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Altered Metabolic Flux due to Deletion of *odhA* causes L-Glutamate Overproduction in *Corynebacterium glutamicum*▽

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L-Glutamate overproduction in *Corynebacterium glutamicum*, a biotin auxotroph, is induced by biotin limitation or by treatment with certain fatty acid ester surfactants or with penicillin. We have analyzed the relationship between the inductions, 2-oxoglutarate dehydrogenase complex (ODHC) activity, and L-glutamate production. Here we show that a strain deleted for *odhA* and completely lacking ODHC activity produces L-glutamate as efficiently as the induced wild type (27.8 mmol/g [dry weight] of cells for the *ohdA* deletion strain compared with only 1.0 mmol/g [dry weight] of cells for the uninduced wild type). This level of production is achieved without any induction or alteration in the fatty acid composition of the cells, showing that L-glutamate overproduction can be caused by the change in metabolic flux alone. Interestingly, the L-glutamate productivity of the *odhA*-deleted strain is increased about 10% by each of the L-glutamate-producing inductions, showing that the change in metabolic flux resulting from the *odhA* deletion and the inductions have additive effects on L-glutamate overproduction. Tween 40 was indicated to induce drastic metabolic change leading to L-glutamate overproduction in the *odhA*-deleted strain. Furthermore, optimizing the metabolic flux from 2-oxoglutarate to L-glutamate by tuning glutamate dehydrogenase activity increased the L-glutamate production of the *odhA*-deleted strain.

Coryneform bacteria are rod-shaped, nonsporulating, gram-positive bacteria with a high GC content. The nonpathogenic species, *Corynebacterium glutamicum*, *Brevibacterium lactofermentum*, and *Brevibacterium flavum*, are used for the fermentative production of nucleotides and amino acids, especially L-glutamate. Recently, *B. lactofermentum* and *B. flavum* have been reclassified as *C. glutamicum* (22), and the genome of *C. glutamicum* ATCC 13032 has been sequenced (12, 30). *C. glutamicum* was originally isolated as an L-glutamate-producing bacterium (20, 36), although the wild type does not overproduce L-glutamate under ordinary culture conditions. Significant L-glutamate production is induced by incubating the biotin-auxotrophic wild type in a biotin-limited medium (32). Although L-glutamate overproduction is suppressed in the presence of an excess of biotin, the addition of certain fatty acid ester surfactants (7, 35) or penicillin (27) to the medium also induces L-glutamate overproduction. For example, as much as 220 mM L-glutamate is produced from 360 mM glucose by wild-type *C. glutamicum* cells under the L-glutamate-producing condition of adding 1 g of Tween 40 liter^{-1}, whereas only 22 mM L-glutamate is produced without Tween 40 addition (Table 1). This means that at least 10-fold more L-glutamate is metabolically synthesized under L-glutamate-producing conditions than under non-L-glutamate-producing conditions. From this perspective, metabolic flux change is expected to be crucial for L-glutamate overproduction in *C. glutamicum*.

The molecular basis of the induction of L-glutamate overproduction remains unclear. One widely held view is that L-glutamate efflux is caused by an alteration in the chemical or physical characteristics of the cell membrane; this is because several changes in the bacterial membrane have been observed under L-glutamate-producing conditions, including a decrease in lipid content, a change in the amount of phospholipid (35), and altered fatty acid composition (31). Recently, alterations of the chemical and physical properties of the cytoplasmic membrane caused by biotin limitation and by genetic modification of lipid biosynthesis were shown to be necessary, but not sufficient, to achieve high L-glutamate efflux (11, 25).

Changes in metabolic flux have not been discussed as often as the basis of L-glutamate overproduction, although there have been suggestions that this process is important. The 2-oxoglutarate dehydrogenase complex (ODHC) catalyzes the oxidative decarboxylation of 2-oxoglutarate to succinyl coenzyme A (succinyl-CoA), and glutamate dehydrogenase (GDH) catalyzes ammonia assimilation of 2-oxoglutarate to form L-glutamate (Fig. 1). These two enzymes compete for 2-oxoglutarate at the branch point of the tricarboxylic acid (TCA) cycle and L-glutamate biosynthesis. It has been reported that ODHC activities are decreased under L-glutamate-producing conditions (14, 33). We and others previously compared ODHC and GDH activities and found a reduction in ODHC activity relative to GDH activity during L-glutamate production (14, 33), suggesting that the change in metabolic flux caused by the reduction in ODHC activity was important for L-glutamate production. The reduction in ODHC activity, however, was not enough to account fully for the high L-glutamate productivity achieved by L-glutamate-producing inductions, as the reductions in ODHC activity ranged from 40 to 90% (14, 33) and did

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TABLE 1. Fatty acid composition of cells[a]

Fatty acid	% of the indicated fatty acid in:		
	ATCC 13869 (wild type)		AJ13133 (*odhA* mutant) without Tween 40
	Without Tween 40	With Tween 40	
12:0	ND[b]	ND	ND
14:0 (myristic acid)	0.3	1.0	0.7
15:0	ND	0.3	ND
16:0 (palmitic acid)	43.6	47.9	39.9
16:1 (palmitoleic acid)	ND	0.2	0.4
17:0	ND	0.7	1.2
17:1	ND	ND	ND
18:0 (stearic acid)	0.7	23.0	1.1
18:1 (oleic acid)	55.0	26.2	54.6
18:2	ND	ND	0.6
18:3 (n−3)	ND	ND	ND
20:0	ND	0.2	ND
22:0	ND	ND	ND
Unknown	0.4	0.5	1.5

[a] Cells from batch fermentation were freeze dried and analyzed by gas chromatography. Tween 40 (1 mg · ml^{-1}) was added only to the wild type to induce L-glutamate overproduction. Glu production levels were 21.8 mM for the wild type without Tween 40, 220.5 mM for the wild type with Tween 40, and 277.7 mM for the *odhA* mutant AJ13133. Similar results were obtained in duplicate independent experiments.

[b] ND, not detected.

not always strictly correspond to the level of L-glutamate production.

In the current study, we analyzed the relationship between L-glutamate-producing inductions, alterations in the fatty acid composition of the membrane, ODHC activity, changes in metabolic flux, and L-glutamate production. We previously cloned the *odhA* gene, which encodes the E1o subunit of the ODHC (37). In the present work, we constructed an *odhA* deletion mutant, which completely lacks ODHC activity, and found that it produced L-glutamate as efficiently as the induced wild-type strain. Surprisingly, this efficient L-glutamate production was achieved without any induction or any alteration of the fatty acid composition of the cells. This indicates that the change in metabolic flux is the direct cause of the L-glutamate production. Next, we analyzed the effects of L-glutamate-producing inductions on the L-glutamate productivity of the *odhA* deletion strain and found that each of these inductions and the loss of ODHC activity had additive effects on L-glutamate production. We analyzed the metabolites in the *odhA* deletion strain under the condition of Tween 40 addition and found that it might induce a drastic metabolic change leading to more efficient L-glutamate biosynthesis.

We also discovered that the cells lacking *odhA* accumulated 2-oxoglutarate due to the loss of ODHC activity, and we demonstrated that optimizing the metabolic flux from 2-oxoglutarate to L-glutamate by tuning GDH activity improved L-glutamate productivity (Fig. 1). To optimize GDH activity, we analyzed the promoter of *C. glutamicum gdh* and showed that mutagenesis of the promoter is a powerful tool for metabolic engineering.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *C. glutamicum* ATCC 13869 (formerly *B. lactofermentum*) was used as a wild-type strain. The *Escherichia coli* K-12 strain JM109 [*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* Δ(*lac-proAB*)/F′ (*traD36 proAB*$^+$ *lacI*^{q} *lacZ*Δ*M15*)] (40) was used in the recombinant DNA procedures. Antibiotics at the following concentrations were used for the selection of cells carrying plasmids: 25 μg kanamycin ml^{-1} or 4 μg chloramphenicol ml^{-1} for *C. glutamicum* and 25 μg kanamycin ml^{-1} or 40 μg chloramphenicol ml^{-1} for *E. coli*. *C. glutamicum* and *E. coli* were grown on CM2B medium (per liter, 10 g yeast extract, 10 g tryptone, 5 g NaCl, and 10 μg biotin) at 30°C and LB medium (per liter, 5 g yeast extract, 10 g tryptone, and 5 g NaCl) at 37°C, respectively.

