THE INHIBITION OF GLYCOGEN PHOSPHORYLASE BY URIDINE DIPHOSPHATE GLUCOSE *

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In the course of studying the relationship of glycogen phosphorylase and glycogen synthetase to the synthesis of glycogen by extracts of Agrobacterium tumefaciens (Madsen, 1961) it was noted that the phosphorylase was inhibited by uridine diphosphate glucose (UDPG), the substrate of the synthetase. The phosphorylases from this bacterium and from rabbit muscle were found to exhibit an inhibition by UDPG which is competitive with respect to glucose-1-phosphate (G-1-P).

METHODS AND MATERIALS

Cells of A. tumefaciens (A-6) were disrupted by sonic oscillation, the cell-free material fractionated with ammonium sulfate and the fraction precipitating at 30% saturation extracted with a half volume of 0.01 M tris (hydroxymethyl) aminomethane buffer containing 0.001 M ethylenediaminetetraacetic acid (EDTA). Nucleic acid was removed from this extract with streptomycin sulfate (Cohen and Lichstenstein, 1960) and the phosphorylase activity concentrated by precipitation with 40% saturated ammonium sulfate.

Enzymatic activity was estimated by determining the release of inorganic phosphate from G-1-P at 30° in a reaction mixture of 0.2 ml. containing 0.3 mg. of bacterial protein, 0.02 M glycerophosphate, pH 6.7, 0.4% glycogen, 0.05 M KF, 0.0001 M EDTA, G-1-P concentration as given in Fig. 1, and 0.0005 M pyridoxal-5-phosphate. The latter compound, which Baranowski et al. (1957) showed to

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be a component of muscle phosphorylase a, stimulated all the bacterial preparations so far tested by approximately 50%.

Phosphorylase a was prepared from rabbit muscle and recrystallized four times (Green and Cori, 1943). Activity was estimated as described above except that the glycogen concentration was 0.68%, pyridoxal-5-phosphate and KF were omitted, 0.001 M adenosine-5'-phosphate was added and only 6 micrograms of protein were used.

UDPG and uridine diphosphate (UDP) were purchased from the Sigma Chemical Company.

RESULTS

Data for the inhibition of the bacterial and muscle phosphorylases by UDPG at various concentrations of G-1-P are plotted in Fig. 1, according to the method of Lineweaver and Burk (1934).

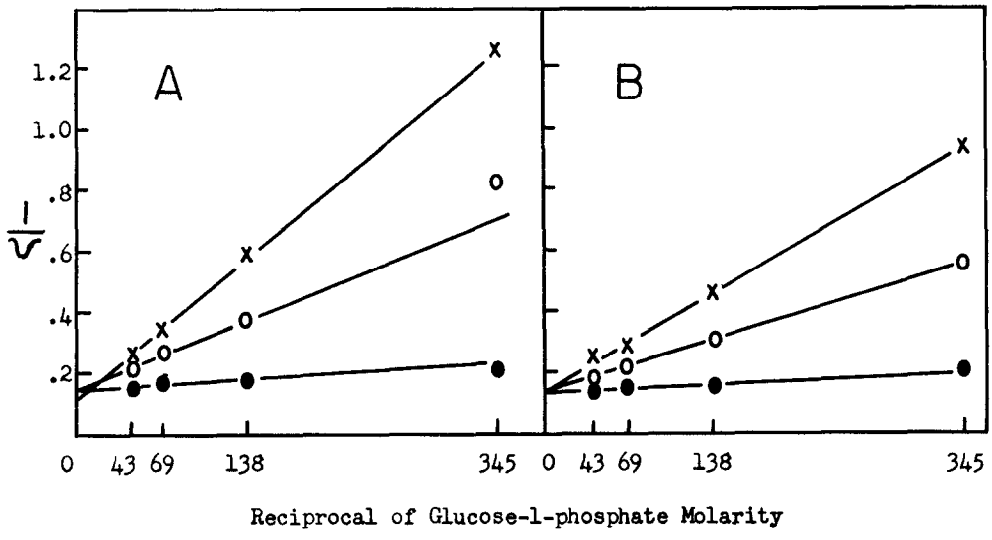

Figure 1, A. The inhibition of bacterial phosphorylase by UDPG. Since the amount of inorganic phosphate released was small, this was used directly as a measure of activity. ●—●, no UDPG; O—O, 4 x 10^{-3} M UDPG; X—X, 8 x 10^{-3} M UDPG.

B. The inhibition of muscle phosphorylase a by UDPG. Enzymatic activity is represented by the inorganic phosphate released in the first minute of incubation, as calculated by the method described by Cori, Cori and Green (1943). ●—●, no UDPG; O—O, 4.6 x 10^{-3} M UDPG; X—X, 9.2 x 10^{-3} M UDPG.

It is obvious that UDPG satisfies the criteria of Michaelis-Menten kinetics for a competitive inhibitor. The data in Fig. 1 yield a K_i of 0.67 x 10^{-3} M UDPG for the bacterial enzyme and 0.92 x 10^{-3} M for the muscle enzyme. The K_m for G-1-P is 1.6 x 10^{-3} M for the bacterial phosphorylase and 1.7 x 10^{-3} M for phosphorylase a. The latter figures are somewhat lower than those previously reported but this may be due to the inhibitory effect of the glycerophosphate buffer (Cori et al., 1943). These authors also reported that glucose is a competitive inhibitor but it is not nearly as effective as UDPG.

Certain aspects of the inhibition by UDPG were studied further, using the crystalline muscle enzyme only. UDPG was found to inhibit phosphorylase a when the enzyme was acting to phosphorylyze glycogen and this inhibition was non-competitive with respect to inorganic phosphate. 8 x 10^{-3} UDPG caused a 73% inhibition of glycogen phosphorolysis. When the inhibition of glycogen synthesis at this concentration of UDPG was plotted against G-1-P concentration, extrapolation to zero G-1-P concentration yielded an inhibition of 71%. Similarily, 4 x 10^{-3} M UDPG caused a 59% inhibition of glycogen synthesis at zero concentration of G-1-P.

UDP did not inhibit glycogen synthesis at low concentrations of G-1-P but did at higher concentrations of the substrate, an effect similar to that reported for glycerophosphate (Cori et al., 1943). At concentrations of 0.01 M UDP and 0.0145 M G-1-P this inhibition was only 15%. UDP caused only a slight inhibition of glycogen phosphorolysis.

DISCUSSION

The inhibition reported here in which a major metabolite from one metabolic sequence is shown to be a competitive inhibitor of an enzyme in an alternative sequence, is similar to other reported cases of which the inhibition of phosphoglucose isomerase by 6-phosphogluconate (Parr, 1956) or by erythrose-4-phosphate (Grazi et al., 1960). Concentrations of UDPG which have been found in various tissues approach the order of 1 x 10^{-3} M (Caputto,

et al., 1950) and inhibition of phosphorylase may therefore be expected to occur to a significant extent in vivo. One may speculate that glycogen phosphorolysis in unicellular organisms may be regulated by the intra-cellular concentration of UDPG. A high level of the latter compound would be expected to increase the rate of glycogen synthesis, since it is the substrate for glycogen synthetase, while limiting the rate of degradation by inhibiting phosphorylase. This postulated control mechanism, which may not be the only one operating, is supplemented in mammals by a more sophisticated hormonal control of some of the enzymes concerned with glycogen metabolism.

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