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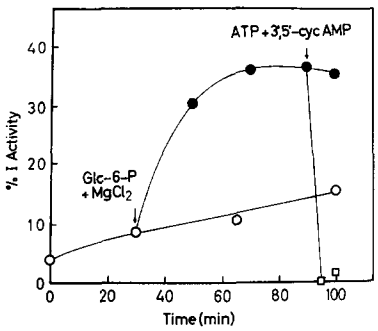
Activation of the D to I form conversion of glycogen synthetase by glucose 6-phosphate in bovine spleen

Two forms of glycogen synthetase (UDP-Glc:glycogen α-4-glucosyltransferase, EC 2.4.1.11), D and I, and their interconversion mediated by phosphorylation and dephosphorylation reactions have been well established by LARNER and coworkers[1-4]. In this communication we deal with conversion of the D form to the I form in bovine spleen enzyme *in vitro*. It was demonstrated that this conversion was activated by Glc-6-*P* and Mg^{2+}.

Fresh bovine spleen placed on ice was transported to the laboratory from a slaughterhouse. The capsule was taken off and the spleen was stored at $-20°$ before use. The frozen spleen was cut into small pieces with scissors and homogenized with ice-cold 50 mM Tris–5 mM EDTA of pH 7.8 (Tris buffer) in a home blender using 4 ml buffer per g tissue. The homogenate was centrifuged for 10 min at $10\,000 \times g$ at 2°. The supernatant (crude extract) contained a considerable portion of I activity (approx. 20%). For the purposes of the present study, the enzyme was converted to the D form by incubation for 10 min at 30° with 2.5 mM ATP, 10 mM $MgCl_2$, 16.7 μM 3',5'-cyclic AMP and 25 mM NaF. After cooling for 10 min, oyster glycogen (4 mg/ml) was added as a carrier of the enzyme. Then it was precipitated 2 times with alcohol, dissolved in Tris buffer and dialyzed against the buffer overnight just before use. The detail preparation and the further purification methods of the enzyme will be described elsewhere. The preparation of which the specific activity (assayed with Glc-6-*P*) was enhanced about 8-fold compared to the crude extract (0.02 unit/mg protein) was used as such, unless otherwise mentioned.

The enzyme activity was assayed by incorporation of [^{14}C]glucose from UDP-[^{14}C]Glc into glycogen as described elsewhere[5], with the following minor modifications; the concentrations of UDP-Glc and Glc-6-*P* (when added) were 5 mM and 10 mM, respectively, the final volume with enzyme was 0.1 ml and temperature was 37°. Total and I activities imply assay with and without Glc-6-*P*, respectively. D activity is the difference between total and I activities. The specific radioactivity of UDP-Glc was 7450 or 16 250 counts/min per μmole. Radioactivity was measured using a gas-flow counter. Enzyme activity was assayed after Sephadex G-25 gel filtration at 0° to remove Glc-6-*P* and other materials which might interfere with the activity. Glc-6-*P* in the filtrate was monitored spectrophotometrically by reduction of $NADP^+$ by Glc-6-*P* dehydrogenase and was as most 2 μM.

The I activity of the above preparation increased slowly when it was incubated at 30° with mercaptoethanol and, of interest, the increase of the I activity was stimulated greatly by the additions of Glc-6-*P* and $MgCl_2$. The total activity increased also but to a much smaller extent than that of the I form. When ATP and 3',5'-cyclic AMP were added at the point at which the I activity was enhanced, a rapid fall of the I activity was noted. These relations are presented in Fig. 1. The increase of the I activity occurred with the highly purified enzyme only when a small aliquot of crude extract was added to it. The boiled crude extract was ineffective. These facts indicate that the same interconversion process acts in the spleen enzyme as in other sources of enzyme and, in addition, that the D to I form conversion mediated by phosphatase



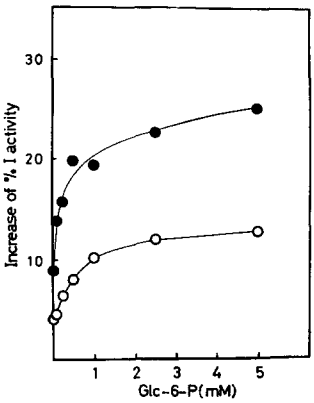


Fig. 1. The interconversion relationship between the D form and the I form. The enzyme in 50 mM Tris–5 mM EDTA (pH 7.8) was incubated at 30° with 50 mM mercaptoethanol (○). At the points indicated, 5 mM Glc-6-*P* and 10 mM $MgCl_2$ (●) and 5 mM ATP and 10 μM 3′,5′-cyclic AMP (□) were added. The incubation was terminated by rapid chilling in an ice-bath.

