PRELIMINARY NOTE

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Interconvertible forms of glycogen synthetase in *Neurospora crassa*

Interconvertible forms of glycogen synthetase (UDPglucose:glycogen α-4-glucosyltransferase, EC 2.4.1.11) have been found in several mammalian tissues[1-4]. In muscle, the enzyme exists in two forms: one is dependent on glucose 6-phosphate (D) and the other independent of this factor (I). It has been found that a conversion of the I to the D form occurs in the presence of ATP–Mg^{2+} and apparently involves a phosphorylation at the protein level. The reverse reaction, *i.e.* a D to I conversion has also been demonstrated[5,6].

In the ascomycete fungus *Neurospora crassa*, an enzymatic activity responsible of the synthesis of glycogen from UDP-glucose has been described by TRAUT AND LIPMAN[5]. The present paper reports preliminary evidence that this enzyme also exists in two interconvertible forms.

A wild-type strain of *N. crassa* (St. L. 74) was grown in Vogel's minimal liquid medium[7] supplemented with 2% sucrose. Culture was started by inoculating a fresh conidial suspension (about $1 \cdot 10^5$ conidia per ml) and was incubated in a rotatory shaker for 48 h at 28°. The cultures were harvested by filtration through coarse filter paper on a Buchner funnel and rinsed with distilled water. Mycelial pads were lyophilized and ground in a mortar. Extracts were prepared by homogenizing the powdered mycelia with seven to ten volumes of 40 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA and 5 mM mercaptoethanol with a glass homogenizer and centrifuged 15 min at $10\,000 \times g$. The supernatant fraction was used as source of enzyme. The extract (0.04 ml) was incubated for different periods with the indicated additions in a total volume of 0.07 ml. Interconversion reactions were stopped by adding 0.4 ml of an ice-cold 40 mM Tris–HCl buffer (pH 7.4) containing 50 mM NaF, 20 mM EDTA and 10 mM mercaptoethanol. Glycogen synthetase was assayed on aliquots of each sample. The assay mixture containing the enzyme (0.02 ml), 1% glycogen, 1 mM UDP-[^{14}C]-glucose (specific activity, 360 000 counts/min per μmole) was incubated for 20 min at 30° with or without the addition of 2.5 mM glucose 6-phosphate. The reaction was stopped by adding 2 ml of 66% ethanol containing 0.1 M ammonium acetate. The precipitated glycogen was isolated as described by PIRAS *et al.*[8] and measured for radioactivity in a liquid scintillation spectrometer with BRAY's[9] solution. Protein was assayed by the method of LOWRY *et al.*[10].

As can be seen in Fig. 1a, incubation of the extract with ATP–Mg^{2+} (*plus* an ATP-generating system) leads to an inactivation of the glycogen synthetase activity assayed either in the presence or absence of glucose 6-phosphate. The opposite effect, *i.e.* an increase in activity, was observed after incubation in the absence of ATP.

The enzymatic extracts obtained from large-scale incubations carried out in the presence or absence of ATP–Mg^{2+} ("inactive" or "active" preparations, respectively) were purified by precipitation with $(NH_4)_2SO_4$ and passage through Sephadex G-25 columns. After that, the enzyme preparations were further incubated as described for activation or inactivation. Fig. 1b shows the results obtained when an "inactive"

