

Oxygen- and Growth Rate-Dependent Regulation of *Escherichia coli* Fumarase (FumA, FumB, and FumC) Activity

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Received 27 March 2000/Accepted 16 October 2000

Escherichia coli contains three biochemically distinct fumarases which catalyze the interconversion of fumarate to L-malate in the tricarboxylic acid cycle. Batch culture studies indicated that fumarase activities varied according to carbon substrate and cell doubling time. Growth rate control of fumarase activities in the wild type and mutants was demonstrated in continuous culture; FumA and FumC activities were induced four- to fivefold when the cell growth rate (k) was lowered from 1.2/h to 0.24/h at 1 and 21% O₂, respectively. There was a twofold induction of FumA and FumC activities when acetate was utilized instead of glucose as the sole carbon source. However, these fumarase activities were still shown to be under growth rate control. Thus, the activity of the fumarases is regulated by the cell growth rate and carbon source utilization independently. Further examination of FumA and FumC activities in a *cya* mutant suggested that growth rate control of FumA and FumC activities is cyclic AMP dependent. Although the total fumarase activity increased under aerobic conditions, the individual fumarase activities varied under different oxygen levels. While FumB activity was maximal during anaerobic growth ($k = 0.6/h$), FumA was the major enzyme under anaerobic cell growth, and the maximum activity was achieved when oxygen was elevated to 1 to 2%. Further increase in the oxygen level caused inactivation of FumA and FumB activities by the high oxidized state, but FumC activity increased simultaneously when the oxygen level was higher than 4%. The same regulation of the activities of fumarases in response to different oxygen levels was also found in mutants. Therefore, synthesis of the three fumarase enzymes is controlled in a hierarchical fashion depending on the environmental oxygen that the cell encounters.

Fumarase, or fumarate hydratase (EC 4.2.1.2), is a component of the citric acid cycle. In facultative anaerobes such as *Escherichia coli*, fumarase also engages in the reductive pathway from oxaloacetate to succinate during anaerobic growth. Three fumarases, FumA, FumB, and FumC, have been reported in *E. coli* (7, 8, 17). Comparison of the *fumA* (FumA), *fumB* (FumB), and *fumC* (FumC) gene sequences and the biochemical characteristics of each gene product reveals that they comprise two biochemically distinct types of fumarase (34). The *fumA* and *fumB* genes are homologous and encode products of identical sizes which form thermolabile dimers of M_r 120,000. FumA and FumB are class I enzymes and are members of the iron-dependent hydrolases, which include acnitate and malate hydratase (17, 25, 34, 36). The active FumA contains a 4Fe-4S center, and it can be inactivated upon oxidation to give a 3Fe-4S center. However, FumA activity can be restored by anaerobic incubation with iron and thiol (4, 6, 36). FumC is a class II enzyme which does not require iron for its activity, and it forms thermostable tetramers containing identical subunits of M_r 50,000 (34). The *fumC* gene does not show any homology to either the *fumA* or *fumB* genes, but it exhibits extensive homology to the fumarase sequences of *Bacillus subtilis*, *Saccharomyces cerevisiae*, and mammals (13, 14, 18, 24, 26, 35). It also shows similarity with the aspartate gene, *aspA*, of *E.*

coli (27, 33). These genes appear to belong to a family that encode structurally related enzymes.

The physiological function of each *E. coli* fumarase has been studied by using a triple mutant transformed with a plasmid containing one of the three *fum* genes (32). The FumA enzyme appeared to be a component of the tricarboxylic acid (TCA) cycle since it was synthesized predominantly under aerobic conditions (34). A decrease in FumA synthesis and catabolite control of the *fumA* promoter were observed when cells were grown in the presence of glucose (20, 32). Crp binding sites have been proposed within the *fumA* promoter region (32). The FumB enzyme was shown to be more abundant under anaerobic conditions, and expression of the *fumB* gene required Fnr as a transcriptional activator (32). However, less is known about the physiological significance of FumC, whose substrate affinity resembles that of FumA. It has been reported that synthesis of FumC increased with the addition of oxidizing agents, and this increase was assumed to be dependent upon the *soxRS* gene products (6, 15). The *soxRS* genes encode positive regulators for controlling synthesis of proteins in response to oxidative stress (5, 28). Recently, it has been demonstrated that both superoxide control and iron starvation control of *fumC* gene expression required the SoxR regulatory protein (20). FumC was thus proposed to substitute for FumA when environmental iron is limiting or when superoxide radicals accumulate.

