THE ACTIVATION OF PHOSPHOGLUCOMUTASE.

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Received August 29, 1960

Phosphoglucomutase (PGM) is the enzyme which catalyses the interconversion of glucose-1-phosphate (G-1-P) and glucose-6-phosphate (G-6-P). It requires a coenzyme, glucose-1-6-diphosphate, which is present in the commercial G-1-P. A pure preparation of the enzyme can be obtained from rabbit skeletal muscle (Najjar, 1948, Jagannathan and Luck, 1949a). The pure enzyme requires Mg^{++} (Jagannathan and Luck, 1949a, Najjar, 1948) and a chelating agent (Najjar, 1948, Sutherland, 1949). In the crude preparations the activation by a "second metal" (Cr^{+++} or Al^{+++}), in addition to Mg^{++}, has also been claimed (Stickland, 1949). PGM is isolated as a phosphoprotein, whose phosphate exchanges with the substrate (Jagannathan and Luck, 1949b). A pentapeptide containing the sequence SerP.His. in its active centre has been recently characterized (Milstein and Sanger, 1960).

PGM has been obtained by the method of Najjar with a specific activity of 27 units per mg. of protein (cf. Najjar, 1948). It runs as a single component in the ultracentrifuge under various conditions. Activity measurements have been made by the procedure of Najjar (1948), determining the amount of acid-stable phosphate formed after incubation of the enzyme in the presence of G-1-P, Mg^{++} and cysteine or another chelating agent.

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Activation by magnesium

The activation of pure PGM by Mg^{++} is essential; that is, no significant activity is detected in the absence of Mg^{++}. This has been shown by Jagannathan and Luck (1949), and in the present work also with the pure PGM prepared according to the method of Najjar. The apparent Michaelis constant for magnesium is 0.5×10^{-4}. A magnesium-enzyme complex was also detected by equilibrium dialysis and the dissociation constant estimated as 0.5×10^{-3} M.

Activation by chelating agents

Chelating agents have two distinct activating effects on PGM. The first is obtained by including a chelating agent in the assay mixture, and the second by pre-incubating the enzyme with EDTA before assay (in a very recent communication in this journal, Robinson and Najjar, 1960, report that preincubation of the enzyme with histidine and Mg^{++} can also activate PGM).

PGM has a very high affinity for heavy metals. The purified preparations cannot be freed from metals by simple dialysis against glass distilled water. In fact "metal free" PGM (Milstein, 1960) obtained by ion exchange purification, can pick up the minute amounts of metal contaminations present in glass distilled water during dialysis. Heavy metals are very strong inhibitors of PGM. Unlike the yeast PGM (McCoy and Najjar, 1959), the rabbit enzyme is completely inactivated even by the presence of Zn^{++}. The inhibition by Zn^{++} and Cu^{++} has been studied by using the "metal buffer" technique (Raaflaub, 1956). Zn^{++} competes with Mg^{++}, with a dissociation constant of 3.9×10^{-14} M. Cu^{++} also competes with Mg^{++} but more than one atom of Cu^{++} per molecule of enzyme is involved in the inactivation. The lower of the inhibition constants, K_i, for Cu^{++} is 2.3×10^{-17}.

It is suggested that the high stability of this type of inactive complex is responsible for the essential requirement of chelating agent in the assay mixture. Partial activity can be obtained in the absence of any chelating agent by a careful purification of all the reagents from metals. In a system purified from metals the efficiency of the activation by

cysteine is about tenfold that obtained in a normal assay, as is shown in Fig. 1. The reagents were purified by shaking with 0.01% dithizone in CCl_4 and then washing out all traces of dithizone by shaking the aqueous layer five times with Zn^{++}-free CCl_4. The enzyme was a "metal free" preparation (Milstein, 1960).

