A COMPARISON OF NONACTIVATED AND ACTIVATED LIVER GLYCOGEN SYNTHETASE*

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The evidence indicating glycogen synthetase of liver to be a site of control of glycogen metabolism has suggested the regulation of enzyme activity to be a result of an interconversion of enzyme forms i.e. G-6-P dependent: G-6-P independent; nonactivated: activated; glycogen synthetase b: glycogen synthetase a (Hizukuri and Larner, 1964; Gold and Segal, 1967; Mersmann and Segal, 1967). Activated enzyme, believed to be independent of G-6-P** for activity, is considered the form present in liver during conditions of increased glycogen deposition (Hornbrook, Burch and Lowry, 1966; Bishop and Larner, 1967; Kreutner and Goldberg, 1967). A recent demonstration of an *in vitro* activation of the enzyme from normal livers has been described (Gold and Segal, 1967). A kinetic comparison of the activated and nonactivated enzyme indicates the *in vitro* activation to result in an enzyme form *not* independent of G-6-P but a form with an increased affinity for G-6-P (Mersmann and Segal, 1967). A comparison of the various methods used for demonstrating either G-6-P dependency or independency has indicated enzyme assay determinations to have been performed with a variety of conditions, especially with respect to the presence of EDTA. In the present report, a

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** Abbreviations are: G-6-P, glucose-6-phosphate; UDP-G, uridine diphosphate glucose.

comparison of the activities of nonactivated and in vitro activated enzyme obtained from the livers of normal animals is presented. The evidence suggests a difference between the two enzyme forms with respect to EDTA and Mg^{++} stimulation of activity.

Materials and Methods. The preparation of nonactivated and activated liver glycogen synthetase has been described (Gold and Segal, 1967). The method for assay of enzyme activity is the same as used previously except the final volume of the assay mixture, after addition of enzyme, was 1 ml and assays were performed in stoppered 12 ml conical centrifuge tubes without shaking. The radioactivity of the glycogen isolated from the assay mixture was determined with a Packard Tri-Carb liquid scintillation counter. The efficiency of counting, estimated by channels ratio, was 60%. Enzyme activity is expressed as CPM incorporated into glycogen and is corrected for residual radioactivity of zero-time control assays. The data presented are the results of experiments performed with single animals (normal male rats, 200-250 g, maintained on a standard laboratory diet) and some variation (± 2-3%) was noted with respect to the level of maximum activity obtained.

Results. In Figure 1, a plot of enzyme activity versus EDTA at several G-6-P concentrations is presented for nonactivated and activated forms of liver glycogen synthetase. It is noted that EDTA stimulates the activity of the activated enzyme (Fig. 1-A) to a level of activity not increased further by G-6-P. At 10 mM EDTA, the "independent" form of glycogen synthetase activity is expressed as a result of little further increase in activity in the presence of G-6-P. In the absence of EDTA, or at low EDTA concentrations, the dependency of the activated enzyme on G-6-P is observed as noted by Mersmann and Segal, 1967. In the case of the nonactivated enzyme (Fig. 1-B), G-6-P stimulates activity at all levels of EDTA and would suggest, with the present assay conditions, the enzyme to be the "dependent" form. Figure 1 also shows a decrease in activity of the nonactivated form with increased EDTA concentrations and suggest EDTA to remove some metal ion which may affect the activity. Since

