Oscillatory behavior of glycolysis in cell-free extracts of rat skeletal muscle involves bursts of phosphofructokinase activity, due to autocatalytic activation by fructose-1,6-P_2. Glucose-1,6-P_2 similarly might activate phosphofructokinase in an autocatalytic manner, because it is produced in a side reaction of phosphofructokinase and in a side reaction of phosphoglucomutase using fructose-1,6-P_2. When muscle extracts were provided with 1 mM ATP and 10 mM glucose, glucose-1,6-P_2 accumulated in a stepwise, but monotonic manner to 0.7 μM in 1 h. The stepwise increases occurred during the phases when fructose-1,6-P_2 was available, consistent with glucose-1,6-P_2 synthesis in the phosphoglucomutase side reaction. Addition of 5–20 μM glucose-1,6-P_2 increased the frequency of the oscillations in a dose-dependent manner and progressively shortened the time interval before the first burst of phosphofructokinase activity. Addition of 30 μM glucose-1,6-P_2 blocked the oscillations. The peak values of the [ATP]/[ADP] ratio were then eliminated, and the average [ATP]/[ADP] ratio was reduced by half. In the presence of higher, near physiological concentrations of ATP and citrate (which reduce the activation of phosphofructokinase by glucose-1,6-P_2), high physiological concentrations of glucose-1,6-P_2 (50–100 μM) increased the frequency of the oscillations and did not block them. We conclude that autocatalytic activation of phosphofructokinase by fructose-1,6-P_2, but not by glucose-1,6-P_2, is the mechanism generating the oscillations in muscle extracts. Glucose-1,6-P_2 may nevertheless play a role in facilitating the initiation of the oscillations and in modulating their frequency.

Oscillatory behavior of the glycolytic pathway occurs under certain conditions when cell-free extracts of rat skeletal muscle are provided with glucose (1–5). The oscillations are generated by repeated bursts of phosphofructokinase activity, involving AMP-dependent activation of the enzyme by its product fructose-1,6-P_2. Such glycolytic oscillations may have advantages in the maintenance of a high [ATP]/[ADP] ratio (4, 5). Additionally, we have proposed that they may be involved in the signaling mechanism of glucose-stimulated insulin release in the pancreaticβ-cell, by causing oscillations in intracellular free Ca^{2+} (6). In this regard, regulatory factors that influence the frequency and/or amplitude of the glycolytic oscillations would modulate that resultant Ca^{2+} oscillations and hormone release. Other activators and inhibitors of phosphofructokinase are prime candidates for such modulatory roles.

Glucose-1,6-P_2 and fructose-2,6-P_2 are naturally occurring analogues of fructose-1,6-P_2 that also activate phosphofructokinase and are important in the control of carbohydrate metabolism in various tissues (7–13). For muscle phosphofructokinase, the apparent affinity for fructose-2,6-P_2 is normally about 10 times greater than for fructose-1,6-P_2, and the affinity for glucose-1,6-P_2 is about 10 times less than for fructose-1,6-P_2 on the basis of kinetic activation assays (14). Although these sugar bisphosphates are thought to bind at the same site on phosphofructokinase, their interactions with other regulatory metabolites are different. Thus, ATP, AMP, and citrate affect the K_{0.5} for fructose-2,6-P_2 with little effect on the maximum activation, whereas they affect the maximum activation rather than the K_{0.5} for fructose-1,6-P_2 (17). Recent work has shown that glucose-1,6-P_2 is more similar to fructose-1,6-P_2 in this regard, although the K_{0.5} is also affected to some degree (18). These kinetic studies also showed that further activation of phosphofructokinase by fructose-1,6-P_2 can be prevented by relatively high concentrations of fructose-2,6-P_2 or glucose-1,6-P_2 (17, 18). In keeping with this, added fructose-2,6-P_2 could block the glycolytic oscillations in muscle extracts at low concentrations of ATP. However, in the presence of physiological concentrations of ATP and citrate, fructose-2,6-P_2 at a physiological concentration (1 μM) did not block the oscillations, but it appeared to increase the oscillation frequency (5). The concentration of glucose-1,6-P_2 in muscle is 50–100 times higher than that of fructose-2,6-P_2, and the free concentration of the fructose-2,6-P_2 may be reduced further by protein binding (19). Hence, glucose-1,6-P_2 may be the more effective modulator in spite of its poorer affinity. Its precise action is difficult to anticipate because of the complexities of the regulatory interactions.