In this study, we examined the three fumarase activities of *E. coli* in batch and continuous cultures at different oxygen levels, cell growth rates, and with various carbon substrates in the medium. The results of these studies demonstrate that the

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activity of the fumarases is regulated independently by the rate of cell growth and carbon source availability. The activity of FumA, FumB, and FumC also shows the hierarchical control which depends on the oxygen availability that the cell encounters. This study further elucidates the different roles of the three fumarases in *E. coli*.

MATERIALS AND METHODS

Bacterial strains and materials. The *E. coli* strains were kindly provided by the *E. coli* Genetic Stock Center at Yale University. W3110 is a *fumA*⁺*B*⁺*C*⁺ parental strain; EJ1535 is a *fumA* *fumC* mutant (34); DJ901 is a *soxRS* deletion strain (15); and CA8306 is a *cya* deletion strain (2). The *cya* mutant strains were constructed by introducing the *cya* mutation into W3110 by P1 transductions and then selecting for the appropriate phenotype (2).

To construct the *fumA* deletion strain, the 1.1-kbp *SacI*-*KpnI* fragment from upstream of the *fumA* gene was inserted into pMAK705. The 1.2-kbp *KpnI*-*SphI* fragment containing the sequence downstream of *fumA* was inserted into vector pUC19 which contained the 1.2-kbp fragment of the kanamycin resistance gene. This 2.4-kbp *KpnI*-*SphI* fragment was then inserted into pMAK705 to form plasmid pMA1KA2. To construct the *fumB* deletion strain, the 1.3-kbp *SacI*-*KpnI* fragment of the *rep* gene, which was upstream of the *fumB* gene, was inserted into pMAK705. The 1.6 kbp of *fumB* downstream gene was inserted into the pCRII-TOPO vector containing the kanamycin resistance gene to form the 2.8-kbp *KpnI*-*SphI* fragment and then inserted into pMAK705 to form plasmid pMB1KB2. To construct the *fumC* mutant, the 0.6-kbp *XbaI*-*HpaI* fragment of the sequence upstream of *fumC* was inserted into the *XbaI* and *SmaI* sites of pBluescript II KS(-) vector to form pBKS-C1. The 0.8-kbp *KpnI*-*HpaI* fragment of the sequence downstream of *fumC* was inserted into the *EcoRV* and *KpnI* sites of pBKS-C1 to form pBKS-C1C2. The 1.2-kbp *PstI* fragment of the kanamycin resistance gene was inserted into pBKS-C1C2 to form pBKS-C1KC2. The 2.7-kbp *SacI*-*SphI* fragment containing the *fumC* and kanamycin resistance genes was then inserted into pMAK705 to form pMC1KC2. To obtain the *fumA*, *fumB*, and *fumC* mutants, plasmids pMA1KA2, pMB1KB2, and pMC1KC2 were integrated into the chromosome as described by Hamilton et al. (8). Single colonies grown on L plates with kanamycin but not on plates with chloramphenicol were checked by PCR and Southern blotting of chromosomal DNA to confirm the mutations.

Cell growth. For continuous culture experiments, a New Brunswick BiofloIII fermentor (New Brunswick Scientific Co., Inc.) was fitted with a 1.5-liter vessel and operated at a 1-liter liquid working volume as previously described (30). A modified Vogel-Bonner medium (pH 6.5) supplemented with Casamino Acids (0.25 mg/liter) and glucose (or acetate) (2.25 mM) was used to limit cell growth (carbon-limited medium). Aerobic continuous culture conditions were maintained by saturating the culture medium with sterile air at a rate of 300 ml/min. Anaerobic conditions were maintained by continuously sparging the vessel with oxygen-free nitrogen at a rate of 300 ml/min. To vary the cell growth rate, the medium addition rate was adjusted accordingly. The medium addition rates ranged from 4 to 20 ml/min ($k = 0.24$ to 1.2/h), which corresponded to cell generation times (g) of 173 and 35 min, respectively. To vary the degree of oxygen in the medium, the vessel was sparged with a stream of premixed gas in which the proportion of compressed air (21% O₂) and compressed nitrogen (99.8%) was combined using a mixing manifold containing individually precalibrated flow meters for each gas (31). The percent oxygen in the medium was monitored with an Ingold oxygen probe (mode 1046), which was calibrated with 100% air (21% O₂) and 99.8% nitrogen (0% O₂) prior to inoculation of the vessel in each experiment. When cells were shifted to a new growth rate or oxygen level, steady state was generally achieved in six reactor residence times. This was confirmed by monitoring cell density at 600 nm and assaying the fumarase activity of harvested cells as an indicator that cells had reached equilibrium. The chemostat was maintained under the same conditions until the fumarase activity values varied no more than 5%. Casamino Acids was purchased from Difco Co., Detroit, Mich. All other chemicals used were of reagent grade.