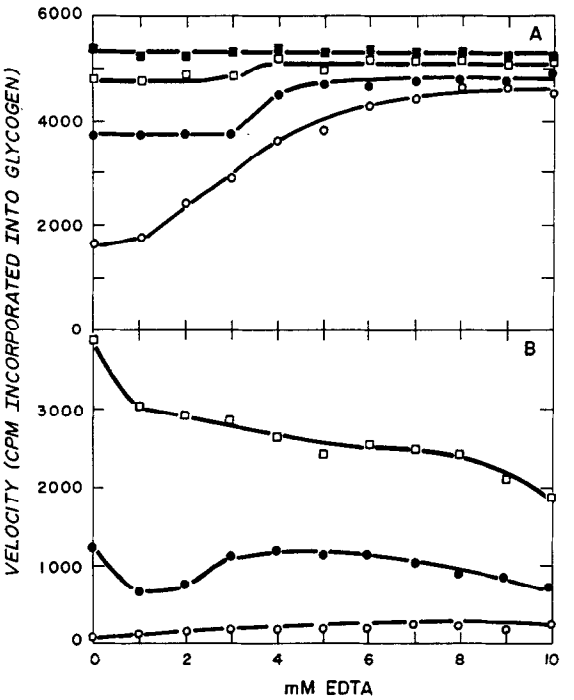

Fig. 1. Activity of activated, A, and nonactivated, B, glycogen synthetase versus EDTA. Activated enzyme was prepared by incubating an 8000 x g extract of liver, blended in 3 volumes of cold 0.1 M glycylglycine, pH 7.4, at 20° for 50 minutes. Nonactivated enzyme was maintained 0°. Assays were initiated by adding 0.05 ml of enzyme to a mixture of 0.25 µmole UDP-G (35,000-40,000 CPM UDPG-^{14}C) 60 µmoles glycylglycine, pH 7.4, 4 mg glycogen and additions as indicated. Final volume was 1 ml. Reactions were stopped with 2 ml hot 30% KOH, after 2 minutes at 37°, glycogen precipitated with 1.1 volume 95% alcohol, the mixture centrifuged, glycogen dissolved in 1.2 ml water and 1 ml counted. Symbols are: A(activated): 0, 0; ●, 0.05; □, 0.2; ■, 2 mM G-6-P respectively. B(nonactivated): 0, 0; ●, 0.05; □, 4 mM G-6-P respectively.

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no decrease in activity of the activated enzyme form is noted with increased EDTA, the possibility of EDTA removal of a metal ion activator in this case is unlikely. However, the increased activity of activated form in the presence of EDTA could be the result of removal of an inhibitor, but does not appear to be the case since it might be expected that EDTA would show similar effects with the nonactivated enzyme.

In view of the possibility that EDTA may remove a divalent metal, the effect of Mg^{++} (added as the chloride) on activity of the activated and

nonactivated forms of the enzyme is presented in Figure 2. A plot of velocity versus Mg^{++} and G-6-P shows Mg^{++} to be almost as effective an activator of activity of the activated form (Fig. 2, circles) as G-6-P. Mg^{++} stimulates the activity of nonactivated form (Fig. 2, squares) about 10 fold when a comparison is made between zero and 2 mM Mg^{++}. In repeated experiments, maximum activity of the activated enzyme in the presence of saturating G-6-P and Mg^{++} ranged from 5600 to 6800 CPM and from 5,000 to 6,500 CPM incorporated into glycogen, respectively. In the experiments where G-6-P and Mg^{++} effects on activity were compared using an enzyme preparation from a single animal (Fig. 2) the maximum activity in the presence of high G-6-P concentrations was always slightly greater than the activity in the presence of saturating amounts of Mg^{++}. In the case of the nonactivated enzyme form, the activity in the presence of saturating amounts of G-6-P ranged from 3000-4000 CPM, values somewhat greater than those reported by

