ISOENZYMES OF GLYCOGEN PHOSPHORYLASE IN HUMAN LEUKOCYTES AND PLATELETS: RELATION TO MUSCLE PHOSPHORYLASE[#]

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Skeletal muscle phosphorylase b requires AMP for activity becoming fully active in the presence of the nucleotide. Inactive liver phosphorylase is not significantly affected by AMP (1, 2).

Previous studies on leukocyte phosphorylase have shown that this enzyme is more closely related to that of liver (3 - 6). Recently, Scott (7) reported that platelet phosphorylase activity was significantly stimulated by AMP, suggesting that this enzyme may be related to that of muscle, thus differing in this respect from the leukocyte enzyme. In the studies to be described, platelet phosphorylase displayed properties intermediate between those of muscle and those of liver phosphorylase. From analysis on acrylamide gel it became apparent that these findings were due to the existence in platelets of phosphorylase isozymes predominant among which is a "muscle type" enzyme.

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Materials and Methods

Yeast AMP crystalline sodium salt, ATP sodium salt, D-glucose-1-phosphate dipotassium salt, glycogen (shellfish) and DEAE cellulose were obtained from Sigma Chemical Company, St. Louis, Mo. Streptomycin sulfate was obtained from Squibb Company, New York, N. Y.

Isolation of platelets and leukocytes. Blood was collected from normal donors in EDTA and sodium fluoride (final concentration of 0.001 M and 0.02 M respectively). Platelets were isolated by differential centrifugation. Leukocytes were isolated by dextran sedimentation, differential centrifugation and lysis of contaminating red cells by hypotonic medium (8). Leukocyte preparations contained less than one platelet for each 10 leukocytes. Platelet preparations were virtually free of any other cells (Fig. 1). Cells were homogenized in 3 volumes of 0.001 M EDTA - 0.05 M NaF in a Virtis Homogenizer.

Human skeletal muscle was obtained at autopsy.

Phosphorylase Assay. - Phosphorylase activity was measured in the direction of polysaccharide synthesis by a modification of the method of Sutherland as previously described (4).

Phosphorylase activation. - Conversion of inactive enzyme to the active form was accomplished by incubating extracts at 37°C in 0.01 M Mg - 0.01 M ATP at pH 7.0 for 30' (4).

Disc. electrophoresis of the active enzyme on acrylamide gel (9) and localization of phosphorylase activity bands by histochemical staining were performed as previously described (5).

Three times crystallized rabbit muscle phosphorylase b was prepared as described by Fischer and Krebs (10). Anti-enzyme globulin was

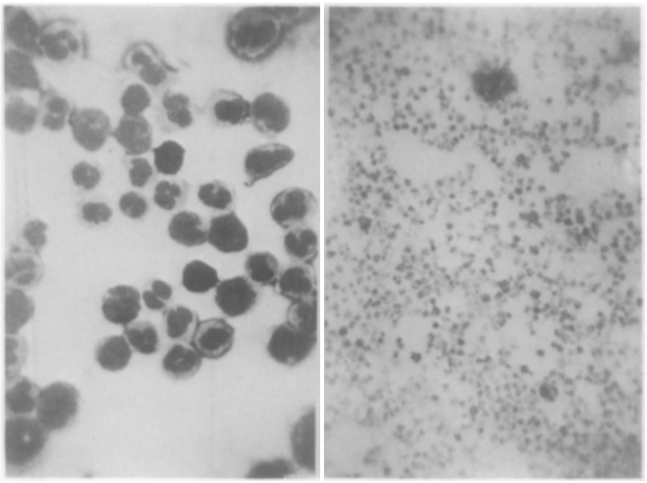

Fig. 1. Wright-stained film of leukocyte (left) and platelet (right) preparations.

prepared in roosters according to the method of Yunis and Krebs (11).