The physiological importance of the three hexose bisphosphates as activators of muscle phosphofructokinase during contraction remains unclear. Fructose-1,6-P_2 and glucose-1,6-P_2 both rise during muscular contraction (20–22), whereas...
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fructose-2,6-P2 has been reported to drop (24) or to rise transiently (20, 25). Furthermore, the concentration of fructose-2,6-P2 is only about 8% of the concentration of phosphofructokinase subunits in muscle (14). Fructose-2,6-P2 is synthesized by a phosphofructo-2-kinase which is different from the glycolytic phosphofructokinase (9-13). On the other hand, it has been proposed that glucose-1,6-P2 is synthesized in part by phosphofructokinase (26). Therefore, glucose-1,6-P2 might participate along with fructose-1,6-P2 in the autocatalytic activation of phosphofructokinase in the glycolytic oscillations. Another pathway for glucose-1,6-P2 synthesis is via phosphoglucomutase using fructose-1,6-P2 or 1,3-diphosphoglycerate as the phosphate donor (27, 28). In this way, too, glucose-1,6-P2 might be involved in amplifying or prolonging the activation of phosphofructokinase in the oscillations, because glucose-1,6-P2 would probably not be removed as rapidly as fructose-1,6-P2. An inverse relationship between glucose-1,6-P2 and fructose-1,6-P2 is another possibility, because fructose-1,6-P2 is an inhibitor of a specific glucose-1,6-P2 synthase, which uses 1,3-diphosphoglycerate (29, 30).

Glucose-1,6-P2 thus may have any of the following roles or effects on the glycolytic oscillations: (a) it may be involved in the oscillatory mechanism; (b) it may block the oscillations by competing with fructose-1,6-P2; and/or (c) it may modulate the frequency of the oscillations. The latter action may be of particular importance in the context of signaling. The objectives of the studies reported here were, first, to examine whether glucose-1,6-P2 is produced by the glycolyzing muscle extracts, and if so, whether the synthesis occurs during certain phases of the oscillations and could play any role in generating the oscillations; and second, to determine the effects of added glucose-1,6-P2 on the oscillating system.

EXPERIMENTAL PROCEDURES

The gel-filtered, high speed supernatant of rat hindleg muscle was prepared as described previously (31), except that the tissue was broken up using a Polytron homogenizer, and EDTA was omitted from the gel filtration buffer. A larger column (5 X 20 cm) of G-25 Sephadex was used to gel filter 50-ml portions of muscle extract at a rate of 1 ml/min. Glycolytic oscillations in reaction mixtures were monitored spectrophotometrically by following changes in the absorbance of ATP and 10 mM glucose, glucose-1,6-P2 accumulated to levels of about 0.7 μM in 1 h (Fig 1). The clear steps in glucose-1,6-P2 accumulation indicate that glucose-1,6-P2 was produced only during certain phases of the oscillation cycle. The beginning of the rise in glucose-1,6-P2 corresponded with the rapid accumulation of fructose-1,6-P2 and the drop in glucose-6-P and fructose-6-P, that is, the time of the triggering of phosphofructokinase activity. However, glucose-1,6-P2 synthesis continued after the burst of phosphofructokinase activity ended, as indicated by the drop in fructose-1,6-P2 and reaccumulation of hexose monophosphates. The periods of accumulation of glucose-1,6-P2 correlate most closely with the presence of at least micromolar levels of fructose-1,6-P2. There is a lesser correlation with the presence of 3-P-glycerate, which in fact did not accumulate significantly in the first oscillation in the experiment shown in Fig. 1. Peaks of P-enolpyruvate (data not shown) matched but were about one-third the size of the 3-P-glycerate peaks, in keeping with the enolase/phosphoglycerate mutase equilibria. Glucose-1,6-P2 levels measured on a number of samples (data not shown) were always in equilibrium with glucose-6-P, the glucose-6-P/glucose-1-P ratio being about 17. Therefore, the rate of glucose-1,6-P2 synthesis was not limited by the availability of hexose monophosphate substrate. These results appear most consistent with synthesis of glucose-1,6-P2 by phosphoglucomutase, dependent on fructose-1,6-P2, together perhaps with phosphofructokinase.