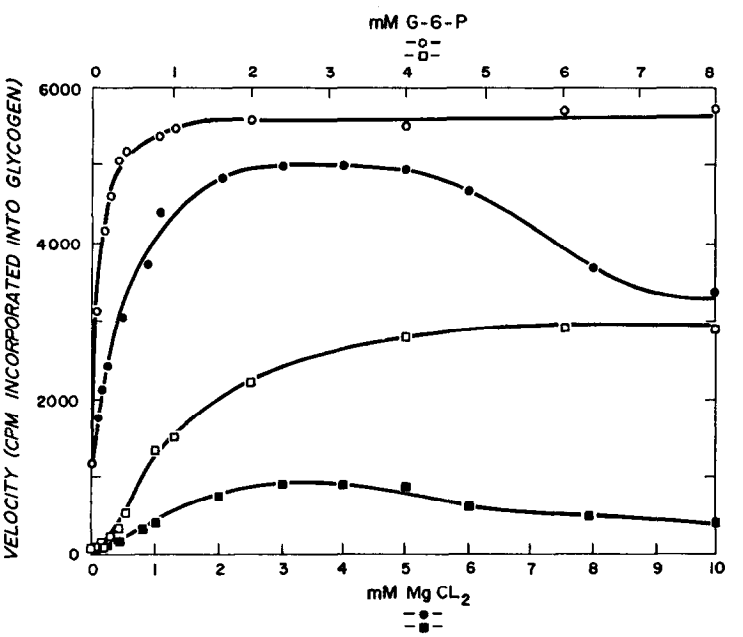

Fig. 2. Activity of activated, circles, and nonactivated, squares, glycogen synthetase versus G-6-P, open symbols, and $MgCl_2$, filled symbols. The conditions for enzyme preparation and assay are the same as Fig. 1.

Mersmann and Segal, 1967. These higher values, as reported here, are probably a reflection of EDTA effect on this enzyme form (Fig. 1). The effect of G-6-P on Mg^{++} stimulation of activity is presented in Figure 3. The results suggest the Mg^{++} increased activity is additive to G-6-P stimulation of activity of activated enzyme when Mg^{++} effect is compared at 0, 0.05, 0.2 and 4 mM G-6-P (Fig. 3-A). The nonactivated enzyme, however, is activated by Mg^{++} in a manner not additive to the G-6-P activation (Fig. 3-B).

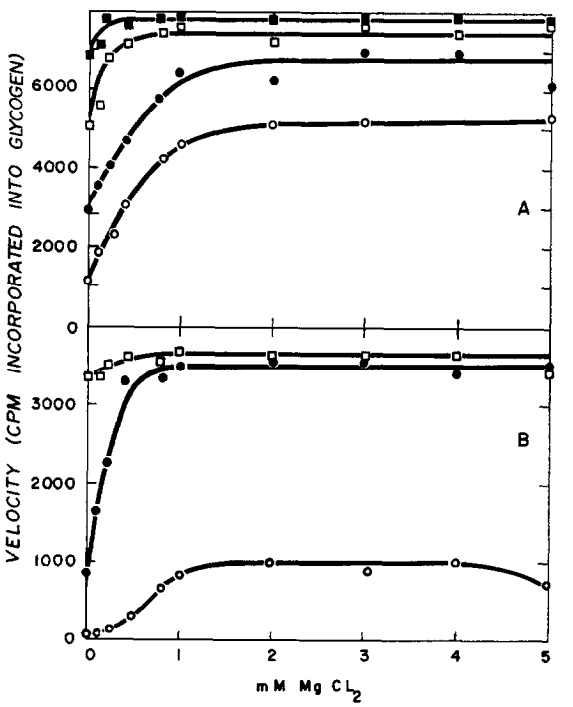

Fig. 3. The effect of G-6-P on $MgCl_2$ activation of activated, A, and nonactivated, B, glycogen synthetase. The conditions for preparation and assay of enzyme are the same as Fig. 1. Symbols are: A(activated): 0, 0; ●, 0.05; □, 0.2; ■, 2 mM G-6-P respectively. B(nonactivated): 0, 0; ●, 0.05; □, 4 mM G-6-P respectively.

Mg^{++}, in the presence of low G-6-P concentrations, appears to augment G-6-P activation of nonactivated enzyme. The effect of Mg^{++} on enzyme affinity for G-6-P is presented in Figure 4. It is noted that Mg^{++} appears to increase the affinity of the nonactivated form for G-6-P (Fig. 4-B), at least at low Mg^{++} concentrations. To rule out the possibility that Mg^{++} was inducing