Enzyme assay. In batch culture, cells were grown aerobically with shaking in 20-ml culture volumes in 150-ml flasks which contained the different carbon substrates at 200 rpm. Flasks were inoculated with overnight cultures grown under the same conditions, and the cells were allowed to double four or five times under mid-log exponential phase prior to harvesting for the fumarase activity assay (11). In continuous culture, the fumarase activities obtained for each condition were independently determined at least twice, and there was less than 10% variation in activity. For cell sampling, 10-ml aliquots of culture were collected and placed on ice. After harvesting the cells by centrifugation, ultra-

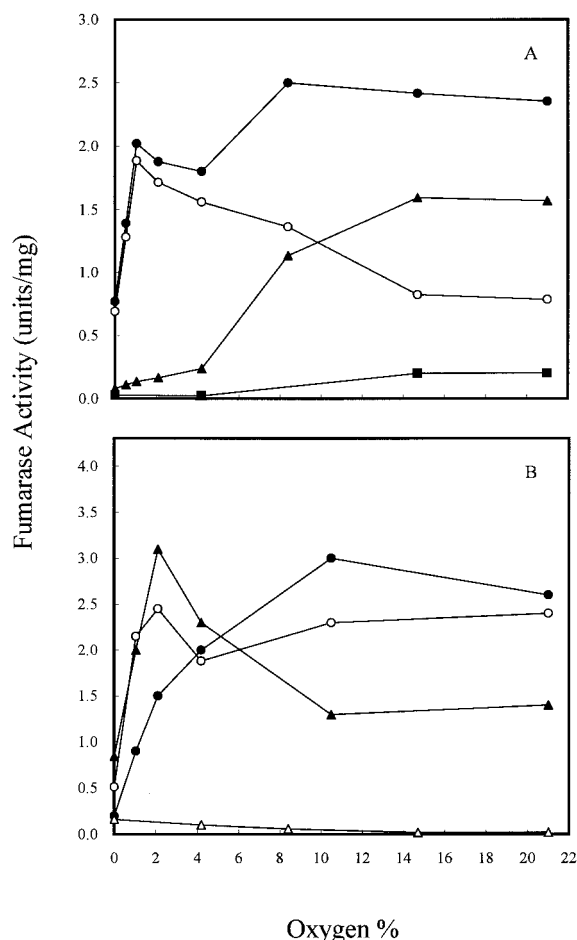


FIG. 1. Effect of oxygen on fumarase activity in continuous culture. Cells were grown in glucose (2.25 mM) minimum medium, and k was 0.6/h. (A) Total fumarase activity in W3110 (●); FumA+B activity in W3110 (○); FumC activity in W3110 (▲); FumC activity in DJ901 (■). (B) Total fumarase activity in EJ1535 (FumA⁻ FumC⁻) (△); total fumarase activity (FumB+C) in the *fumA* mutant (●); total fumarase activity (FumA+C) in the *fumB* mutant (○); total fumarase activity (FumA+B) in the *fumC* mutant (▲). Units of fumarase activity are expressed as micromoles of fumarate formed per minute at 25°C and pH 7.3.

sonicated extracts were prepared, and the supernatants were obtained after centrifuging at 15,000 × g for 20 min. Fumarase assays were performed at 25°C as described (11, 34). The protein concentration of cell extracts was determined by the method of Bradford (1) with Bio-Rad dye reagent using bovine serum albumin (Sigma) as a standard. One unit is defined as 1 μ mol of fumarate formed per min at 25°C and pH 7.3. Since FumA and FumB are thermostable enzymes, FumC activity was obtained by incubation of total enzymes at 18°C for 16 h; this treatment inactivates the fractions attributed to FumA and FumB, whereas the FumC activity is unaffected (15, 16, 34).