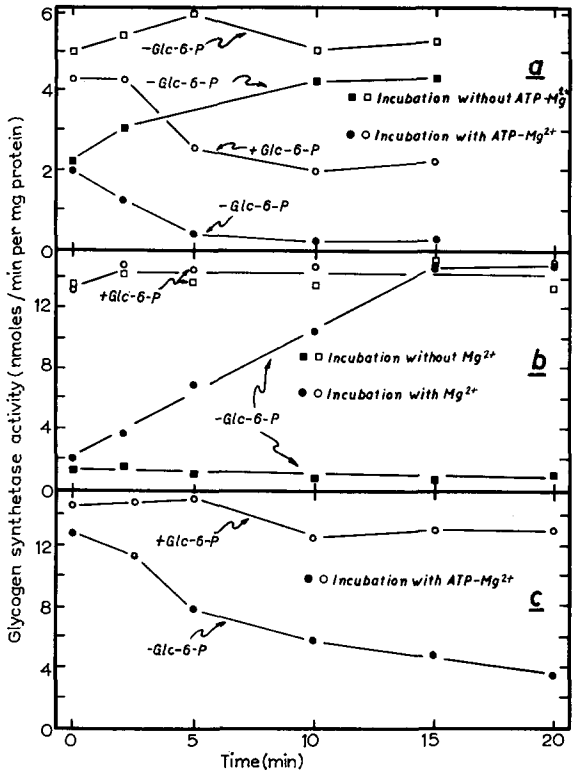

Fig. 1. a. Activation and inactivation of glycogen synthetase. The extract was incubated at 30° in the presence (○, ●) or absence (□, ■) of 2.5 mM ATP–3.5 mM phosphoenolpyruvate–12 mM $MgCl_2$–140 mM KCl. b. D to I conversion of glycogen synthetase. The extract (0.4 ml) was incubated for 10 min at 30° with ATP–phosphoenolpyruvate–Mg^{2+}–K^+ at the concentrations indicated above. The total volume was 0.7 ml. The reaction was stopped by the addition of 0.3 ml of 50 mM Tris–HCl buffer, pH 7.4, containing 1 mM EDTA and 5 mM mercaptoethanol *plus* 1.5 ml of an ice-cold satd. $(NH_4)_2SO_4$ solution. The precipitate was collected by centrifugation at 10 000 × *g* for 10 min and was resuspended in 0.3 ml of the EDTA–mercaptoethanol–buffer solution. After that the sample was passed through a G-25 Sephadex column (0.7 cm × 10 cm) equilibrated with the same buffer solution. Aliquots of the eluate were incubated in the presence (○, ●) or absence (□, ■) of 10 mM $MgCl_2$. c. I to D conversion of glycogen synthetase. The extract (1.2 ml) was incubated for 15 min at 30° in the presence of 10 mM $MgCl_2$. The total volume was 1.3 ml and the reaction was stopped by the addition of 1.8 ml of an ice-cold satd. $(NH_4)_2SO_4$ solution. The precipitate was collected and passed through a Sephadex G-25 column as described above. Aliquots of the eluate were incubated at 30° in the presence of 2.5 mM ATP and 8.5 mM $MgCl_2$. Glycogen synthetase activity was assayed in the presence (○, □) or absence (●, ■) of glucose 6-phosphate as described in the text.

preparation was incubated in the presence or in the absence of Mg^{2+}. As can be seen, Mg^{2+} is required for the conversion of a glucose 6-phosphate-dependent form of the enzyme to the corresponding independent form. The conversion occurred without significant change in the activity measured in the presence of glucose 6-phosphate. Fig. 1c shows the result of the incubation of an "active" preparation of glycogen synthetase with ATP–Mg^{2+}. It can be observed that the enzyme was converted to a glucose 6-phosphate-dependent form. No important changes in the activity measured in the presence of glucose 6-phosphate were detected.

