Metabolic adaptation of *Trichomonas vaginalis* to growth rate and glucose availability

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The parasitic protist *Trichomonas vaginalis* adapted the specific activities of twelve of the enzymes involved in glucose metabolism to the growth rate and glucose availability. These changes in enzyme activities were induced by culturing *T. vaginalis* in chemostats with glucose, present in rate-limiting or excess concentrations, as carbon and energy source. The specific activities were measured in pelleted cells at each steady state, while metabolic end products were determined in filtered culture fluid. The specific activities were lower in cells grown on growth-rate-limiting concentrations of glucose and higher in organisms cultured in the presence of excess glucose. In both cases enzyme activities were higher at increasing growth rates. For most enzymes the difference between the highest and lowest activities was an order of magnitude. The specific activities of eleven of the enzymes were strongly correlated to each other (correlation coefficients 0.83-0.99), the exception being lactate dehydrogenase. The rates of production of the three major end products, lactate, acetate and glycerol, increased with increasing growth rates. Alanine was not formed in measurable quantities. The ratio of the end products formed was strongly influenced by the growth rates and glucose availability. The rates of formation of acetate and glycerol correlated best with the specific activities of the enzymes catalysing the final reactions of their respective pathways. This suggests that the production of acetate and glycerol is rate-limited by these final steps. In contrast, the formation of lactate did not correlate with the specific activity of lactate dehydrogenase but was determined by the rate of glucose consumption.

**Keywords**: energy metabolism, glucose, chemostat, metabolic control analysis, *Trichomonas vaginalis*

**INTRODUCTION**

The hydrogenosome-containing amitochondriate protist *Trichomonas vaginalis* is a parasite of the human genito-urinary tract. In man its presence is often asymptomatic, but it can cause heavy infections of the vagina. In the vagina it is exposed to long-term variations in environmental conditions due to the menstrual cycle and the extent of the infection (Fouts & Kraus, 1980; Müller, 1988). The energy metabolism of *T. vaginalis* has been studied extensively in cultures and in washed cell suspensions (reviewed in Müller, 1988, 1989, 1991), but the conditions in culture and in its primary habitat differ widely. Among the environmental factors that have been investigated are glucose concentrations (Mack & Müller, 1980) and the effects of oxygen and carbon dioxide (Lloyd & Paget, 1991). Increasing levels of O₂ decrease the growth rates of *T. vaginalis* (Mack & Müller, 1978). In one study the presence or absence of O₂ affected only H₂ production and did not alter the formation of the other end products significantly (Lloyd & Kristensen, 1985). Subsequent more detailed research revealed that traces of O₂ and high levels of CO₂ exert a strong influence on growth rates, enzyme activities and the ratio of end products formed (Paget & Lloyd, 1990).

In addition to changing concentrations of O₂ and CO₂, the levels of glucose and its oligomers also vary in the vagina. The changing availability of suitable carbohydrates necessitate metabolic adaptation in order to maintain internal homeostasis (Hochachka & Somero, 1984). Although such adaptation cannot be studied in situ, chemostats can provide the controlled and adjustable conditions needed for this type of investigation (Lehker & Alderete, 1990; Ter Kuile, 1994a, b). Initially it was shown that *T. vaginalis* remains viable at very low growth
rates (Lehker & Alderete, 1990). A characterization of the overall carbon and energy utilization of T. vaginalis indicated that its carbohydrate metabolism is not optimally efficient, because an unusually large proportion of its carbohydrate source was used for non-growth purposes (Ter Kuile, 1994a). T. vaginalis has the ability to adapt its energy metabolism extensively, as indicated by changes in both the rate of glucose transport across the plasma membrane and the specific activity of glucokinase (Ter Kuile, 1994b). It was not investigated whether the metabolic adaptation is limited to the transport step and glucokinase only or the specific activity of other enzymes changes in response to different environmental conditions.