Partial Purification. - Extracts were fractionated with saturated ammonium sulfate as previously described (5). The fraction between 33 and 50 percent saturation was collected and dialyzed against 0.005 M Tris - 0.001 M EDTA - 0.05 M NaF pH 7.5.

Results

Phosphorylase activity with AMP expressed as μmoles P_i/30'/mg. of protein in the extract varied from 3.2 - 4.5 in leukocytes and from 2.5 - 4.0 in platelets. The initial activity ratio $\frac{\text{without AMP}}{\text{with AMP}}$ was 0.37 for platelets and 0.5 for leukocytes (Fig. 2). Incubation in 0.001 M EDTA, pH 7.0 at 37°C resulted in almost complete conversion to the inactive form. Inactive platelet enzyme was markedly stimulated by AMP exhib-

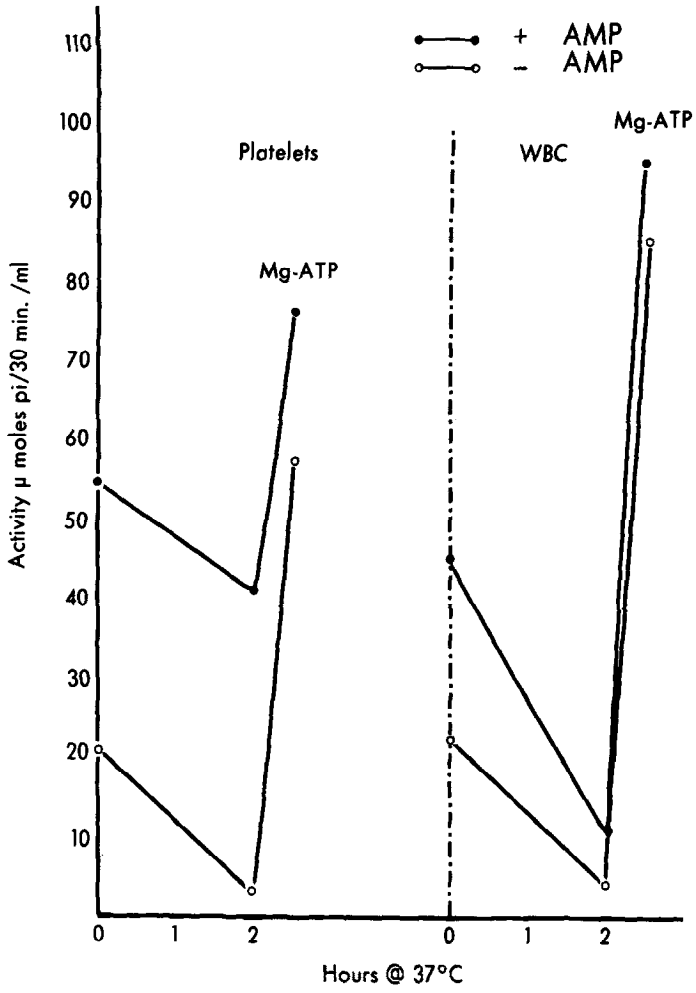

Fig. 2. Effect of AMP on the activity of inactive phosphorylase of platelets and leukocytes. Cell extracts were prepared in 0.001 M EDTA. Incubation was carried out as shown. (Activation with Mg. ATP was done as described in text).

iting approximately 60 percent of its maximal activity in the presence of the nucleotide (Fig. 2). However, the increase of activity (with AMP) of approximately 50 percent after activation with Mg - ATP is more than would be expected for inactive muscle enzyme suggesting the existence in platelets of an inactive enzyme species which is not stimulated by

AMP. Inactive leukocyte phosphorylase was only slightly stimulated by AMP; activation with Mg - ATP resulted in a 10-fold increase in activity. Active enzyme of both platelets and leukocyte exhibited 70-90 percent of its activity without AMP.

Cysteine stimulated platelet phosphorylase activity by approximately 30 percent but had no effect on the leukocyte enzyme (not illustrated).

