A REEVALUATION OF THE PATHWAY BY WHICH GLUCOSE IS CONVERTED INTO GLYCOGEN IN A LIVER HOMOGENATE

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1. Introduction

Although there has been for long a general agreement that the conversion of glucose to glycogen occurs by the successive formation of glucose 6-phosphate, glucose 1-phosphate and UDPG, several investigators, working either on isolated muscle [1,2], liver homogenates [3], liver slices [4] or the liver of intact rats [5] have put in doubt that glucose 6-phosphate it an intermediary in the pathway. Smith et al. [6] have even hypothesized that glucose 1-phosphate could be formed by transphosphorylation from glucose 1,6-diphosphate to glucose.

In this work, we have reinvestigated each step of the conversion of glucose to glycogen, as it occurs in a mouse liver homogenate, incubated for a short time at 25°.

2. Material and methods

All experiments were performed with 20% mouse liver homogenates, prepared with a Potter and Elvehjem homogenizer in 0.15 M KCl containing 0.05 M pH 7.4 glycylglycine buffer. 0.1 ml of this homogenate was incubated in a total volume of 0.15 ml at 25° with rapid shaking. The ^{14}C-labelled substrates were obtained from the Radiochemical Centre (Amersham, England). ^{32}P-labelled glucose 1-phosphate was obtained by the action of crystalline phosphorylase [7] on glycogen in the presence of labelled phosphate. The formation of UDP-^{14}C-glucose was measured as described by Zancan and Hers [8]. Labelled liver glycogen was isolated and counted by conventional methods. A preparation of rat liver microsomes [9] was used as a source of glucose 6-phosphatase. Hexokinase was purchased from Boehringer (Mannheim, Germany). Other technical details are given in the text.

2. Results and discussion

In a first series of experiments, we have investigated the possibility that glucose might be phosphorylated into glucose 1-phosphate without its prior convertion to glucose 6-phosphate. The homogenate was incubated with a trace amount of ^{14}C-glucose together with 2 μmoles of glucose 1-phosphate added as a trapping agent. The experiment lasted only 1 min and it has been verified that during this time, only 0.4 μmole of glucose 1-phosphate was converted into an acid stable phosphoric ester. The reaction was stopped by the addition of 0.2 ml of 0.15 M $ZnSO_4$ and 0.2 ml of 0.15 M $Ba(OH)_2$. The hexose phosphates were adsorbed on the precipitate which was washed several times with a 0.5 M glucose solution, resuspended in 2 ml of water and mixed with 300 mg of Amberlite IR 120 H^+; after centrifugation, the phosphoric esters were recovered in the supernate. Incubation with glucose 6-phosphatase or heating for 10 min in N HCl liberated the ^{14}C-glucose from glucose 6-phosphate or glucose 1-phosphate, respectively. After adsorption of the remaining esters by the Zn/Ba mixture, the free sugar was counted in a scintillation counter. 5 and 95% of the radioactivity were recovered from glucose 1-phosphate and glucose 6-phosphate, respectively, totalling 130 μmoles formed from glucose per minute and per g liver. There is therefore no indication that glucose 1-phosphate is formed from glucose

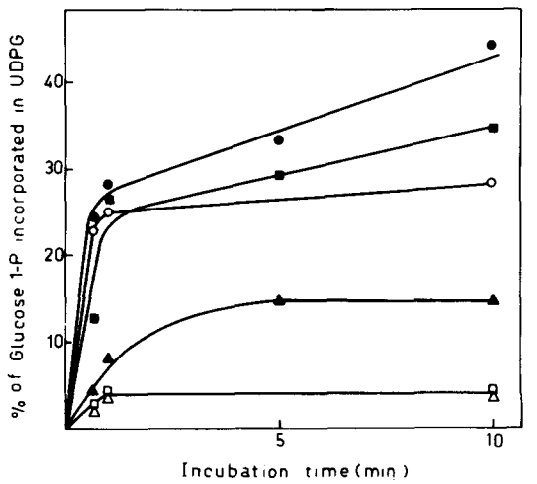

Fig. 1. Influence of uridine nucleotides and of oligomycin on the incorporation of ^{14}C-glucose 1-phosphate in UDPG. After a 5 min preincubation with (open symbols) or without (black symbols) oligomycin (10 μg/ml), the homogenates were enriched with UTP (○, ●), UDP (□, ■) or UMP (△, ▲) at a final concentration of 1.3 mM and incubated with a trace amount of ^{14}C-glucose 1-phosphate.

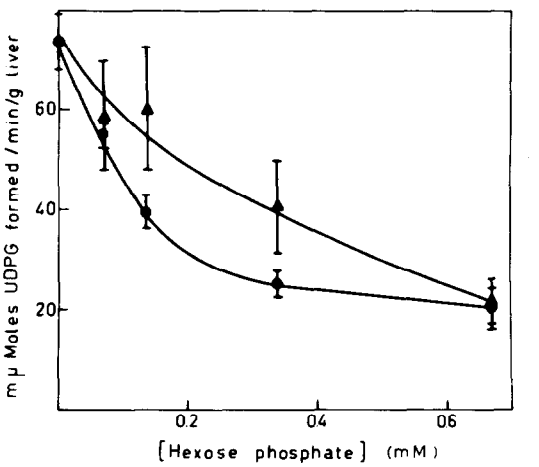

Fig. 2. Influence of hexose-phosphates on the incorporation of ^{14}C-glucose in UDPG. The homogenates were incubated for 2 min in the presence of 1.3 mM UTP and different concentrations of glucose 6-phosphate (●) or glucose 1-phosphate (▲). Each point represents the mean of 3 experiments ± the standard error of the mean.

by an other mechanism than the one involving hexokinase and phosphoglucomutase.