Batch fermentation. Batch fermentations were conducted at 30°C in 1,000-ml stirred vessels (1,200 rpm) containing 300 ml of a solution comprising (per liter) 65 g glucose, 1.5 g KH_2PO_4, 1 g $MgSO_4$, 0.01 g $FeSO_4$, 0.01 g $MnSO_4$, 0.45 mg vitamin B_1, 0.45 mg biotin, and 40 ml soybean protein hydrolysate. They were maintained at pH 7.0 by the automatic addition of ammonia gas. The fermentations were initiated by inoculating cells grown overnight on CM2B agar at an optical density at 660 nm (OD_{660}) of 0.15. During fermentation, samples were removed to measure OD_{660}, L-glutamate, and glucose. When all the glucose had been consumed, the fermentation was stopped, and fatty acid proportions and dry weight of cells were determined.

Flask culture. To analyze the clones isolated from the originally obtained *odhA* deletion strain AJ13133, to investigate the effects of L-glutamate-producing inductions on L-glutamate production in AJ110214 cells, and to assay enzyme activities, cells were cultured in a 500-ml flask containing 20 ml of a medium comprising (per liter) 30 g glucose, 1 g KH_2PO_4, 0.4 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO4 \cdot 7H_2O$, 0.01 g $MnSO_4 \cdot 4H_2O$, 15 g $(NH_4)_2SO_4$, 200 μg vitamin B_1 hydrochloride, 300 μg biotin, and 40 ml soybean protein hydrolysate (pH 8.0). To inoculate the flask cultures, cells grown overnight on CM2B agar were added to an OD_{660} of 0.15. Tween 40 (Wako, Tokyo, Japan) or penicillin (Wako) was added to the cultures at the desired concentrations. For biotin-limiting conditions, 1-ml aliquots of precultures grown in the appropriate concentrations of biotin were inoculated into 20 ml of the main culture medium without biotin, so

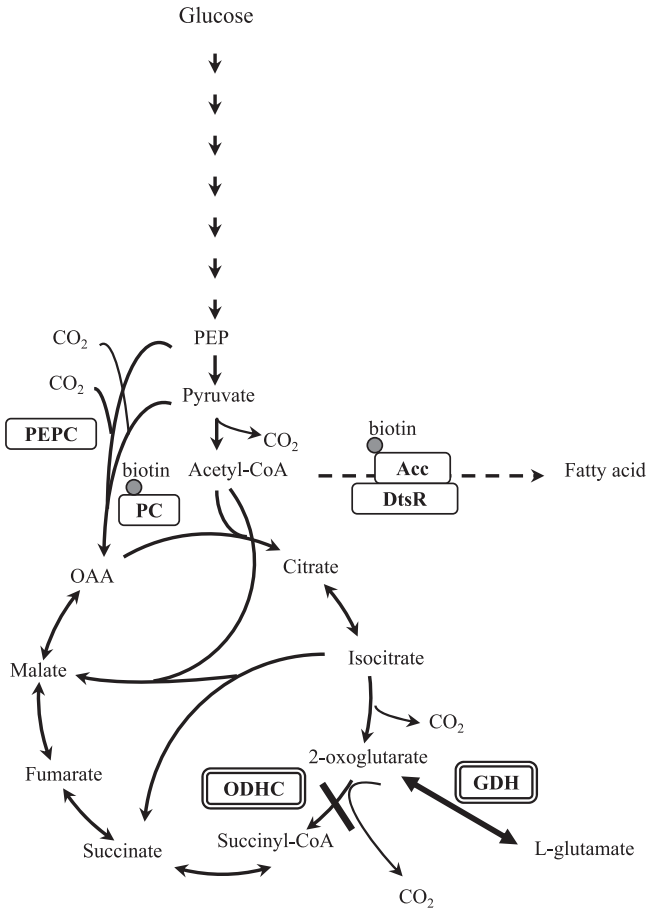


FIG. 1. Summary of the metabolic pathway from glucose to L-glutamate in *C. glutamicum*.

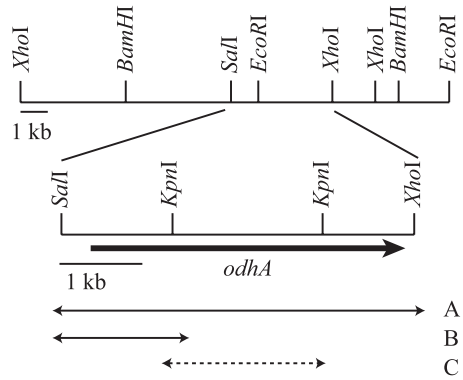


FIG. 2. Map of the *odhA* gene. Double-headed arrows indicate the fragment used. Arrow A, 4.4-kbp SalI-XhoI fragment containing the *odhA* gene cloned into pPKSX4.4. Arrow B, 1.5-kbp 5′-terminal fragment used for Southern hybridization. Double-headed dashed arrow C indicates the 1.9-kbp KpnI-KpnI region deleted in the *odhA* deletion strain.

that the biotin was depleted during subsequent growth. Samples were removed every 2 to 3 h to measure OD_{660}, L-glutamate, and glucose.

Analysis of fatty acids. Cells were harvested by centrifugation, washed twice in H_2O, and freeze dried. Fatty acids were analyzed by gas chromatography (Japan Food Research Laboratories' Custom Service, Tokyo, Japan).

Analysis of growth, L-glutamate production, and glucose consumption. Growth was monitored by measuring OD_{660}. L-Glutamate and glucose concentrations were measured with a Biotech-Analyzer AS-210 (Sakura Seiki, Tokyo, Japan) using a glutamate oxidase and a glucose oxidase sensor, respectively. Specific rates of L-glutamate production and glucose consumption (expressed as grams per gram [dry weight] of cells per hour) were calculated. The dry weight of cells was calculated from the OD_{660} by the formula obtained experimentally by measuring the dry weight of cells and the OD_{660}s of six samples with different cell densities (OD_{660}, 36 to 60); dry weight of cells (expressed in grams per liter^{-1}) = $26.052 \times (OD_{660}/101)^{1.1224}$.

Analysis of organic acids and amino acids. Organic acid concentrations were analyzed by high-performance liquid chromatography using an ULTRON DW-80 AD0415 column with phosphate buffer (pH 1.9) as the mobile phase (1 ml^{-1}; 45°C) and were detected at 210 nm. Amino acid concentrations were measured by ninhydrin reaction followed by high-performance liquid chromatography using an L-8500 automatic amino acid analyzer (Hitachi, Tokyo, Japan).

Analysis of intracellular metabolites. Cells of a 500-μl sample were separated from the medium by centrifugation on 500 μl of KF-54 silicon oil (specific gravity, 1.07) (Shinetsu Kagaku Kogyo, Tokyo, Japan) as the separation layer and 300 μl 21% $HClO_4$ as an acid fixation layer (64,000 × *g*, 5 min, and 4°C) (13). The sedimented cells in the acid layer were further disrupted using a freeze-thaw procedure, and the resulting extracts were neutralized in the cold by adding 225 μl of 2 M Na_2CO_3. The supernatant obtained by centrifugation (64,000 × *g*, 5 min, and 4°C) was used for determination of intracellular amino acids and organic acids. The cytoplasmic volume was assumed to be 1.6 μl (mg [dry weight] of cells)$^{-1}$ (10).

Analysis of N-terminal amino acids. Approximately 200 μg of protein in the crude cell extract was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto a polyvinylidene fluoride membrane. The membrane was stained with Coomassie brilliant blue R-250, the region corresponding to the 136-kDa band was cut out, and its N-terminal sequence was analyzed by automated Edman degradation employing a gas phase protein sequencer (TaKaRa Custom Service Center, Kyoto, Japan).