RESULTS

Effect of oxygen on FumA, FumB, and FumC activity. To determine how the three fumarase activities vary in the cell during growth at different oxygen levels, strains W3110, EJ1535, and DJ901 were grown in continuous culture at the same cell growth rate ($k = 0.6$ /h) but the oxygen level was varied from 0 to 21% (Fig. 1). Strain W3110 is a parent strain which contains three fumarase genes. Strain EJ1535 was

known to bear a mutation in the *fumA* gene that also abolishes expression of the *fumC* gene (34). It has been suggested that all fumarase activity in EJ1535 is due to FumB (16). In continuous culture, we found that the pattern of the three fumarase activities in strain W3110 varied significantly at different oxygen levels (Fig. 1A). Over the range from 0% (anaerobic) to 21% O₂, the total fumarase activity was at a low basal level under anaerobic conditions and induced threefold at 21% O₂. As the oxygen level was increased, FumA and FumB (FumA+B) activity increased until it achieved a maximum at 1% O₂ (ca. 2.5-fold induction over the anaerobic level). However, when the oxygen level was increased to 15% O₂, the activity was lowered to a level similar to the anaerobic level. Further increasing the oxygen to 21% did not lower FumA+B activity. The decrease in FumA+B activity may be due to oxidation by O₂ under high oxygen levels (4). FumC activity was determined after inactivation of FumA+B activity at 18°C for 16 h as described by Woods et al. (34) and Liohevand and Fridovich (15, 16). The pattern of FumC activity at different oxygen levels differed from that observed for FumA+B. FumC activity remained at the lowest level when the oxygen level was below 4%, but it increased as the oxygen level was raised and achieved maximum activity when the oxygen level was 15%. Further increasing the oxygen to 21% did not change either FumA+B or FumC activities. FumC activity was also determined in a *soxRS* deletion strain, which showed that activity was maintained at a low level over the range of oxygen levels examined (Fig. 1A). The effect of oxygen tension on the activities of the three fumarases was also determined in *fumA*, *fumB*, and *fumC* deletion strains. Although the fumarase activities increased 30% in the *fumA* and *fumC* mutants, a reciprocal role for FumA and FumC was still seen in two mutants over the range of oxygen levels examined (Fig. 1B). In contrast to the pattern of varied fumarase activity in strain W3110, the maximum level of FumB activity in strain EJ1535, in which FumA and FumC were inactivated (16), was observed under completely anaerobic conditions (Fig. 1B). As the level of oxygen in the premixed gas stream was elevated above 4%, the FumB activity of strain EJ1535 was reduced by half. The basal level was about 10% of the maximum level of total fumarase activity seen under completely aerobic conditions. Even though FumB activity in strain EJ1535 was the highest under anaerobic conditions, it was relatively low compared to the FumA+B activity of strain W3110 and the *fumC* mutant under anaerobic growth conditions. A comparison of the fumarase activities of strains W3110, EJ1535, and the *fumC* mutant suggests that FumA is the major enzyme synthesized under anaerobic and microaerophilic conditions (ca. 0 to 4% O₂), whereas FumC becomes more important under highly oxidized growth conditions.

Effect of different carbon substrates on fumarase activity. In aerobic batch cultures, FumA+B activities varied over a 50-fold range in strain W3110, but FumC activity did not vary more than 8-fold in all media tested (Fig. 2A). These observations are consistent with the *fumA* and *fumC* gene expression levels found during growth with different carbon substrates (20). The fumarase activity of strain EJ1535 was relatively low compared to the activity in strain W3110 (less than 5% in batch minimum medium with glucose as the carbon source) (Table 1). Therefore, the activity of FumA in W3110 is the major

