Metabolic flux analysis of *Escherichia coli* in glucose-limited continuous culture. II. Dynamic response to famine and feast, activation of the methylglyoxal pathway and oscillatory behaviour

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The metabolic dynamics of the *Escherichia coli* K-12 strain TG1 to feast and famine were studied in glucose-limited steady-state cultures by up- and downshifts of the dilution rate, respectively. An uncoupling of anabolic and catabolic rates was observed upon dilution rate upshifts, apparent through immediately increased glucose uptake rates which were not accompanied by an immediate increase of the growth rate but instead resulted in the temporary excretion of methylglyoxal, D- and L-lactate, pyruvate and, after a delay, acetate. The energetic state of the cell during the transient was followed by measuring the adenylate energy charge, which increased within 2 min after the upshift and declined thereafter until a new steady-state level was reached. In the downshift experiment, the adenylate energy charge behaved inversely; no by-products were formed, indicating a tight coupling of anabolism and catabolism. Both dilution rate shifts were accompanied by an instantaneous increase of cAMP, presaging the subsequent changes in metabolic pathway utilization. Intracellular key metabolites of the Embden–Meyerhof–Parnas (EMP) pathway were measured to evaluate the metabolic perturbation during the upshift. Fructose 1,6-diphosphate (FDP) and dihydroxyacetone phosphate (DHAP) increased rapidly after the upshift, while glyceraldehyde 3-phosphate decreased. It is concluded that this imbalance at the branch-point of FDP induces the methylglyoxal (MG) pathway, a low-energy-yielding bypass of the lower EMP pathway, through the increasing level of DHAP. MG pathway activation after the upshift was simulated by restricting anabolic rates using a stoichiometry-based metabolic model. The metabolic model predicted that low-energy-yielding catabolic pathways are utilized preferentially in the transient after the upshift. Upon severe dilution rate upshifts, an oscillatory behaviour occurred, apparent through long-term oscillations of respiratory activity, which started when the cytotoxic compound MG reached a threshold concentration of 1·5 mg l\(^{-1}\) in the medium.

INTRODUCTION

The flexible nature of bacterial metabolism is reflected by the redundancy of single enzymes and entire pathways which are active under specific conditions, resulting in a metabolic flux distribution that allows the cell to couple energy generation and catabolic carbon utilization with the anabolic requirements for optimal growth. When cells are exposed to unbalanced conditions, such as sudden changes in nutrient supply, the catabolic and anabolic rates can become uncoupled, forcing the cell to respond by rearranging the metabolic flux distribution (Russell & Cook, 1995).

One important group of regulatory compounds involved in the coupling of catabolic and anabolic reactions are the adenosine nucleotides AMP, ADP and ATP. The level of these nucleotides reflects the energetic status of the cell. High ATP levels in conjunction with low AMP levels will inhibit enzymes of the ATP-replenishing pathways and vice versa (Atkinson, 1968). cAMP, in conjunction with the cAMP receptor protein, is another adenosine-nucleotide-derived molecule that plays a key regulatory role. Synthesis of cAMP is catalysed by adenylate cyclase and, after the first discovery of cAMP in *Escherichia coli* (Makman &
Sutherland, 1965), numerous studies have been carried out to unravel its not yet fully understood function in the regulation of gene expression and anaobic and catabolic pathway utilization [for a review, see Saier et al. (1996) and references cited therein, for example]. Most notably, cAMP formed after depletion of a preferred carbon substrate such as glucose plays a key role in restructuring carbon catabolism to allow the utilization of less-preferred carbon substrates. Under conditions of glucose excess, adenylate cyclase activity is repressed but expression of the gene encoding adenylate cyclase, cyA, is maximal, thereby increasing the potential for cAMP formation once glucose is depleted.