Growing anaerobically in batch cultures T. vaginalis produces equimolar quantities of glycerol, acetate, $\text{H}_2$ and $\text{CO}_2$, while lactate makes up the balance of the glucose consumed (Chapman et al., 1985; Steinbucbchel & Muller, 1986a). The pathways leading to the various end products of glucose metabolism by T. vaginalis are outlined in Fig. 1. The specific activities of the glycolytic enzymes have been measured in 100,000 g supernatants of lysed T. vaginalis, providing information on the relative activities of the different metabolic steps in cells grown at unrestricted rates (Arese & Cappuccinelli, 1974). In euclimia the ratio of the end products formed can change due to shifts in redox balance (reviewed in Thauer et al., 1977). Non-proportional adaptation of enzymes of the central pathway and those involved in the formation of only one or part of the end products can also lead to changes in the ratio of the end products formed. The metabolic adaptation described earlier (Ter Kuile, 1994b) may cause such non-proportional adaptation. This phenomenon was observed in the yeast Saccharomyces cerevisiae (Sierkstra et al., 1994). The specific activities of three out of seven metabolic enzymes measured changed as a function of the growth rate as did the ratio of the products formed (Sierkstra et al., 1994). The specific activities of metabolic enzymes of three species of kinetoplastids, parasitic protists harbouring glycosomes, changed during aging of the culture (Cazzulo et al., 1985), probably as an effect of the changing composition of the medium.

Earlier studies (Ter Kuile, 1994a, b) showed that T. vaginalis can adapt its metabolism to the concentration and/or flux of glucose. The aim of the present study was threefold: (1) to document the rate of formation of each of the end products as a function of growth rate and glucose availability; (2) to study the accompanying changes in the specific activities of metabolic enzymes and (3) to identify as far as possible the steps that control the fluxes through the different parts of the metabolic pathway. The results indicate that the levels of the end products formed and their ratios are influenced by the growth rate and the availability of the carbon source, lactate being the product of overflow metabolism. The specific activities of the metabolic enzymes of T. vaginalis increased with increasing flux of carbon. The activities of most enzymes changed proportionally, with the exception of lactate dehydrogenase. The rates of production of glycerol and acetate were controlled to a great extent by the specific activities of the enzymes catalysing the final step of their formation.

METHODS

Culture conditions and sampling. Trichomonas vaginalis (strain NIH-CI, ATCC 30001), a long-term established laboratory isolate, was grown in chemostats at varying dilution rates (D) on glucose as carbon and energy source, which was either rate-limiting or available in excess. The purpose of the chemostats in this study was to adapt T. vaginalis to constant, defined conditions. The chemostats used were of the single-stage type with pH control as described previously (Ter Kuile & Opperdoes, 1991; Ter Kuile, 1994a). The flow was controlled by the pumping rate and N$_2$ [Matheson, 99.99% (v/v) pure, manufacturers data] was used as driving gas, rendering the system anaerobic. The phosphate-buffered tryptone/yeast extract medium (Diamond, 1957) without agar contained 5 or 54 mM glucose and was supplemented with 10 or 65% (v/v) horse serum in the low and high sugar medium, respectively (Ter Kuile, 1994a). Preliminary experiments for an earlier study (Ter Kuile, 1994a) suggested that when the carbon source was in excess, the growth rate was limited by one of the components of the serum. The purpose of the lowered serum content of the excess glucose medium was to keep the density of the cultures under this set of conditions similar to those of glucose-limited cultures. Still higher densities were reached without causing instability of the culture, indicating that the metabolic end products do not inhibit growth rates. The pH was maintained at 6.40 ± 0.02 and the temperature at 37 °C. The establishment of a steady state was monitored by counting cells in a haemocytometer. The culture was assumed to be in steady state after the conditions, including the pumping rate, had been constant for at least five volume changes and cell density remained unchanged for a minimum of 24 h.

At each steady state one sample of 10 ml was rapidly (within 20 s) filtered (pore size 0.45 μm) and divided into approximately
1 ml aliquots for determination of residual substrate and end product concentrations. In addition, a 20 ml sample was divided into 18 aliquots of 1 ml which were centrifuged for 30 s at 13000 g. The pellets were used for measurement of cellular parameters and enzyme activities. All samples were stored at −20 °C and processed simultaneously for each measurement. Protein was measured by the Bradford method (Bradford, 1976). Preliminary experiments showed that the specific activities of the enzymes measured for this study (Table 1) remained constant even after prolonged storage at −20 °C. The specific activity of pyruvate kinase decreased drastically during multiple cycles of freezing and thawing but the other enzymes tested were far less affected.

