Design of Expression Systems for Metabolic Engineering: Coordinated Synthesis and Degradation of Glycogen

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Received 23 February 1996; accepted 20 December 1996

Abstract: In metabolic engineering, systems which allow coordinated control of two metabolic pathways can be useful. We designed two expression systems and demonstrated their application by coordinating glycogen synthesis and degradation. The first expression vector pMSW2 expressed the glycogen synthesis genes in one operon and the glycogen degradation gene in a separate, coordinately regulated operon. The plasmid was designed to switch off expression of the first operon and activate expression of the second operon on addition of IPTG. As an alternative means to control glycogen synthesis and degradation pathways, we constructed expression vector pGTSD100, which contains the native *Escherichia coli* glycogen synthesis and degradation operon under control of the *tac* promoter. Both expression vectors work successfully to control the net synthesis and degradation of glycogen. In cultures of the *E. coli* strain TA3476 carrying the plasmid pMSW2, before the addition of IPTG, glycogen continued to accumulate in the culture. About three hours after IPTG was added, glycogen levels began to decrease. When no IPTG was added to cultures of TA3476:pMSW2, glycogen accumulated in the cells as before but the rate of degradation of glycogen was much lower. When IPTG was added to TA3476:pMSW2, the total cell protein at the end of batch cultivation was approximately 15% higher compared to cultures without IPTG addition. The extra biomass was formed during the glycogen degradation phase. © 1997 John Wiley & Sons, Inc. *Biotechnol Bioeng* **55**: 419–426, 1997.
Keywords: metabolic engineering; glycogen synthesis; glycogen degradation; carbon metabolism

INTRODUCTION

Controlled synthesis and degradation of glycogen is a potential strategy to manage carbon flow through the central carbon pathways (Dedhia et al., 1996). Glycogen synthesis during exponential phase can store part of the flux which would otherwise flow to unwanted toxic acid products. In principle, controlled degradation of glycogen during the deceleration growth phase or the stationary phase can be used to channel the stored carbon to biomass or metabolite products. Many important secondary metabolites are produced during stationary phase in certain microorganisms. A large class of important antibiotics are synthesized using hexose phosphate as precursor (Bailey and Ollis, 1986). By coupling the degradation of a storage compound such as glycogen to the antibiotic production pathway, the stored carbon might be routed to secondary metabolite synthesis.

Glycogen biosynthesis in *E. coli* requires the activities of ADPG pyrophosphorylase [EC 2.7.7.27], glycogen synthase [EC 2.4.1.2.1], and the branching enzyme [EC 2.4.1.18], which are encoded by the *glgC*, *glgA* and *glgB* genes respectively (reviewed in Preiss, 1984; Preiss and Romeo, 1989). Glycogen degradation requires the activity of glycogen phosphorylase [EC 2.4.1.1], which is encoded by the *glgP* gene (Choi et al., 1989; Romeo et al., 1988; Yu et al., 1988). The *glgC* and *glgA* genes, along with the glycogen degradation enzyme *glgP*, are apparently cotranscribed in an operon *glgCAP*. There are no reports of transcriptional regulation of the *glgP* gene. However, since the *glgP* gene is located only 18 bp distal to the *glgA* stop codon, it has been suggested that the expression of glycogen phosphorylase may be coordinately regulated with that of ADPG pyrophosphorylase and glycogen synthase as part of a glycogen biosynthetic/catabolic operon (Romeo et al., 1988).

The design of the first expression vector to control the pathway of glycogen synthesis and degradation is an application of the ''metabolic switch'' concept described previously (Chen and Bailey, 1994). Expression systems based on the ''metabolic switch'' concept potentially enable the cell to express one set of proteins initially and, following addition of IPTG to the medium, enable the cell to switch off expression of this first set of proteins and to switch on expression of a second set of proteins. In general, controlled switching from synthesis of one set of enzymes to expression of another set of enzymes could be used to shift the cells at a designed stage of the bioprocess from utilizing one metabolic pathway or physiological process to the activation of a different metabolic pathway or physiological process.

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Contract grant sponsor: National Science Foundation