One pathway that is often neglected in the evaluation of unbalanced conditions is the methylglyoxal (MG) pathway, which exists in many organisms including E. coli (Tempest & Neijssel, 1992; Inoue & Kimura, 1995; Ferguson et al., 1998; Kalaposs, 1999). In Klebsiella aerogenes, it was shown that MG formation is activated when a slow-growing culture is pulsed with glucose (Teixeira de Mattos et al., 1984). In E. coli, activation of the MG pathway has been confirmed when the intracellular uptake of glucose 6-phosphate or other carbon substrates such as xylose, lactose, arabinose, glycerol or gluconate are deregulated by mutation and/or by addition of cAMP (Ackerman et al., 1974; Puskas et al., 1983; Kadner et al., 1992). MG is produced from dihydroxyacetone phosphate (DHAP) and the MG pathway represents an energetically unfavourable bypass to the glycolytic reactions of the lower Embden–Meyerhof–Parnas (EMP) pathway (Cooper & Anderson, 1970). Synthesis of MG is induced by high concentrations of DHAP and inhibited by high phosphate concentrations (Hopper & Cooper, 1971). It has been suggested that utilization of the MG pathway relieves the cells from stress caused by elevated levels of sugar phosphates (Cooper & Anderson, 1970; Kadner et al., 1992; Ferguson et al., 1998; Tötemeyer et al., 1998). However, MG is a very toxic compound that arrests growth of E. coli at millimolar concentrations (Egyud & Szent-Györgyi, 1966). It can react with the nucleophilic centres of macromolecules such as DNA, RNA and proteins (Lo et al., 1994, Papoulis et al., 1995) and it has been proposed that MG inhibits growth by interfering with protein synthesis and consequently preventing initiation of DNA replication (Fraval & McBrien, 1980). Due to the cytotoxicity of MG, its production is tightly controlled. Even a 900-fold overexpression of MG synthase in E. coli caused the accumulation of only very low levels of MG (Tötemeyer et al., 1998).

In this study, the metabolic dynamics of E. coli in response to feast and famine were studied in glucose-limited continuous culture described in the accompanying article (Kayser et al., 2005). The dynamic experiments were started by a step change of the dilution rate after steady-state conditions were established (at least eight residence times prior to the dynamic experiment). Details are specified in the figure legends.

Analytical techniques. For analysis of MG, 9–10 ml culture liquid was sprayed into vacuum-sealed precooled test tubes using a rapid sampling technique as described in the accompanying article (Kayser et al., 2005). The test tubes were filled prior to sampling with 1 ml of a precooled solution of 5 mol perchorlic acid 1−1 (0°C). Conversion of MG to 2-methylquinoxaline with o-phenylenediamine was performed according to Chaplen et al. (1996a, b). 2-Methylquinoxaline was analysed by HPLC using a C-18 column (Alltech) with 5-methylquinoxaline as internal standard. The column was eluted at 20°C with 68% v/v 10 mmol KH2PO4 1−1 and 32% v/v acetonitrile at a flow rate of 1 ml min−1.

For analysis of DHAP, GAP and FDP, 3–4 ml culture liquid was sprayed into precooled test tubes filled with 1 ml of 5 mol perchorlic acid 1−1 (−25°C). After one freeze–thaw cycle, the extracts were neutralized on ice with a solution containing 2 mol KOH and 0–4 mol imidazole 1−1 (pH 7.2 ± 0.1). The KClO4 precipitate was removed by centrifugation (14 000 r.p.m., 30 s) and filtration (pore size 0.45 μm). Quantification of DHAP, GAP and FDP was carried out according to Michal (1984). For conversion to intracellular concentrations, an intracellular volume of 2·15 ml corresponding to 1 g cell dry mass was assumed (Pramanik & Keasling, 1997).

Analytical techniques for the determination of glucose, cell dry mass, organic acids, adenosine nucleotides (AMP, ATP, AMP and cAMP) and outlet-gas composition were described in the accompanying article (Kayser et al., 2005). For analysis of pyruvic acid, test kits from Roche Diagnostics were employed. D- and L-lactic acid were also analysed separately by enzymatic test kits from Roche Diagnostics.

