THE EFFECT OF GLUCOSE ON THE SEDIMENTATION AND CATALYTIC ACTIVITY OF GLYCOGEN PHOSPHORYLASE*

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To understand the role of subunits in enzymic catalysis, it is essential to know the state of aggregation of the protein molecule under the conditions where enzymic activity is measured. The present work shows that glucose, an inhibitor of phosphorylase, (Cori et al. 1943) can also serve as an activator. Since activation and ultracentrifugal experiments are carried out under identical conditions, the data described herein suggest that activation is directly related to enzyme dissociation. Similar conclusions were reached on the basis of kinetic experiments with phosphorylase a in solutions of high ionic strength or low protein concentration (Wang and Graves, 1964).

Materials and Methods: Preparation of AMP free phosphorylase, estimation of protein concentration and ultracentrifugation were as described previously (Wang and Graves, 1963). Shellfish glycogen was obtained from Sigma Chemical Co., St. Louis, Missouri, and purified according to Sutherland and Wosilait (1956). Phosphorylase activity was measured as described by Krebs et al. (1964) but expressed as μ moles of phosphate released after 30 seconds of reaction.

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Results and Discussion

Although inclusion of 0.025 M glucose in the assay inhibits phosphorylase a activity approximately 60% at 20°, stimulation of enzymic activity can also be demonstrated if enzyme is preincubated in glucose. Figure 1A shows that incubation of enzyme (1.2 mg/ml) with 0.05 M glucose at 20° results in approximately a three fold increase in enzymic activity. No increase in activity could be demonstrated with incubation in the absence of glucose. To relate alterations of enzymic activity to physical structure of glycogen phosphorylase, ultracentrifugation was carried out under the exact conditions of activation. Figure 1B, lower curve, shows that two diffuse boundaries with $s_{20,w}$ of 8.9 and 13.9 S appear during

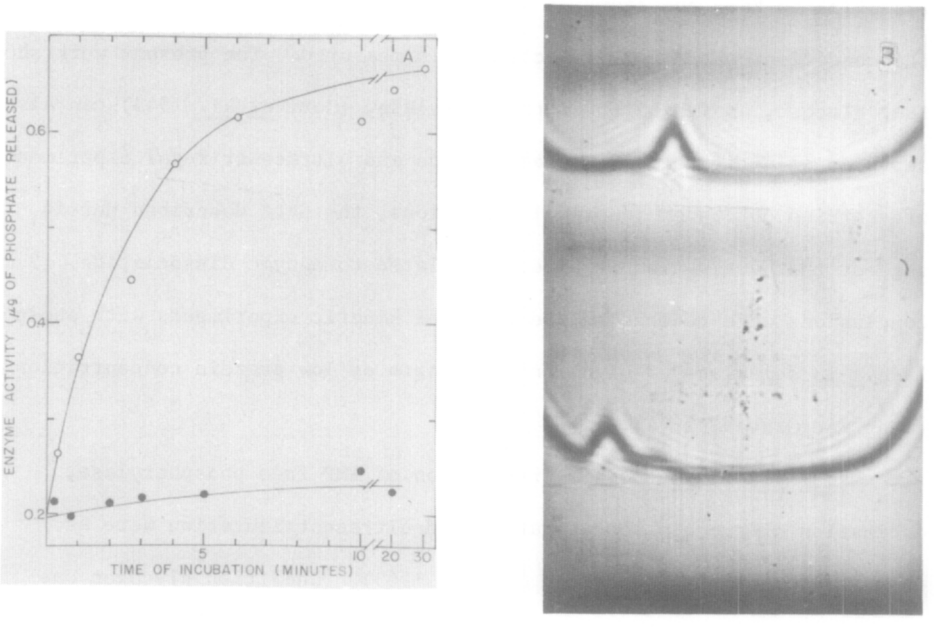
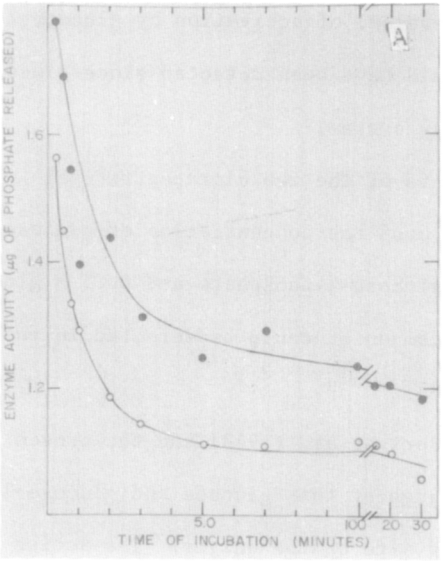


Figure 1 Effect of glucose on the activity and sedimentation of phosphorylase a. (A) phosphorylase a (12 mg/ml) was diluted 10 fold at 20° in 0.03 M cysteine -- 0.04 M glycerophosphate, pH 6.8, solid circles; in buffer containing 0.056 M glucose, open circles. Aliquots were removed at various intervals for measurement of enzymic activity (30 second assay) at 20°. The assay contained 0.6 mg/ml of enzyme, 0.016 M glucose-1-phosphate, 1% glycogen, and 0.025 M glucose. (B) ultracentrifugation at 20° of enzyme (1.2 mg/ml) in glycerophosphate-cysteine, upper curve; in buffer with glucose, lower curve. Picture was taken at 18 minutes after attainment of 59,780 r.p.m. Sedimentation is from left to right.

the course of centrifugation at 20°C with enzyme in 0.05 M glucose. In the absence of glucose only a component with an $s_{20,w}$ of 13.9 S is evident (Fig. 1B, upper curve). Since activation may be correlated with the formation of a component with an $s_{20,w}$ of 8.9 S, a sedimentation constant similar to that obtained for the dimeric form of phosphorylase a, these data suggest that activation is directly related to the conversion of the tetrameric form of phosphorylase a, mw 495,000 (Keller and Cori, 1953) to a dimeric species.