**Enzyme and end product determinations.** The concentrations of glucose and its metabolic end products in the fresh medium and in the filtered and thus cell-free culture fluid were measured enzymically using published methods. Glucose was determined according to Bergmeyer (1974). Lactate and glycerol were measured as described by Gutmann & Wahlefeld (1974) and Eggstein & Kuhlmann (1974), respectively. Acetate was determined using acetate kinase (Boehringer Mannheim) to form ADP from acetate and ATP. The ADP formed was measured by converting phosphoenolpyruvate to pyruvate and ATP, catalysed by pyruvate kinase. The subsequent reaction of pyruvate and NADH forming lactate and NAD (lactate dehydrogenase) was followed spectrophotometrically. Preliminary experiments indicated that this method was more exact than a method using acetate kinase and hydroxylamine. Alanine was measured using alanine dehydrogenase (Williamson, 1974). The gaseous end products, CO₂ and H₂, were not measured. All enzyme activities were measured according to established procedures on Triton X-100 (0.1 %)-solubilized cells: glucokinase (GK) as hexokinase, Mertens & Müller (1990); phosphogluconate isomerase (PGI), Misser & Opperdoes (1984); pyrophosphate-dependent phosphofructokinase (PPi-PFK), Mertens et al. (1989); aldolase, glycerol-3-phosphate dehydrogenase (G-3-PDH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Misser & Opperdoes (1984); glycerol-3-phosphatase (G-3-Pase), Steinbüchel & Müller (1986a); pyruvate kinase (PK), Misser et al. (1987); malate dehydrogenase (MDH), Hrdy et al. (1993); malic enzyme, Avilan & Garcia (1994); lactate dehydrogenase (LDH), Markos et al. (1993); acetate/ succinate CoA-transferase (ASCT), Steinbüchel & Müller (1986b). Throughout the study the data are presented as milliunits (mU) per mg total cellular protein; 1 mU = 1 nmol substrate converted per minute.

**RESULTS**

**Data analysis**

A total of 19 parameters were determined for each steady state: dilution rate (D); at steady state D equals the growth rate), cell density, protein, glucose consumed, glycerol, lactate and acetate produced and the 12 enzyme activities listed above. An evaluation of the correlation coefficients between the different parameters was conducted using the Excel (Microsoft) spreadsheet program. The concentrations of alanine in each of the batches of medium and in the corresponding culture fluids were equal within the precision of the measurement and were approximately 6 mM. This indicates that alanine was never produced in significant quantities as an end product of carbon metabolism, hence it was not included in the statistical evaluation of the dataset. The following aspects were selected for detailed analysis and are presented below: (1) the rates of end product formation (Fig. 2); (2) the range of specific activities of the enzymes, indicating the extent of adaptation (Table 1); (3) influence of the growth rate (D) on the enzyme activities; the two enzymes with the lowest (LDH) and highest (PPi-PFK) correlation with D are shown as examples in Fig. 3; (4) the correlation of the rate of formation of an end product with the activity of the enzyme catalysing the final step of its pathway (Fig. 4); (5) the degree of proportionality of the changes in the specific activities (Fig. 5); (6) changes in the ratios of the end products formed as a function of the growth rate (Fig. 6). Comparison of the specific activities of different enzymes was not undertaken. Such comparisons could, at least in theory, identify the rate-limiting step(s) of the energy metabolism. However, it may not be possible to draw firm conclusions from the absolute numbers because the assay conditions rarely correspond to the conditions in the cell. The changes in the specific activities of metabolic enzymes with varying growth rates and glucose levels can be interpreted with much more confidence as the assay conditions were kept identical for each sample and the intracellular conditions are unlikely to vary extensively.

**Metabolic rates**

The rate of formation of the three non-gaseous end products formed by T. vaginalis increased with increasing growth rate (D) (Fig. 2). The rate of glucose consumption varied from 5.3 to 105 nmol min⁻¹ (mg protein⁻¹). The rates of total end product formation of excess-glucose-grown T. vaginalis were about double those of glucose-limited cells at similar growth rates. Cells growing on limiting concentrations of glucose formed hardly any glycerol at low growth rates. Glycerol production increased rapidly at D exceeding half of the maximum growth rate. Acetate production increased linearly with D. At low growth rates acetate and lactate production were similar, but the rate of lactate formation increased rapidly in the same range of D that showed increased glycerol production. When glucose was present in excess, thus limiting neither the growth rate nor the rate of its metabolism, lactate accounted for about half the total carbon excreted. The rate of its formation increased biphasically linear with D. Acetate and glycerol were produced in approximately equimolar quantities. Production rates increased with increasing growth rate, again with biphasic linearity, gradually at low D and more rapidly at D exceeding half the maximum growth rate.