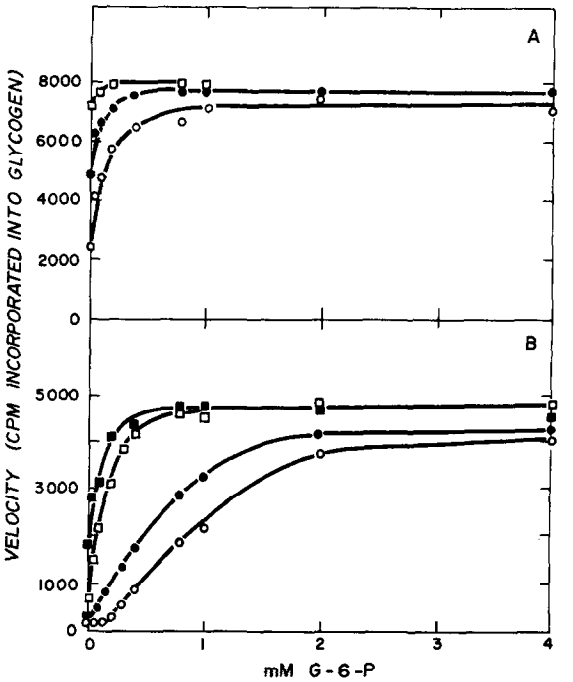

Fig. 4. The effect of $MgCl_2$ on enzyme affinity for G-6-P. The conditions for preparation and assay of enzyme are the same as Fig. 1. Symbols are: A(activated): 0, 0; ●, 0.4; □, 4 mM $MgCl_2$ respectively. B(nonactivated): 0, 0; ●, 0.1; □, 0.5; ■, 2 mM $MgCl_2$ respectively.

G-6-P formation from ATP and glucose present in the enzyme preparation, duplicate assays were performed in the absence and presence of Mg^{++} at the G-6-P concentrations indicated in Figure 3. The reaction was stopped with cold perchloric acid, neutralized with KOH and G-6-P determined with TPN^+ and G-6-P dehydrogenase. No change in G-6-P concentration from that originally present could be observed, either in the absence or presence of Mg^{++}, with either the activated or nonactivated enzyme form.

Discussion. The dependency of the activated form of glycogen synthetase on G-6-P for activity is clearly observed when the activity is determined in the absence of EDTA. In the past, either the preparation of the enzyme or the assay for activity has been done in buffer solutions containing relatively high EDTA concentrations (5-10 mM) which would mask G-6-P activation of this

enzyme form. This supports the observation that the in vitro activation of the enzyme does not result in the conversion to a G-6-P-independent enzyme, but does result in the appearance of a form with an increased sensitivity to G-6-P activation. It seems unlikely that EDTA would act as an enzyme activator similar to G-6-P since opposite effects are observed with the nonactivated form of the enzyme.

A comparison of Mg^{++} effect on activity of nonactivated and activated enzyme suggest the in vitro activation of the enzyme to result in a form with an increased affinity for the metal ion. Mg^{++}, in the presence of $SO_3^{=}$, has been suggested to induce the interconversion of the enzyme to the G-6-P "independent" form (Hizukuri and Larner, 1964). Preliminary experiments in this laboratory have indicated Mg^{++} has no effect on the interconversion of the enzyme with conditions described for the in vitro activation (Gold and Segal, 1967). Of particular interest is the apparent change in kinetic characteristics of the nonactivated enzyme with respect to G-6-P saturation in the presence of low Mg^{++} concentrations. Under these conditions, Mg^{++} apparently increases the affinity of the enzyme for G-6-P resulting in the appearance of kinetic properties, at least with respect to affinity for G-6-P, similar to the activated enzyme form. These results do not rule out the possibility that Mg^{++} effect is due to an alteration in enzyme affinity for UDP-G or glycogen. Studies are in progress to clarify this question.

References

Bishop, J. S. and Larner, J., J. Biol. Chem., 242, 1354 (1967).
Gold, A. H. and Segal, H. L., Arch. Bioch. Biophys. 120, 359 (1967).
Hizukuri, S. and Larner, J., Biochemistry, U.S., 3, 1783 (1964).
Hornbrook, K. R., Burch, H. B. and Lowry, O. H., Mol. Pharmacol., 2, 106 (1966).
Kreutner, W. and Goldberg, N. D., Proc. Nat. Acad. Sci., U.S., 58, 1515 (1967).
Mersmann, H. J. and Segal, H. L., Proc. Nat. Acad. Sci., U.S., 58, 1688 (1967).