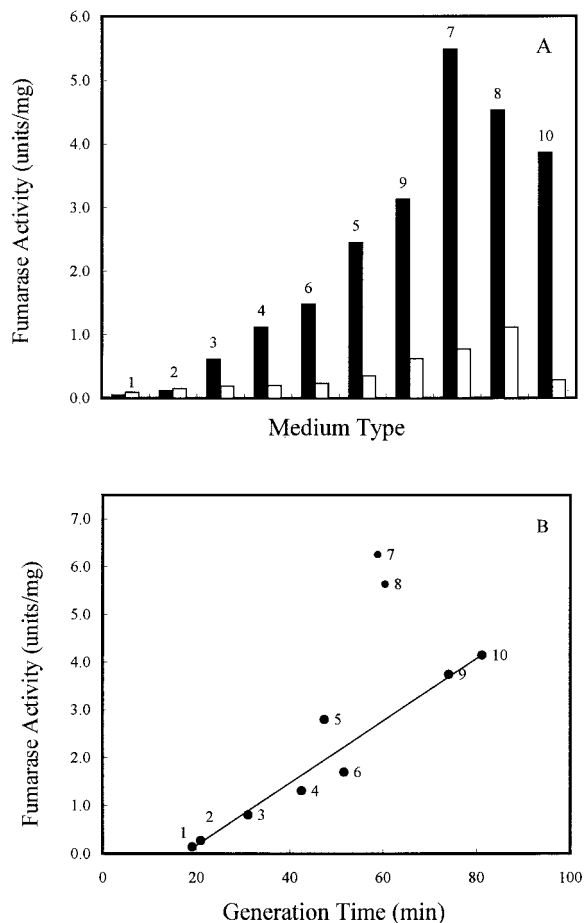


FIG. 2. Effect of cell growth rate and carbon substrate on total fumarase activity in batch culture. (A) W3110 cells were grown in minimal medium with the indicated carbon compounds (40 mM) or in buffered LB. Solid bars, FumA+B activities; open bars, FumC activities. (B) W3110 cells were grown in the indicated medium, and the cell generation time as well as the total fumarase activities were recorded. Medium and carbon compound (40 mM) used for cell growth: 1, LB plus glucose; 2, LB; 3, glucose plus Casamino Acids; 4, glucose; 5, glycerol; 6, xylose; 7, fumarate; 8, succinate; 9, galactose; 10, acetate. Units of fumarase activity are expressed as micromoles of fumarate formed per minute at 25°C and pH 7.3.

component of the FumA+B activity. Interestingly, when cells were grown in batch culture in different media, the total fumarase activity was higher in minimal medium containing two-, three-, or four-carbon compounds than in rich medium (Fig. 2B). The cell growth rates were determined for each type of medium, and when the generation times were graphed versus the level of the total fumarase activities, cell growth rate was inversely proportional to the total fumarase activity but generation time was directly proportional (Fig. 2B). Therefore, fumarase activity is proposed to be growth rate controlled.

Effect of cell growth rate on fumarase activities. To determine if the variation in fumarase activity with different of carbon substrates was caused directly by the type of carbon compound used or indirectly by the change in cell growth rate, we examined the fumarase activity of cells grown in continuous culture (Fig. 3), where cell growth rate could be controlled by the specific medium flow rate when carbon is limited (i.e., by

TABLE 1. Total fumarase activity of strain EJ1535 in different media in batch culture

Medium ^a	Fumarase activity ^b (U/mg)	Generation time ^c (min)
LB + glucose	0.02	21
LB + pyruvate	0.02	23
LB	0.03	23
Glucose + Casamino Acids	0.03	40
Glucose	0.03	47
Xylose	0.03	71
Glycerol	0.03	84
Acetate	—	NG
Succinate	—	NG
Fumarate	—	NG

^a Cells were grown aerobically in a minimal basal medium (pH 7.0) with the indicated carbon compounds added (40 mM).

^b Units are expressed as micromoles of fumarate formed per minute at 25°C and pH 7.3.

^c NG, no growth.

glucose or acetate). For cultures grown at 1% O₂, which gave the maximum activity of FumA+B, both FumA+B and FumC activities were elevated by fourfold when the cell growth rate was shifted from 1.2/h to 0.24/h (Fig. 3A). In contrast, the growth rate control of FumB in strain EJ1535 remained constant: it did not vary by more than twofold at the different growth rates examined. Therefore, growth rate control of FumA+B activity in strain W3110 essentially reflects the growth rate regulation of FumA, since FumB activity was constant in strain EJ1535 under different growth rates. In addition, when strain W3110 was grown in acetate medium, the total fumarase and FumA+B activities still exhibited a fourfold growth rate control. The FumA+B activity was about twofold higher when cells were grown with acetate than with glucose at the same growth rates (Fig. 3B). This finding demonstrates that the growth rate control of FumA and FumC activities is independent of carbon substrate control. When cells were grown under higher agitation (21% O₂), the total fumarase and FumC activities were still growth rate dependent. FumC activity was elevated about fourfold when the cell growth rate was decreased from 1.2/h to 0.24/h. However, FumA+B activity was growth rate independent, and its activity remained constant at the lowest level over the range of growth rates examined (Fig. 3C). The shift of FumA activity from growth rate dependent to independent at high oxygen levels may be caused by inactivation of FumA activity by the high oxidative state (4, 36). Growth rate-dependent regulation of fumarase activities was further demonstrated in the *fumA*, *fumB*, and *fumC* mutants. For cultures grown at 1% O₂, the total fumarase activity of the *fumA*, *fumB*, and *fumC* mutants was elevated by two-, four-, and eightfold, respectively, when the cell growth rate was shifted from 1.2/h to 0.24/h (Fig. 4A). When cells were grown under higher agitation conditions (21% O₂), the total fumarase activity in each of the three mutants remained growth rate dependent. Fumarase activities in the *fumA* and *fumB* mutants were each elevated about fourfold when the cell growth rate was decreased from 1.2/h to 0.24/h. However, the fumarase activity of the *fumC* mutant was only increased 50% over the range of growth rates examined (Fig. 4B). As the fumarase activities were repressed by glucose in batch and continuous cultures (Fig. 2A, 3A, and 3B), we tested whether the *cya* gene