There was little if any degradation of glucose-1,6-P2 during the phases when fructose-1,6-P2 was low. Hence, the accumulation of glucose-1,6-P2 was nearly monotonic. This indicates that the large changes in phosphofructokinase activity in the course of the oscillations are not due to changes in the level of glucose-1,6-P2. In contrast, fructose-1,6-P2 levels varied 50-fold or more in the course of an oscillation cycle, and AMP at least 20-fold (Fig. 1).

To estimate the relative importance of phosphofructokinase in synthesizing glucose-1,6-P2, experiments with different concentrations of NAD were performed. Because the Km of glyceraldehyde-3-P dehydrogenase for NAD is about 90 μM (35), increased NAD in this range should promote the metab-
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FIG. 1. Accumulation of glucose-1,6-P$_2$ in muscle extracts undergoing oscillations of glycolysis. The reaction mixture initially contained 1 mM ATP, 0.3 mM GTP, 4 mM aspartate, 10 mM glucose, 30 mM NAD, 7.5 mM orthophosphate, 25 mM imidazole-HCl buffer, pH 6.9, 8.3 mM MgCl$_2$, 0.15 mM citrate, 0.06 unit/ml crystalline yeast hexokinase, and rat muscle extract equivalent to 0.9 mg of protein/ml. The protein extract contributed 47 mM KCl, 17 pM dithiothreitol, and 2.5 mM of the total orthophosphate to the reaction mixture. The temperature was 30 °C. Samples were taken every minute for analysis of the metabolites, beginning 8 min after the start of the reaction. NDP = ADP + GDP. G6P, glucose-6-P; F6P, fructose-6-P; FIGBP, fructose-1,6-P$_2$; G6GBP, glucose-1,6-P$_2$; 3PG, 3-phosphoglycerate.

FIG. 2. Effect of increased NAD concentration on the accumulation of glucose-1,6-P$_2$, fructose-1,6-P$_2$, 3-P-glycerate, and lactate during glycolytic oscillations. Conditions were similar to those described for Fig. 1, except that the buffer pH was 6.8, citrate was omitted, and NAD was added at the concentrations indicated. Muscle protein concentration was 1.3 mg/ml. Shown), which as shown previously (5) largely prevents accumulation of fructose-1,6-P$_2$ and blocks the oscillations under these conditions; overall lactate accumulation was about the same in the presence and absence of added fructose-2,6-P$_2$. These experiments suggest that phosphofructokinase plays a minor role in glucose-1,6-P$_2$ synthesis under these conditions.

Modulation of Glycolytic Oscillations by Glucose-1,6-P$_2$—Addition of 5-20 pM glucose-1,6-P$_2$ increased the frequency of the oscillations in a dose-dependent manner (Fig. 3). Increasing concentrations of glucose-1,6-P$_2$ also progressively shortened the time interval before the first burst of phosphofructokinase activity, as indicated by the first plateau or shoulder in the traces of Fig. 3. The combined result of these effects was that the number of bursts of phosphofructokinase activity in the course of the experiment was increased from 5, in the absence of added glucose-1,6-P$_2$, to 6, 7, 8, and 9 in the presence of 5, 10, 15, and 20 pM glucose-1,6-P$_2$, respectively. These levels of glucose-1,6-P$_2$ are considerably higher than the levels that were synthesized in the experiments of Figs. 1 and 2.