Contract grant number: BCS-891284

CCC 0006-3592/97/020419-08

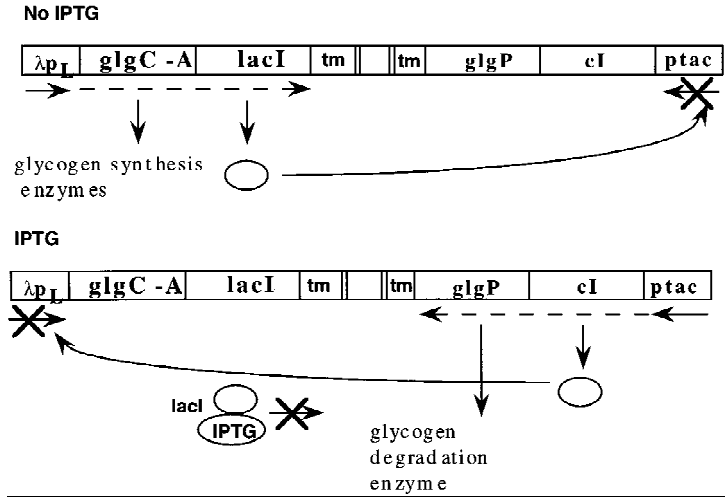

Figure 1. Operation of the ''metabolic switch''. λp_L is a constitutive promoter which expresses *glgC, glgA* and *lacI.* The lacI protein represses the *tac* promoter. When IPTG is added, IPTG inactivates the lacI protein. The *tac* promoter is turned on, *glgP* and *cI* are expressed. cI protein represses the λp_L promoter. *tm* represents the transcriptional terminator sequence.

The metabolic switch plasmid designed for this study has two operons. The first operon contains the glycogen synthesis genes, *glgC* and *glgA*, along with *lacI* under the control of the λ*pL* promoter; the second operon consists of the glycogen degradation gene, *glgP*, along with *cI* under the control of the *tac* promoter. In the absence of IPTG, the first operon should be expressed constitutively. When IPTG is added, the λ*pL* promoter of the first operon should be repressed and the *tac* promoter of the second promoter should be activated (Figure 1).

We compared the functioning of the ''metabolic switch'' expression system with a second expression system, which represents an alternative means to control the synthesis and degradation of glycogen. This expression system contains the native *E. coli glgC-glgA-glgP* operon under the control of the *tac* promoter. The operon includes 52 bp upstream of the *glgC* start codon. The operon ends 521 bp downstream of the *glgP* gene. Induction of this operon is controlled by the addition of IPTG. We report here the functioning and effectiveness of these vectors in controlled synthesis and degradation of glycogen and the concomitant effects on culture physiology.

MATERIALS AND METHODS

Strains and Plasmids

The genotype of strain TA3476 and characteristics of the plasmids used in this study are shown in Table 1. TA3476 is a mutant defective in acetate biosynthesis due to mutations in the *ack* (acetate kinase) and *pta* (phosphotransacetylase) genes. Plasmid maps are shown in Figure 2. The construction of plasmids is described below.

Polymerase Chain Reaction (PCR)

The PCR reaction was carried out in a 50 μl final reaction volume containing 2.5 ng of template DNA, 5 μl of 10× reaction buffer (100 mM Tris-HCl pH 8.3, 500 m*M* KCl, 15

Table I. Description of strains and plasmids.

Strain or plasmid	Description	Reference
TA3476	*E. coli* K12 *his*Δ (*pta-ack-dhAhisJ-hisQ-hisP*)	7
pGTC12	contains *glgP* gene.	4
pPR2	contains *glgC*-glgA gene.	15
pSL1180	commercial cloning vector.	Pharmacia, Piscataway, NJ
pλlacI	contains the lac repressor gene under the control of the λP_L promoter (parent vector pPL-Lambda from Pharmacia, NJ)	2
pKQV4	cloning vector derived from Pharmacia's cloning vector pKK223-3	16
pGT100	contains *glgC* and *glgA* genes under the control of the *tac* promoter (parent vector pKQV4).	5
pGTSD100	contains *glgC, glgA* and *glgP* genes under the control of the *tac* promoter (parent vector pKQV4).	this study
pMSW2	contains *glgC, glgA, lacI* under the λ*pL* promoter and *glgP, cI* under the *tac* promoter (parent vector pBR322).	this study

m*M* $MgCl_2$, 0.01% (w/v) gelatin), 8 μl of dNTPs mix (1.25 m*M* each), 2 m*M* each of the primers, and 0.5 μl of Taq DNA polymerase (1 U/μl) (Perkin Elmer-Cetus, USA). The amplification was carried out for 36 cycles in a DNA thermal cycler (Perkin Elmer-Cetus, USA). The DNA was denatured at 92°C for 1 min, annealed at 42°C for 2 min, and extended at 72°C for 5 min.