Construction of the *odhA* deletion strain. A 4.4-kbp SalI-XhoI fragment (Fig. 2) from plasmid pPKSX4.4 (37) containing the *odhA* gene was cloned into the SalI site of *E. coli* vector plasmid pHSG299 (GenBank accession no. M19415; TaKaRa, Kyoto, Japan), yielding plasmid pHSGodhASX. A 1.9-kbp KpnI-KpnI region in the middle of *odhA* was deleted by digesting pHSGodhASX with KpnI, followed by self-ligation. Then a temperature-sensitive *C. glutamicum* plasmid replicon, a 2.9-kbp BamHI-KpnI fragment from pHSC4 (34), which is derived from pHM1519 (23) and cannot replicate at 34°C in *C. glutamicum*, was ligated into its BamHI site to generate plasmid pdodhA. It harbors the deleted *odhA*, and its replication is blocked at the nonpermissive temperature of 34°C. A *C. glutamicum* derivative lacking *odhA* (designated AJ13133) was obtained by homologous recombination replacing the chromosomal *odhA* gene with the deleted *odhA* on pdodhA by the following procedures. The *C. glutamicum* wild-type strain ATCC 13869 was transformed with pdodhA at 30°C, cultured at 34°C with kanamycin, and the clones resistant to kanamycin at 34°C were selected. They had their chromosomal *odhA* gene integrated with pdodhA. The cells were then cultured at 34°C without kanamycin, and the clones sensitive to kanamycin at 34°C were selected. The deletion in chromosomal *odhA* was confirmed by dideoxy sequencing and Southern hybridization, in which chromosomal DNA was digested with BamHI and probed with the 1.5-kbp 5′-terminal region of *odhA* (Fig. 2). A 9.5-kbp BamHI restriction fragment hybridized to an *odhA* probe was observed in the mutant lacking *odhA*, whereas this restriction fragment was 11.4 kbp in the wild type. Spontaneous mutants from the *odhA* deletion strain AJ13133 that grew faster on CM2B agar were isolated. One of these, named AJ110214, had no ODHC activity.

Construction of AJ110214 derivatives with various GDH activities. A 2.2-kbp DNA fragment containing the GDH coding region and its putative promoter (2) was amplified by PCR with oligonucleotides 5′-GCTAGCCTCGGGAGCTC T-3′ and 5′-GATCTTTCCCAGACTCTG-3′ as the primers and with *C. glutamicum* ATCC 13869 chromosomal DNA as the template. The resulting fragment was cloned into the SmaI site of the *E. coli* vector plasmid pSTV28 (TaKaRa), yielding plasmid pSTVgdhwt. The *C. glutamicum* plasmid pHM1519 (23) was then ligated into the SalI site of pSTVgdhwt to produce plasmid pgdhwt. The *gdh* promoter variants of pgdhwt (Table 2) were obtained by site-directed mutagenesis of the putative promoter region, as follows. The first PCR was performed with oligonucleotides 5′-GCTAGCCTCGGGAGCTCTAGGAGAT-3′ and 5′-ACGTTCAATTATAGCAGTGTCGCAC-3′ (primer 2) as the primers and with *C. glutamicum* ATCC 13869 chromosomal DNA as the template. The second PCR was performed using the amplified DNA fragment as one of the

TABLE 2. GDH activities of promoter variants of *gdh*

Strain	Sequence of the following putative region:		Relative activity		
	−35	−10	Sp act (μmol L-glutamate formed/min/mg of protein)	Relative to AJ110214 or AJ110214/pSAC4	Relative to AJ110214/pgdhwt
AJ110214/pSAC4 (vector)			2.3	1.0	
AJ110214/pgdh2	TGGTCA	TATAAT	136.3	58.6	4.3
AJ110214/pgdh3	TTGACA	TATAAT	221.1	95.0	7.0
AJ110214/pgdh4	TTGTCA	TATAAT	180.7	77.6	5.8
AJ110214/pgdh7	TTGCCA	TATAAT	221.4	95.1	7.1
AJ110214/pgdhwt	TGGTCA	CATAAT	31.4	13.5	1.0
AJ110214	TGGTCA	CATAAT	2.6	1.0	
AJ110214gdh2	TGGTCA	TATAAT	11.7	4.5	
AJ110214gdh3	TTGACA	TATAAT	18.5	7.1	
AJ110214gdh7*	TTGCCA	CATAAT	14.2	5.4	
AJ110214gdh7	TTGCCA	TATAAT	15.6	6.0	

primers, oligonucleotide 5′-GATCTTTCCCAGACTCTG-3′ as another primer, and *C. glutamicum* ATCC 13869 chromosomal DNA as the template. The resulting fragment was cloned into the SmaI site of the *E. coli* vector plasmid pSTV28 (TaKaRa), yielding plasmid pSTVgdh2. The *C. glutamicum* plasmid pHM1519 (23) was then ligated into the SalI site of pSTVgdhwt to produce plasmid pgdh2. Oligonucleotides 5′-ACGTTCAATTATAGCAGTGTCGCAC AGATATGTCAACAAAGAATTAAAATTGTTTGAAA-3′, 5′-ACGTTCAA TTATAGCAGTGTCGCACAGATATGACAACAAAGAATTAAAATTGTT TGAAA-3′, and 5′-ACGTTCAATTATAGCAGTGTCGCACAGATATGGCA ACAAAGAATTAAAATTGTTTGAAA-3′ were used instead of primer 2 to produce pghd3, pdhh4, and pghd7, respectively. The direction of *gdh* transcription on the plasmid was opposite that of the *lacZ* transcription originally located on pSTV28. Sequences were confirmed by dideoxy sequencing whenever PCR was performed. The resulting plasmids were introduced into *C. glutamicum* AJ110214 by electroporation to generate strains with various GDH activities (Table 2).

Chromosomal *gdh* promoter variants were also obtained from AJ110214 by homologous recombination, replacing the putative promoter region with the various nucleotide sequences shown in Table 2, by the following procedures. A 1.9-kbp DNA fragment containing the putative *gdh* promoter (2) was amplified by PCR with oligonucleotides 5′-CAGTTGTGGCTGATCCGCCAAGGCC-3′ and 5′-TCGCCCTTAGCCTTGATCATTTCAC-3′ as the primers and with *C. glutamicum* ATCC 13869 chromosomal DNA as the template. The resulting fragment, which had the promoter region in its middle so that two homologous crossovers would occur equally on both sides of the promoter region, was cloned into the SmaI site of the *E. coli* vector plasmid pHSG299 (GenBank accession no. M19415; TaKaRa), yielding plasmid pHSGgdhprwt. Into the XbaI site of pHSGgdhprwt, a temperature-sensitive *C. glutamicum* plasmid, comprising a 2.96-kbp BamHI-KpnI fragment from pHSC4 (34), which is derived from pHM1519 (23), was ligated to give plasmid pgdhprwt. Then, the NheI-BglII region of pgdhprwt containing the promoter within the insert of pHSGgdhprwt was replaced by the corresponding region of pgdh2, pgdh3, or pgdh7 (Table 2) to give plasmid pgdhpr2, pgdhpr3, or pgdhpr7, respectively. AJ110214 was transformed with pgdhpr2, pgdhpr3, or pgdhpr7 by electroporation, and the clones with the chromosomal *gdh* promoter replaced by the corresponding region on the temperature-sensitive plasmid pghdpr2, pgdhpr3, or pgdhpr7 were selected as in the construction of the *odhA* deletion strain. AJ110214gdh2 was obtained using pghdpr2, AJ110214gdh3 was obtained using pghdpr3, and AJ110214gdh7 and AJ110214gdh7′ were obtained using pghdpr7. The chromosomal *gdh* promoter sequences were confirmed by dideoxy sequencing.

Enzyme assays. OHDC and GDH were assayed as described previously (2, 14) using the cells from flask cultures.

RESULTS

The N-terminal amino acid sequence of OdhA. We previously cloned the *C. glutamicum odhA* gene, which encodes the E1o subunit of ODHC. Its deduced amino acid sequence revealed that OdhA has a unique N-terminal extension similar to the catalytic domain of the E2o subunit of ODHC (37). In order to identify the precise protein-coding region of the *odhA* gene, we determined the N-terminal amino acid sequence of OdhA. As we reported previously (37), a 136-kDa band was enhanced by sodium dodecyl sulfate-polyacrylamide gel electrophoretic analyses of cell extracts of *C. glutamicum* harboring plasmid pPKSX4.4 carrying the *odhA* gene. The sequence of 11 N-terminal amino acids was NH_2-Xaa-Xaa-Ala-Ser-Thr-Phe-Gly-Asn-Ala-Xaa-Leu (the first, second and tenth residues could not be identified). This sequence was identical to the sequence from ^{38}Ser to ^{49}Leu of the *odhA* open reading frame in D84102. The translation start was found to be ^{551}GTG. *odhA* encodes a 1,221-amino-acid polypeptide with a molecular mass of 134,623 Da. A ribosome binding-like sequence, GAGG, is located 12 bp upstream of the initiation codon.

