Effect of fructose 1-phosphate on the activation of liver glycogen synthase

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1. The activation (dephosphorylation) of glycogen synthase and the inactivation (dephosphorylation) of phosphorylase in rat liver extracts on the administration of fructose were examined. The lag in the conversion of synthase *b* into *a* was cancelled, owing to the accumulation of fructose 1-phosphate. A decrease in the rate of dephosphorylation of phosphorylase *a* was also observed. The latency re-appeared in gel-filtered liver extracts. 2. Similar latency was demonstrated in extracts from glucagon-treated rats. Addition of fructose 1-phosphate to the extract was able to abolish the latency, and the activation of glycogen synthase and the inactivation of phosphorylase occurred simultaneously. 3. Fructose 1-phosphate increased the activity of glycogen synthase *b* measured in the presence of 0.2–0.4 mM-glucose 6-phosphate. According to kinetic investigations, fructose 1-phosphate increased the affinity of synthase *b* for its substrate, UDP-glucose. 4. The accumulation of fructose 1-phosphate resulted in glycogen synthesis in the liver by inducing the enzymic activity of glycogen synthase *b* in the presence of glucose 6-phosphate *in vivo* and by promoting the activation of glycogen synthase.

INTRODUCTION

Glycogen synthase (EC 2.4.1.11) is known to catalyse the rate-limiting step in glycogen synthesis. In liver this is a highly phosphorylated enzyme (Nuttall *et al.*, 1981) and, as in other tissues, the activity is regulated by the state of phosphorylation. Glycogen synthase is activated by dephosphorylation when the concentration of phosphorylase *a* is lowered below a threshold value. This energy-conserving situation may result from the fact that *in vitro* the activation of glycogen synthase is preceded by the dephosphorylation of phosphorylase *a* (Stalmans *et al.*, 1974).

A number of monosaccharides are known to result in glycogen synthesis in the liver. The administration of fructose leads to the accumulation of fructose 1-phosphate in liver (Burch *et al.*, 1969; Woods *et al.*, 1970; Iles *et al.*, 1980) and kidney (Burch *et al.*, 1980). The effect of fructose administration on the enzymes of glycogen metabolism has been investigated. A load of fructose provokes the inactivation of phosphorylase *a*, which occurs only 10–20 min after the intravenous administration of the ketose to intact animals (Thurston *et al.*, 1974; Van den Berghe *et al.*, 1973). It is now evident that this sugar can evoke the rapid accumulation of fructose 1-phosphate with a simultaneous increase in phosphorylase *a* activity in perfused rat liver (Walli *et al.*, 1975; Jakob, 1976; Miller, 1978; Nuttall *et al.*, 1983), in isolated hepatocytes (Van de Werve & Hers, 1979; Ciudad *et al.*, 1979, 1980) and in intact animals (Regan *et al.*, 1980; Nuttall *et al.*, 1983). It was also reported that the treatment of rats *in vivo* with fructose results in hepatic glycogen synthase activation (Whitton & Hems, 1975; Miller, 1978). The latter was demonstrated in isolated rat hepatocytes (Ciudad *et al.*, 1979, 1980), where fructose provokes the simultaneous activation of glycogen synthase and phosphorylase. On the other hand, fructose causes a striking decrease in glycogen synthase *a* in intact animals (Regan *et al.*, 1980; Nuttall *et al.*, 1983).

The aim of the present study is to describe the activation–inactivation pattern of glycogen synthase and phosphorylase in crude liver extracts. When gel-filtered liver extracts are incubated, the conversion of synthase *b* into *a* is preceded by a pronounced lag, which corresponds to the time required to convert phosphorylase *a* into the *b* form. This latency was abolished by the presence of fructose 1-phosphate. Our previous work showed that fructose 1-phosphate inhibits the dephosphorylation of purified liver and muscle phosphorylase *a* (Bot *et al.*, 1982). The present paper demonstrates that fructose 1-phosphate also enhances the enzymic activity of glycogen synthase *b* measured in the presence of glucose 6-phosphate in low concentration.

MATERIALS AND METHODS

Materials and buffers

D-Fructose 1-phosphate (dicyclohexylammonium salt), D-glucose 6-phosphate (disodium salt) and glucagon were obtained from Sigma, Sephadex G-25 was supplied by Pharmacia, and UDP-[U-^{14}C]glucose was purchased from Amersham International. All other chemicals used were of the highest grade commercially available.