Construction of Plasmid pMSW2

The polymerase chain reaction was used to synthesize both the *glgC-glgA* and *glgP* structural gene with plasmids pPR2 (Romeo and Preiss, 1989) and pGTC12 (Choi et al., 1989) as templates, respectively. The two primers used to amplify the *glgC-glgA* gene were: (i) 5′-GGGAGCTCGGAAGGAAGGAGTTAGTCATGGTTAG-3′ and (ii) (5′-CCGAGCTCCCCACTATTTCGAGCGATAGTAAAGCTC-3′). These two primers contained only the Shine-Dalgarno sequence of the *glgC* gene but not the transcriptional stop sequence so that the amplified fragment can be used to create a λ*pL-glgC-glgA-lacI* operon. Restriction sites *SacI* were created at both ends for subcloning. To synthesize the structural portion of the *glgP* gene, the two primers used were: (i) 5′-GGATGCATGGCTATCGCTTACAAGCTGATG-3′ and (ii) 5′-CCATGCATCCCTTACAATCTCACCGGATCG-3′. As in the previous two primers, only the Shine-Dalgarno sequence of the *glgP* gene is included. *NsiI* sites were created to facilitate subcloning.

The 2.76 kb *glgC-glgA* fragment generated from PCR amplification was digested with *SacI* overnight. After gel purification, the resulting fragment was subcloned into pUC18 to create pUCglgCA. The functionality of the *glgC-glgA* fragment was confirmed by comparing the glycogen content of *E. coli* TA3476 with and without this plasmid (results not shown). This plasmid was then cleaved with *SacI* and the *glgC-glgA* fragment was inserted into the same site of plasmid pλlacI to give pλCAI.

Similarly, the 2.36 *glgP* PCR fragment was cleaved with *NsiI* and subcloned into pSL1180 to yield pSLglgP. To construct plasmid pTCIP, the *glgP* fragment was cleaved from pSLglgP and inserted into pTCI. Finally, the glycogen metabolic switch construct was obtained by transferring a 5.8 kb *NdeI/BamHI* fragment from pλCAI into the *NdeI/SmaI* sites of pTCIP with blunt end ligation at the *BamHI/SmaI* sites.

Construction of Plasmid pGTSD100

Plasmid pGT100 (Dedhia et al., 1994), which carries the *glgC* and *glgA* genes under the control of the *tac* promoter, was digested with *PstI*, treated with Klenow enzyme to create blunt ends and then digested with *NcoI*. This released a 7.6 kb fragment which included the *tac* promoter, the *glgC* gene and part of the *glgA* gene. Plasmid pGTC12 (Choi et al., 1989; kindly donated by Dr. Komano, Department of Agricultural University, Kyoto University, Japan) was digested with *BsaAI* and *NcoI*, which released a 3.9 kb fragment containing the remaining part of the *glgA* gene and the *glgP* gene. The 7.6 kb and 3.9 kb fragment were ligated to create plasmid pGTSD100. The plasmid pGTSD100 contains the tac promoter followed by the *glgC-glgA-glgP* operon as it exists naturally in the chromosome of *E. coli.*

Medium and Culture Conditions

The fermentations were carried out in a BioFlo III benchtop fermentor (New Brunswick Scientific, Edison, NJ) containing 2.5 L of medium. The medium used was LB medium buffered with phosphate and supplemented with 0.2% glucose. The medium contained in g/l: bactotryptone, 10; yeast extract, 5; NaCl, 5; KH_2PO_4, 3; K_2HPO_4, 1. The culture conditions were: temperature, 37°C; pH, 7.0; air flow rate, 2.0 liters per minute. pH was controlled with 2N NaOH and 2N HCl. Ampicillin (50 mg/L) was added during fermen-

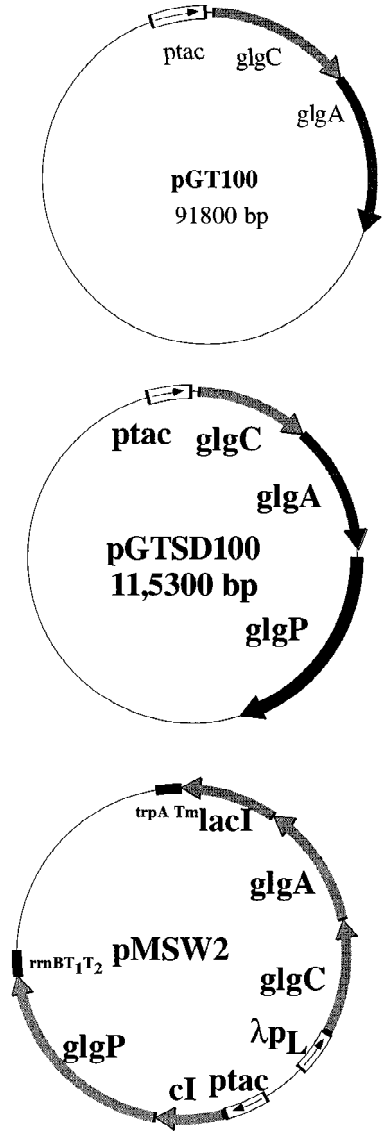

Figure 2. Schematic maps of plasmids: (a) pGT100 (b) pGTSD100 (c) pMSW2.

tations of plasmid-bearing strains. Foam was controlled by the addition of Medical Antifoam AF solution (Dow Corning, Midland, MI).

