LEUKOCYTE CREATINE KINASE OF RABBIT PERITONEAL EXUDATE

I. P. Ashmarin, S. N. Lyzlova, L. S. Nersesova, and I. S. Freidlin

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The presence of creatine kinase was established in monocytes and polymorphs of the rabbit peritoneal exudate. Creatine kinase activity in monocytes was more than six times higher than in the polymorphs. Michaelis constants were determined and their values were: for the enzyme from monocytes, for creatine phosphate $2.66 \cdot 10^{-3}$ M, for Mg-ADP $1.98 \cdot 10^{-4}$ M; for the enzyme from polymorphs, for creatine phosphate $3.50 \cdot 10^{-3}$ M, and, for Mg-ADP $1.72 \cdot 10^{-4}$ M. Electrophoretic fractionation of extract of monocytes revealed only one enzyme whose electrophoretic mobility corresponds to that of the isoenzyme of brain type.

The high energy metabolism of leukocytes [15] and the fact that they contain a mysoin-like adenosine triphosphatase [17] suggested that these cells must also contain another enzyme which is a usual participant in energy utilization and accumulation processes, namely creatine kinase (ATP: creatine-phosphotransferase; 2.7.3.2). No information on the creatine kinase of leukocytes could be found in the literature. Indeed there is direct evidence of the absence of this enzyme in granulocytes and erythrocytes of human blood [16].

To test the hypothesis stated above the first essential step was to use a sensitive method to determine creatine kinase activity and the second was to use cells in the most active state as the test object.

EXPERIMENTAL METHOD

Leukocytes from rabbit peritoneal exudate were obtained by the method of Cohn et al. [8-10] with slight modifications. The formation of exudate was induced by intraperitoneal injection of 300 ml of 0.5% glycogen solution in 0.9% NaCl solution or of a 2% solution of starch in 0.9% NaCl into male rabbits weighing 2.5-3 kg. The solutions were first sterilized. To obtain polymorphs the exudate was removed 3 h after injection of the inducing solution. The yield of exudate was 150-200 ml. The cell count in the exudate varied between $2 \cdot 10^8$ and $7 \cdot 10^8$.

To prevent clotting heparin solution (5 units to 1 ml exudate) was added to the exudate. The exudate withdrawn was filtered through four layers of gauze and centrifuged at 760 g for 15 min to deposit the cells. The residue was washed twice with ice-cold physiological saline. If the cells were contaminated with erythrocytes, these were removed by hemolysis with 0.83% NH_4Cl solution, which does not damage leukocytes [15]. The leukocytes isolated in this way contained 99% of polymorphs [8, 9].

To obtain monocytes the exudate was withdrawn after 3 days. The cells were washed out with 200 ml 0.02% sterile versene solution which was injected under pressure into the rabbit's peritoneal cavity. Usually the volume of exudate was 150-170 ml and it contained $1 \cdot 10^8$–$9 \cdot 10^8$ cells, of which 90% were monocytes [10].

To prepare the extracts the residue of washed cells was homogenized in water with cooling for 5 min. The homogenates were centrifuged for 20 min at 20,000 g. The freshly obtained supernatant was used for the estimation of creatine kinase activity [3, 11].

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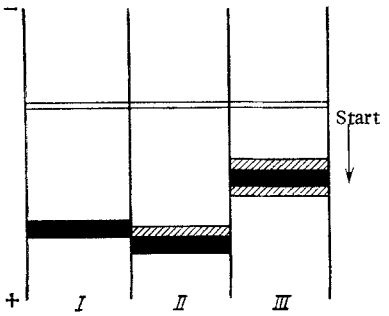

Fig. 1. Distribution of creatine kinase isoenzymes after starch gel electrophoresis of extracts of monocytes (I), brain (II), and skeletal muscle (III) of rabbit.

The reaction velocity remained constant for the first 3-6 min. The quantity of creatine formed also was directly proportional to the volume of cell extract taken. The creatine kinase isoenzymes were fractionated by electrophoresis of the proteins of the leukocyte extracts in starch gel [18].

EXPERIMENTAL RESULTS

The results show that leukocytes contain substantial creatine kinase activity, which differed in the monocytes and polymorphs of the rabbit peritoneal exudate. In the monocytes its activity was about six times higher than in the polymorphs $\bar{x}$ (the corresponding values of $\bar{x}$ were 31,070 and 4613 μmoles creatine/h/10^{11} cells). These differences were evidently attributable to differences in the functional activity of the different types of leukocytes. Inequality of the activity of the enzymes from different types of leukocytes is also mentioned in the literature [9, 14].

The Michaelis constant (K_m) for Mg-ADP for creatine kinase from the two types of cells was about equal ($1.98 \cdot 10^{-4}$ M for monocytes and $1.72 \cdot 10^{-4}$ M for polymorphs). In the case of K_m for creatine phosphate, this was a little higher ($3.50 \cdot 10^{-3}$ M) for the creatine kinase from polymorphs than for the enzyme from monocytes ($2.66 \cdot 10^{-3}$ M). It is interesting to note that in both cases K_m for the substrates tested agreed closely with the values of K_m for creatine kinase from rabbit brain [12].

The electrophoretic mobility of creatine kinase from monocytes was then determined and compared with the electrophoretic mobility of the enzyme from rabbit muscles and brain. The results showed that the enzyme from leukocytes moves in an electric field toward the anode at the same rate as creatine kinase from the brain (Fig. 1). No other creatine kinase isoenzymes were found in these tests. Leukocyte creatine kinase thus resembles brain creatine kinase in its K_m value and electrophoretic mobility. Presumably this type of isoenzyme in nerve tissue is the phylogenetically oldest form of creatine kinase.

The question arises whether creatine kinase plays such an important role in the energy metabolism of leukocytes as in muscle tissue. All that can be said at the moment is that the existence of a mechanochemical system linked with ATP as the source of energy has been postulated in leukocytes [17] and that an actomyosin-like protein participates in the movements of leukocytes [5, 17]. If granulocytes are deprived of glucose for a short time there is no particularly sharp decrease in their concentrationor rate of renewal of labile phosphorus of ATP [4, 13]. The possibility cannot therefore be ruled out that the necessary ATP level can be maintained in leukocytes in the absence of glucose by glycogenolysis and the creatine kinase reaction.

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