Zancan and Hers [8] have previously described the energy requiring formation of UDPG from glucose, glucose 6-phosphate or glucose 1-phosphate by a liver homogenate incubated in the presence of UTP, UDP or UMP; as these nucleotides are rapidly interconverted, it had not been possible to demonstrate which one was the closest precursor. By reducing the incubation period to 1 min we have now been able to show that only UTP is a precursor of UDPG in the presence of oligomycin which presumably prevents the phosphorylation of UMP and UDP (fig. 1). This result indicates that UDPG is formed from glucose 1-phosphate and UTP by the corresponding pyrophosphorylase. The same conclusion is also supported by experiments in which ^{14}C- or ^{32}P-labelled glucose 1-phosphate was incubated with UDP and gave rise in both cases to a similar proportion of labelled UDPG (extracted with methanol and isolated by paper chromatography), confirming therefore that the glucose and the phosphate moieties of glucose 1-phosphate are simultaneously incorporated in the nucleotide.

In the presence of 1.3 mM UTP, 60 to 80 mμmoles of UDPG/min/g liver were formed from glucose and 3 to 4 times more from glucose 1-phosphate or glucose 6-phosphate. When unlabelled esters were added, they markedly inhibited the incorporation of ^{14}C-glucose into UDPG (fig. 2) despite the fact that their own incorporation in the nucleotide was roughly proportional to their concentration; we therefore assume that their action was due to an inhibition of hexokinase by glucose 6-phosphate. Nevertheless, the addition of an excess of hexokinase (9 U/g liver) caused a 3-fold increase in the rate of glucose to UDPG conversion and in these conditions, the concentration of glucose 6-phosphate in the medium reached 0.9 mM at the end of the incubation. These results confirm the importance of glucose 6-phosphate as a necessary intermediary in the glucose to UDPG conversion.

The transfer of a glucosyl unit from UDPG to glycogen is catalyzed by glycogen synthetase. The participation of this enzyme in glycogen synthesis has never been seriously questioned and the recent work by De Wulf and Hers [10] has clearly demonstrated that liver glycogen synthesis *in vivo* depends on the amount of enzyme which is in its active or *a* form.

Table 1
Influence of various additions on the rate of incorporation of ^{14}C-glucose into glycogen by a mouse liver homogenate.

Additions	mμmoles of ^{14}C-glucose incorporated/min/g liver	
	Without hexokinase	With hexokinase (8.8 U/g liver)
None	3.3 ± 0.3	67 ± 8.6
1.3 mM UTP	1.3 ± 0.1	55 ± 10.3
3.3 mM ATP, 3.3 mM $MgCl_2$	3.1 ± 0.5	98 ± 8.3
1% glycogen	10 ± 0.1	134 ± 32
1.3 mM UTP, 3.3 mM ATP, 3.3 mM $MgCl_2$	2 ± 0.2	73 ± 8.3
1.3 mM UTP, 1% glycogen	8 ± 0.7	125 ± 7.3
3.3 mM ATP, 3.3 mM $MgCl_2$, 1% glycogen	15 ± 2.3	233 ± 22
1.3 mM UTP, 3.3 mM ATP, 3.3 mM $MgCl_2$, 1% glycogen	11 ± 0.3	209 ± 18

The homogenate was incubated for 4 min at 25°. Values shown are the means of 4 experiments ± standard error of the means.

Finally we have investigated the condition in which ^{14}C-glucose can be incorporated into glycogen by a mouse liver homogenate; it is known that, in contrast to pigeon liver, the mammalian liver is nearly inactive in this process [11]. It must first be pointed out that, with time, as much as 1% of the glucose present in the homogenate can be incorporated into glycogen by the action of amylo-1,6-glucosidase only [12] and it appears that some of the previous investigations have been misinterpreted due to the ignorance of this fact. We show in table 1 that, in a mouse liver homogenate, the incorporation of ^{14}C-glucose into glycogen was negligeable in the absence of added hexokinase. The small amount incorporated in these conditions may be explained by the action of amylo-1,6-glucosidase and the stimulating action of glycogen is in agreement with the known affinity constant (33 mg/ml) of glycogen for this enzyme. The amount of radioactive glycogen formed was greatly increased by the addition of hexokinase alone and still further by ATP and glycogen. In the best conditions, a rate of 0.23 μmole/min/g liver was reached which, taking into account the difference in temperature, is close to the maximal physiological rate *in vivo* (0.5 to 1 μmole/min/g). The action of hexokinase can be explained by the increase of the glucose to UDPG conversion (see above) and by the accumulation of glucose 6-phosphate which stimulates glycogen synthetase. In agreement with this interpretation, it has been recently observed [13] that in the pigeon liver, glycogen synthetase is mostly *a*. The action of glycogen is presumably to prevent the degradation of the newly formed radioactive polysaccharide by α-amylase.

We have therefore obtained no evidence suggesting that a pathway different from the generally accepted one is operating for the synthesis of glycogen from glucose.

Acknowledgements

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