Inocula were grown in two stages; this was done to condition the glycogen overproducing strain to growth in liquid medium. The first stage consisted of inoculating a single colony from a freshly streaked plate in 4 ml of medium. After nearly 8 hours, 1:200 dilutions of the first-stage culture were inoculated in 50 ml medium. The second-stage culture was allowed to grow overnight and used to inoculate the main fermentation in fermentor or shake flask. The amount of inoculum added was adjusted to give a starting OD_{600} of 0.1. Bioreactor cultivations were repeated twice. The difference in total protein content during duplicate runs was +/− 3%.

Analytical Methods

Protein was measured as described by Peterson (1977). Protein was measured in duplicate samples. The difference in the duplicate samples was routinely less than 3%. Glycogen was assayed essentially as described in Reference 6. Glycogen was also measured in duplicate samples. The difference in the duplicate samples was routinely between 5% to 2%.

Assay of Glycogen Phosphorylase

15 ml of culture from the bioreactor were sampled and centrifuged. The pellets were washed twice with 50 m*M* potassium phosphate, pH 7.5 and finally suspended in 1 ml of 25 m*M* potassium phosphate buffer, pH 7.5 containing 0.5% Triton X-100. After sonication for 90 seconds with intermediate cooling, the debris was removed by centrifugation. The cell extracts were assayed in duplicate for glycogen phosphorylase activity as described by Mendicino et al. (1975).

Assay of ADPG Pyrophosphorylase

15 ml of culture from the bioreactor were sampled and centrifuged. The pellets were washed with 25 m*M* Tris, pH 8.0 and finally the pellets were suspended in 50 m*M* glycylglycine buffer, pH 7.0 including 5 m*M* DTT. After sonication for 90 s with intermediate cooling, the cell extracts were assayed in duplicate for ADPG pyrophosphorylase as described by Ozaki and Preiss (1972) using PP^{32}.

RESULTS

Dynamics of Glycogen Net Synthesis and Degradation with the Metabolic Switch Plasmid

To test the ability of pMSW2 to control synthesis and degradation of glycogen, we measured the variation with time of glycogen in *E. coli* cultures, with and without addition of IPTG. The bacterium used in this study was the *E. coli* strain TA3476, which is impaired in the acetate synthesis pathway. We have shown in a previous paper (Dedhia et al., 1994) that TA3476 engineered for glycogen overproduction, with no significant glycogen net degradation, grows to about 15–20% higher cell culture densities than a control strain. TA3476 was transformed with the plasmid pMSW2. Cultivations of TA3476:pMSW2 were carried out in a bench-top bioreactor at constant pH, temperature and impeller speed. Cells were inoculated into buffered LB supplemented with 0.2% glucose, and after 4.66 hours, IPTG (to a final concentration of 500 μM) was added.

We measured the glycogen profiles in cultures of TA3476 carrying pMSW2 to which IPTG was added and

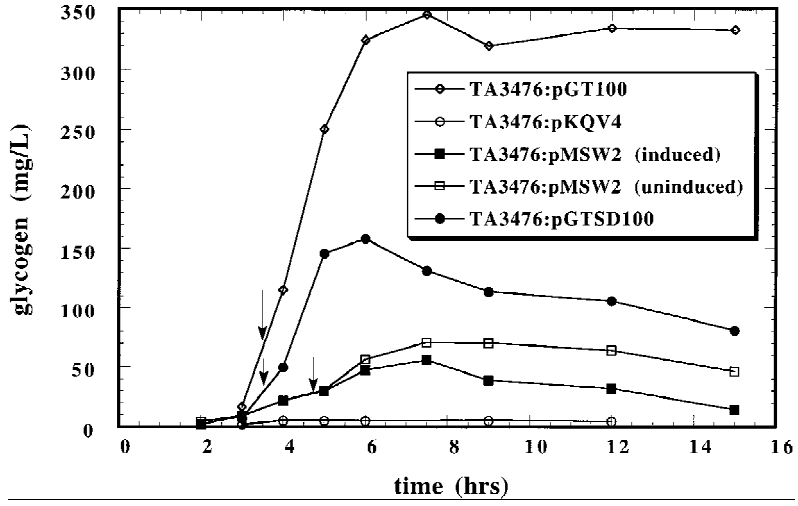

Figure 3. Glycogen trajectories during batch cultivations of different constructs. The cultures were cultivated in a bioreactor in LB supplemented with 0.2% glucose. The arrows indicate the time when IPTG was added to cultures of *E. coli* TA3476:pGT100, TA3476:pMSW2 and TA3476:pGTSD100 at a final concentration of 500 μM.