This N-terminal amino acid analysis confirmed that OdhA was translated from its N-terminal extension. Recent genome analysis has revealed that the open reading frame of the *odhA* gene of *Corynebacterium efficiens* starts with GTG, which corresponds to the initiation codon ^{551}GTG of *C. glutamicum odhA* (26). An E2o-like N-terminal extension is commonly found in the OdhA proteins of gram-positive bacteria with high GC contents, such as *Mycobacterium tuberculosis* (3), *Mycobacterium leprae* (4), and *Streptomyces coelicolor* (1). *C. glutamicum* has another gene annotated as an E2o subunit within its genome (gene ID, 1020158), which has the characteristic feature of E2 subunits: three copies of a lipoyl-binding domain followed by the catalytic domain (12, 30). It would be interesting to know the function of the N-terminal extension of OdhA.

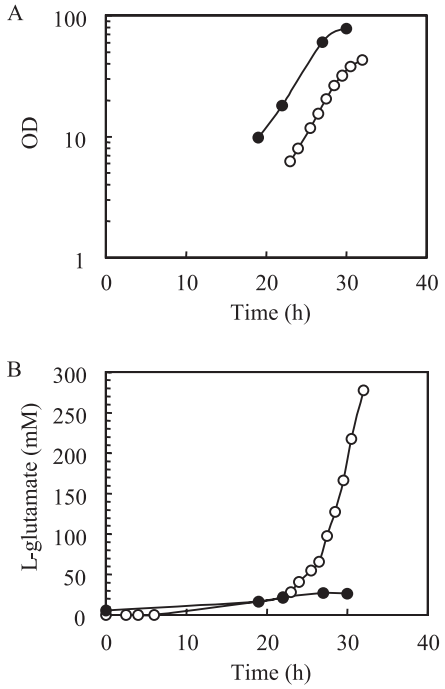


FIG. 3. Growth (A) and L-glutamate production (B) of the *C. glutamicum* wild-type strain ATCC 13869 and its derivative lacking *odhA*. Batch fermentations were conducted in stirred vessels at 30°C and pH 7.0 until all the glucose was consumed. AJ13133 produced a remarkably high level of L-glutamate. It accumulated L-glutamate during growth, which continued to the end of the fermentation. Solid circles, wild type; open circles, *odhA* deletion strain.

Construction of the *odhA* deletion strain. To construct a strain that completely lacked ODHC activity, we deleted the region between two KpnI sites in *odhA*, which corresponds to the segment from ^{265}Thr to ^{905}Gly in OdhA (see Materials and Methods). As expected, no ODHC activity was detected in the resulting *odhA* deletion strain, AJ13133, whereas the ODHC-specific activity of the wild-type strain was 2.7 nmol min^{-1} (mg of protein)$^{-1}$.

Growth and L-glutamate production of the *odhA* deletion strain. AJ13133 produced a remarkably high level of L-glutamate without any inductions (Fig. 3). This was as high as the level of the wild type under L-glutamate-producing conditions: 27.8 mmol (g [dry weight] of cells)$^{-1}$ compared with 1.0 mmol (g [dry weight] of cells)$^{-1}$ for the wild type without any induction (Fig. 3). Moreover, AJ13133 accumulated L-glutamate during growth, unlike the wild type under L-glutamate-producing conditions, where L-glutamate production takes place after

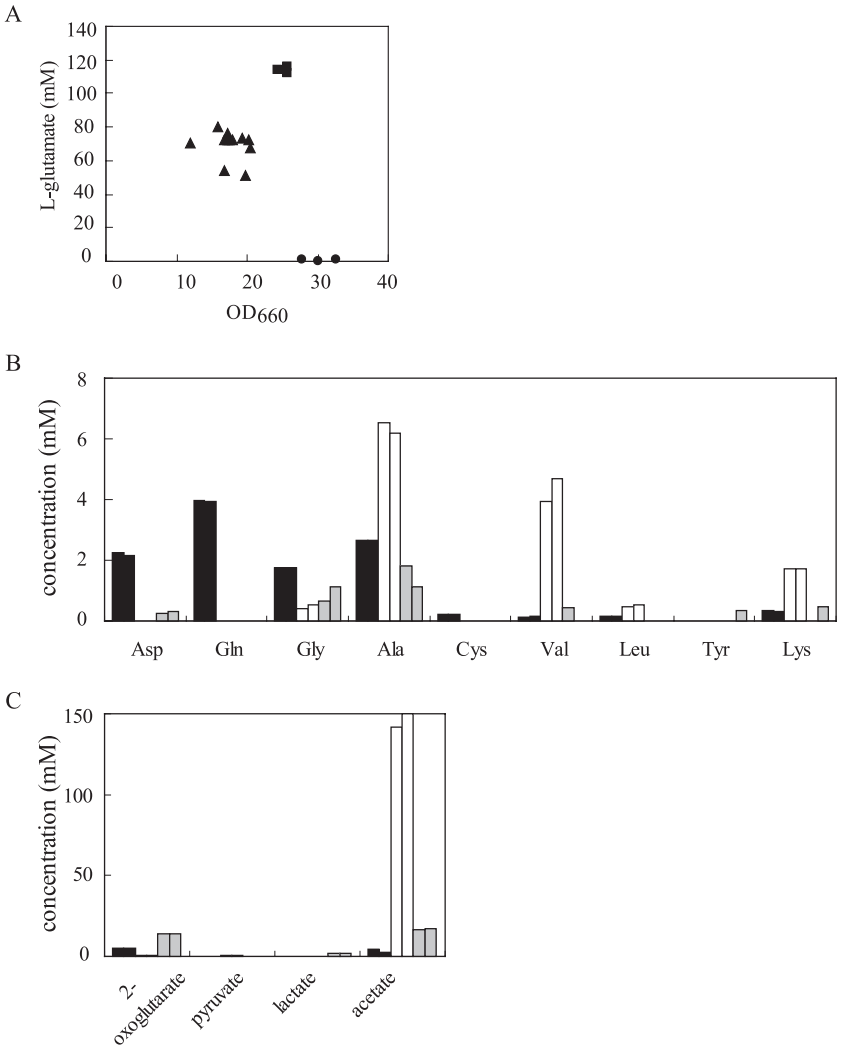


FIG. 4. Clones isolated from the original strain AJ13133 lacking *odhA*. (A) Growth and L-glutamate production of clones isolated from the original *odhA* deletion strain AJ13133. The isolated clones could be divided into three groups: the first, rapidly growing clones that produced L-glutamate as efficiently as the parent strain (squares); the second, rapidly growing clones that did not overproduce L-glutamate (circles); and the third, clones whose growth was slower than that of the parent and whose L-glutamate production was 60 to 70% of that by the parent (triangles). OD_{660} and L-glutamate levels were measured after glucose was completely consumed, when the cultivation time was 16 to 20 h for the clones in the first and second groups and 40 to 46 h for the clones in the third group. (B and C) By-products of the isolated clones. (B) Amino acids; (C) organic acids. Results for two clones from each group are shown. Solid bars, clones in the first group; open bars, clones in the second group; shaded bars, clones in the third group.

growth arrest due to the induction treatments (14, 33). L-Glutamate production by AJ13133 continued until all the glucose was consumed.

Fatty acid composition. To determine whether alteration of the fatty acid composition of the membrane was necessary for L-glutamate production, we analyzed the fatty acid composition of whole cells of AJ13133 (Table 1). We assumed that the fatty acid composition of whole cells paralleled that of the membrane, since almost all fatty acid exists in the membrane.

