Specificity of uridine diphosphate glucose–glycogen glucosyltransferase

The specificity of UDPG–glycogen glucosyltransferase (E.C.2.4.1.11) for the acceptor has been reported to be very similar to that of animal glycogen phosphorylase[1]. In both cases glycogen is the best acceptor. Recently ILLINGWORTH *et al.*[2] have shown that muscle phosphorylase can form amylose chains in the absence of any added acceptor, but at a very low rate. They also reported that when muscle phosphorylase acts in the direction of phosphorolysis it can degrade substrates down to maltotetraose, but not further.

The action of rat-muscle UDPG–glycogen glucosyltransferase on different acceptors and donors has been studied in more detail. The results obtained corroborate the similarity in the behaviour of UDPG–glycogen glucosyltransferase and α-glucan phosphorylase (E.C.2.4.1.1) towards acceptors. An enzyme preparation, inactive under the conditions of the experiment in the absence of added acceptor, was obtained as follows. Rat muscle was homogenized in a Waring blendor with 3 vol. of cold 1 m*M* EDTA and left at 37° for 20 min. The pH was maintained at about 7 by intermittent addition of NaOH. The extract was then centrifuged at 0° for 10 min at 12000 × *g* and the supernatant fluid was mixed with 1 vol. of 50% w/v ammonium sulfate (pH 7). After centrifuging for 10 min at 25000 × *g* the precipitate was washed with 25% ammonium sulfate, redissolved in one-tenth of the original volume of 0.05 *M* Tris–maleate buffer (pH 7.5) containing 0.001 *M* EDTA, and dialyzed at 4° for 2 h against pure glycerol. The dialysis reduced the volume of the extract 4–5 fold. The enzyme preparation was more stable than the isoelectric precipitate used in previous studies[1] and retained 80% of its activity after storage at —15° for 15 days. The ammonium sulfate precipitate was still more stable if kept undissolved. It could be stored for 3 weeks at —15°, and yielded a 90–100% active preparation when dissolved and dialyzed before use.

The malto-oligosaccharides were obtained from an amylose hydrolysate by chromatography on a charcoal-Celite column and gradient elution with ethanol[3]. Some of the homologues were separated by chromatography on washed Whatman No. 17 paper. The paper was washed and assembled as described by CABIB AND CARMINATTI[4] and irrigated with butanol–pyridine–water (6:4:3).

The results obtained with oligosaccharides as acceptors are shown in Fig. 1. In order to get comparable rates, the concentration of oligosaccharides had to be much higher than in the case of glycogen. The Michaelis constant is $7 \cdot 10^{-2}$ *M* for maltotetraose, maltohexaose and maltoheptaose, and much lower for glycogen. The values for the latter are: $1 \cdot 10^{-3}$ *M* when expressed in glucose units and $0.9 \cdot 10^{-4}$ *M* if the concentration is calculated as end groups (assuming 9% end groups[6]). Maximal velocity is higher for the oligosaccharides than for glycogen, as obtained from the Lineweaver–Burk plot: values of 1.8 and 1.5 were found for maltotetraose and maltopentaose, respectively, compared to glycogen as unity. It is evident that the enzyme binds tightly to the glycogen molecule, and probably in many points; yet it can be assumed that some of these attachments are not the most favorable in every instant, and thence lead to a sub-optimal maximal velocity. Oligosaccharides would behave in the opposite way.

It can be observed in Fig. 1 that the rates obtained with maltopentaose and maltooctaose were somewhat lower than for the other homologues. The same results

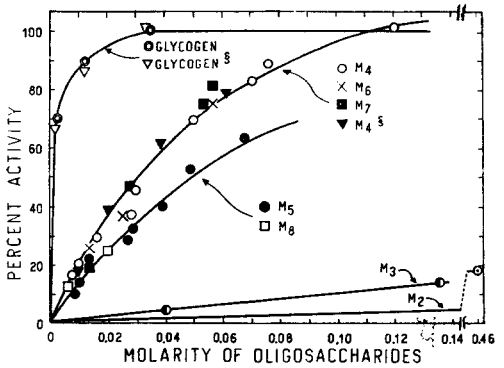

Fig. 1. Malto-oligosaccharides as acceptors for glycogen synthesis. Test system as described in ref. 5, with suitable dilutions of the glycerol extract. Incubation for 15 min at 37°. The reaction was linear with time under the conditions of the experiment. The molarity of glycogen is expressed in glucose units. Values shown in the graph are an average of several determinations. M_2, maltose; M_3, maltotriose; M_4, maltotetraose; M_5, maltopentaose; M_6, maltohexaose; M_7, maltoheptaose; M_8, maltooctaose; §, maltotetraose and glycogen as acceptors for muscle α-glucan phosphorylase; this enzyme was tested as previously described[5].

