take place even at its storage at −20 °C. During the storage the quantity of molecule of 73 kD gradually decreases, and that of the 68 kD increases in the solution.

DISCUSSION

The pathobiological role of secretory phospholipases A_2 has been proved in a series of diseases, especially in inflammations [10]. The potent mediators, PAF and the derivatives of arachidonic acid (e.g. $PGF_{1\alpha}$, $PGF_{2\alpha}$ and leukotrine B_4) play central roles in the actions of phospholipases A_2 [3]. Our finding that PLA_2-73 produ-

ces not only PAF (6) but also $PG_{1\alpha}$, $PG_{2\alpha}$ and LTB_4 in the suspensions of human neutrophils underlines the importance of this new type of PLA_2 enzymes in the regulation of inflammations.

The large scaled purification procedure of this enzyme needs still further biochemical efforts. As our data show the inactivation and degradation of PLA_2-73 can take place even at a storage temperature of -20 °C. We suggest to regard the molecule of 68 kD to be the enzymatically inactive fragment of the complete, active enzyme of 73 kD.

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