The data on the wild-type cells were in good agreement with published results; we noted a relative decrease of about 50% in oleic acid levels, and a relative increase of a similar amount in stearic acid levels, with Tween 40 addition (11, 31). The fatty acid composition of the cells lacking *odhA*, which were producing L-glutamate as efficiently as the wild-type cells with Tween 40 added, was almost the same as that of wild-type cells under the non-L-glutamate-producing condition. Similar results were obtained in two independent experiments. Evidently, L-glutamate production takes place without alterations in the fatty acid composition of the membrane. We therefore conclude that L-glutamate production in *C. glutamicum* can be caused by metabolic flux change due to *odhA* deletion alone, without alteration of the cell membrane.

Clones isolated from the *odhA* deletion strain. The original *odhA* deletion strain, AJ13133, appeared to be genetically unstable, because the colonies formed on CM2B agar differed in size after storage at 4°C for about 2 weeks. Although the reason was unclear, a cell lacking *odhA* might accumulate considerable L-glutamate, which could be harmful to the cell. It might also accumulate intermediates of glycolysis or the TCA

cycle (such as 2-oxoglutarate, acetate, or pyruvate), which could inhibit some important enzymes of glycolysis or the TCA cycle or could be toxic due to acidity. Mutants in which such problems are somehow overcome might arise spontaneously, for example, mutants in which the organic acids might be metabolized more rapidly, or mutants in which L-glutamate might be excreted more actively. We isolated 6 clones that grew rapidly and 12 that grew slowly on CM2B agar and cultured them in flasks in order to know their growth and L-glutamate production characteristics (Fig. 4). OD_{660} and L-glutamate levels were measured after glucose was completely consumed, when the cultivation time was 16 to 20 h for the first six clones and 40 to 46 h for the last 12 clones. The first six clones were of two types: three clones produced L-glutamate as efficiently as the original strain, while the other three clones did not produce L-glutamate and accumulated metabolites such as 145.8 mM acetate, 6.3 mM alanine, 4.3 mM valine, 1.7 mM lysine, and 0.7 mM 2-oxoglutarate (Fig. 4 A, B, and C). The 12 clones that grew slowly on CM2B agar produced 60 to 70% of the L-glutamate of the original strain. We picked one clone from the first group, which produced L-glutamate as efficiently as the original strain, named it AJ110214, and used it in further experiments. The growth rates of the wild type, AJ13133, and AJ110214 on CM2B liquid medium were 0.47 ± 0.03 h^{-1}, 0.22 ± 0.04 h^{-1}, and 0.40 ± 0.01 h^{-1}, respectively (averages ± standard deviations of three independent experiments). No ODHC activity was detected in AJ110214, and it was not unstable like the original mutant, AJ13133. AJ110214 transformed by plasmid pPKSX4.4, containing the *odhA* gene, did not overproduce L-glutamate.

Effect of L-glutamate-producing inductions on L-glutamate production by strain AJ110214 lacking *odhA*. To clarify the relationship between L-glutamate-producing inductions, ODHC activity, and L-glutamate production, we analyzed the effects of L-glutamate-producing inductions, biotin limitation, and the addition of Tween 40 or penicillin on L-glutamate production in AJ110214.

Under biotin-limited conditions, where the total amount of biotin supplied to the culture was 0.014 μg/ml or less, L-glutamate production was induced in the wild type, as reported previously. In AJ110214, significant L-glutamate production was observed, as in AJ13133, even in the presence of biotin levels above 0.3 μg/ml, at which the wild type does not overproduce L-glutamate. When biotin was limited to 0.0014 μg/ml or less, the L-glutamate yield (millimoles of glutamate produced per millimole of glucose at the end of the experiment) of AJ110214 increased; when biotin was limited to 0.0005 μg/ml, the L-glutamate yield of AJ110214 increased by as much as 8.3% over that under biotin-rich conditions (average of three independent experiments) (Fig. 5C). In addition, the L-glutamate yield of AJ110214 was 11.8% higher than that of the wild type (average of three independent experiments) when the biotin concentration was 0.0014 μg/ml. These findings show that *ohdA* deletion plus biotin limitation gave a higher L-glutamate yield than either *ohdA* deletion alone or biotin limitation alone.

Furthermore, under biotin-rich conditions, the specific glucose consumption rate of AJ110214 was much higher than that under biotin-limited conditions (Fig. 5B). The specific L-glutamate production rate under biotin-rich conditions was also

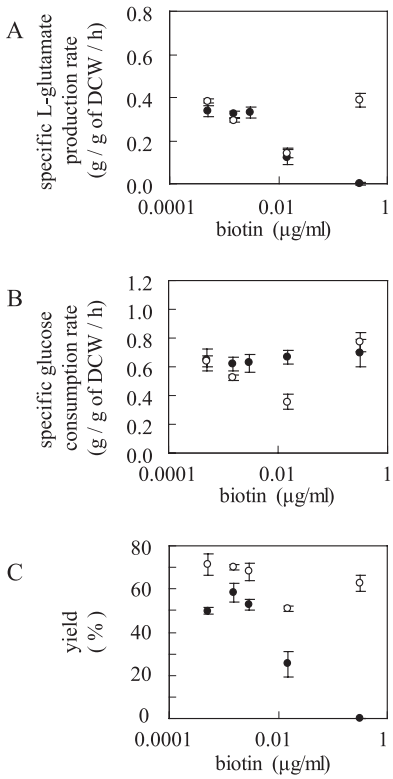


FIG. 5. Effects of the L-glutamate-producing condition of biotin limitation on L-glutamate production in AJ11024. Wild-type (solid circles) and AJ110214 (open circles) cells were cultured under biotin-limited conditions. Biotin concentrations are shown along the *x* axis. For biotin-limited conditions, seed cultures were prepared with 10, 30, 60, 100, and 300 μg of biotin $liter^{-1}$, and 1 ml was inoculated into 20 ml of the main medium lacking biotin so that biotin was depleted during the main cultures. For biotin-rich conditions, a seed culture was prepared in 300 μg of biotin $liter^{-1}$, and 1 ml was inoculated into 20 ml of the main medium containing 300 μg of biotin $liter^{-1}$. The biotin concentration was calculated by dividing the total amounts of biotin supplied to the seed and main cultures by the volume of the main culture. The specific L-glutamate production rate (A), the specific glucose consumption rate (B), and the L-glutamate yield (C) are shown. Specific rates were calculated during the periods when they were approximately constant. L-Glutamate yield is defined as millimoles of L-glutamate produced per millimole of glucose at the end of the experiment. Error bars, standard deviations from the means, calculated from three independent experiments.

high, probably as a consequence of the high glucose consumption rate (Fig. 5A), although the L-glutamate yield was lower than that under biotin-limited conditions. The specific glucose consumption rate was more sensitive to the biotin concentration in AJ110214 than in the wild type (Fig. 5B).

When Tween 40 was added, L-glutamate production was induced in the wild type, as reported previously (7, 35) (Fig. 6). In AJ110214, significant L-glutamate production was observed even without Tween 40 addition, as in AJ13133, and when 0.1 g of Tween 40 $liter^{-1}$ was added, both the specific L-glutamate production rate and the specific glucose consumption rate increased (Fig. 6A and B), and the L-glutamate yield also increased, by as much as 7.1% (average of three independent experiments). With 0.3 g of Tween 40 $liter^{-1}$, the L-glutamate yield of AJ110214 was much higher than that of the wild-type

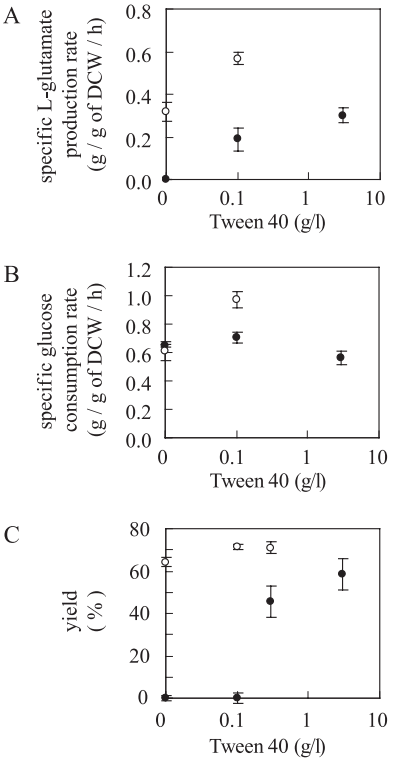


FIG. 6. Effects of the L-glutamate-producing condition of Tween 40 addition on L-glutamate production in AJ110214. Wild-type (solid circles) and AJ110214 (open circles) cells were cultured with Tween 40 addition; Tween 40 was added to 0, 0.1, 0.3, or 3 g liter^{-1}. The specific L-glutamate production rate (A), the specific glucose consumption rate (B), and the L-glutamate yield (C) are shown. Specific rates were calculated during the periods when they were approximately constant. L-Glutamate yield is defined as millimoles of L-glutamate produced per millimole of glucose at the end of the experiment. Error bars, standard deviations from the means, calculated from three independent experiments.