Fig. 2. The effect of Glc-6-*P* on the conversion of the D form to I form. The enzyme was incubated at 30° for 15 min with Tris buffer containing Glc-6-*P* (at indicated concentrations), 50 mM mercaptoethanol, and with (●) or without (○) 10 mM $MgCl_2$.

is activated by Glc-6-*P*. TRAUT AND LIPMANN[6] reported a similar increase of the I activity by Glc-6-*P* in the lamb muscle enzyme, but the role of the sugar phosphate was obscure. In liver enzyme, Glc-6-*P* served as an effector to convert the D form to the I form through the inactive intermediate[7]. In the spleen enzyme, however, the inactive intermediate could not be found.

The activation of the conversion was noted by Glc-6-*P* alone but much more by Glc-6-*P* and Mg^{2+} together. It was dependent on the concentration of Glc-6-*P* as shown in Fig. 2. The half-maximum activation of the conversion by Glc-6-*P* was in a range of 0.35–1 mM. The activation by Mg^{2+} alone (Fig. 2) has not yet been clarified, since a small amount of Glc-6-*P* (less than 20 μM) was produced in the incubation. But the activation in the presence of Glc-6-*P* was clear since the accelerative activation

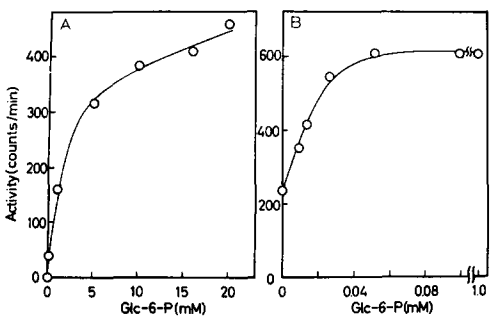


Fig. 3. The Glc-6-*P*-dependent activity of the D form (before conversion, A) and the converted (B) enzyme. The conversion was carried out by incubating the D form with 50 mM mercaptoethanol, 10 mM $MgCl_2$ and 5 mM Glc-6-*P* at 30° for 2 h. After the conversion, it was passed through Sephadex G-25 to remove Glc-6-*P*. The assay conditions are the same as in the routine assay except Glc-7-*P* concentration which is indicated in the figure.

with Mg^{2+} was noted at high concentrations of Glc-6-*P*. Glutathione could be replaced by mercaptoethanol but not by SO_3^{2-}, which was effective in liver enzyme[7]. The pH optimum of the conversion was about 7.5. No conversion took place at 0–10° over a 60-min incubation.

The conversion attained its limit in 1–2 h of incubation at 30° with all components, Glc-6-*P*, Mg^{2+} and mercaptoethanol. The limit of conversion was 33–36% of the I activity by the present assay method. The limit value was lower than some muscle and liver I form enzymes[5] which were fully independent at the similar assay conditions. the conversion, however, seems to be complete, since the enzyme showed a marked difference in terms of the activation by Glc-6-*P* before and after the incubation as shown in Fig. 3. After the incubation, the portion of the D activity of the enzyme was activated fully by the 50 μM Glc-6-*P* while before the incubation the enzyme was not activated fully even at the concentration of 20 mM Glc-6-*P*. If the conversion was not complete and, in part, the enzyme remained as it was, the enzyme would not show the full activation at 50 μM Glc-6-*P* after the incubation. The properties of the enzyme are under investigation.

The Glc-6-*P* sensitivity of the enzymes may indicate that glycogen synthesis *in vivo* is carried out mainly by the I form, as was mentioned by MERSMANN AND SEGAL[8]. From this point of view, the present results imply that Glc-6-*P* enhances glycogen synthesis by activating the conversion of the D form to the I form *in vivo* rather than by activation of the enzyme itself. This implication is in agreement with the earlier finding of an insulin-mediated increase of Glc-6-*P* and of the I form of glycogen synthetase in rat diaphragm by LARNER[1] and with the recent finding of an increase in the I activity at high levels of Glc-6-*P* after tetanic contraction of rat posterior muscle by PIRAS AND STANELONI[9]. The hormonal and nonhormonal effects on linking with enhancement of the I form could be explained in terms of an increase of Glc-6-*P*. It is of particular interest that Glc-6-*P* not only activates both phosphatases of phosphorylase[10] and glycogen synthetase but also inhibits phosphorylase b[11] and activates glycogen synthetase[12].

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