THE TIME DEPENDENT ACTIVATION OF CRYSTALLINE PHOSPHOGLUCOMUTASE BY MAGNESIUM IONS AND IMIDAZOLE

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The mechanism of action of phosphoglucomutase involves the alternate formation of phospho- and dephospho-forms of the enzyme (Najjar and Pullman, 1954).

Glucose 1-phosphate + Phosphoenzyme

1. ↓ ↑

Glucose 1,6-diphosphate + Dephosphoenzyme

2. ↓ ↑

Glucose 6-phosphate + Phosphoenzyme

The equilibrium constants were found to be 4.58 and 3.76 respectively (Sidbury and Najjar, 1957).

It has long been recognized that the presence of Mg^{++} in the reaction mixture causes considerable stimulation of the enzyme activity (Cori, Colowick, and Cori, 1938). It was, therefore, of special interest to determine the role that Mg^{++} plays with respect to these two forms of enzyme.

Studies on phosphoenzyme. We have observed that preincubation of the crystalline phosphoenzyme with Mg^{++} and imidazole or histidine at pH 7.5, causes considerable activation of the enzyme. The enzyme so treated

is many fold more active than the untreated controls. Activity measurements were made under standard conditions of glucose 1-phosphate 4×10^{-3}M, Mg^{++} 1×10^{-3}M, imidazole 4×10^{-2}M at pH 7.5 and 30°. Activation by preincubation occurs at 0° reaching near maximum in a few minutes and is faster at 30°. Mg^{++} alone or imidazole alone in concentrations of 0.04M or less did not result in any significant activation. However, imidazole at 0.4M alone produces significant activation. This may well be due to minimal Mg^{++} contamination. The extent of activation increases with the increase in concentrations of Mg^{++} and imidazole. At constant Mg^{++} concentration of 1×10^{-3}M, the activation in 4×10^{-2}M imidazole is about five times as large as that obtained with 4×10^{-3}M. Similarly, at constant concentrations of imidazole of 4×10^{-2}M, the activation with Mg^{++} at 1×10^{-2}M is about twice that obtained with 1×10^{-4}M. These findings suggest that the activation is effected by a magnesium-imidazole complex. A similar activating effect by Fe^{++} and cysteine has been shown for aconitase (Dickman and Cloutier, 1951). Prolonged dialysis of the phosphoenzyme against imidazole 0.04M, pH 7.5, or acetate 0.15M, at pH 5.0 at 4°, did not significantly change the magnitude or characteristics of activation. The possible removal of inhibitory metals by imidazole cannot, therefore, be related to its effect in this system.

All preparations of crystalline phosphoenzyme tested so far proved to be readily and substantially susceptible to activation. However, there was some variation in the extent and rate of activation in fresh as well as aged samples (Table 1). Freshly prepared twice crystallized enzyme gave activities in the range of $4\text{-}5 \times 10^{2}$ μmoles of glucose 6-phosphate formed per mg of enzyme per minute. This was obtained in enzyme preparations, activated by preincubation in Mg^{++} 1×10^{-2}M and imidazole 4×10^{-2}M at 0° for 10 minutes and assayed in similar concentrations under otherwise standard

conditions. This is about 2.5-3.5 times as high as was obtained earlier with identical preparations of crystalline enzyme (Najjar, 1948). At that time, activities were measured without preincubation, using cysteine instead of imidazole.

Optimally activated phosphoenzyme requires the continuous presence of optimal concentrations of Mg^{++} and imidazole to remain activated. A reduction of either effects a reduction of the level of activation. When maximally activated, by preincubation of the phospho-form with these two substances, the enzyme maintains its high rate of enzymatic activity during the course of the reaction under the standard conditions defined above. Furthermore, suboptimally activated and nonactivated phosphoenzyme preparations exhibit no increase in rate during the course of the reaction. Thus, while substantial activation is obtained during the first 30 seconds under the conditions of preincubation at 0°, the nonactivated enzyme shows no measurable activation during ten minutes of enzymatic activity. Since the enzymatic reactions are carried out under conditions that differ from those of preincubation only by the presence of substrate, it is clear that the substrate in some manner causes maintenance of the state of activation at a constant level. One possible explanation could involve the participation of the enzyme in phosphate transfer. From the mechanism of the reaction and the equilibrium constants of the two steps, it can be seen that in the reaction steady state, the bulk of the enzyme is in the dephospho-form. It appeared possible, therefore, that the dephosphoenzyme was not activatable under the standard conditions. A study of the dephospho-form was, therefore, indicated.

The activation mixture consisted of Mg^{++} 1×10^{-3}M, imidazole 4×10^{-2}M and twice crystallized enzyme preparations A, B, and C. Activation was carried out by preincubation at zero degrees and assayed for activity

TABLE 1

The activation of phosphoglucomutase by Mg^{++} and imidazole

Activation Mixture	Activation time in minutes					
	0	1	2	3	4	5
	μmoles glucose 6-phosphate formed/mg protein/minute					
Phosphoenzyme A, Mg^{++}	3.2	—	5.0	5.0	4.5	4.5
Phosphoenzyme A, Imidazole	4.4	6.3	5.0	5.0	7.5	4.4
Phosphoenzyme A, Mg^{++}, Imidazole	4.4	34.0	55.3	59.7	63.6	70.8
Phosphoenzyme B, " "	12.0	39.0	49.2	50.0	52.6	55.2
Dephospho " " " "	40.3	37.4	41.6	41.6	41.6	41.2
Phosphoenzyme C, " "	55.0	122.0	133.0	—	152.0	166.0

at the times indicated under standard conditions as described in the text. A and B were aged preparations that had already shown considerable loss of activity, while C was freshly prepared. All preparations show definite activation with Mg^{++} and imidazole with the exception of the dephosphoenzyme.

Studies on Dephosphoenzyme. Dephosphoenzyme was prepared as usual by repeated treatment of the enzyme with glucose 6-phosphate, in the presence or absence of Mg^{++}, followed by a 20 hour dialysis against repeated changes of imidazole 0.04M at 6° (Najjar and Pullman, 1954). Such preparations of dephosphoenzyme were found to be in a maximally activated state and hence could not be activated further by preincubation with Mg^{++} and imidazole (Table 1).

On the basis of all these findings, it can be assumed that during the reaction, the state of activation of the enzyme is strictly maintained at a constant level by both forms of enzyme for a considerable time during activ-

ity. This may be inherent in the configuration of the catalytic site in the phospho-form which remains unaltered in the dephospho-state. The other possibility is that the presence of substrate in some manner exerts this effect. A similar effect of substrate on phosphatase activation has been previously shown (Cloetens, 1941).

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