Glycogen synthase *b* was purified from the liver of normally fed rabbits given glucagon (Tan & Nuttall, 1983). Specific enzymic activity was 12.6 units/mg in the presence of 10 mM-glucose 6-phosphate, and the activity ratio (activity in the absence/activity in the presence of 10 mM-glucose 6-phosphate) was less than 0.05.

Buffer A contained 250 mM-sucrose, 0.5 mM-dithiothreitol and 50 mM-glycylglycine, and was adjusted to pH 7.4.

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Assays

Phosphorylase *a* was assayed as described by Stalmans & Hers (1975) and glycogen synthase *a* as described by Doperé *et al.* (1980), for liver extracts. The activity of purified rabbit liver glycogen synthase *b* was measured by the method of Camici *et al.* (1984). One unit of activity of these enzymes converts 1 μmol of substrate into product/min at 30 °C. Protein was determined as described by Bradford (1976), with bovine serum albumin as a standard.

Handling of animals and livers

Preparation of liver extracts. Animal experiments were performed *in vivo* on normally fed Wistar rats weighing about 200 g. The rats were anaesthetized by intraperitoneal injection of pentobarbital (100 mg/kg). The abdomen was opened and the liver exposed. Glucagon (100 μg/kg) or fructose (2 g/kg) were injected intravenously at 20 min after the beginning of anaesthesia. Glucagon and fructose are capable of maintaining high activities of phosphorylase *a* and synthase *b* in the liver. Decapitation was performed 7 min after the injection. The livers were immediately removed and homogenized in a Potter–Elvehjem tube in 2.5 vol. of ice-cold buffer A. The homogenate was centrifuged for 10 min at 8000 *g* in the cold, and 2.5 ml of the supernatant was filtered through a column of Sephadex G-25 (40 cm × 1.5 cm) equilibrated in buffer A.

Activation–inactivation of endogenous synthase and phosphorylase. Gel filtration caused some dilution (about 1.5-fold), therefore the protein concentrations of the supernatant and gel-filtered extract were adjusted to 45 mg/ml by the addition of buffer A. The extracts were then incubated at 25 °C in the presence of 1 mM-magnesium acetate and 5 mM-$(NH_4)_2SO_4$ as described by Mvumbi *et al.* (1983). The phosphorylase and glycogen synthase activities were determined in samples of this mixture at regular intervals.

RESULTS

Activation of glycogen synthase in liver extracts

When a crude extract from the liver of a fructose-treated rat was incubated in the presence of 1 mM-Mg^{2+} and 5 mM-sulphate, no latency was apparent in the conversion of glycogen synthase *b* into *a*. The inactivation of phosphorylase *a* and the activation of synthase *b* occurred simultaneously (Fig. 1). In contrast, when the gel-filtered extract was incubated in the presence of 1 mM-Mg^{2+} and 5 mM-sulphate, a pronounced lag preceded the activation of glycogen synthase *b*. This latency corresponded to the time required for the dephosphorylation of phosphorylase *a*. The latency in the activation of synthase *b* also disappeared when the gel-filtered extract was incubated with 20 mM-fructose 1-phosphate, 1 mM-Mg^{2+} and 5 mM-sulphate (Fig. 1). The patterns of the gel-filtered extract, as illustrated in Fig. 1, correspond basically to observations made with mouse or rat liver preparations (Stalmans *et al.*, 1971; Mvumbi *et al.*, 1983). A significant increase in the dephosphorylation rate of phosphorylase *a* was observed in the gel-filtered extract. This increase can be attributed to the removal of fructose 1-phosphate, an inhibitor of the dephosphorylation of phosphorylase *a* (Bot *et al.*, 1982).

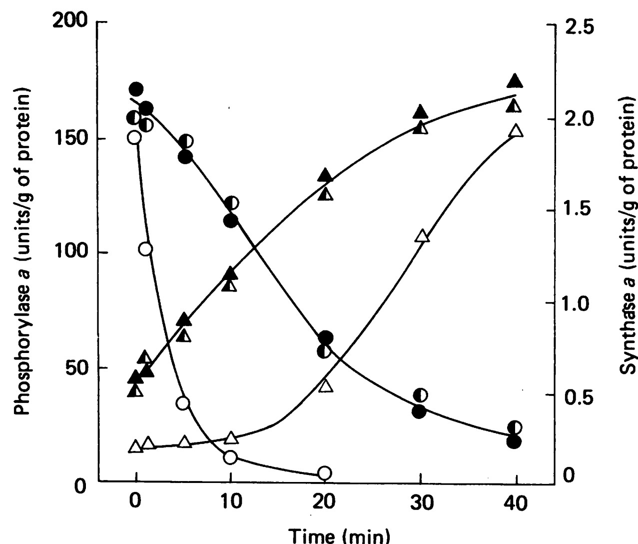

Fig. 1. Inactivation of phosphorylase and activation of glycogen synthase in liver extracts of fructose-treated rats