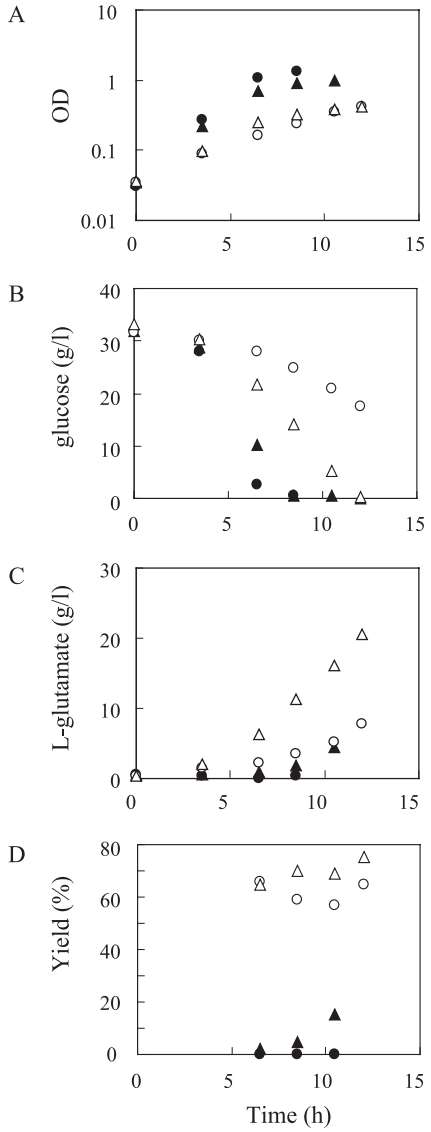


FIG. 7. Effects of the L-glutamate-producing condition of penicillin addition on L-glutamate production in AJ11024. Wild-type (solid symbols) and AJ110214 (open symbols) cells were cultured without penicillin (circles) or with 0.2 U of penicillin ml^{-1} (triangles). (A) Time courses of growth; (B) glucose consumption; (C) L-glutamate production; (D) L-glutamate yield.

strain (70.9% and 45.5%, respectively [averages of three independent experiments]). These findings show that *ohdA* deletion plus Tween 40 addition yielded higher L-glutamate production than either *ohdA* deletion alone or Tween 40 addition alone.

When penicillin was added, L-glutamate production was induced in the wild type, as reported previously (7, 35). In AJ110214, significant L-glutamate production was observed even without penicillin addition, as in AJ13133, and when 0.2 U of penicillin ml^{-1} was added, though the growth rate and the OD remained approximately unchanged (Fig. 7A), both the glucose consumption rate and the L-glutamate production rate increased (Fig. 7B and C), and the L-glutamate yield also increased (Fig. 7D). The L-glutamate yield of AJ110214 was much higher than that of the wild-type strain (75.2% versus 15.2%) when 0.2 U of penicillin ml^{-1} was added (Fig. 7D). These findings show that *ohdA* deletion plus penicillin addition yielded higher L-glutamate production than either *ohdA* deletion alone or penicillin addition alone.

In summary, AJ110214 produced L-glutamate without L-glutamate-producing inductions, and its productivity was increased by biotin limitation, addition of Tween 40, or addition of penicillin. These results show that each of the L-glutamate-producing inductions and the loss of ODHC activity have additive effects with respect to L-glutamate production.

Analysis of the *C. glutamicum gdh* promoter. We found that the *odhA* deletion strains, AJ13113 and AJ110214, accumulated 2-oxoglutarate in the medium; AJ13113 accumulated about 12 mM, and AJ110214 accumulated about 5 to 6 mM 2-oxoglutarate when cultured in flasks (Fig. 4C). This suggests that the GDH activities of these strains are not high enough to deal with the increased intracellular pool of 2-oxoglutarate resulting from the loss of ODHC activity. To investigate the GDH activity that optimizes the balance between the metabolic flux to 2-oxoglutarate via the TCA cycle and the flux from 2-oxoglutarate to L-glutamate, we constructed AJ110214 deriv-

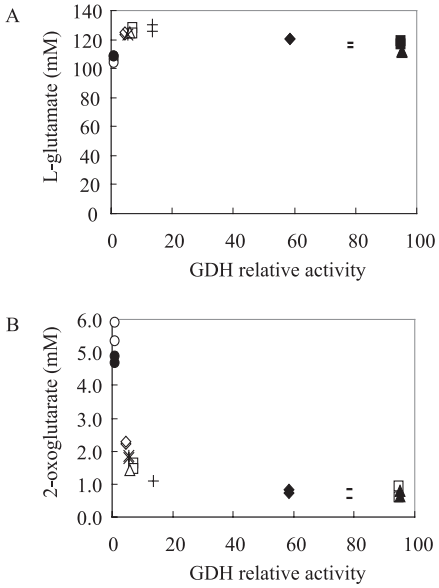


FIG. 8. Optimization of GDH activity for L-glutamate production in AJ110214. Derivatives of AJ110214 with various GDH activities were cultured in flasks, and production of L-glutamate (A) and 2-oxoglutarate (B) was analyzed at 15 to 16 h, when glucose was completely consumed. GDH activities are shown relative to that of AJ110214 (taken as 1). L-Glutamate production was maximally increased from 108 to 130 mM, at which concentration GDH activity was 7.1- to 13.5-fold higher than that in AJ110214. Two independent experiments were performed. Open diamonds, AJ110214gdh2; open squares, AJ110214gdh3; open triangles, AJ110214gdh7; asterisks, AJ110214gdh7′; solid diamonds, AJ110214/pgdh2; solid squares, AJ110214/pgdh3; small bars, AJ110214/pgdh4; solid triangles, AJ110214/pgdh7; crosses, AJ110214/pgdhwt; open circles, AJ110214; solid circles, AJ110214/pSAC4 (vector).

atives with GDH activities ranging from 1-fold to 95.1-fold higher than that of the parent strain. For this purpose, we analyzed the promoter of *gdh* (Table 2). The sequence upstream of the GDH-coding region in *C. glutamicum* ATCC 13869 was slightly different from the sequence reported for *C. glutamicum* ATCC 13032 (2); however, we were able to locate the −35 TGGTCA and −10 CATAAT motifs 33 and 10 bp upstream of the transcription start, respectively, as reported previously (2). The regions between −10 and the transcription start and between −35 and −10 were shorter by 1 nucleotide in ATCC 13869 than in ATCC 13032. The extended −10 motif "TGn" found in *E. coli* (21) was also present just upstream of the putative −10 hexamer. We mutated the putative −35 and −10 regions to make them more similar to the *E. coli* or *C. glutamicum* consensus promoter motifs (Table 2): TTGACA for the −35 sequence in *E. coli*, TTGCCA for the −35 sequence in *C. glutamicum* (28), and TATAAT for the −10 sequence in both species (28). The −35 region is reported to be much less conserved in *C. glutamicum* than in *E. coli* or *Bacillus* spp. (28, 29). Replacement of the putative −10 region by TATAAT enhanced GDH activity 4.5-fold. In addition, replacement of the putative −35 region by TTGCCA enhanced GDH activity sixfold, and replacement by TTGACA enhanced it sevenfold. T was more favorable than G for the second nucleotide in the −35 hexamer.