No activation of enzyme (0.03 mg/ml) could be demonstrated by preincubation with 0.05 M glucose at 30°. Since kinetic experiments (Wang and Graves, 1964) suggested that phosphorylase a exists predominately as a dimer at low protein concentrations at 30°, the lack of activation



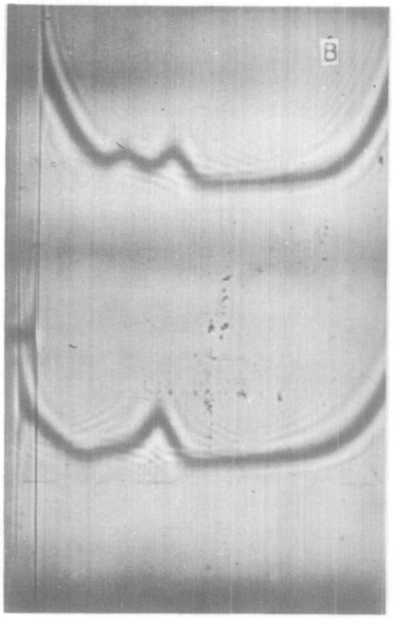


Figure 2 Effect of glucose-1-phosphate and AMP on activation and dissociation of phosphorylase a by glucose. (A) phosphorylase a (1.33 mg/ml) was pre-incubated at 20° for 30 min. in 0.03 M cysteine -0.04 M glycerophosphate, pH 6.8, containing 0.056 M glucose before addition of glucose-1-phosphate (solid circles) or AMP (open circles) to a final concentration of 0.032 M and 10^{-3} M, respectively. Aliquots were withdrawn at various intervals and assayed for activity as in Fig. 1 with 10^{-3} M AMP. (B) ultracentrifugation at 20° of enzyme (1.2 mg/ml) in 0.03M cysteine -0.04M glycerophosphate, pH 6.8, containing 0.05 M glucose. Upper curve, with 0.032 M glucose-1-phosphate. Lower curve, with 10^{-3} M AMP. Picture was taken as in Fig. 1B.

further supports the view that activation by glucose is directly related to enzyme dissociation.

Since inhibition by glucose was found by Cori et al. (1943) to be overcome by glucose-1-phosphate and AMP, the effect of these two compounds on the activation by glucose was tested. The results illustrated in Figure 2 show that addition of 0.032 M glucose-1-phosphate or 10^{-3} M AMP to enzyme which had been incubated for 30 minutes with glucose results in a slow decrease of catalytic activity and a decrease in amount of the component with $s_{20,w}$ of 8.9 S. Figure 2A, lower curve, shows that 10^{-3} M AMP had a greater effect on reversal of activation by glucose than 0.032 M glucose-1-phosphate and that the extent of reversal is paralleled by a further decrease in amount of the slow moving component (Fig. 2B, lower curve). It should be noted that if reversal of activation by glucose-1-phosphate were fast, no activation would have been detected since glucose-1-phosphate is a component of the assay system.

Activation and the relative amounts of the two ultracentrifugal components were found to be dependent upon the concentration of glucose and glucose-1-phosphate; with 0.16 M glucose-1-phosphate and 0.05 M glucose at 20°, no activation or slow moving component could be detected in the ultracentrifuge.

Inhibition kinetics reported by Cori et al. (1943) and the present experiments with glucose-1-phosphate suggest that glucose and glucose-1-phosphate compete for a single binding site on the enzyme. The action of AMP on inhibition (Cori et al. 1943) and activation may be explained by binding of this nucleotide to a different site which results in a decreased affinity of enzyme for glucose and/or a decrease in Km for glucose-1-phosphate (Helmreich and Cori, 1964; Lowry et al. 1964). Binding of AMP at the substrate site for glucose-1-phosphate would not be expected since AMP potentiates rather than inhibits phosphorylase a activity.

In contrast to results obtained with phosphorylase a, incubation of

phosphorylase b, a dimer which is inactive in the absence of AMP, (Cori et al. 1938) at 1.0 mg/ml in 0.05 M glucose at 20° did not yield higher enzymic activity. No alteration of the ultracentrifugal characteristics of phosphorylase b (2.0 mg/ml) could be detected in 0.05 M glucose at 20°.

The relationship between subunit structure and the catalytic activity of glycogen phosphorylase has been examined in the presence of glucose. The present experiments provide physical and catalytic evidence that the dimeric form of phosphorylase a is more active than the tetrameric species, a conclusion reached earlier on the basis of kinetic experiments (Wang and Graves, 1964).

References

Cori, G. T., Colowick, S. P., and Cori, C. F., (1938), J. Biol. Chem., 123, 381.
Cori, C. F., Cori, G. T., and Green, A. A., (1943), J. Biol. Chem., 151, 39.
Helmreich, E., and Cori, C. F., (1964), Proc. N. A. S., 51, 131.
Keller, P. J., and Cori, G. T., (1953), Biochim. et Biophys., Acta, 12, 235.
Krebs, E. G., Love, D. S., Bratvold, G. E., Trayser, K. A., Meyer, W. L., and Fischer, E. H., (1964) Biochemistry, 3, 1022.
Lowry, O. H., Schulz, D. W., and Passonneau, J. V., (1964), J. Biol. Chem. 239, 1947.
Sutherland, E. W., and Wosilait, W. D., (1956), J. Biol. Chem., 218.
Wang, J. H., and Graves, D. J., (1963), J. Biol. Chem., 238, 2386.
Wang, J. H., and Graves, D. J., (1964), Biochemistry, In Press.