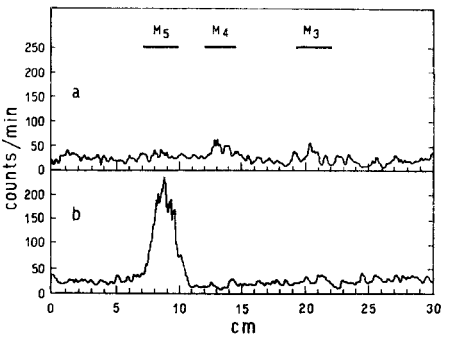

Fig. 2. Reaction product ing maltotetraose as acceptor. The enzyme preparation was incubated with (in μmoles): 10 of glycine buffer (pH 8.6); 0.25 of EDTA; 0.50 of glucose 6-phosphate; 0.12 of [^{14}C]UDPG, 5200 counts/min, and acceptor. a, 0.83 μmole (as glucose) of glycogen; b, 2.5 μmoles of maltotetraose. Total volume was 0.05 ml. After 30 min at 37°, 1.2 mg of carrier glycogen and 0.5 ml of 80% ethanol were added, and the suspension was centrifuged. The supernatant, containing the oligosaccharides, was further treated as described by LELOIR *et al.*[7]. The chromatograms were scanned automatically with a Nuclear-Chicago model D-47 gas-flow counter fitted to C-100A actigraph II (½″ in collimator). M_3, maltotriose; M_4, maltotetraose; M_5, maltopentaose.

were found with samples of maltopentaose obtained directly from the charcoal column, separated on paper, and/or passed through a column of mixed-bed resin (Amberlite MB_3); nevertheless it is possible that some inhibitory contaminants might account for the results.

Maltose and maltotriose at high concentrations acted as acceptors but with very low efficiency. Thus 0.64 *M* maltose or 0.13 *M* maltotriose were as effective as $7 \cdot 10^{-4}$ *M* glycogen (the latter expressed in glucose units). The formation of new glycogen molecules does not necessarily require a preformed molecule of same structure; it can start, though at a very reduced rate, with saccharide acceptors as small as maltose.

As shown in Fig. 2, incubation of [^{14}C]UDPG labeled in the glucose moiety, maltotetraose and enzyme, led to the formation of the next higher homologue. The same result was obtained with unlabeled UDPG, and radioactive maltotetraose. This fact, as well as the identical values of K_m obtained for maltotetraose, maltohexaose and maltoheptaose, point to a multi-chain pattern of enzyme action.

It was also observed that glycogen from different sources: shell fish, rabbit liver, rat liver, or corn, served equally well as acceptors in the reaction. The oligosaccharides of the α-1,6 series, potato starch grains, and dextran, were ineffective as acceptors, while amylose and amylopectin were 15% as efficient as glycogen of the same concentration (expressed in glucose units). Amylopectin inhibited the reaction when added together with glycogen.

Some experiments were carried out with muscle α-glucan phosphorylase. Either a purified preparation[8] or the above-mentioned glycerol extract (which also has phosphorylase activity) were used, and as shown in Fig. 1, the results were very similar to those obtained with UDPG–glycogen glucosyltransferase.

Several synthetic nucleoside diphosphate sugars were tested as donors. ADP-glucose was found to be 50% as effective as UDPG of the same molarity, while CDP-glucose, IDP-glucose, and ADP-maltose did not serve as substrates for the enzyme.

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Instituto de Investigaciones Bioquímicas Fundación Campomar, and Facultad de Ciencias Exactas y Naturales, Obligado 2490, Buenos Aires (Argentina)

SARA H. GOLDEMBERG*

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* Investigator of the Instituto Nacional de Microbiología.

The inhibition of haemolysis by phloridzin

Delayed haemolysis in hypotonic glucose solutions is inhibited by phloridzin[1]; since delayed haemolysis also occurs in hypotonic solutions of malonamide[2] the effect of phloridzin on this system was examined by methods already described[3,4].

Fig. 1 illustrates the action on malonamide-induced haemolysis of 0.1% (w/v) phloridzin, in which concentration the osmotic contribution of the inhibitor is approximately equivalent to 0.05 atm.; the graphs show that although the osmotic contribution of phloridzin is about one tenth that of glucose and glycine, the inhibition it produces is much greater. The effect of temperature on the inhibited process was also examined and the combined results are recorded in Table I.

Glucose stabilizes the water lattice through the promotion of extensive hydrogen bonding and because a system of this nature is very heat sensitive, glucose inhibition