Glucose-1,6-P$_2$ at High Concentrations Blocks Glycolytic Oscillations—Under the conditions of the experiment shown in Fig. 3, addition of 30 pM glucose-1,6-P$_2$ appeared to block the
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Glucose 1,6-Bisphosphate (Glc-1,6-P₂) and its role in modulating glycolytic oscillations.

**Fig. 3. Modulation of glycolytic oscillations by added glucose-1,6-P₂.** The changes in absorbance are due to oscillatory operation of the purine nucleotide cycle which reflects the changes in the [ATP]/[ADP] ratio associated with the glycolytic oscillations; decreases and increases in absorbance are indicative of low and high [ATP]/[ADP] ratios, respectively. Conditions were the same as described for Fig. 2, except that the NAD concentration was 10 µM. Glucose-1,6-P₂ was added at the concentrations indicated. The dashed line at about 9 min marks the approximate time of the first burst of phosphofructokinase activity in the absence of added glucose-1,6-P₂.

Oscillations. This was examined in more detail in the experiment shown in Fig. 4, in which repeated samples were taken for metabolite analysis from reaction mixtures run concurrently with and without 30 µM glucose-1,6-P₂. In the presence of the added glucose-1,6-P₂, the glycolytic oscillations were indeed blocked. Glucose-6-P and fructose-6-P accumulated at a constant rate and corresponded to the maximum levels reached in the oscillating system. Little fructose 1,6-P₂ accumulated, and a constant low level of triose-P was maintained. There was little, if any, appearance of phosphoglycerate or P-enolpyruvate (data not shown). Similar amounts of lactate accumulated with glucose-1,6-P₂ (not shown) as without (see Fig. 2). In the presence of glucose-1,6-P₂, intermediate levels of ATP, NDP (ADP + GDP), and AMP were maintained, compared with the oscillating system (Fig. 4). In the absence of glucose-1,6-P₂, the [ATP]/[ADP] ratio oscillated between low values of 3 to 5 and high values of up to approximately 40 (Fig. 5). In the presence of glucose-1,6-P₂, the [ATP]/[ADP] ratio rose gradually from 5 up to about 8 in the course of the experiment, perhaps because of the rise in fructose-1,6-P. The high peak values of the oscillating system were eliminated in the steady state system. The average [ATP]/[ADP] ratio in the experiment of Fig. 5 was 13.8 for the oscillating system and 7.1 for the steady state system.

**Fig. 4. Blockage of glycolytic oscillations by 30 µM glucose-1,6-P₂.** Conditions were similar to those described for Fig. 3. Samples were taken for metabolite analysis every minute for the reaction mixture lacking glucose-1,6-P₂ and every 2 min for the reaction mixture containing glucose-1,6-P₂ (30 µM), beginning 5 min after the start of the reaction. The glucose-1,6-P₂ concentration gradually declined from 30 to 22 µM over the duration of the experiment. NDP = ADP + GDP; G6P, glucose-6-P; F6P, fructose-6-P.

**Fig. 5. Effect on the [ATP]/[ADP] ratio of blocking glycolytic oscillations with glucose-1,6-P₂.** The experiment was the same as that shown in Fig. 4. The [ATP]/[ADP] ratios were calculated from the assayed concentrations of ATP and NDP (=ADP + GDP), and the relationships [GTP] + [GDP] = 0.3 mM and [ATP]/[ADP] = [GTP]/[GDP] (2).