compared these to the glycogen profiles in cultures to which no IPTG was added. As shown in Figure 3, plasmid pMSW2 when induced by IPTG addition enabled the cultures to degrade glycogen. If no IPTG was added to the cultures, glycogen accumulated in the earlier portion of the cultivation was degraded at a much lower rate. The glycogen trajectory of strain TA3476:pMSW2 shows three phases with different specific glycogen net synthesis and degradation rates (see Table 2). In the first phase (0 to 4 hour post inoculation), we see the maximum specific net rate of glycogen synthesis. In the second phase (5 to 7.5 hour post inoculation), the net rate of glycogen synthesis is reduced. Finally, in the third phase (7.5 hour to end of cultivation, 15 hour), net glycogen degradation is observed. When no IPTG is added to cultures of TA3476:pMSW2, there is one phase of net glycogen synthesis (0 to 7.5 hour post inoculation) and a second phase of glycogen degradation (7.5 hour to 15 hour) with a very low rate of net glycogen degradation.

We measured also the trajectories of the specific activities of the glycogen synthesis enzyme, ADPG pyrophosphorylase, and the glycogen degradation enzyme, glycogen phosphorylase (Figures 4 and 5). Glycogen phosphorylase activity (Figure 5) is higher in the induced cultures after induction. Although it is unclear why ADP pyrophosphorylase activity shows an initial increase immediately after induction with IPTG, figure 4 indicates that after the initial increase, glgC activity stabilizes in the induced cultures, whereas it increases in the uninduced cultures.

Coordination of Glycogen Net Synthesis and Degradation Using the Plasmid pGTSD100

Plasmid pGTSD100 was constructed to examine an alternative configuration for regulated expression of glycogen synthesis. Plasmid pGTSD100 contains the native *E. coli* glycogen synthesis and degradation operon. We replaced the native promoter with the inducible *tac* promoter, so that addition of IPTG to cultures harboring pGTSD100 will induce transcription of the *glgC-glgA-glgP* operon.

Cultures of strain TA3476 transformed with plasmid pGTSD100 were grown in a bioreactor under conditions similar to that described for pMSW2, except IPTG was added 3.5 hours after inoculation. In cultures of TA3476:pGTSD100, within 30 minutes after addition of IPTG, the specific activity of glgC increased from 1500 cpm/mg-min to ca. 60,000 cpm/mg-min. The glycogen profiles in cultures of TA3476 carrying pGTSD100 show that the plasmid pGTSD100 also enables the cell to control the degradation of glycogen. When IPTG is added to cultures of pGTSD100 3.5 hours after inoculation, there is a phase of rapid net glycogen synthesis from hours 3.5 to 6, and the glycogen net degradation phase follows during hours 6 to 15 post inoculation (Figure 3 and Table 2).

To compare the glycogen profiles of TA3476:pGTSD100 with a strain overexpressing only the glycogen synthesis genes, we studied the glycogen trajectories of TA3476 cultures carrying the plasmid pGT100. pGT100 and pGTSD100 were constructed from the same parent vector, pKQV4; thus, the only difference between pGT100 and pGTSD100 is the addition of the glycogen degradation gene, *glgP*, downstream of the glycogen synthesis genes, *glgC* and *glgA*. The strain TA3476:pGT100 was grown under conditions identical to that of TA347:pGTSD100 (IPTG was added 3.5 hours post-inoculation). As shown in Figure 3, the level of glycogen produced in TA3476:pGT100 was nearly 2-fold higher than the maximum level of glycogen produced in TA3476:pGTSD100. No net glycogen degradation was observed in TA3476:pGT100.

Table II. Specific rates of glycogen synthesis and degradation in different phases of growth.

Construct	Phase	Time post-inoculation, hrs	Synthesis or degradation rate (g glycogen/g protein-hr)
pMSW2 (induced)	Synthesis phase 1	0 to 4 hrs	0.0432
	Synthesis phase 2	5 to 7.5 hrs	0.0163
	Degradation phase	7.5 to 15 hrs	−0.0032
pMSW2 (uninduced)	Synthesis phase	0 to 7.5 hrs	0.0248
pGTSD100	Synthesis phase	3.5 to 5 hrs	0.114
	Degradation phase	5 to 15 hrs	−0.0041

Effect of Glycogen Accumulation and Degradation on Batch Growth

We studied the effect of net glycogen degradation on batch growth of *E. coli* TA3476 cultures by following the total protein trajectory of strain TA3476 transformed with pMSW2, with and without addition of IPTG. As shown in Figure 6, when IPTG is added to TA3476 transformed with pMSW2, the amount of total cell protein at the end of the batch cultivation is about 15% higher compared with the amount when no IPTG is added. The growth curves of TA3476 cultures carrying pGTSD100 or pGT100 are also compared in Figure 6.