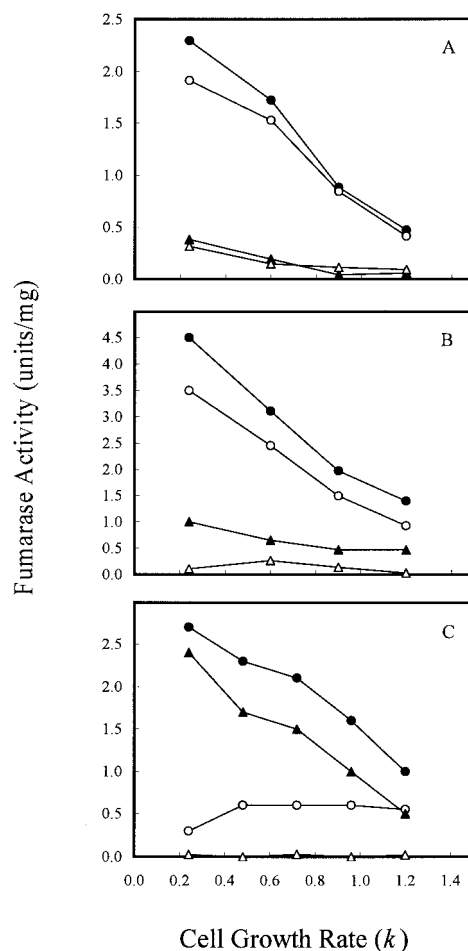


FIG. 3. Effect of cell growth rate on fumarase activity in continuous culture. (A) Cells were grown in glucose (2.25 mM) minimal medium at the indicated growth rates. The oxygen level was 1%. (B) Cells were grown in acetate (2.25 mM) minimal medium, and the oxygen level was 1%. (C) Cells were grown in glucose (2.25 mM) minimal medium, and the oxygen level was 21%. Total fumarase activity in W3110 (●), FumA+B activity in W3110 (○), FumC activity in W3110 (▲), and total fumarase activity in EJ1535 (FumA⁻ FumC⁻) (△) were determined.

product contributes to this control. The results showed that fumarase activity decreased and became growth rate independent at various growth rates (Fig. 5). Therefore, the growth rate control of FumA and FumC activities was proposed to be cyclic AMP (cAMP) dependent.

DISCUSSION

The existence of three genetically and biochemically distinct fumarases in *E. coli* poses questions concerning their physiological functions. Their differing affinities for fumarate and malate suggest that they function in reciprocal roles either in the overall oxidation of fumarate via the citric acid cycle (by FumA) or in the ultimate reduction of malate by providing fumarate as an anaerobic electron acceptor (by FumB) (32, 34). Also, it was previously shown that the *fumA* and *fumC* genes were expressed at higher levels under aerobic than anaerobic conditions, whereas *fumB* was expressed under anaerobic