The concentration of glucose-1,6-P₂ in resting muscle is about 30 µM and may double with exercise (see "Discussion"). The results presented in Figs. 3 and 5 do not necessarily imply that these concentrations would prevent glycolytic oscillations in vivo, for the kinetics of activation of phosphofructokinase by glucose-1,6-
FIG. 6. Effect of glucose-1,6-P₂ on oscillations at near-physiological concentrations of ATP and citrate. The changes in absorbance are due to oscillatory operation of the purine nucleotide cycle which reflects the changes in the [ATP]/[ADP] ratio associated with the glycolytic oscillations. Conditions were similar to those described for Fig. 1, except for initial concentrations of 10 mM ATP, 0.2 mM citrate, 15 mM MgCl₂, 20 mM glucose, 0.5 mM glucose-6-P, 50 mM imidazole-HCl buffer, pH 7.1, 150 mM KCl, 0.1 mM NaCl, 0.1 unit/ml of hexokinase, 0.17 unit/ml of potato apyrase, and the indicated concentrations of glucose-1,6-P₂. The pH was 7.0, and the muscle protein concentration was 1.3 mg/ml. The three reaction mixtures were recorded simultaneously using three sample positions of the HP 8450 spectrophotometer. Analyses of deproteinized samples taken at 20 min showed that prior deamination had reduced the ATP concentration to 6 mM. The glucose-1,6-P₂ concentrations measured at that time were 0.75, 45, and 194 μM, respectively, in the three reaction mixtures. At 52 min, the ATP concentration was about 4.5 mM, and the glucose-1,6-P₂ concentrations were 1.3, 45, and 94 μM.

P₂ are affected strongly by the concentrations of the inhibitors ATP and citrate (18). Fig. 6 shows spectral tracings for an experiment starting with nearly physiological concentrations of ATP (10 mM) and citrate (0.2 mM). Addition of 50 or 100 μM glucose-1,6-P₂ did not block the oscillations. The added glucose-1,6-P₂ did cause a small increase in frequency of the oscillations and did reduce the time interval before the first burst of phosphofructokinase activity. The ATP concentration had declined to about 6 mM at the beginning of the oscillations.

DISCUSSION

Glucose-1,6-P₂ has been proposed to be an important regulatory metabolite for glycolysis (7, 8). Studies presented here show that glucose-1,6-P₂ can promote the earlier initiation of glycolytic oscillations in muscle extracts, as well as increasing the frequency of the oscillations in a dose-dependent manner (Figs. 3 and 6). Glycolytic oscillations have been studied previously by others in yeast cells and extracts (36–39), ascites cells (40), and heart extracts (41); however, the effect of glucose-1,6-P₂ was not studied. In glycolyzing yeast extracts, where levels of fructose-1,6-P₂ were very high (millimolar), presumably glucose-1,6-P₂ would have little effect.

There has been limited study of the effects of other activators or inhibitors of phosphofructokinase on glycolytic oscillations, with the exception of the adenine nucleotides that participate in the oscillation mechanism. Thus, addition of AMP or ADP caused a phase shift and sometimes a change in frequency in yeast and heart extracts (36, 38, 42, 43). It was suggested that AMP may have acted by relieving product inhibition of glyceraldehyde-3-P dehydrogenase. Increased concentrations of phosphate, an activator of phosphofructokinase, shortened the time before the first burst of activity and increased the frequency of the oscillations in muscle extracts (1); however, this may have been due in part to inhibition of AMP deaminase, thus allowing more rapid accumulation of AMP. For heart and yeast extracts, the only effect reported for added phosphate was the initiation of oscillations under some conditions when they did not occur spontaneously (98, 99, 44). Recently, we have found that increasing concentrations of citrate, a physiologically relevant inhibitor of phosphofructokinase, have modulatory effects opposite to those shown here for glucose-1,6-P₂. Citrate was reported to block oscillations in heart extracts (at the very high concentration of 4 mM) (44) and to have no effect on the oscillations in yeast extracts (36).