The extracts were incubated in the presence of 1 mM-Mg^{2+} and 5 mM-sulphate. Activity of phosphorylase *a* (○, ◐, ●) and glycogen synthase *a* (△, ◭, ▲) in crude (●, ▲) or gel-filtered (○, △) extracts or in gel-filtered extracts + 20 mM-fructose 1-phosphate (◐, ◭).

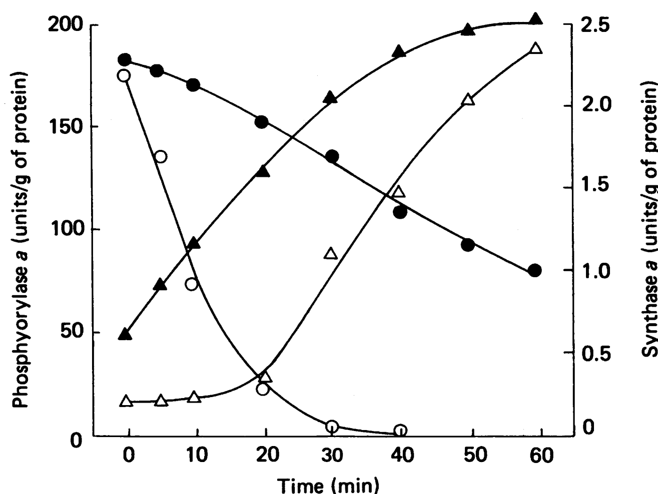

Fig. 2. Effect of fructose 1-phosphate on the activation of glycogen synthase in gel-filtered extract from glucagon-treated rat liver

The gel-filtered extracts were incubated without (○, △) or with 20 mM-fructose 1-phosphate (●, ▲). Activities of phosphorylase *a* (○, ●) and glycogen synthase *a* (△, ▲) were measured. Incubation was performed in the presence of Mg^{2+} and sulphate.

The sequential inactivation of phosphorylase *a* and activation of glycogen synthase *b* is also observed in the gel-filtered extracts of glucagon-treated rats (Fig. 2). In the presence of Mg^{2+} and sulphate, the inactivation of liver phosphorylase *a* started immediately, inducing a pronounced lag in the activation of synthase *b*, which lasted until the near-complete inactivation of phosphorylase. The conversion of glycogen synthase *b* into *a* started without any lag and at a rate identical with that

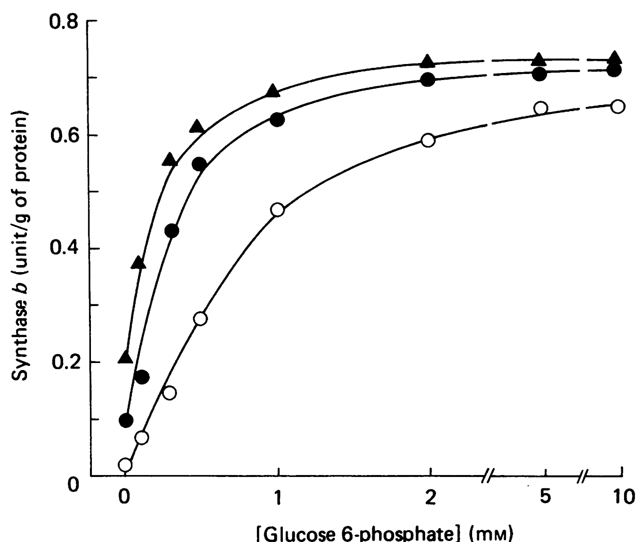

Fig. 3. Dependence of glycogen synthase *b* activity on glucose 6-phosphate concentration in the presence of fructose 1-phosphate

The activity of purified liver glycogen synthase *b* as a function of glucose 6-phosphate concentration was determined in the presence of 0.2 mM-UDP-glucose and 0.6% glycogen as described in the Materials and methods section. Additions: ○, none; ●, 2 mM-fructose 1-phosphate; ▲, 10 mM-fructose 1-phosphate.