Both platelet and leukocyte phosphorylase activities were stimulated by 0.6 M Na_2SO_4. However, a much greater stimulating effect was noted with the leukocyte enzyme (not illustrated).

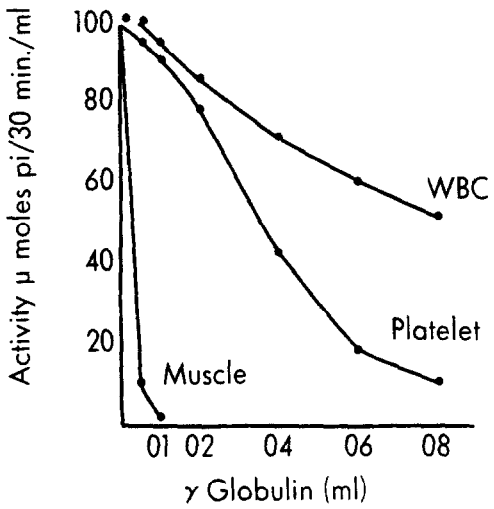

Fig. 3. The effect of anti-rabbit muscle phosphorylase globulin on the activity of the enzyme from rabbit muscle, leukocytes and platelets.

The inhibitory effect of anti-crystalline rabbit muscle phosphorylase b globulin was tested on the activity of phosphorylase in extracts of muscle, platelets, and leukocytes prepared from rabbits (Fig. 3). As little as 0.01 ml. of globulin caused complete inhibition of muscle phosphorylase, the specific antigen. At a concentration of globulin below

0.02 ml., leukocyte and platelet enzymes were equally inhibited. At higher globulin concentrations, the inhibition of platelet phosphorylase activity was much more pronounced (80 percent inhibition by 0.06 ml. globulin compared to 40 percent inhibition of the leukocyte enzyme).