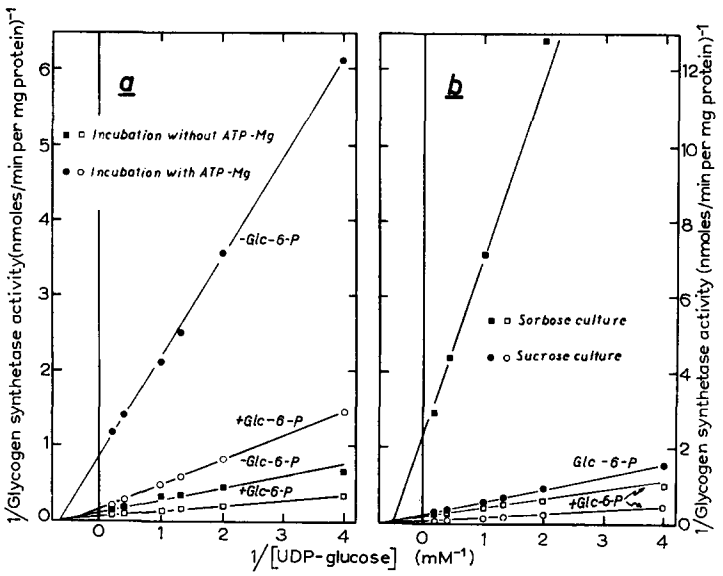

Fig. 2. Effect of UDP-glucose concentration on the activity of glycogen synthetase. a. Lineweaver–Burk plots of the results obtained with the enzymes prepared by incubation of 0.4 ml of the extract in the presence (○, ●) or absence (□, ■) of 2.5 mM ATP–3.6 mM phosphoenolpyruvate–12 mM $MgCl_2$–140 mM KCl. After incubation for 10 min at 30° in a total volume of 0.7 ml, the reactions were stopped by the addition of 0.3 ml of a solution containing 50 mM Tris–HCl buffer, pH 7.4, 50 mM NaF, 10 mM EDTA and 20 mM mercaptoethanol *plus* 1.5 ml of an ice-cold satd. $(NH_4)_2SO_4$ solution. The precipitate was collected by centrifugation at 10 000 × *g* for 10 min and resuspended in 0.3 ml of the NaF–EDTA–mercaptoethanol–buffer solution. After that the sample was passed through a Sephadex G-25 column (0.7 cm × 10 cm) equilibrated with the same solution. b. Lineweaver–Burk plots of the results obtained with the extract prepared from mycelia grown for 30 h in sorbose (□, ■) or sucrose (○, ●) supplemented cultures. The concentration of sorbose in the Vogel's minimal medium was 1 %. Conditions of the cultures were as those indicated in the text. The lyophilized mycelia were homogenized in 15 vol. of NaF–EDTA–mercaptoethanol–buffer solution and the supernatant fluids obtained after centrifugation at 10 000 × *g* for 10 min were passed through Sephadex G-25 columns (0.7 cm × 10 cm) equilibrated with the same solution. Glycogen synthetase activity was assayed at different concentrations of UDP-glucose in the presence (○, □) or absence (●, ■) of 2.5 mM glucose 6-phosphate as described in the text.

The inactivation of glycogen synthetase by ATP–Mg^{2+} leads to a decrease in the maximum velocity when the enzyme was assayed at high concentration of UDP-glucose, either in the presence or absence of glucose 6-phosphate. No appreciable change in the apparent K_m for UDP-glucose was observed (Fig. 2a).

Some evidences may indicate that these conversions also occur *in vivo*. It has been shown that the substitution of sucrose by sorbose as the carbon source in the growth medium leads to gross morphological and physiological changes in *N. crassa*[11,12]. Moreover, it was observed that mycelia grown in the presence of sorbose accumulated much less glycogen than those grown in a sucrose-supplemented medium (unpublished results). Crude extracts obtained from sorbose-grown mycelium showed lower levels of glycogen synthetase activity than those grown in sucrose. In addition, the ratio between the glycogen synthetase activity measured in the absence of glucose 6-phosphate to that measured in the presence of this activator is 3 to 6 times higher in the

extracts prepared from mycelium grown in sucrose than in those extracts prepared from sorbose-grown mycelium. Fig. 2b shows the effect of the UDP-glucose concentration on the glycogen synthetase activity of the extracts prepared from sorbose- or sucrose-grown mycelium. As can be seen, these enzymes differ in the maximum velocity.

The results reported in this paper indicate that glycogen synthetase in *N. crassa* has at least two interconvertible forms. One is dependent on glucose 6-phosphate and the other independent of this phosphoric ester. Conversion of the I to D form requires *in vitro* ATP–Mg^{2+}; the reverse, D to I, requires Mg^{2+}.

In conclusion it appears that glycogen metabolism in *N. crassa* is regulated in a fashion similar to that described for mammalian tissues and is different from that in bacteria in which regulation is believed to occur at the nucleotide–sugar pyrophosphorylase step[13]. In Neurospora mycelia extract, UDP-glucose pyrophosphorylase activity (UTP:α-D-glucose 1-phosphate uridylyltransferase, EC 2.7.7.9) is 10–30 times greater than that of glycogen synthetase (unpublished results). It is suggested that the type of regulation of glycogen metabolism found in this fungus may occur in other eucaryotic organisms.

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