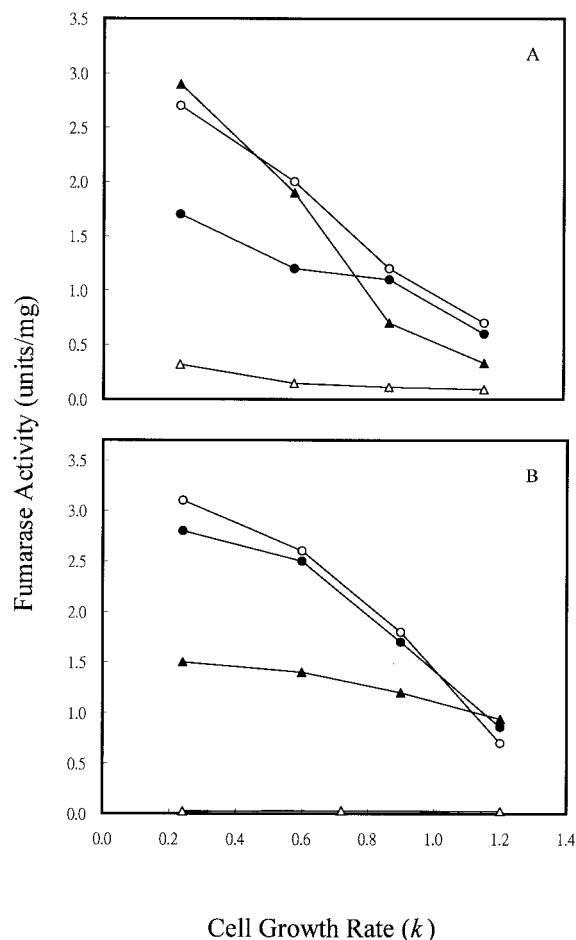


FIG. 4. Effect of cell growth rate on fumarase activity in *fumA*, *fumB*, and *fumC* mutants. (A) Cells were grown in glucose (2.25 mM) minimal medium at the indicated growth rates. The oxygen level was 1%. (B) Cells were grown in glucose (2.25 mM) minimal medium, and the oxygen level was 21%. Total fumarase activity (FumB+C) in the *fumA* mutant (●), total fumarase activity (FumA+C) in the *fumB* mutant (○), total fumarase activity (FumA+B) in the *fumC* mutant (▲), and total fumarase activity in EJ1535 (FumA⁻ FumC⁻) (Δ) were determined.

obic condition (20, 29, 32). In this study, we performed a more detailed analysis of three fumarase activities under different oxygen levels. The results indicate that FumA provides the major anaerobic fumarase activity (more than 70% of the total fumarase activity under anaerobic conditions), while the activity of FumB was also fully elevated under anaerobic conditions (Fig. 1). These physiological functions coincide with the results of studies using various *fum-lacZ* reporter fusions in which the level of *fumA* gene expression was seven- to eightfold higher than that of *fumB* even under anaerobic condition (20, 29). As the three fumarase activities in *E. coli* are regulated by oxygen differently, the cell utilizes a complex strategy for adaptation to diverse environmental oxygen levels. First, FumA activity was about fivefold higher than FumB activity under anaerobic growth conditions, while FumB activity was highest during anaerobiosis. FumA+B activities were elevated to the maximal level under microaerophilic conditions (1 to 2% O₂) and then decreased back to anaerobic levels under aerobic conditions

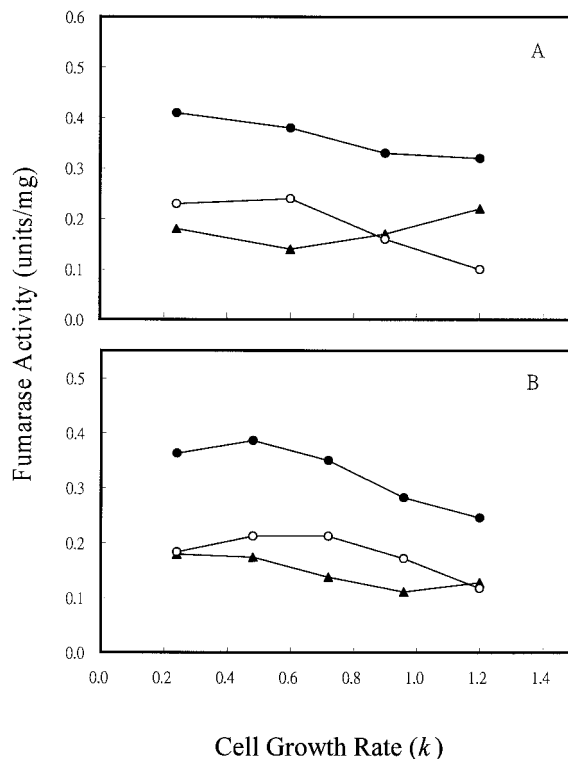


FIG. 5. Effect of cell growth rate on fumarase activity in a *cya* mutant strain. (A) Cells were grown in glucose (2.25 mM) minimal medium at the indicated growth rates. The oxygen level was 1%. (B) Cells were grown at 21% O₂. Total fumarase activity (●), FumA+B activity (○), and FumC activity (▲) in the *cya* mutant strain were determined.