Calculation of rates. The glucose uptake and biomass formation rates were calculated from dynamic mass balances. For the j-th compound, it can be written as:

\[ r_j = \frac{dc_j}{dt} + \left( c_j - c_{j,0} \right) D \]  

where \( r_j \) is the reaction rate of \( j \), \( c_j \) is the concentration of \( j \) in the bioreactor, \( c_{j,0} \) is the concentration of \( j \) in the feeding solution and \( D \) is the dilution rate. All rates can be expressed in either mass or molar units. The two units can be easily converted by using the molecular mass of the corresponding compound, e.g. for glucose, 180 g mol−1. For the calculation of the biomass formation rate on a molar basis, a ’molecular mass of the biomass’ was defined as 100 g mol−1 (see Kayser et al., 2005).

Flux analysis. Metabolic flux analysis applying a linear programming technique was used to predict by-product formation during

METHODS

Micro-organism and cultivation conditions. The E. coli K-12 strain TG1 was grown in glucose-limited continuous culture as described in the accompanying article (Kayser et al., 2005). The dynamic experiments were started by a step change of the dilution rate after steady-state conditions were established (at least eight residence times prior to the dynamic experiment). Details are specified in the figure legends.

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Flux analysis. Metabolic flux analysis applying a linear programming technique was used to predict by-product formation during
carbon-overflow conditions and to simulate the activation of the MG pathway. The metabolic model and the methodology are described in the accompanying article (Kayser et al., 2005). To simulate pathway reorganization in response to carbon-overflow conditions, an upper limit for the biomass formation rate was given and, with known glucose and oxygen uptake and carbon dioxide formation rates, the metabolic model determined the excreted by-products as a function of the discrepancy between the catabolic and anabolic reactions.

RESULTS AND DISCUSSION

Dynamics of catabolic and anabolic carbon utilization during adaptation to dilution rate shifts

The dynamic response of E. coli TG1 to changes in the dilution rate was studied in glucose-limited continuous cultures. The transients were followed during the adaptation period from low to high dilution rate and vice versa (Fig. 1). During both transients, the most obvious event was the sudden increase in the respiratory activity in response to the dilution rate upshift or decrease in case of the downshift (Fig. 1a, b). This overshoot response within the first 10 min was followed by an opposite reaction; a gradual decrease or increase for a period of about 2 h in the up- or downshift experiment, respectively. Thereafter, the respiratory activity started to increase or decrease slowly again, respectively, until a new steady-state level was reached.

During the upshift experiment, the biomass concentration decreased as long as the growth rate $\mu$ was slower than the dilution rate $D$ and, after $\mu$ approached $D$, increased slowly to a value characteristic of the new steady state (Fig. 1c). During this adaptation phase, the glucose was not consumed completely by the cells and instead accumulated in the medium. However, the glucose uptake rate increased more rapidly to the new steady-state value than the biomass formation rate, demonstrating the uncoupling of anabolic and catabolic reactions during this period (Fig. 1c). In contrast, no uncoupling of anabolism and catabolism was observed in the downshift experiment (Fig. 1d). In this case, an instantaneous and immediate adaptation of the biomass formation rate as well as the glucose uptake rate to the new conditions occurred.

Changes in the specific concentrations of the adenosine nucleotides (AMP, ADP, ATP and cAMP) were also followed during the adaptation period after the up- and downshift of the dilution rate (Fig. 2). After the upshift, the ATP level increased instantaneously, reaching a peak within 2 min and subsequently decreased during the next hour until a new steady-state level was reached (Fig. 2a). The adenylate energy charge (AEC) closely followed the time profile of the ATP concentration, also peaking 2 min after the dilution rate upshift and then decreasing within the next hour until the new steady-state value of 77% was reached (Fig. 2c; see Kayser et al., 2005). The rapidly increasing AEC within the first 2 min after the dilution