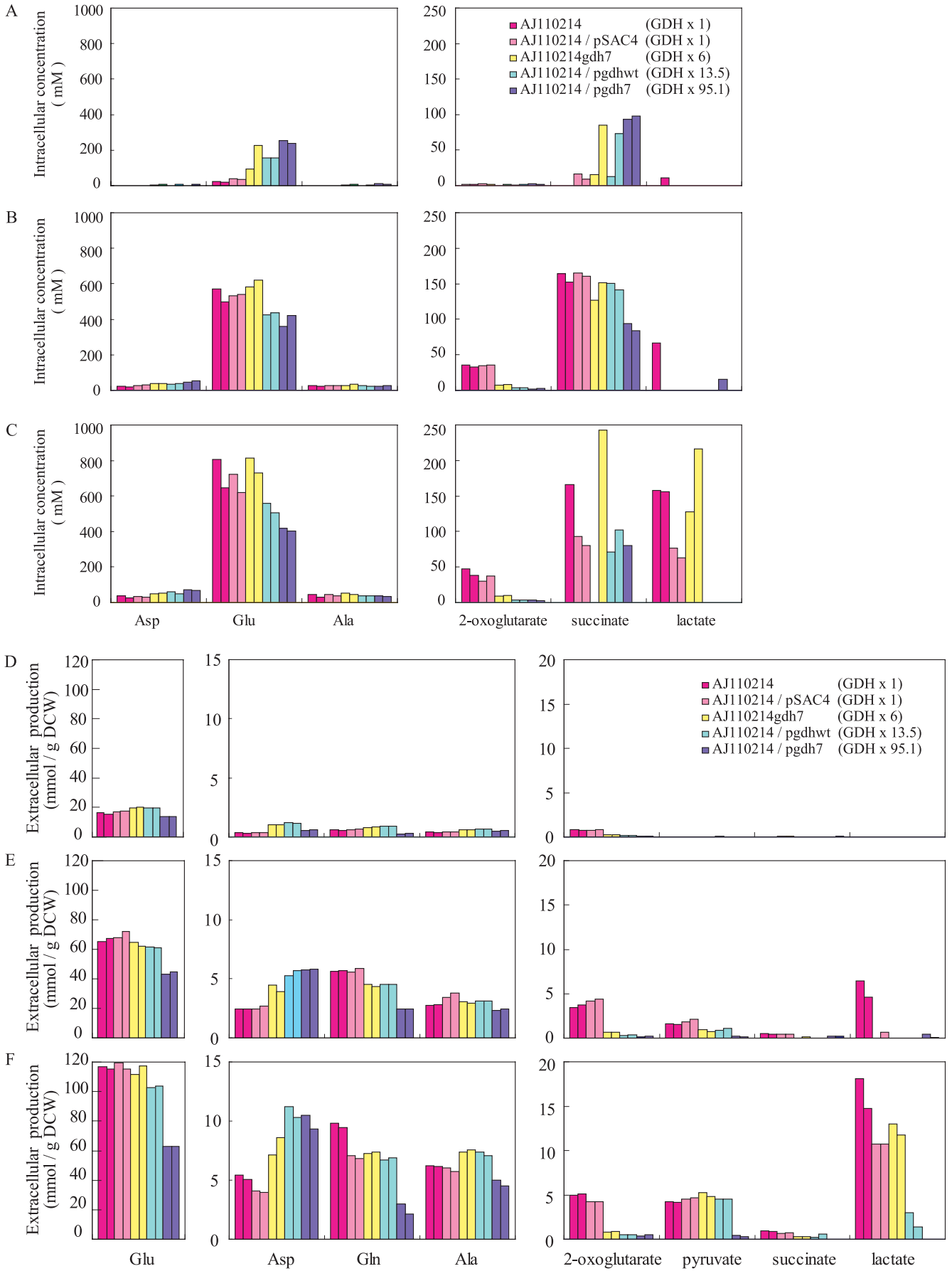
Optimizing metabolic flux for L-glutamate production by tuning GDH activity. To identify the GDH activity that optimizes the metabolic flux from 2-oxoglutarate to L-glutamate, we analyzed the effect of the GDH activity level on L-glutamate production (Fig. 8). When GDH activity was 7.1- to 13.5-fold higher than that in AJ110214, 2-oxoglutarate accumulation fell to 1.1 mM and L-glutamate production was maximally increased, from 108 to 130 mM (average of two independent experiments). When the GDH activity was increased more than 13.5-fold, L-glutamate production decreased. Thus, further metabolic changes optimizing the flux from 2-oxoglutarate to L-glutamate improved L-glutamate production resulting from loss of ODHC activity.

In addition, we noted that the accumulation of 2-oxoglutarate also fell when the GDH activity was increased more than 13.5-fold (Fig. 8B). We investigated whether the intracellular concentrations of 2-oxoglutarate and L-glutamate would reflect the extracellular production of the two metabolites under conditions of wild-type-level or high-level (6.0-, 13.5-, and 95.1-fold higher than the wild-type level) GDH activity (Fig. 9A). Intracellular 2-oxoglutarate was detected in several samples, but we could not determine the precise concentrations, because they were too low. The intracellular L-glutamate concentration was elevated in parallel with the increase in the GDH activity level from 22.5 mM to 245.5 mM (average of two independent experiments) (Fig. 9A), unlike extracellular L-glutamate production (Fig. 8 and 9D), indicating that the L-glutamate efflux is limiting when the GDH activity level is high.

Analysis of the effects of Tween 40 addition on L-glutamate production in the *odhA* deletion strain. We show that each of the L-glutamate-producing inductions had an additive effect on L-glutamate production brought about by loss of ODHC activity. To understand the effects of those inductions, we investigated the intracellular and extracellular production of L-glutamate, other amino acids, and organic acids including 2-oxoglutarate under the condition of Tween 40 addition, using *odhA* deletion strains with wild-type-level or high-level (6.0-, 13.5-, and 95.1-fold higher than that of the parent) GDH activity.

For AJ110214, intracellular concentrations of lactate, aspartate, alanine, succinate, and 2-oxoglutarate were increased by the addition of 0.1 g of Tween 40 liter^{-1}, and this increase was enhanced when the Tween 40 concentration was elevated to 0.3 g liter^{-1}, except for succinate. Accordingly, the intracellular L-glutamate concentration of AJ110214 rose from 22.5 mM to 553.6 mM and 726.1 mM (averages of two independent experiments) (Fig. 9A) by addition of 0.1 g (Fig. 9B) and 0.3 g (Fig. 9C) of Tween 40 liter^{-1}, respectively. This indicates that addition of Tween 40 might dramatically change the metabolic flux, leading to more-efficient L-glutamate synthesis. A similar result was obtained in the same experiment using the wild-type: increases in the intracellular alanine (from 0.5 ± 0.2 mM to 10.9 ± 0.9 mM [average ± standard deviation of three independent experiments]), succinate (from 26.1 ± 7.3 mM to 87.1 ± 9.4 mM), and 2-oxoglutarate (from 0.75 ± 0.14 mM to 4.96 ± 0.54 mM) concentrations were also observed by the addition of 0.3 g of Tween 40 liter^{-1}, besides the increase in arginine concentrations (from 0.4 ± 0.1 mM to 10.0 ± 0.9 mM), when the intracellular L-glutamate concentration was elevated from 35.6 ± 10.6 mM to 202.9 ± 28.0 mM.

As for the effects of GDH activity levels on intracellular metabolites, 2-oxoglutarate concentrations decreased in paral-



lel with the increase in the GDH activity level in the presence of 0.1 g and 0.3 g of Tween 40 liter^{-1}. The stronger the GDH activity, the more strongly the metabolic flux would be pulled to L-glutamate synthesis, as expected. The intracellular L-glutamate concentration rose with the increase in the GDH activity level in the absence of Tween 40. In the presence of 0.1 and 0.3 g of Tween 40 liter^{-1}, however, when the metabolic flux was drastically altered toward more-efficient L-glutamate synthesis, intracellular L-glutamate concentrations increased only slightly, from 553.6 mM to 601.9 mM under 0.1 g of Tween 40 liter^{-1} (Fig. 9B) and from 726.1 mM to 772.3 mM under 0.3 g of Tween 40 liter^{-1} (Fig. 9C) (average of two independent experiments), when the GDH level was elevated 6.0-fold. The intracellular L-glutamate concentration decreased when the GDH level was higher than 13.5-fold. These findings might be attributed to the result of metabolic balance; for example, the intracellular aspartate and alanine concentrations were increased with the increase in the GDH activity level at either of the Tween 40 concentrations, indicating altered equilibrium in aspartate aminotransferase and alanine aminotransferase reactions. In accordance with this, extracellular aspartate and alanine production was elevated, and extracellular L-glutamate production was decreased, when the GDH activity level was higher than 13.5-fold at either of the Tween 40 concentrations.