(>15% O₂) (Fig. 1). These results suggest that FumA is the major fumarase enzyme under microaerophilic conditions (1 to 2% O₂) and is constitutively synthesized under fermentation and aerobic growth conditions. FumB is an alternative enzyme during anaerobiosis, since it has a higher affinity for L-malate than for fumarate (34). Second, the observation that FumC activity remains quite low during anaerobic growth supports the model that the product of the *fumC* gene is not needed by the cell under these conditions because the cell can synthesize FumA and FumB to metabolize malate to fumarate (34). However, during aerobic growth (21% O₂), FumC activity was elevated by 20-fold compared to the level under anaerobic conditions (Fig. 1). This elevated level of FumC activity reflects a strategy of the cell to produce an active fumarase, FumC, instead of iron-dependent FumA, which is inactivated under highly oxidative conditions (>4% O₂) (4, 36). Higher fumarase activities in the *fumA* and *fumC* mutants than in the wild-type strain may be due to the products of the *fumA* and *fumC* genes involved in feedback regulation of the promoter of the *fumA* gene, which drives both *fumA* and *fumC* mRNA expression (20). Thus, the results of these studies imply that the three fumarase enzymes are controlled in a hierarchical manner depending on the oxidative conditions that the cell encounters. Therefore, *E. coli* appears to utilize highly responsive regulatory elements to adjust the levels of the fumarase enzymes during different cell growth conditions for energy generation.

Expression of the *fumA* and *fumC* genes has been previously found to be affected by the type of carbon used for cell growth (20). The same pattern of variation is also seen at the biochemical level for fumarase activity (Fig. 2A). Further examination showed that fumarase activity is controlled independently by cell growth rate and carbon substrate availability (Fig. 3 and 4). Prior studies reported that the β -galactosidase activity of *fumA-lacZ* varies over 20-fold in different media (20), which is similar to the variation of FumA activities in batch culture (Fig. 2A). This result suggests that the growth rate-dependent regulation of FumA activity occurred at the transcriptional level. However, in contrast to a more than 10-fold variation in *fumAC-lacZ* expression, the β -galactosidase activity of *fumC-lacZ* remained relatively constant in different media (20). These results suggest that the growth rate regulation of FumC activity is effected via readthrough from the transcription of the upstream *fumA* promoter instead of transcription from the internal *fumC* promoter.

Growth rate regulation of succinate dehydrogenase, 2-ketoglutarate dehydrogenase, and NADH dehydrogenase activities has been reported in *E. coli* (12). Growth rate control of several TCA cycle genes has also recently been reported (21, 22, 30), but the regulation is still unclear. All of them show the same regulatory pattern as the FumA and FumC activities in continuous cultures. It is well known that during rapid aerobic growth, *E. coli* produces acetate as a by-product. The metabolism of *E. coli* switches from respiration to fermentation when cells grow at a high specific growth rate with a low glucose concentration even under aerobic conditions (3, 10). It has been suggested that an increase in cell growth rate presumably increases glucose uptake rate and intracellular glucose concentration (23). Recently, the intracellular cAMP concentration has been found to decrease as the dilution rate increases in glucose-limited chemostat (19). Expression of the *fumA* gene is catabolite controlled (20); in this study, the substrate glucose also caused a twofold suppression of the fumarase activities in batch (Fig. 2A) and continuous (Fig. 3) cultures. In addition, the fumarase activities decreased sixfold at a low growth rate ($k = 0.24/\text{h}$) and did not appreciably alter in a *cya* mutant at all cell growth rates examined (Fig. 5). Since Crp binding sites have been proposed to reside within the *fumA* promoter region (32), it is suggested that growth rate-dependent regulation of fumarase activity and catabolite inhibition at high growth rates are controlled by changing intracellular cAMP levels. In addition, the growth rate control of the fumarase activities parallels the previous report that *E. coli* decreases the respiratory quotient at high growth rates (12). Thus, the decrease in fumarase activity at a high cell growth rate could also account for the decrease in the respiratory quotient values.

ACKNOWLEDGMENTS

This work was supported by grants NSC86-2321-B-009-001 and NSC87-2321-B-009-001 from the National Science Council of the Republic of China.

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