HORMONAL CONTROL OF PHOSPHOGLUCOMUTASE ACTIVITY

Takashi Hashimoto, Hiroo Sasaki* and Haruhisa Yoshikawa

Department of Physiological Chemistry and Nutrition,
Faculty of Medicine, the University of Tokyo, Tokyo.

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During the course of a comparative study of phosphoglucomutases from various biological sources (Handler, et al., 1965, 1966), one of the present authors noticed that the enzyme activities of crude extracts from rabbit muscle or liver, differred significantly among different preparations from the respective tissue when assayed without pretreatment with histidine plus Mg^{++}. The simplest explanation appeared to be variation in the efficiency of the extraction procedure. However, these differences in activity between individual preparations became much less apparent if assayed after a preincubation with histidine and Mg^{++} (Robinson and Najjar, 1960). This phenomenon was confirmed in a series of careful experiments using mouse liver and muscle extracts in this laboratory.

Although there has been some disagreement concering the mechanism of the activation of the enzyme under these conditions, (Milstein, 1961: Najjar et al., 1965 a, b: Ray and Roscelli, 1966), we may assume that each enzyme molecule, after activation is fully capable of catalyzing the reaction at the maximal rate in a given assay system. If so, the level of activity after pretreatment with histidine plus Mg^{++} indicates the number of the enzyme molecules in the extract. Furthermore, it was observed that the level of activity in an unactivated preparation, was constant even after storage of the tissue, or of an homogenate prepared therefrom for more than one hour.

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* A research student from Tokyo College of Science, Tokyo.

These data suggested the possibility that the physiological status of an animal at the time of sacrifice, may be one of the main reasons for the observed variation in enzyme activity. To test this, we have studied the effects of hormones on phosphoglucomutase activity.

Young male mice (20 to 25 g) were fed ad libitum on a laboratory chow (RC 5, Oriental Yeast Co., LTD., Tokyo) and water for a week in one cage. They were transferred to small separate cages where they were held for about one hour before experimental use. They were sacrificed, after treatment with hormone, as indicated. Liver or leg muscle was homogenized with 5 volumes of 0.02 M Tris-Cl buffer, pH 7.4. The homogenates were centrifuged at 10,000 x g for 10 minutes and the precipitates were discarded. The supernatants were diluted with Tris buffer. For activation prior to use, the extracts were incubated in 0.02 M histidine buffer, pH 7.4 and 1 mM $MgCl_2$ at 30^o for 10 minutes. Enzyme activity was determined at 30^o in a reaction mixture which contained, in a final volume of 0.5 ml, the following components: histidine buffer, pH 7.4, 20 μmoles; $MgCl_2$, 2 μmoles; glucose 1-phosphate, 2μmoles; glucose 1,6-diphosphate, 2.5 mμmoles; the freshly prepared enzyme extract. The reaction was terminated by addition of 1 ml of 5 N H_2SO_4 after a 10 minute incubation. Enzyme activities were expressed as μmoles of decrease in acid-labile phosphate per 5 minutes.

As shown in Fig. 1 in liver extract the reaction proceeds with an intial lag phase of 2 to 3 minutes. Enzyme activity evident without the activation treatment was increased by injection of insulin and decreased by adrenalin and by adenosine 3,5-phosphate. All of the preparations exhibited the same activity if assayed after activation with histidine and Mg^{++}. This rapid change in enzyme activity by treating with adrenalin or insulin was observed with both liver and muscle. The effect of adrenalin is summarized in Fig. 2. Quite the opposite change in blood sugar level and enzyme activity has been observed with insulin.

The activation mechanism of mammalian phosphoglucomutase in vivo and

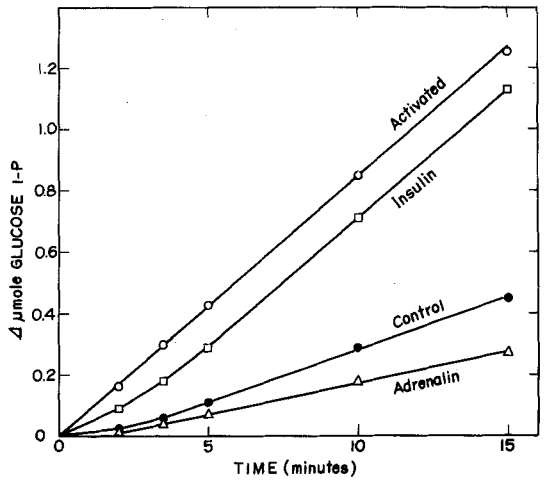

Fig. 1. The time course of the reactions. Insulin (0.2 unit) or adrenalin tartrate (0.1 mg) was injucted subcutaneously. After one hour, livers were excised from mice and homogenized as described in Text. The reactions were started by addition of the extract (0.9 mg tissue equivalent). All of the preparations gave the same activity as that of the control when activated with histidine plus Mg^{++}.

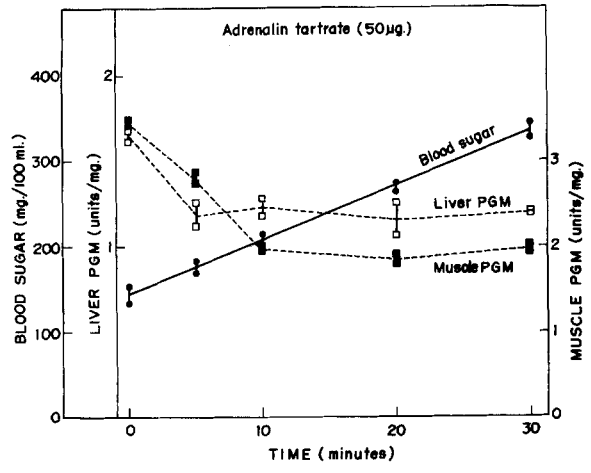

Fig. 2. The effect of adrenalin. Mice were sacrificed at intervals after injection of adrenalin tartrate (0.05 mg). Enzyme activities was assayed without pretreatment. The activities are expressed as units per mg. of protein of the supernatant. The activities of the pretreated preparations are 2.36 $\pm$ 0.04 (standard deviation) for the liver and 11.9 $\pm$ 0.2 for the muscle. Blood sugar was determined colorimetrically (Nelson, 1944).

in vitro, is now under investigation. From the data of our preliminary experiments, the change in activity induced by hormones seems likely to be due to some conformational change of the enzyme molecule or to some firmly bound effector, but not to a dissociable one.

It is also of interest in this connection that the concentration of glucose 1-phosphate and glucose 6-phosphate in liver and muscle are not those of the phosphoglucomutase reaction at equilibrium. The data suggest that the mutase reaction sometimes proceeds at a relatively low rate in cells: this must be governed by some other conditions, one of which may be the Mg-chelation by ATP. Therefore, the hormonal control of the enzyme activity may have a physiological role in regulating the rate of glycolysis.

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