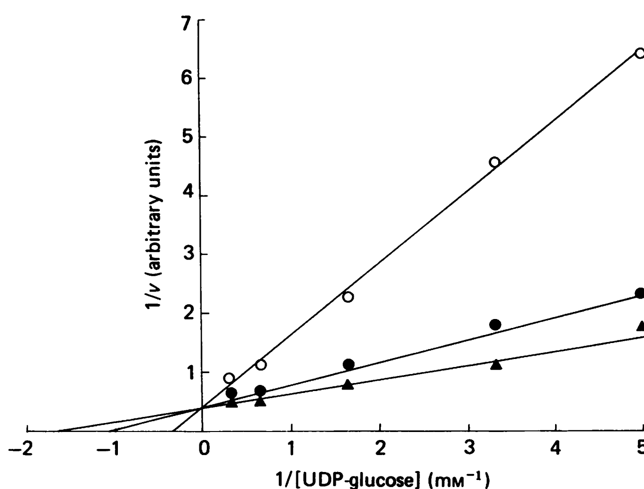

Fig. 4. Stimulation of glycogen synthase *b* activity by fructose 1-phosphate

The activity of purified liver glycogen synthase *b* was assayed in the presence of 0.3 mM-glucose 6-phosphate, 0.6% glycogen and various concentrations (0.2–6.6 mM) of UDP-glucose as described in the Materials and methods section. Additions: ○, none; ●, 2 mM-fructose 1-phosphate; ▲, 10 mM-fructose 1-phosphate.

when fructose 1-phosphate was added to the gel-filtered liver extract (Fig. 2). This metabolite also inhibited the dephosphorylation of phosphorylase *a*.

Effect of fructose 1-phosphate on the activity of liver glycogen synthase *b*

This was investigated at different concentrations of glucose 6-phosphate. The enzymic activity, assayed with physiological concentrations of UDP-glucose, was also dependent on glucose 6-phosphate (Fig. 3). The addition of fructose 1-phosphate significantly increased the specific activity of synthase *b*, when the concentration of glucose 6-phosphate was lower than 1 mM. Fructose 1-phosphate behaves as an activator of glycogen synthase *b*, since it also induces some activity in the absence of glucose 6-phosphate. The Lineweaver–Burk plot (Fig. 4) demonstrated that the addition of fructose 1-phosphate decreased the K_m for UDP-glucose without altering $V_{max.}$. The values of K_m for UDP-glucose were 3 mM in the absence of fructose 1-phosphate, 0.9 mM in the presence of 2 mM-fructose 1-phosphate and 0.6 mM in the presence of 10 mM-fructose 1-phosphate.

Glycogen synthase *b* is active only in the presence of glucose 6-phosphate, but this activity is cancelled by P_i and ATP. Consequently, this enzyme form remains inactive, owing to the intracellular concentrations of P_i and ATP (De Wulf *et al.*, 1968). We have demonstrated that fructose 1-phosphate still stimulates the activity of purified synthase *b* in the presence of negative effectors such as P_i and ATP. The specific activity of synthase *b* was 0.04 unit/mg when assayed with 0.3 mM-glucose 6-phosphate in the presence of 2.5 mM-ATP and 5 mM-P_i. This activity was stimulated 5-fold by adding 2–10 mM-fructose 1-phosphate. It is known that loading a liver with fructose causes a rapid loss of ATP and P_i (Woods *et al.*, 1970). The stimulatory effect of fructose 1-phosphate was more pronounced in the presence of 0.3 mM-glucose 6-phosphate, 0.5 mM-ATP and 2 mM-P_i, the specific activity of synthase *b* being within the range 0.3–0.4 unit/mg.

The stimulatory effect of fructose 1-phosphate on the activity of glycogen synthase *b* was also demonstrated in rat liver extracts. When a gel-filtered liver extract of a glucagon-treated rat was incubated, the activation of glycogen synthase took place in every case (see Fig. 2). Active synthase *a* was generated in the course of dephosphorylation, and this process could be monitored with the activity ratio measured in the absence and presence of 10 mM-glucose 6-phosphate. Thus the three different samples in Table 1 corresponded to the inactive synthase *b* and the partially and fully active forms of synthase *a* respectively. It is seen that fructose 1-phosphate can stimulate the activity of the inactive *b* form only.