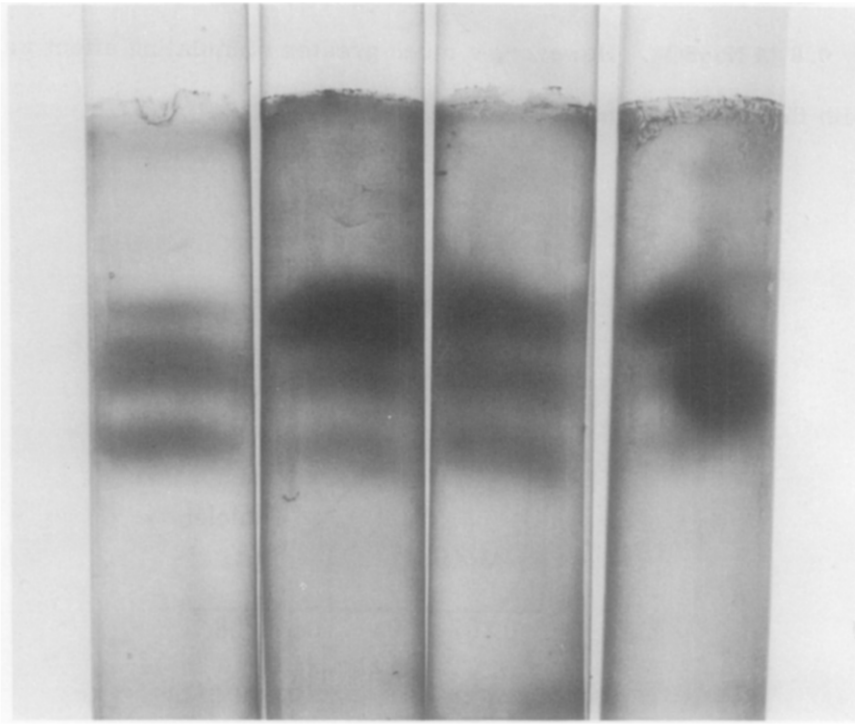

Fig. 4. Electrophoresis of phosphorylase on acrylamide gel. From left to right - 1. platelet enzyme - sample 15 μl. of 33-50% ammonium sulfate fraction: activity 4 μmoles P_i/30'. 2. Leukocyte enzyme sample 15 μl. of 33-50% ammonium sulfate fraction, activity 5 μmoles P_i/30'. 3. A mixture of platelet and leukocyte fraction (10 μl. of each). 4. A plastic divider was placed on top to separate samples, allowing a separate run on each half of the column. Left half: 7 μl. of leukocyte enzyme fraction; right half: 10 μl. of a 41 percent ammonium sulfate fraction of skeletal muscle phosphorylase; activity 3.0 μmoles P_i/30'.

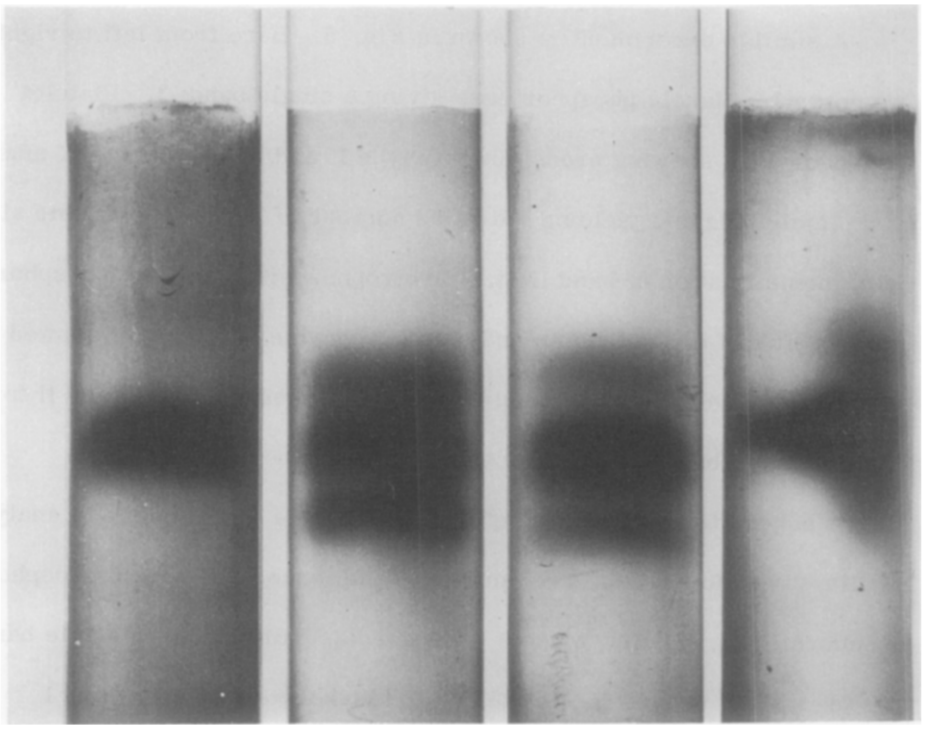

Fig. 5. Electrophoresis of phosphorylase on Acrylamide Gel. From left to right: 1. Muscle enzyme, 5 μmoles P_i/30'. 2. Platelet enzyme 6 μmoles P_i/30'. A mixture of muscle and platelet protein (2.5: 3.0). 4. Left half of column, muscle enzyme (2.5 μmoles P_i/30'); right half: platelet enzyme 3.5 μmoles P_i/30').

Both platelets and leukocytes contained 3 activity bands as shown on acrylamide gel (Fig. 4 and 5) (types I, II, and III, type I having the highest electrophoretic mobility). In Fig. 4 from left to right the following could be noted: 1. Predominance of type II and I in platelets; 2. Predominance of type III in leukocytes; 3. An equal mixture of leukocyte and platelet enzyme yielded the same number of bands; 4. Electrophoresis of leukocyte phosphorylase on the left half of column and muscle

phosphorylase on the right side revealed that the muscle enzyme band corresponded to band II of leukocytes.