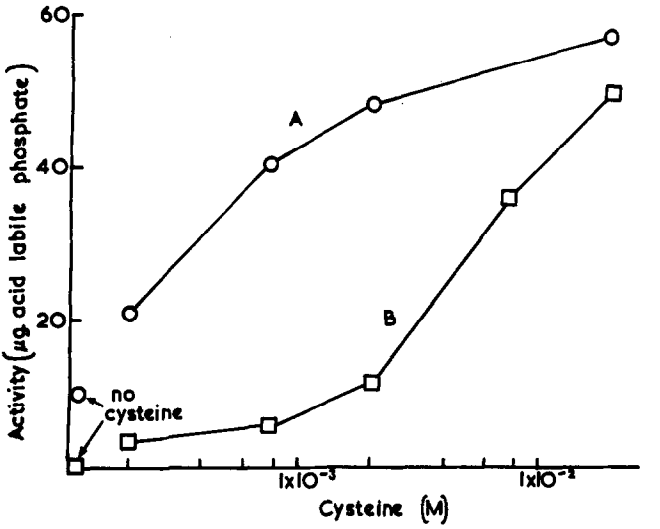

Fig. 1. The activation of PGM in standard and heavy-metal-freed assay mixture.

2.1 μg of PGM incubated with 6.5 mM G-1-P and 1.5 mM $MgSO_4$ for 5 min. at 30° (final volume 0.4 ml).

Curve A. G-1-P and $MgSO_4$ purified by dithizone treatment. Cysteine was added as indicated, together with metal free tris-HCl to maintain ionic strength and pH.

Curve B. As curve A but reagents were not freed from heavy metals.

The suggestion that the activation by chelating agents is due to binding of the chelating agent to the active site of the enzyme has been advanced for some other enzymes (Mounter et al., 1953, Sauer and Rapoport, 1959). In PGM, however, this is not the case, and removal of the inhibitory metals is a better explanation of the mechanism of the essential activation by chelating agents.

Preactivation by EDTA.

Preincubation of PGM with EDTA increases its specific activity by a factor of 2.5 to 3. This further activation is time-dependent, and is complete after 45 min at 30°C. It is not obtained with other chelating agents such as cysteine, histidine or 8-hydroxyquinoline (Fig. 2).

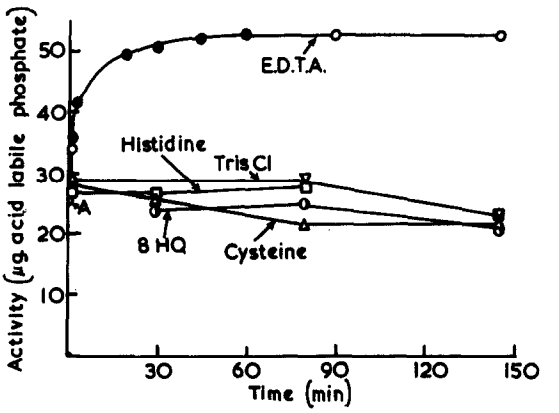

Fig. 2. The activation of PGM by preincubation with EDTA.

89.2 µg/ml of PGM were incubated at 30° in the presence of 1 mM concentrations of the stated substances. In all cases pH was adjusted to 7.5 with tris. After the stated times 0.02 ml were assayed for activity in the presence of 25 mM cysteine, 6.5 mM G-1-P and 1.5 mM Mg^{++} (2.5 min incubation at 30°). Point A was determined with enzyme which was not preincubated with EDTA, in an assay mixture containing 5×10^{-5} M EDTA in addition to the other components.

Robinson and Najjar (1960) reported that when Mg^{++} is present together with histidine a similar time-dependent activation is obtained. In their experiment shorter times and a much lower temperature were necessary for complete activation.

EDTA preactivation can also be obtained with "metal free" PGM. Aged preparations give an increase in activity of up to 6 times. EDTA preactivation can be reversed in a time-dependent fashion by restricted dialysis against water and dilution at 0°C. The process of reversal takes less than 10 min at 0°C for completion, by which time the specific activity has returned to its original value. EDTA preactivation affects neither the Michaelis constant for magnesium nor the sedimentation pattern of the enzyme.

A full account of this work will be published elsewhere.

I am indebted to Dr. M. Dixon (F.R.S.) for his advice and encouragement and to the British Council for a Scholarship.

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