**Fig. 1.** Glucose-limited continuous cultures of E. coli TG1. Dynamic response to an upshift (a, c) and downshift (b, d) of the dilution rate between 0.066 and 0.23 h$^{-1}$. Time-dependent changes of the oxygen (OTR; dashed lines) and carbon dioxide (CTR; solid lines) transfer rates (a, b) and biomass (■) and glucose (□) concentrations and specific rates of glucose uptake $r_s$ (solid lines) and biomass formation $r_x$ (dashed lines) (c, d) during the transient are shown.
rate upshift coincided with the immediate increase in the glucose uptake rate, which was not accompanied by a corresponding increase in the biomass formation rate (compare Fig. 1c). Thus, the production of ATP in the catabolic pathway exceeded the consumption of ATP for anabolic purposes. This is also confirmed by the immediate increase in the respiratory activity (Fig. 1a), which reflects the ATP formation rate, since most of the ATP is generated through oxidative phosphorylation in the respiratory chain. Subsequently, pathways generating less energy were activated and the ATP level and, correspondingly, the AEC decreased again.

During the downshift experiment, the time-course of the adenosine nucleotide concentrations and the AEC revealed inverse behaviour compared with the upshift experiment (Fig. 2b, d, respectively). Due to the sudden reduction of the glucose uptake rate, the ATP consumption in the anabolic pathway was transiently higher than ATP production during catabolic breakdown. Again, this is corroborated by the immediate decrease in respiratory activity (Fig. 1b). The resulting decline in the ATP concentration and, correspondingly, the rapid decline of the AEC within the first 2 min in turn caused the activation of the enzymes of ATP-replenishing pathways. As a result, the respiratory activity partly recovered prior to the subsequent decrease, thereby causing an increase in the ATP level and AEC until they reached their new steady-state values (Fig. 2b, d; cf. Kayser et al., 2005).

The cAMP level rose quickly after the dilution rate downshift (Fig. 2d), but also increased after the dilution rate upshift within the first 2 min from zero to more than 1 μmol (g cell dry mass)$^{-1}$, a third of the level that was reached during the downshift experiment (Fig. 2c). In both experimental set-ups, the cAMP level declined again after the change in the dilution rate and a steady-state level of zero was reached in all experiments. Thus, the immediate response of the cells to a sudden carbon-overflow situation resembles the typical response that cells exhibit when they need to reorganize metabolism while adapting to the utilization of a less-preferred carbon substrate. Apparently, cAMP is involved in restructuring carbon catabolism not only after nutrient downshift but also after nutrient upshift.

**Accumulation of extra- and intracellular metabolites in response to feast conditions**

During the dilution rate downshift experiment, extracellular by-product formation was not detectable, in agreement with the finding that there was no uncoupling of anabolic and catabolic reactions during the adaptation period to reduced carbon supply. During the upshift experiment, however, D- and L-lactate, acetate and, in small quantities, pyruvate and formate were excreted into the culture fluid (Fig. 3). Acetate accumulated to significantly higher concentrations than the other by-products; a maximum of 450 mg acetate l$^{-1}$ was reached during the transient in comparison to about 100 mg lactate l$^{-1}$ (sum of D- and L-lactate).
However, the excretion of acetate started considerably later than the excretion of the other organic acids. The most rapid by-product formation was observed for lactate and MG; just 14 min after the dilution rate upshift, more than 0.6 mg MG l\(^{-1}\) was detected in the culture fluid (Fig. 3). The maximum concentration of MG was 0.9 mg l\(^{-1}\); this is well below the threshold value of 20 mg l\(^{-1}\) that is reported to cause growth inhibition (Ferguson et al., 1996). The accumulation of MG indicates the activation of the MG pathway, which bypasses the EMP pathway at the level of DHAP (Fig. 4). DHAP is converted to pyruvate via MG and D- and L-lactate, suggesting that excretion of D- and L-lactate may originate from degradation of MG.