In summary, addition of Tween 40 was indicated to induce a drastic alteration in metabolic flux that might lead to more-efficient L-glutamate synthesis.

DISCUSSION

We have shown that L-glutamate overproduction in *C. glutamicum* can be brought about by the change in metabolic flux due to loss of ODHC activity alone, without any change in the fatty acid composition of the bacterial membrane. It was previously reported that an *E. coli* mutant in which the pathway between 2-oxoglutarate and succinate was blocked overproduced L-glutamate (5). In that mutant, ODHC, or both ODHC and succinyl-CoA synthetase, could be blocked. Thus, the situation where the change in metabolic flux due to loss of or reduction in ODHC activity causes L-glutamate overproduction might be common in various bacterial species, although the resulting level of production might differ. We found that *C. glutamicum* accumulated 2-oxoglutarate as a result of *odhA* deletion, and we demonstrated that an additional metabolic change optimizing the flux from 2-oxoglutarate to L-glutamate, brought about by tuning GDH activity, further increased L-glutamate production. Clearly, metabolic flux change is important for L-glutamate production in *C. glutamicum*. Moreover, further metabolic tuning to optimize each step in the flux from the carbon source to L-glutamate should increase L-glutamate production further. For this purpose, promoter mutagenesis should be a powerful tool. It was thought that the −35 regions of many of the promoters of *C. glutamicum* played only marginal roles in transcription, because the −35 motif is poorly conserved (29). Our observations showed for the first time, however, that the −35 region is recognized by RNA polymerase in a manner similar to that seen in *E. coli*, even though its role in promoter function might be relatively small.

On the other hand, *C. glutamicum* has a very interesting, unique feature in that the biotoin-auxotrophic wild type overproduces L-glutamate under conditions of biotin limitation, addition of certain fatty acid ester surfactants, or penicillin addition. All the conditions that lead to overproduction of L-glutamate are thought to cause chemical or physical alterations in the bacterial membrane influencing L-glutamate efflux activity—for example, permeability and/or exporter activity if it exists (8). These conditions also lead to a reduction in ODHC activity. Our work has revealed that the metabolic flux change resulting from the reduction in ODHC activity causes L-glutamate overproduction and that each L-glutamate-producing induction and the metabolic flux change caused by the reduction in ODHC activity works additively for overproduction of L-glutamate.

The molecular events underlying the action of the L-glutamate-producing inductions remain elusive, although some studies of biotin limitation and Tween 40 addition have been performed (15, 16, 19). In previous work, we showed that the amount of DtsR protein, a homolog of the β subunit of the biotin enzyme acetyl-CoA carboxylase complex that is involved in fatty acid synthesis (9, 17), declined in response to biotin limitation or Tween 40 addition (19). A *dtsR* gene disruptant, which is an oleic acid auxotroph, overproduced L-glutamate (18). Therefore, it was thought that biotin limitation and Tween 40 addition might lead to inactivation of the biotin enzyme complex that includes DtsR and that this might somehow cause L-glutamate production (18). A reduction in ODHC activity was observed for the *dtsR* gene disruptant (19), and this might contribute to L-glutamate production.

Tween 40 addition would inactivate fatty acid synthesis by decreasing the level of the DtsR protein, which might supply the TCA cycle with a larger amount of acetyl-CoA. Our result of increases in intracellular lactate, succinate, and 2-oxoglutarate concentrations with addition of Tween 40 might agree with this. Tween 40 addition simultaneously leads to a reduction in cell growth for the *odhA* deletion strain as for the wild type, which might be related to the metabolic changes. Lactate excretion is also observed in the L-glutamate-producing phase in an L-glutamate-producing strain of *C. glutamicum*, likely due to the decrease in pyruvate dehydrogenase activity (38).

The case of biotin limitation is more complex, because *C.*

FIG. 9. Effects of Tween 40 addition on L-glutamate production in the *odhA* deletion strains (AJ110214) with wild-type levels of GDH activity. Intracellular and extracellular metabolites were analyzed under conditions of no Tween 40 addition (A and D) or addition of 0.1 (B and E) or 0.3 (C and F) g of Tween 40 liter^{-1}, using the *odhA* deletion strains (AJ110214) with 1-, 6.0-, 13.5-, and 95.1-fold-higher levels of GDH activity. (A, B, and C) Intercellular concentrations of amino acids (left panels) and organic acids (right panels); (C, D, and E) extracellular production of L-glutamate (left panels), other amino acids (center panels), and organic acids (right panels) per gram of cells (dry weight). Results of two independent experiments are shown. Magenta, AJ110214; pink, AJ11024/pSAC4 (vector); yellow, AJ110214gdh7 (with 6.0-fold-higher GDH levels); light blue, AJ11024/pghdwt (13.5-fold-higher GDH levels); violet, AJ11024/pgdh7 (95.1-fold-higher GDH levels).

glutamicum has more than one biotin enzyme. The specific glucose consumption rate is more dependent on the biotin concentration in the *odhA* deletion strain AJ11024 than in the wild type, pointing to the involvement of biotin enzymes, in addition to DtsR, which affect glucose metabolism, such as pyruvate carboxylase (PC). Smooth glucose consumption would need a sufficient supply of oxaloacetic acid (OAA) to provide the glycolytic flux to the TCA cycle. In the *odhA* deletion strain, OAA could not be supplied by the TCA cycle, and the supply of OAA would depend on anaplerotic pathways or the glyoxylic acid cycle. *C. glutamicum* is known to possess two anaplerotic enzymes supplying OAA: phosphoenolpyruvate carboxylase (PEPC) and PC (30). PEPC is inhibited by asparate, 2-oxoglutarate, and L-glutamate (6, 24). Because aspartate, 2-oxoglutarate, and L-glutamate accumulated in cells lacking *odhA* (Fig. 9A), and they should inhibit PEPC, the supply of OAA should be largely dependent on PC. When the biotin concentration is high enough to thoroughly activate PC, the glucose consumption rate should be high; however, when the amount of biotin supplied to the cultures is reduced, PC activity should fall, leading to a decline in the glucose consumption rate. When the biotin concentration was limited to 0.014 μg/ml, the specific glucose consumption rate in AJ11024 fell, probably because of the lower PC activity, and as a consequence, the specific L-glutamate production rate declined. When biotin is more limited (0.0014 μg/ml or less), however, L-glutamate production should be activated due to inactivation of DtsR, and this should elevate the glucose consumption rate.

In the case of penicillin addition, DtsR is not affected (19). Penicillin-binding proteins involved in peptidoglycan synthesis are thought to be the targets of penicillin (39); however, whether the interaction of a penicillin-binding protein with penicillin is enough to induce L-glutamate production is not clear.

Thus, we showed that each L-glutamate-producing induction and the metabolic flux change caused by loss of ODHC have an additive effect on L-glutamate overproduction. Moreover, the effect of Tween 40 addition was indicated to induce metabolic alteration for more-efficient L-glutamate synthesis in cells lacking *odhA*. This implies that Tween 40 triggers a very drastic metabolic change, including inactivation of ODHC, toward L-glutamate synthesis. In addition, Tween 40 has been supposed to stimulate L-glutamate excretion (8). The drastic metabolic alteration toward efficient L-glutamate synthesis and the activation of L-glutamate excretion would together lead to L-glutamate overproduction.

The molecular basis of these L-glutamate-producing inductions and how they reduce ODHC activity are interesting questions that we are currently addressing. In the future, we aim to investigate whether there is a metabolic linkage between fatty acid synthesis and L-glutamate synthesis. We also need to understand the global metabolic changes that occur under L-glutamate-producing conditions.

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