DISCUSSION

The biological significance of the latency that precedes the activation of glycogen synthase in gel-filtered extracts was demonstrated a decade ago (Stalmans *et al.*, 1974) and the characteristics were subsequently discussed in more detail (Mvumbi *et al.*, 1983). Fructose 1-phosphate accumulating after fructose administration in the liver increases the amounts of active phosphorylase *a* and glycogen synthase *a* (see Ciudad *et al.*, 1980). The presence of glycogen synthase in the *a* form is apparently in contrast with the theory of latency, since the activation of glycogen synthase has occurred, but the amount of phosphorylase *a* is still high. This discrepancy may be explained by the presence of fructose 1-phosphate. Fructose 1-phosphate can bind to muscle or liver phosphorylase *a*, inhibiting its enzymic activity (Kaufmann & Froesch, 1973; Thurston *et al.*, 1974) and its

Table 1. Effect of fructose 1-phosphate on the activity of liver glycogen synthase

The gel-filtered liver extracts of glucagon-treated rats were incubated in the presence of 1 mM-Mg^{2+} and 5 mM-sulphate for increasing time periods, and glycogen synthase activity was assayed in each incubation mixture in order to calculate the state of phosphorylation (−/+ glucose 6-phosphate) and the stimulating effect of fructose 1-phosphate. The maximal activity of glycogen synthase, assayed with 10 mM-glucose 6-phosphate, was 2.6 units/g.

		Specific activity of synthase in the presence of 0.3 mM-glucose 6-phosphate (units/g of protein)	
Incubation time (min)	−/+ Glucose 6-phosphate	Without fructose 1-phosphate	With 2 mM-fructose 1-phosphate
0	0.13	0.11	0.32
30	0.43	1.93	2.01
60	0.92	2.55	2.50

dephosphorylation by protein phosphatase-1 (Bot *et al.*, 1982). The complex of phosphorylase *a* and fructose 1-phosphate is resistant to the action of phosphatase, and is then apparently non-inhibitory to synthase phosphatase. The removal of this sugar phosphate by gel-filtration results in the appearance of latency. The suppression of the lag period by AMP was also demonstrated. It has been shown that AMP counteracts the inhibition of synthase phosphatase by phosphorylase *a* (Stalmans *et al.*, 1971).

It is also noteworthy that fructose 1-phosphate significantly decreases the rate of dephosphorylation of phosphorylase in liver extract, without affecting the activation rate of glycogen synthase. When the dephosphorylation of the purified synthase *b* by the catalytic subunit of protein phosphatase-1 was examined, fructose 1-phosphate was a strong inhibitor, with K_i of about 4 mM (B. Tóth & P. Gergely, unpublished work). However, this inhibitory effect of fructose 1-phosphate did not occur in crude liver extracts. A plausible explanation has been offered for the lack of inhibition, i.e. that the dephosphorylation of synthase is catalysed by another phosphatase existing in liver. The significance of phosphatases in the sequential order of the dephosphorylation of hepatic phosphorylase and synthase is a long-standing and challenging question in this field. It seems that two different phosphatases are responsible for the dephosphorylation of phosphorylase *a* and synthase *b* (Tan & Nuttall, 1978; Gilboe & Nuttall, 1984), and our results support this view. Furthermore, synthase phosphatase has two, a cytosolic and a glycogen-bound, components (Mvumbi *et al.*, 1983), and both are important in the development of latency.

Fructose 1-phosphate can also control the enzymic activity of glycogen synthase *b*. The hepatic concentrations of UDP-glucose and glucose 6-phosphate are 0.2 mM and 0.15 mM respectively (Niewoehner *et al.*, 1984). The concentration of glucose 6-phosphate can reach 0.3–0.4 mM after fructose administration, while the concentrations of ATP and P_i respectively decreased to 0.5 mM and 2 mM (Woods *et al.*, 1970). Fructose 1-phosphate significantly enhances the activity of inactive glycogen synthase by increasing its affinity for UDP-glucose in the presence of 0.1–0.3 mM-glucose 6-phosphate, as we have demonstrated. Fructose 1-phosphate still stimulates the activity of synthase *b* in the presence of ATP and P_i, which normally keep the enzyme inactive even in the presence of physiological concentrations of glucose 6-phosphate.

In conclusion, the effect of fructose 1-phosphate on the glycogen metabolism of the liver is twofold. The accumulation of fructose 1-phosphate in the liver leads to high activities of phosphorylase *a*, owing to inhibition of the dephosphorylation process. It also gives rise to hypoglycaemia (Van den Berghe, 1977), since fructose 1-phosphate inhibits the enzymic activity of hepatic phosphorylase *a*, thus preventing the breakdown of glycogen. Fructose 1-phosphate stimulates glycogen synthesis by activating the inactive hepatic synthase and promoting the conversion of synthase *b* into *a*.

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