Formation of MG is carried out by MG synthase, which is regulated by allosteric activation by the substrate DHAP and inhibited by high concentrations of the product phosphate (Cooper, 1984). Accordingly, inhibition of GAP dehydrogenase by low phosphate concentrations would predispose the cell to accumulate DHAP (Cooper, 1984; Ferguson et al., 1998). The triosephosphates DHAP and GAP are formed in equimolar amounts through enzymic conversion of FDP by aldolase. Thus, the intracellular pools of DHAP and GAP as well as the FDP pool were analysed after the upshift of the dilution rate (Fig. 5). The intracellular concentration of FDP increased rapidly from 3.2 to 4.5 mmol l\(^{-1}\) after the dilution rate upshift and decreased within the next hour to a new steady-state value similar to that before the shift. The intracellular concentration of DHAP also increased within 2 min after the upshift from a steady state value of 1.7 mmol l\(^{-1}\) to more than

![Fig. 3. By-product excretion by E. coli TG1 in response to an upshift of the dilution rate from 0.066 to 0.23 h\(^{-1}\). Time-courses of the extracellular concentrations of acetate (○), D-lactate (■) and L-lactate (□) (a) and formate (△), pyruvate (□) and MG (●) (b) are shown.](http://mic.sgmjournals.org)

![Fig. 4. The MG pathway in E. coli embedded in the central network of glucose catabolism. The network is shown in a simplified manner; potentially reversible reactions, not necessarily catalysed by the same enzyme, are indicated by double-headed arrows (see the detailed stoichiometric model given in the appendix to Kayser et al., 2005). ATP-generating steps through substrate-level phosphorylation are indicated and NADH, FADH and NADPH are considered as reducing equivalents ‘H\(_2\)’ in this network. P\(_S\), Phosphate.](http://mic.sgmjournals.org)
3 mmol l\(^{-1}\) and subsequently decreased within the next hour to a new steady-state value similar to that before the shift. The intracellular GAP concentration revealed a completely different time-course; it decreased immediately from a steady-state preshift value of 2-1 mmol l\(^{-1}\) to a new steady-state value of 1-2 mmol l\(^{-1}\). These data show that the excess glucose created limitations in the upper part of the EMP pathway which caused the accumulation of FDP and DHAP and, in turn, caused the activation of MG synthase, the key enzyme of the MG pathway.

Little information exists on the intracellular concentrations of FDP, DHAP and GAP in *E. coli* in relation to environmental conditions. However, there are reports that cells accumulate FDP and DHAP while phosphoenolpyruvate (PEP) levels go down when glucose is added to a culture growing on a poor substrate such as acetate (Lowry *et al*., 1971). More recent studies on the cellular response to a glucose pulse in glucose-limited continuous culture have also been reported by other groups (Buchholz *et al*., 1999). Increasing concentrations of sugar phosphates and decreasing concentrations of PEP were observed during the first seconds after the glucose pulse, intracellular concentrations of FDP and DHAP increase and those of GAP and PEP decrease (Schaefer *et al*., 1999). Increasing concentrations of sugar phosphates and decreasing concentrations of PEP during the first seconds after the addition of a glucose pulse to a continuous glucose-limited culture have also been reported by other groups (Buchholz *et al*., 2002; Chassagnole *et al*., 2002).

A decrease in GAP with a concomitant increase in DHAP can be explained by a strong increase in the flux of the upper part of the EMP pathway up to the triose level, where equal amounts of GAP and DHAP are produced from FDP by aldolase, concomitant with a reduced drain in the lower part of the EMP pathway (from GAP to PEP) and the induction of the MG pathway. The short-term increase in the AEC and elevated levels of FDP contribute to the activation of MG synthase and inhibition of GAP dehydrogenase by decreasing the level of available free phosphate.

The triosephosphate-isomerase-catalysed conversion of GAP to DHAP and vice versa favours the formation of DHAP (95% DHAP at equilibrium; Veech *et al*., 1969; Richard, 1993), thereby contributing to an increased DHAP level and induction of the MG pathway at the expense of the lower part of the EMP pathway. Prior to the dilution rate upshift, intracellular GAP and DHAP concentrations did not differ very much (2-1 \(\pm\) 0-1 mmol GAP l\(^{-1}\); 1-67 \(\pm\) 0-04 mmol DHAP l\(^{-1}\)); during the transient, however, intracellular GAP levels decreased to 1-2 mmol l\(^{-1}\) whereas DHAP levels reached 3 mmol l\(^{-1}\), thus approaching more closely, with 70% DHAP, the favourable formation of DHAP (Fig. 5).