A similar experiment is shown in Fig. 5. Here from left to right we note: 1. Muscle phosphorylase giving a single band; 2. Platelet phosphorylase showing predominantly type II and I. A mixture of muscle and platelet enzyme yielding the same number of bands as platelets alone with intensification of band II; 3. Electrophoresis of muscle phosphorylase on the left half of the column and platelet enzyme on the right showed that the muscle enzyme merged with platelet phosphorylase band II to form a single band indicating identical electrophoretic mobility.

A schematic presentation of these results of acrylamide gel analysis is illustrated in Fig. 6. Both platelets and leukocytes have 3 phosphorylase isoenzymes: I, II, III. Muscle phosphorylase appears as a single band corresponding to type II of platelets and leukocytes. Type II and I (II>I) predominate in platelets. In leukocytes type III is predominant.

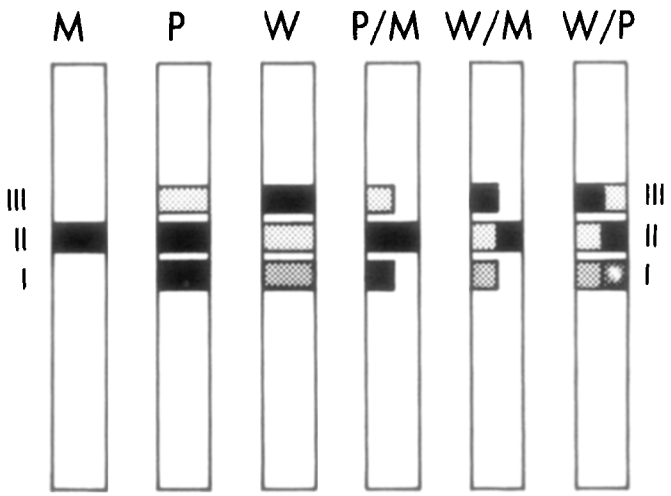

Fig. 6. A schematic presentation of the results of electrophoretic analysis of muscle, platelet, and leukocyte phosphorylase on acrylamide gel. M - muscle, P - platelets, W - leukocytes.

Discussion

The observation that platelet phosphorylase activity was significantly stimulated by AMP was particularly interesting since it pointed out an important difference between this enzyme and that of another blood cell, the leukocyte. The studies described herein have demonstrated other similarities between platelet and muscle phosphorylase. Thus, like the muscle enzyme, the platelet enzyme requires cysteine for maximal activity and is significantly inhibited by antimuscle phosphorylase antibody. However, none of these properties is displayed to the same extent as by the muscle protein. Furthermore, inactive platelet phosphorylase was significantly stimulated by high salt concentration, a property shared by liver and leukocyte phosphorylase (12, 4). Because platelet phosphorylase displayed intermediate properties between the muscle enzyme on the one hand and the enzyme from liver and leukocytes on the other, and in view of previous reports describing the existence of phosphorylase isoenzymes in heart muscle (13) and subsequently in rat chloroma (5), it was postulated that platelet phosphorylase was also heterogenous. This was clearly demonstrated by analysis on acrylamide gel. Thus the similarities in the properties of platelet phosphorylase to those of muscle phosphorylase are accounted for by the preponderance in these cells of a "muscle type" enzyme. Further comparative studies on the purified isoenzymes are necessary to determine the extent of structural similarity between the enzymes from platelet and muscle.

The preponderance of a "muscle type" phosphorylase in platelets is in keeping with the existence in these cells of a contractile protein, thrombosthenin (14), akin to actomyosin of muscle. It is possible that for

the proper function of this contractile protein, a control system of activation-inactivation of phosphorylase similar to that of muscle is necessary.

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