Oscillations in NADH fluorescence, which are associated with glycolytic oscillations in all the above systems, have also been observed in intact muscle following strong stimulation (45, 46), in lens (47) and corn (48) in vivo and in vitro, and most recently in single pancreatic β-cells on stimulation with glucose (49). We have proposed that glycolytic oscillations are involved in the stimulus-secretion coupling for insulin release in pancreatic islets, by causing periodic elevations of intracellular free Ca²⁺ (6). Oscillations in the [ATP]/[ADP] ratio (see Fig. 5) should cause opening and closing of ATP-sensitive potassium channels. Closure of the potassium channels at high [ATP]/[ADP] ratios leads to depolarization and resultant influx of Ca²⁺ through voltage-sensitive channels. Glucose-induced Ca²⁺ oscillations have been observed in single isolated β-cells (49–51), as well as in single mouse (52) and rat* islets. Importantly, the initial rise in NADH in single β-cells preceded that of Ca²⁺, consistent with Ca²⁺ changes being secondary to metabolic coupling factors (49). Oscillations in intracellular free Ca²⁺ in single cells have been seen in a variety of cell types on stimulation with a receptor-mediated agonist (53–57). Various mechanisms have been proposed to account for these Ca²⁺ oscillations, including oscillatory production of inositol trisphosphate (which releases Ca²⁺ from intracellular stores) and Ca²⁺-induced Ca²⁺ release (55, 58). Glucose stimulation of islet cells is a special case in that the stimulus-secretion coupling requires the metabolism of the glucose; however, no metabolite has been pinpointed as the effector (59). Our proposal is that the coupling is provided by the oscillatory behavior of glycolysis and the [ATP]/[ADP] ratio. This could account for the observed pulsatile release of insulin in vivo (60, 61) and in perfused groups of isolated islets (62, 63), and the loss of this pulsatility in Type II diabetes (60, 64). The recent report of oscillatory release of lactate from perfused islets, with a period similar to that for insulin release, supports the existence of glycolytic oscillations and a linkage to secretion (65). The glucose-1,6-P₂ concentration is increased in glucose-stimulated islets, to about 20 μM, and this has been proposed to be involved in the stimulation of glycolytic flux and insulin secretion (67). The experiments presented here with the muscle extract system suggest that in islets the rise in glucose-1,6-P₂ could facilitate the initiation of glycolytic oscillations and the repeated closing of the ATP-sensitive potassium channels and Ca²⁺ influx. A graded rise in glucose-1,6-P₂ might also have the effect of increasing the frequency of the oscillations in glycolysis and free Ca²⁺. Berridge (68, 69) and Rapp (70) have emphasized the advantages

K. Tornheim, V. Andréé, and V. Schultz, manuscript in preparation.

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Calculated assuming that the dry weight of an islet is 0.8 μg, that the wet weight/dry weight ratio is 4.2 (66), and that intracellular water is 67% of the wet weight.
of oscillatory behavior and such frequency modulation in signal transduction.

Several paths have been suggested for the synthesis of glucose-1,6-P2: (a) a dismutase reaction of glucose-1-P, probably by phosphoglucomutase; (b) phosphorylation of glucose-1-P (or glucose-6-P) with ATP, by phosphofructokinase (28); (c) phosphorylation of glucose-1-P by phosphoglucomutase, using fructose-1,6-P2 or 1,3-diphosphoglycerate instead of glucose-1,6-P2 as the cosubstrate (27, 28); and (d) phosphorylation of glucose-1-P by 1,3-diphosphoglycerate by a specific synthase (29, 30). The striking characteristic of the glucose-1,6-P2 accumulation in the glycolyzing muscle extract is its stepwise nature (Fig. 1); that is, glucose-1,6-P2 was synthesized only at specific phases of the oscillation cycle. Comparison of the profile of other metabolites with the time interval of glucose-1,6-P2 synthesis suggests the primary involvement of the phosphoglucomutase reaction using fructose-1,6-P2. First, the dismutase reaction is unlikely to be responsible for the bursts of glucose-1,6-P2 synthesis, since levels of glucose monophosphates were similar or higher in periods with no glucose-1,6-P2 synthesis. In contrast, the appearance of fructose-1,6-P2 strongly corresponded with the rises in glucose-1,6-P2, suggesting either phosphoglucomutase- or phosphofructokinase-catalyzed synthesis. The lesser contribution of phosphofructokinase is suggested by the observations that glucose-1,6-P2 accumulation continued after phosphofructokinase was inhibited, and increased NADH, or addition of glucose-1,6-P2 accumulation continued after phosphofructokinase was inhibited. This is probably an underestimate, because the maintenance of the glucose-5-P/glucose-1-P equilibrium means that the forward and back reaction rates are considerably higher than the net flux. Assayed phosphoglucomutase activity was 200 and 580 μmol/min/g wet weight in mouse and rabbit muscle (where the factor of 0.015% for the side reaction was determined) (27). Thus the phosphoglucomutase side reaction could account for the rate of glucose-1,6-P2 synthesis, and its increase during exercise could be secondary to the rise in fructose-1,6-P2. However, Passonneau et al. (27) have raised the thermodynamic question that the equilibrium concentration of glucose-1,6-P2 should be limited to about one-third that of fructose-1,6-P2. With regard to the specific glucose-1,6-P2 synthase, Lee and Katz also reported that concentrations of inhibitors of the enzyme increased when glucose-1,6-P2 increased (22), whereas the substrate 1,3-diphosphoglycerate declined, when calculated assuming equilibrium of the phosphoglycerate kinase and creatine kinase reactions (71). Hence, an unknown regulator of the synthase must be postulated if it is to be responsible for the increase in glucose-1,6-P2 in muscle.