**Simulation of MG pathway activation and excretion of by-products**

The key event for the activation of the MG pathway is the excessive uptake of glucose, which leads to an imbalance of catabolic and anabolic reactions. This imbalance is manifest as increasing respiration and glucose uptake rates, while the biomass formation rate did not increase accordingly (Fig. 1). To simulate the activation of the MG pathway and the excretion of by-products during carbon-overflow conditions, by-product excretion rates normalized with respect to the glucose uptake rate were calculated at various fixed ratios of biomass formation to glucose uptake rates (Fig. 6). The ratio variation was used to approach the dynamic response that cells exhibit when going through a carbon-overflow transient, characterized by more rapid adaptation of catabolic reactions compared with anabolic reactions. All excreted by-products that were determined

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**Fig. 5.** Time-course of intracellular concentrations of FDP (□), DHAP (○) and GAP (△) in *E. coli* TG1 after the upshift of the dilution rate from 0-066 to 0-23 h\(^{-1}\). Interpolation curves serve for visualization.

**Fig. 6.** Prediction of by-product excretion by restricting the formation of biomass. Fluxes towards by-products under conditions of restricted biomass formation were estimated from the detailed stoichiometric model (Kayser *et al*., 2005) applying linear programming with ‘optimize growth’ as the objective function. All rates \(r\) are expressed in molar units (mmol l\(^{-1}\) h\(^{-1}\)) and are normalized with respect to the glucose uptake rate. The normalized rates can be expressed in mass units by using the molecular mass of the corresponding compound, e.g. for glucose 180 g mol\(^{-1}\) and for biomass 100 g mol\(^{-1}\) (for example; \(r_s r_s^{-1}\) or \(Y_{X//Glucose} = 0-17 g g^{-1}\)).
experimentally were also predicted to accumulate by the metabolic model. The model predicted that, at high ratios of biomass formation to glucose uptake rate, $r_x r_s^{-1}$, formate would be formed and, at lower values of $r_x r_s^{-1}$, pyruvate, acetate, MG and finally D- and L-lactate (in equal amounts) would be excreted. To see how the entire network responds to the restricted anabolic rate, a flux distribution map for $r_x r_s^{-1} = 30\%$ mol mol$^{-1}$ is shown as an example (Fig. 7). The calculation revealed that triosephosphate isomerase (r6) favours the formation of DHAP, which is subsequently converted to MG (r42). Half of the MG is degraded through the MG reductase and aldehyde dehydrogenase system (r43 and r49) and the other half through the glyoxylase I/II and glyoxylase III pathways (r44/r45 and r46). The fluxes through the glyoxylase I/II or glyoxylase III pathways cannot be discriminated by the model. The resulting D- and L-lactate is the major source for pyruvate in addition to the phosphotransferase system (PTS; r1); the excess of pyruvate is excreted. These results also indicate that the carbon flow from pyruvate to DL-lactate (r47 and r50) is smaller than the flow from DL-lactate to pyruvate (r48 and r51), suggesting that the observed excretion of DL-lactate is connected to the activation of the MG pathway (r43, r44 and r46). The pyruvate kinase (r9) of the EMP pathway carries no flux. In the PTS system, glucose enters the cell with concomitant consumption of PEP to form glucose 6-phosphate and pyruvate (r1). The high PEP consumption due to the high glucose uptake rate is balanced by PEP synthase (r10), which converts pyruvate to PEP at the cost of 1 mol ATP. In general, it is found that pathways are utilized that yield less ATP: (i) the MG bypass is used, which circumvents substrate-level phosphorylation; (ii) the glyoxylate bypass (r26–r29) is active instead of the entire TCA cycle; (iii) the less-energy-yielding enzymes of the respiratory chain, i.e. NADH dehydrogenase without proton-translocating capability (r32), FADH reductase (r34) and formate dehydrogenase (r35), exhibit elevated activities; and (iv) ATP-dissipating futile cycles are observed, e.g. between PEP carboxylase (r11) and ATP-consuming PEP carboxykinase (r12). Recently, the existence of the PEP carboxylase and PEP carboxykinase futile cycle has been established experimentally (Sauer et al., 1999). The preferential formation of MG and lactate instead of acetate predicted by the model at low $r_x r_s^{-1}$ is in line with the measured late formation of acetate during the transient, indicating that low-energy-yielding reactions are utilized preferentially in the very first part of the adaptation period, since acetate formation (r15) is the only by-product

