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LEUCINE AMINOPEPTIDASE AS AN ECTO-ENZYME OF POLYMORPHONUCLEAR NEUTROPHILS

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Summary

Intact polymorphonuclear neutrophils were modified chemically by a poorly permeable reagent, diazotized sulfanilic acid, and the changes in the activity of 5′-nucleotidase, alkaline phosphodiesterase, and leucine aminopeptidase were examined. Among three plasma membrane enzymes, 5′-nucleotidase activity was hardly detected in the human neutrophils. The activity of alkaline phosphodiesterase was observed in all the neutrophils examined, but was not inhibited by diazotized sulfanilic acid in the guinea-pig neutrophils. On the other hand, the activity of leucine aminopeptidase was not only found but also inhibited by diazotized sulfanilic acid without the inhibition of lactate dehydrogenase, a cytosol enzyme, in all the neutrophils, suggesting that leucine aminopeptidase is located generally on the plasma membrane as an ecto-enzyme in the neutrophils.

The preparation of the plasma membrane has been needed to elucidate the mechanism of the chemotactic inhibition of the neutrophils by a chemical modification [1, 2]. At present, 5′-nucleotidase, alkaline phosphodiesterase, Mg^{2+}-ATPase and leucine aminopeptidase are generally reported to be the marker enzymes of the plasma membrane [3, 4]. As for the neutrophils, among these enzymes, 5′-nucleotidase [5] and Mg^{2+}-ATPase [6] have been fairly well studied and proved to be ecto-enzymes by a chemical modification of intact cells with diazotized sulfanilic acid. However, 5′-nucleotidase is not widely distributed in all the neutrophils, i.e. it is not found in the human neutrophils [7, 8]. Alkaline phosphodiesterase is known to be associated with the plasma membrane [4] but it is not clear whether alkaline phospho-

diesterase is an ecto-enzyme or not. There is little information on leucine aminopeptidase except for the observations of Davies et al. [9] that leucine aminopeptidase activity was involved in the postgranular fraction of the rabbit neutrophils and of Wachsmuth [10] that no aminopeptidase activity was found immunohistochemically in lymphocytes and granulocytes.

In this communication, therefore, the presence of leucine aminopeptidase in the neutrophils and its subcellular localization were examined with reference to 5′-nucleotidase and alkaline phosphodiesterase and the results were obtained that leucine aminopeptidase was located on the plasma membrane as an ecto-enzyme in not only guinea-pig but also human and rabbit.

The guinea-pig neutrophils were obtained from glycogen-induced peritoneal exudates as described previously [11]. The rabbit neutrophils were prepared from glycogen-induced peritoneal exudates as described previously [11] with a slight modification: acid citrate dextrose was used as an anticoagulant instead of sodium citrate. The human neutrophils were prepared from acid citrate dextrose-anticoagulated venous blood by means of dextran-sodium metrizoate sedimentation followed by the hypotonic lysis of erythrocytes. A chemical modification of the neutrophils was carried out by incubating $1 \cdot 10^7$ cells/ml with diazotized sulfanilic acid at a concentration of 1 mM for guinea-pig, 2.5 mM for human or 0.25 mM for rabbit at 37° C in a final volume of 4.0 ml. At indicated time intervals, each neutrophil suspension was washed twice with ice-cold 0.9% NaCl to stop the reaction. The washed neutrophils were suspended in 2 ml of 0.9% NaCl, homogenized with a Teflon-glass homogenizer at 0° C for 20 min to disrupt completely and finally assayed for leucine aminopeptidase, 5′-nucleotidase, alkaline phosphodiesterase and lactate dehydrogenase. Leucine aminopeptidase activity was measured by the method of Goldbarg and Rutenburg [12] with a slight modification. Alkaline phosphodiesterase and 5′-nucleotidase activities were measured by a modification of the method of Touster et al. [13]. Lactate dehydrogenase activity was measured by the disappearance of NADH at 340 nm upon conversion of pyruvate to lactate [14]. Diazotized sulfanilic acid was prepared as outlined by DePierre and Karnovsky [5] and the concentration of diazotized sulfanilic acid was determined by the method of Edelson and Erbs [15].

The activities of enzymes known to be the marker for the plasma membrane are shown together with lactate dehydrogenase, a cytosol marker, in Table I. As reported already [7, 8], 5′-nucleotidase was hardly involved in the human neutrophils and alkaline phosphodiesterase was present in all the neutrophils examined. Leucine aminopeptidase was found in not only rabbit but also human and guinea-pig, suggesting that leucine aminopeptidase as well as alkaline phosphodiesterase would be widely distributed in the neutrophils. Next, the question as to whether these enzymes are present as ecto-enzymes was studied. As can be seen in Fig. 1A, when the intact neutrophils were treated at 37° C with diazotized sulfanilic acid, the ability of the neutrophils to exclude the trypan blue was not affected at all until at least 15 min, indicating no death of cells with a chemical modification. Lactate dehydrogenase, a soluble cytoplasmic enzyme, used as an internal indicator, was unaffected until 5 min and then inhibited with increasing modification time, i.e.