The nearly monotonic accumulation of glucose-1,6-P2 in the muscle extract strongly suggests that it is not part of the mechanism generating the glycolytic oscillations. In contrast, levels of fructose-1,6-P2 oscillated over a 50-fold range (Fig. 1). Because glucose-1,6-P2 and fructose-1,6-P2 appear to compete for the same site on phosphofructokinase, and no further activations of glucose-6-P by 1,3-diphosphoglycerate occurred when the substrate 1,3-diphosphoglycerate was eliminated (22), glucose-1,6-P2 accumulation continued after phosphofructokinase was inhibited. The stepwise production of glucose-1,6-P2 might also involve oscillations in the availability of 1,3-diphosphoglycerate, which might regulate production via either phosphoglucomutase or the specific synthase. Concentrations of 1,3-diphosphoglycerate were too low to measure, but would most likely correspond to the changes in 3-P-glycerate and P-enolpyruvate, the other glycolytic high energy phosphate donor. The observations that little 3-P-glycerate accumulated during some periods of glucose-1,6-P2 synthesis (such as the first step in Fig. 1) and that glucose-1,6-P2 accumulation sometimes stopped even though 3-P-glycerate still remained suggest that the reactions using 1,3-diphosphoglycerate probably play less of a role here than does the synthesis via phosphoglucomutase using fructose-1,6-P2. Furthermore, much of the synthesis of glucose-1,6-P2 occurred during times when fructose-1,6-P2 was high and the specific synthase should have been inhibited strongly, because fructose-1,6-P2 is an inhibitor of that enzyme, with a Ki of 0.7 μM (30).

The relative importance of these alternate reactions in muscle in vivo is less clear. Katz and Lee (21) argued that the phosphofructokinase side reaction would be negligible under physiological conditions because of the high Kn for glucose-1-P and strong inhibition by ATP. They also calculated that the phosphoglucomutase side reaction with fructose-1,6-P2 could only account for one-third of the glucose-1,6-P2 rise they observed in human muscle, assuming that this side reaction was 0.015% of the rate of glucose-6-P production. However, in their calculation they assumed that the phosphoglucomutase rate was equal to the glycolgenolytic rate (70 μmol/min/g dry weight, or about 20 μmol/min/g wet weight). This is probably an underestimate, because the maintenance of the glucose-6-P/glucose-1-P equilibrium means that the forward and back reaction rates are considerably higher than the net flux. Assayed phosphoglucomutase activity was 200 and 580 μmol/min/g wet weight in mouse and rabbit muscle (where the factor of 0.015% for the side reaction was determined).
oscillations (39, 45, 46). The rise in glucose-1,6-P₂ that occurs during exercise may have a role in modulating oscillations in glycolytic flux under these conditions, by promoting earlier activation of phosphofructokinase and increasing the frequency of the oscillations.

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Oscillatory synthesis of glucose 1,6-bisphosphate and frequency modulation of glycolytic oscillations in skeletal muscle extracts.
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