Fig. 7. Metabolic network of *E. coli* grown on glucose (a) and a flux distribution for a selected glucose uptake rate when the anabolic rates are restricted and the MG pathway is active (b). In (a), reactions are represented by numbers as given in the appendix to Kayser et al. (2005). Not all reactions are displayed. For flux towards excreted by-products, refer to Fig. 6. In (b), a flux distribution map for $r_x r_s^{-1} = 30\%$ mol mol$^{-1}$ demonstrates how the entire network responds to the restricted anabolic rate. All fluxes are normalized with respect to the glucose uptake rate.
formation reaction that results in ATP generation. However, these calculations represent a rough estimate, since the pseudo-steady-state approximation for intracellular metabolites is not warranted under these conditions.

Oscillatory behaviour in response to excessive feast

When the dilution rate was increased from 0·066 h\(^{-1}\) to values beyond 0·28 h\(^{-1}\), oscillations were observed, apparent through periodic changes in the respiratory activity. The amplitude of the oscillations was constant and independent of the magnitude of the dilution rate change; however, no clear correlation was apparent between the duration of an oscillation period and the extent of the dilution rate shift (Table 1). The oscillations in respiratory activity continued for several days; thereafter they levelled off to reach new steady-state values.

As an example, the cellular response after a dilution rate shift from 0·066 h\(^{-1}\) to 0·28 h\(^{-1}\) is shown in Fig. 8. Initially, the dynamics of respiratory activity revealed a pattern similar to that observed in the previously described upshift experiment (Fig. 1a); the first rapid overshoot increase was followed by a transient decrease for a period of about 2 h with a subsequent increase over the next few hours (Fig. 8a). In contrast to the previously described experiment, however, the respiratory activity did not approach a new steady-state value but started to oscillate around 8 h after the upshift, with an oscillation period of 4·5 h.

The concentration of MG increased immediately after the upshift and revealed a time profile similar to that in the experiment described earlier (Fig. 3b) until a concentration of approximately 1·5 mg l\(^{-1}\) (20 μmol l\(^{-1}\)) was reached (Fig. 8b). Thereafter, the concentration of MG also exhibited oscillatory behaviour, with a profile similar to that of the respiration rate. When the concentration of MG reached about 1·5 mg l\(^{-1}\), the respiratory activity dropped and the MG concentration started to decrease. Concomitant with the recovery of respiratory activity, the concentration of MG increased again until it reached 1·5 mg l\(^{-1}\); thereafter, a new oscillatory cycle started, with

### Table 1. Oscillatory behaviour in glucose-limited continuous cultures of *E. coli* TG1

The period and amplitude of oscillations of the respiration rates (OTR and CTR) after the upshift of the dilution rate from 0·066 h\(^{-1}\) to the indicated dilution rates are shown.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( D (\text{h}^{-1}) )</th>
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<tr>
<td></td>
<td>0·28</td>
</tr>
<tr>
<td>Number of experiments</td>
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</tr>
<tr>
<td>Period (h(^{-1}))</td>
<td>4·5 ± 0·35</td>
</tr>
<tr>
<td>Amplitude (mol l(^{-1}) h(^{-1}))</td>
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**Fig. 8.** Oscillatory behaviour of *E. coli* TG1 in response to an upshift of the dilution rate from 0·066 to 0·28 h\(^{-1}\). The CTR (solid line) and OTR (dashed line) (a) and the MG concentration (■, dashed line) (b) and AEC (■, dashed line) (c) in comparison with the OTR (solid lines) are shown.
a decrease in respiratory activity and MG concentration and a subsequent recovery. The AEC oscillated with a 30 min delay compared with the respiration rate, indicating an uncoupling of ATP production and consumption in the catabolic and anabolic pathways (Fig. 8c).

