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Enzyme Activity in Human Mononuclear Blood Cells

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Summary. Two different kinds of mononuclear blood cell samples (mixed blood cell population consisting of lymphocytes, monocytes, and platelets; pure lymphocyte population) were tested for enzyme activity of mitochondrial malic enzyme, peptidase A, and α-fucosidase. None of the three enzymes was demonstrable in the pure lymphocyte preparation; the mixed cell population, however, exhibited a distinct enzyme activity. Positive reactivity is thus obviously dependent upon monocytes and/or platelets.

Key words: Enzyme activity, malic enzyme, peptidase A and α-fucosidase – Malic enzyme, activity – Peptidase A, activity – α-fucosidase, activity

Zusammenfassung. Zwei unterschiedliche Präparationen mononuclearer Blutzellen (eine gemischte Zellpopulation, bestehend aus Lymphozyten, Monozyten und Thrombozyten in einem Fall, eine Präparation reiner Lymphozyten im anderen) wurden auf die Aktivität der Enzyme Malic enzyme, Peptidase A und α-Fucosidase überprüft. Keines der drei Enzyme konnte in der reinen Lymphozytenpräparation nachgewiesen werden. In der gemischten Zellpopulation war der Nachweis in jedem Fall erfolgreich. Die Expression dieser Enzyme ist also offenbar an Mono- bzw. Thrombozyten gebunden.

Schlüsselwörter: Enzymaktivität, Malic enzyme, Peptidase A und α-Fucosidase – Malic enzyme, Aktivität – Peptidase A, Aktivität – α-Fucosidase, Aktivität

Introduction

The existence of genetically determined polymorphisms of various enzymes in different cell populations is as well known as is the variety of expressions and activities of enzymes in different cell populations. Moreover, Radzun et al. (1980) recently reported that different isozymes may exist in different blood cell

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populations, especially in lymphocytes and monocytes. It seemed logical, therefore, to type enzymes in isolated blood cell populations.

In this article we compare enzyme activities and their distribution in an isolated lymphocyte population with those in a mixed blood cell population (i.e., lymphocytes, monocytes, platelets). The enzymes determined were malic enzyme (mitochondrial) (ME 2: E.C. 1.1.1.40), peptidase A (PEP A: E.C. 3.4.11), and α-fucosidase (FUCA: E.C. 33.2.1.51). These enzymes have already been demonstrated in a variety of tissues (Rapley et al. 1971; Saha et al. 1978). Siebert et al. (1979), in particular, tested the presence of ME 2 in polymorphnuclear leukocytes.

Materials and Methods

Isolation of Blood Cells

Two samples of fresh heparinized blood (2×7 ml) were obtained from each of five healthy individuals. White blood cell populations were isolated immediately after venous puncture. The cell isolation methods as applied to each of the two samples are described below.

To obtain pure lymphocyte populations one sample was treated according to the method given by Böyum (1976): The blood was defibrinated with glass beads, layered on top of 3 ml Lymphopräp (Nyegaard Co., Oslo, Norway), and centrifuged at 800 *g* for 20 min.

Mixed cell populations consisting of lymphocytes, monocytes, and platelets were found in the layer at the top of the Lymphopräp solution using plastic EDTA-tubes to prevent clotting. The leukocyte containing layer was taken off and centrifuged again.

The final cell concentrations in each of the cell populations ranged from 0.5 to 1.0×10^{-5} cell per milliliter. A smear made from the resulting sediment was stained according to May-Grünwald/Giemsa. Cell viability was tested using trypan blue stain. The remainder of the sediment was stored at $-70°$ C until use.

Preparation of Samples

Preparation of white cell lysates was done according to the procedure described by Siebert et al. (1979). For typing of FUCA 20 µl of the white cell lysate was incubated with an equal volume of 1 mg/ml Neuraminidase (Boehringer) for 2 h at 37° C. Fifteen microliters of this mixture was applied to the gel surface by means of filter papers (Whatman No. 1).

Detection of Enzyme Activity

Electrophoresis and staining of ME 2 and PEP A was carried out as described by Kömpf et al. (1979) and Siebert et al. (1979). Fucosidase was typed by isoelectric focusing (IEF) (Kömpf 1980).

The methods applied are described briefly.

PEP A and ME 2 were typed using horizontal starch gel electrophoresis. PEP A: Bridge buffer consisted of 0.165 *M* Tris, 0.2 *M* His-HCl, adjusted to pH 6.0 with 1 *N* HCl. Gel buffer was a 1 : 10 dilution of bridge buffer. 18% starch (Connaught). Time of experiment: 18 h (9 V/cm), at 4° C. Staining mixture: 2 mg% Leu-Ala, 2 mg% α-ketoglutarate-Na_2 salt, 1 mg% NADH, 0.5 mg% MTT, 3 µl/ml LDH in 0.5 *M* Tris HCl buffer, pH 8.2.

ME 2: Bridge buffer: 0.2 *M* Tris, 0.2 *M* His-HCl, pH 7.6. Gel buffer was a 1 : 7 dilution of the bridge buffer, 18% starch. Time of experiment: 14 h, 5 V/cm, at 4° C. Staining mixture: 3 g% malic acid, 6 g% Tris, 0.5 g% $MgCl_2$, 50 mg% NADP, 25 mg% MTT, and 100 µl% of a 1% solution of Meldolablau (Boehringer).

FUCA was typed on PAG plates, pH range 3.5–9.5 (LKB). Conditions of run were according to the recommendations of LKB. Staining mixture: 0.3 mg% 4-methylumbelliferyl-L-fucoside (Sigma) in 0.1 *M* potassium phosphate-citric acid buffer, pH 4.9. To demonstrate the different fucosidase gene products the middle part (5×24 cm) of the PAG plates was carefully covered with

CA-electrophoresis foils (CA 251/0, Schleicher & Schüll), soaked with the staining mixture, incubated for 30 min at 37° C, and inspected under UV light.

Results and Discussion

Viability tests were positive each time for about 95% of the cells. Virtually, a 100% lymphocyte population was obtained after defibrination with beads.

The EDTA blood showed a mixed cell population with numerous platelets as well as lymphocytes and monocytes in a 1:1 ratio.

ME2 activity is well detectable in the mixed cell population (platelets, monocytes, and lymphocytes), but is absent in the pure lymphocyte preparations. This result is consistent with the observation of Siebert et al. (1979) that the ME2 polymorphism can be clearly demonstrated from crude white cell preparations. It supports also previous findings of Cohen and Omenn (1972) who could not detect any ME2 activity in phytohemagglutinin (PAH)-stimulated lymphocytes either.

The common polymorphism of peptidase A was described by Lewis (1973) when he subjected samples to electrophoresis which were made from the total of the white cell population. The results from this investigation indicate that from pure lymphocyte preparations no PEP A-activity can be demonstrated. Samples containing platelets and monocytes, however, show normal enzyme activity. That the staining is just too weak to be detected due to the substrate used is improbable; in our separation and staining system the best results have been obtained by using Leu-Ala as dipeptide.

As was the case with ME2 and PEP A, FUCA activity was only demonstrable from mixed white cell preparations. Turner et al. (1974) found FUCA activity in leukocytes, fibroblasts, and long-term lymphoid cell lines. Since these lines were established by culture after PAH stimulation and partial incubation with Epstein-Barr virus, the demonstration of FUCA activity from this cell population does not seem to be comparable with the findings in freshly prepared lymphocyte samples.

On the basis of these findings it can be concluded that the enzyme activity of ME2, PEP A, and FUCA observed in the mixed blood cell samples can be attributed to monocytes and/or platelets.

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