During the growth of all strains (TA3476:pMSW2 with and without IPTG, TA3476:pGTSD100 and TA3476:pGT100), glucose is exhausted from the medium by 4.0 hours post inoculation (Figure 7). Thus, all the strains accumulate most of the glycogen after glucose has been exhausted from the medium. Considering the nutrients available in LB medium, it is likely that gluconeogenic pathways contribute to the stored glycogen. Pyruvate is the major organic acid secreted by all strains and the maximum levels are about 0.5 g/L (Figure 7). After the exhaustion of glucose, pyruvate is completely consumed within 5.5 hours post inoculation. Other acids such as acetate, lactate and succinate are not accumulated in significant quantities (<0.05 g/l) (data not shown). Glycogen levels in all the strains reach a peak around 6 hours. It is possible that the consumed pyruvate may contribute to glycogen synthesis.

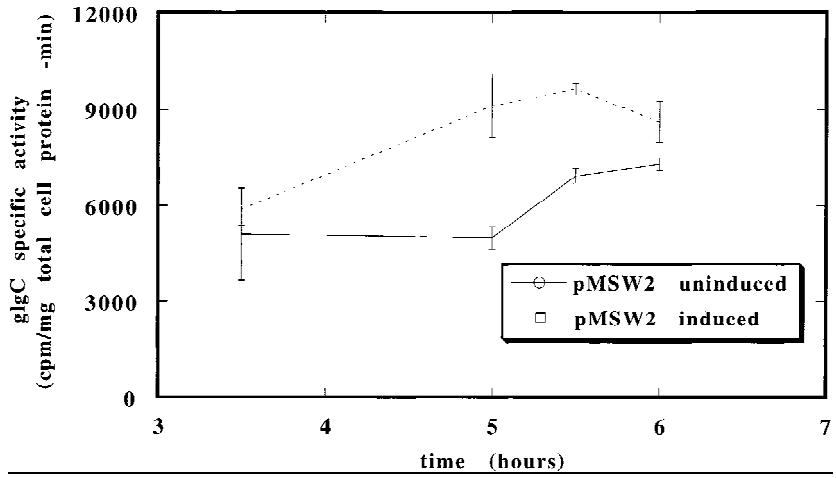

Figure 4. Specific activity of ADPG pyrophosphorylase in cultures of induced and uninduced TA3476:pMSW2. IPTG was added at 4.66 hours post inoculation. The growth conditions were as described in caption of figure 3.

DISCUSSION

Both TA3476:pMSW2 and TA3476:pGTSD100 were able to synthesize and degrade glycogen in a controlled fashion dependent on the time point of addition of IPTG. The time trajectories of net glycogen accumulation and degradation in the presence of pGTSD100 were different from those in presence of pMSW2: the specific net rate of glycogen synthesis was higher in TA3476:pGTSD100 and the glycogen net degradation started earlier. The rate of net glycogen synthesis may be higher in TA3476:pGTSD100 because the activity of ADPG pyrophosphorylase is 6-fold higher in TA3476:pGTSD100 compared to that in TA3476:pMSW2. This larger amount could be due to the difference in the strength of the promoters of *glgC-glgA*, the different copy numbers of pMSW2, whose parent vector is pBR322, and also due to pGTSD100, whose parent vector is pKQV4 (the copy number of pKQV4 is higher than that of pBR322 because it has the *lacI* gene inserted within the *rop* gene which controls copy number (Strauch et al., 1989)). The difference in glycogen amounts could also be due to the difference in the timing of induction. The earlier depolymerization of glycogen in TA3476:pGTSD100 may possibly reflect the effect of the larger amount of glycogen synthesized in TA3476:pGTSD100 on the activity of ADPG pyrophosphorylase or it may reflect a different ratio of degradation enzyme levels to synthesis enzyme levels. The specific rate of net glycogen degradation in TA3476:pGTSD100 is similar to that in TA3476:pMSW2.

The difference in ADPG pyrophosphorylase enzyme levels between TA3476:pMSW2 and TA3476:pGTSD100 in-