The maximum MG concentration excreted into the medium upon the upshift was dependent on the extent of the dilution rate change (Fig. 9). At dilution rate shifts where no oscillations were observed (from $D = 0.066$ h$^{-1}$ to $D < 0.28$ h$^{-1}$), the maximum concentration of MG which was reached during the adaptation period increased nearly linearly with the dilution rate change. At dilution rate shifts beyond 0.28 h$^{-1}$, where oscillations of respiratory activity occurred, the maximum MG concentration did not surpass 1.5 mg L$^{-1}$. This seems to be the threshold value that affects the cellular vitality of *E. coli* at a given biomass concentration, resulting in an unstable oscillatory behaviour. Oscillations in cell density in connection with MG formation have been reported so far only for the ruminal bacterium *Prevotella ruminicola* grown in nitrogen-limited continuous culture with an excess of glucose (Russell, 1993). In this case, the cell density changed between 0.5 and 2.5 OD units with an oscillatory cycle of approximately 3 days.

**Conclusions**

The coupling between anabolism and catabolism in *E. coli* was investigated in glucose-limited continuous culture by up- and downshifts of the dilution rate. No uncoupling of anabolic and catabolic rates was observed after a dilution rate downshift, indicating that cells can adapt quickly to low nutrient supply. In contrast, when cells were exposed to a dilution rate upshift, an uncoupling of anabolism and catabolism was observed, apparent through a delayed adaptation of the anabolic fluxes. The more-rapidly-increasing catabolic activities resulted in the activation of the MG pathway, thereby circumventing energy generation through substrate-level phosphorylation in the EMP pathway. In general, analysis of the by-product formation pattern and a metabolic flux modelling approach using linear programming revealed that, during adaptation to feast conditions, the most energy-inefficient pathways are utilized first. This concerns mainly the utilization of the MG pathway, the glyoxylate shunt, energy-inefficient reactions of the respiratory chain and ATP-dissipating futile cycles. By utilizing these pathways, the cell has a mechanism to control the rate of energy generation relative to the overall catabolic rate. However, once adapted to rapid growth, cells can use the carbon source even more energy-efficiently than slower-growing cells (Kayser et al., 2005).

The metabolic reorganization during the transient also became apparent through the time profiles of the intracellular concentrations of key glycolytic pathway intermediates (FDP, GAP, DHAP) and co-metabolites (adenosine nucleotides). The rapidly increasing concentration of FDP reflects the bottleneck in the upper part of the EMP pathway, while the simultaneously increasing level of DHAP is considered to play a key role in the activation of the MG pathway. Activation of the MG pathway may also be stimulated by the transient increase in the cAMP concentration and by the increase in the AEC, resulting from elevated ATP concentrations, which are most likely accompanied by a transiently reduced level of intracellular available inorganic phosphate. The depletion of the intracellular inorganic phosphate pool is probably further amplified by the elevated level of sugar phosphates.

With a more substantial increment in the dilution rate upshift, the culture exhibited oscillatory behaviour, most likely caused by critical levels of MG in the culture broth. In this context, activation of the MG pathway must be regarded as a high-risk strategy, since formation and consumption of MG must be balanced very precisely. However, it has been shown for many substrates that their uptake capabilities are not fully exploited in low-nutrient environments (Teixeira de Mattos & Neijssel, 1997); for example, *E. coli* and *K. aerogenes* can rapidly increase the glucose transport capacity when grown in a glucose-limited chemostat culture even when growing close to the maximum growth rate (Neijssel et al., 1977), indicating that cells always maintain a reserve in capacity to pick up a substrate that will then become unavailable to a potential competitor. It is thus tempting to speculate that cells are predisposed to harm themselves rather than to share a feast with a rival organism.

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REFERENCES