TABLE I

ENZYME ACTIVITY OF PLASMA MEMBRANE MARKERS OF GUINEA-PIG, HUMAN AND RABBIT NEUTROPHILS WITHOUT AND WITH TRITON X-100

Enzyme assays were carried out using homogenates prepared as in the text. In the case of Triton added, homogenates were exposed to 0.1% Triton X-100 at 0°C for 10—20 min and then assayed for enzyme activity. Leucine aminopeptidase activity (LAP) was measured by incubating homogenate (corresponding to $4 \cdot 10^5$ neutrophils) at 37°C for 2 h in a total volume of 1.5 ml of 67 mM phosphate buffer (pH 7.0) with 0.46 mM L-leucyl-β-naphthylamide hydrochloride, 5′-Nucleotidase (5′-N) was determined after 1 h of incubation of homogenate ($2 \cdot 10^6$ neutrophils) at 37°C with 5 mM AMP in 50 mM glycine buffer (pH 9.1) containing 10 mM $MgCl_2$ in a total volum of 0.5 ml. Alkaline phosphodiesterase (APD) was assayed by incubating homogenate ($4 \cdot 10^6$ neutrophils) with 0.71 mM *p*-nitrophenyl 5′-thymidylate at 37°C for 2 h in a total volume of 0.7 ml of 14.3 mM Tris-HCl buffer, pH 9.0. Values give mean activity in milliunits per $1 \cdot 10^7$ cells ± S.D.; number of determinations in parentheses. One unit of activity is defined as the amount of enzyme that split 1 μmol of substrate in 1 min under the condition given. In the case of Triton added, the activity (the mean of two experiments) is expressed as the percent of control activity (no Triton). LDH, lactate dehydrogenase.

	Enzyme activity											
	Guinea-pig				Human				Rabbit			
	No Triton			Triton added	No Triton			Triton added	No Triton			Triton added
LAP	14.9	± 1.2	(7)	109	19.1	± 4.8	(3)	97	4.6	± 0.2	(3)	76
APD	0.263	± 0.050	(6)	129	0.183	± 0.023	(3)	172	1.417	± 0.333	(3)	99
5′-N	34.0	± 8.0	(6)	219	0.87	± 0.56	(5)	—	9.0	± 2.8	(3)	140
LDH	476.0	± 55.7	(5)	100	411.2	± 81.0	(3)	102	298.8	± 71.0	(3)	106

approx. 5% inhibition was observed in 10 min and 10% inhibition in 15 min. However, lactate dehydrogenase was more sensitive to diazotized sulfanilic acid than the plasma membrane marker enzymes if homogenate was modified, indicating that diazotized sulfanilic acid is too poorly permeable to inhibit lactate dehydrogenase during a short modification time in the intact neutrophils. Leucine aminopeptidase was markedly inhibited within 5 min when lactate dehydrogenase was unaffected, suggesting the possibility that leucine aminopeptidase is an ecto-enzyme. 5′-Nucleotidase was less inhibited than leucine aminopeptidase under our conditions but approx. 50% inhibition was obtained in 10 min, implying that 5′-nucleotidase is also an ecto-enzyme. On the other hand, alkaline phosphodiesterase, known to be an ecto-enzyme in macrophages [15], was not inhibited at all even after 30-min modification, although not shown. If homogenates were used, however, alkaline phosphodiesterase was completely inhibited. Therefore, the active site of alkaline phosphodiesterase does not seem to face the external medium in the guinea-pig neutrophils. The activation by detergents of enzymes associated with the membrane is well known [5, 16]. As seen in the Table I, lactate dehydrogenase, a soluble enzyme, was not activated by Triton X-100 and leucine aminopeptidase activity was hardly affected by the detergent, supporting the fact that the active site of leucine aminopeptidase would be exposed to the external medium. The existence of leucine aminopeptidase as an ecto-enzyme on the plasma membrane of the neutrophils other than those of guinea-pig was investigated with reference to alkaline phosphodiesterase and 5′-nucleotidase. As shown in Fig. 1B, both leucine aminopeptidase and alkaline phosphodiesterase were similarly inhibited with a chemical modification, whereas lactate dehydrogenase was hardly inhibited, suggesting that leucine amino-

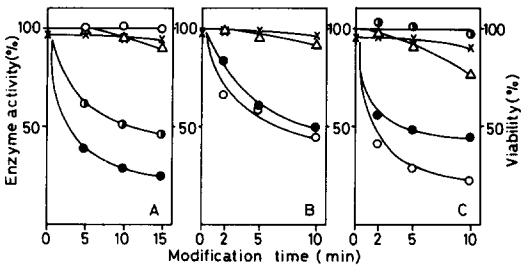

Fig. 1. Time course of the effect of diazotized sulfanilic acid on enzymes of guinea-pig, human and rabbit intact neutrophils. A, guinea-pig; B, human; C, rabbit. Enzyme activities are expressed as percentages of those measured for untreated cells. The key to the figures is as follows: ●——●, leucine amino peptidase; ◐——◐, 5′-nucleotidase; ○——○, alkaline phosphodiesterase, △——△, lactate dehydrogenase; X——X, viability checked by the trypan blue exclusion test [11]. For details, see the text.

peptidase and alkaline phosphodiesterase are both present as ecto-enzymes in the human neutrophils. With the rabbit neutrophils, leucine aminopeptidase and alkaline phosphodiesterase were rapidly inhibited by a modification, whereas the 5′-nucleotidase activity remained unchanged (Fig. 1C). Rabbit lactate dehydrogenase was a little inhibited by a chemical modification compared with the other neutrophils, suggesting that diazotized sulfanilic acid was more permeable in the rabbit neutrophils.

Among three plasma membrane markers examined, 5′-nucleotidase was hardly detected in human. Alkaline phosphodiesterase was found in guinea-pig, human and rabbit, but no evidence for the ecto-enzyme was obtained in guinea-pig. On the other hand, leucine aminopeptidase was observed with all the neutrophils examined and proved to be present as an ecto-enzyme. Therefore, leucine aminopeptidase seems to be an even better marker for the plasma membrane of the neutrophils than the other enzymes.

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