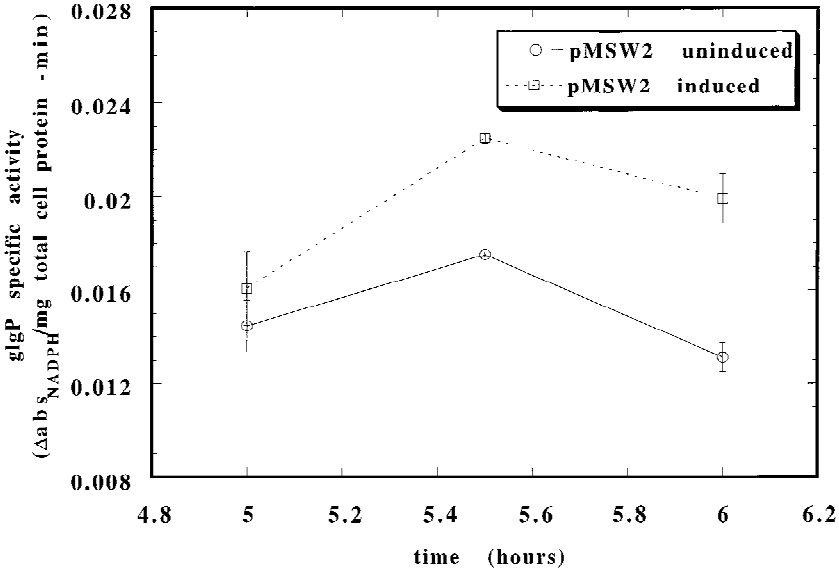

Figure 5. Specific activity of glycogen phosphorylase in cultures of induced and uninduced TA3476:pMSW2. IPTG was added at 4.6 hours post inoculation.

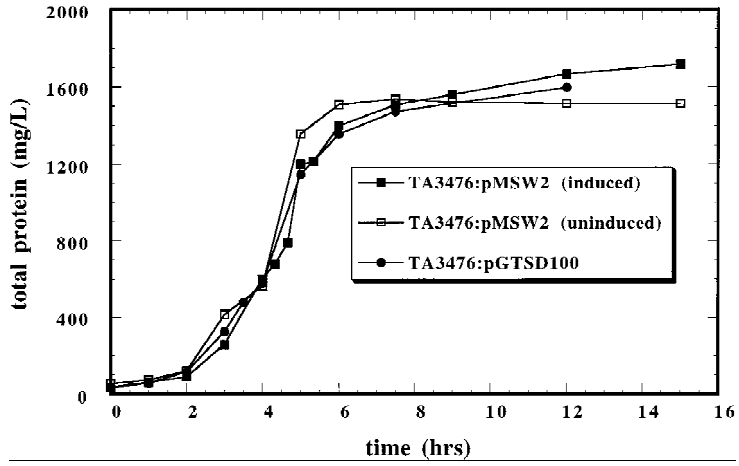

Figure 6. Total protein trajectories during batch cultivations of different constructs. The growth conditions are described in caption of Figure 3.

dicates that, to increase the level of expression of the encoded genes, the next generation of ''metabolic switch'' plasmids should have a replicon with a higher copy number (the current replicon is from pBR322).

There exists a time delay between the time points of addition of IPTG and the initiation of glycogen degradation in both TA3476:pMSW2 and TA3476:pGTSD100. In the cultivation in which IPTG is added to cultures of TA3476:pMSW2, although IPTG is added 4.66 hours post inoculation, we observe a net rate of glycogen degradation only 7.5 hours post inoculation. Similarly, in the cultivation of TA3476:pGTSD100, IPTG is added 3.5 hours post inoculation while a net rate of glycogen degradation is observed only 6 hours post inoculation. Cultures of TA3476:pMSW2 contain both ADPG pyrophosphorylase and glycogen phosphorylase in phase two of glycogen synthesis (5 to 7.5 hours post inoculation). By the very nature of the design of plasmid pGTSD100, which is intended to transcribe the *glgC*, *glgA* and *glgP* genes in an operon, both ADPG pyrophosphorylase and glycogen phosphorylase should be present after the addition of IPTG. Thus due to coexistence of ADPG pyrophosphorylase and glycogen phosphorylase after addition of IPTG, the net rate of glycogen synthesis or degradation will be strongly influenced by the specific activity of ADPG pyrophosphorylase relative to the glycogen phosphorylase specific activity. It is known that ADPG pyrophosphorylase is allosterically activated by fructose 1,6 diphosphate and inhibited by 5′-AMP while glycogen phosphorylase is inhibited by ADPG and activated by AMP (reviewed in Preiss, 1984; Preiss and Romeo, 1984). The changing levels of these metabolites will control the specific activities of ADPG pyrophosphorylase and glycogen phosphorylase. In wild-type *E. coli*, it has been suggested that allosteric regulation of ADPG pyrophosphorylase and glycogen phosphorylase determines whether glycogen is accumulated, as in the early stationary phase, or degraded, as occurs later in stationary phase (Romeo et al., 1988).

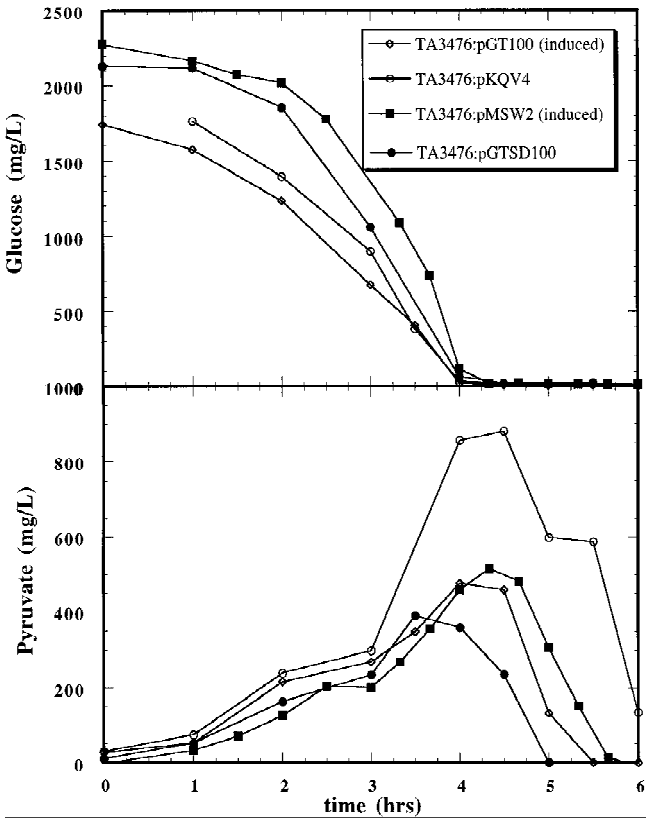

Figure 7. Glucose and pyruvate trajectories. The growth conditions are described in caption of Figure 3.

Did the switch in enzyme expressions occur in TA4376:pMSW2 and if so, what role did it play in the pattern of glycogen levels we observe under induced and uninduced conditions? After addition of IPTG to cultures of TA3476:pMSW2, glgP activity increases as expected. Activity of glgC stabilizes in the induced cultures after an initial increase for reasons we do not understand yet, whereas it continues to increase in the uninduced cultures. Thus until 6 hours post inoculation, a complete switch in enzyme expressions had not occurred: glgP levels had in-

Table III. Total protein content at end of batch cultivation.

Strain	Final total protein (mg/L)
TA346:pKQV4	1310
TA3476:pGT100	1590
TA3476:pMSW2(uniduced)	1540
TA3476:pMSW2	1710

creased and glgC levels were about equal. The rate of glycogen synthesis begins to decline in the induced cultures between 5 and 6 hours post inoculation. Glycogen degradation appears to be triggered by the appearance of glgP activity and the passage of time.

Comparing the glycogen trajectories of TA3476:pGT100 and TA3476:pGTSD100 (which is constructed by the addition of *glgP* gene to pGT100), we can observe the effect of glycogen phosphorylase on the dynamics of glycogen accumulation. The glycogen net synthesis rate in TA3476: pGTSD100 is slightly lower than in TA3476:pGT100. Glycogen levels peak about 90 minutes earlier in TA3476: pGTSD100, and the maximum quantity of glycogen accumulated in cultures of TA3476:pGTSD100 is nearly half the maximum quantity observed for TA3476:pGT100.

All strains which overproduce glycogen (TA3476: pMSW2 induced and uninduced, TA3476:pGTSD100, induced TA3476:pGT100) accumulate more biomass than the control strains TA3476:pKQV4 and uninduced TA3476: pGT100 (Table 3). The difference in biomass achieved between induced and uninduced TA3476:pMSW2 shows that glycogen degradation can contribute to growth. The degraded glycogen can provide energy or biosynthesis precursors or both. When induced, cultures of TA3476: pMSW2 accumulate an extra 170 mg/L protein and degrade about 40 mg/L glycogen. This indicates that inputs other than glycogen have also contributed to the extra biomass. We do not know definitely how glycogen over accumulation affects biomass synthesis in *E. coli* cells deficient in acetate synthesis. For a review of our various hypotheses, see Dedhia et al. (1994).

Vectors analogous to pMSW2 can be constructed by replacing the glycogen synthesis cassette and the glycogen degradation cassette with other dual sets of genes. By choosing appropriate genes, these constructs can be used to shift from one physiological process to another, provided that metabolic regulation of specific activity does not dominate. In such cases, appropriate mutant or heterologous enzymes will also be required to accomplish inducible switching between different metabolic pathways.

The vectors pMSW2 and pGTSD100 can be used to study the channeling of stored glycogen carbon to metabolites, especially in the stationary phase. Similar vectors can be designed for performing metabolic engineering in microorganisms which produce secondary metabolites. It has been suggested that recombinant proteins can be made in the stationary phase of *E. coli* cultures by using carbon starvation promoters (Matin, 1992). In such cases, the glycogen management system described here may be a useful adjunct.

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