**Changes in the specific activities**

The ranges of the specific activities of the enzymes measured for this study are given in Table 1. A comparison of the specific activities measured with the fluxes of metabolites suggests that only GAPDH, PK and, to a much lesser extent, ASCT had in vitro specific activities that cannot fully account for the flows observed in vivo. The difference for GAPDH and PK was a factor of approximately 2, which can probably be accounted for by non-optimization of the in vitro assay. The influence of the
growth rate ($D$) on the specific activities of the enzymes is shown for two examples, PPI-PFK and LDH, in Fig. 3. In both cases the activities were higher in excess-glucose-grown cells than in glucose-limited cells at similar growth rates. The difference, both in absolute numbers and in ratios, increased with increasing growth rates. The dependence of PPI-PFK on $D$ was biphasic linear with greater increase at the higher growth rates (Fig. 3a), very similar to the relationship between the rate of metabolite formation and growth rate observed in four out of six cases in Fig. 2. The only major difference was that in cells grown under glucose limitation the specific activity remained at an apparent base value at low growth rates and increased only at the higher $D$ values. No consistent relationship between LDH activity and the growth rate could be discerned (Fig. 3b). The relationship between the growth rate and the specific activities of most of the metabolic enzymes measured resembled that of PPI-PFK (Fig. 3a) rather than that of LDH (data not shown).

A change in molar ratios of the end products can be caused by differences in the extent of changes in the activities of enzymes in different parts of the pathway. A high degree of correlation between the rate of formation of an end product and the enzyme catalysing the final step of its pathway is to be expected if changes in enzyme activities rather than redox balance caused the change in the ratios of the end products. The rates of production of

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**Table 1.** List of the enzymes measured in this study, their abbreviations, EC numbers, range of specific activities observed and references for the assay used

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Abbreviation</th>
<th>EC no.</th>
<th>Range of specific activities [mU (mg protein)$^{-1}$]*</th>
<th>Reference for assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucokinase</td>
<td>GK</td>
<td>2.7.1.2</td>
<td>41-1-442</td>
<td>Mertens &amp; Müller (1990)</td>
</tr>
<tr>
<td>Phosphoglucose isomerase</td>
<td>PGI</td>
<td>5.3.1.9</td>
<td>561-3913</td>
<td>Misset &amp; Opperdoes (1984)</td>
</tr>
<tr>
<td>Pyrophosphate-dependent phosphofructokinase</td>
<td>PPI-PFK</td>
<td>2.7.1.90</td>
<td>80-254</td>
<td>Mertens et al. (1989)</td>
</tr>
<tr>
<td>Aldolase</td>
<td></td>
<td>4.1.2.13</td>
<td>40-7-286</td>
<td>Misset &amp; Opperdoes (1984)</td>
</tr>
<tr>
<td>Glycerol-3-phosphate dehydrogenase</td>
<td>G-3-PDH</td>
<td>1.1.1.8</td>
<td>330-195</td>
<td>Misset &amp; Opperdoes (1984)</td>
</tr>
<tr>
<td>Glycerol-3-phosphatase</td>
<td>G-3-Pase</td>
<td>3.1.3.21</td>
<td>190-151</td>
<td>Steinbüchel &amp; Müller (1986a)</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>GAPDH</td>
<td>1.2.1.12</td>
<td>5-9-82</td>
<td>Misset &amp; Opperdoes (1984)</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>PK</td>
<td>2.7.1.40</td>
<td>5-2-85</td>
<td>Misset et al. (1987)</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>LDH</td>
<td>1.1.1.27</td>
<td>19-0-299</td>
<td>Markos et al. (1993)</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>MDH</td>
<td>1.1.1.37</td>
<td>1015-16208</td>
<td>Hrdy et al. (1993)</td>
</tr>
<tr>
<td>Malic enzyme</td>
<td></td>
<td>1.1.1.40</td>
<td>66-8-313</td>
<td>Avilan &amp; Garcia (1994)</td>
</tr>
<tr>
<td>Acetate/succinate CoA-transferase</td>
<td>ASCT</td>
<td>2.8.3.8</td>
<td>9-5-58</td>
<td>Steinbüchel &amp; Müller (1986b)</td>
</tr>
</tbody>
</table>

* The highest and lowest values measured independent of the growth conditions are reported.
glycerol and acetate correlated well [correlation coefficient \( r^2 > 0.95 \)] with the specific activities of G-3-Pase and ASCT, respectively (Fig. 4). Neither end product correlated better with the specific activity of any other enzyme. In contrast, the specific activity of LDH correlated only weakly with the rate of lactate production \( (r^2 = 0.39) \). Of the three end products, the production of lactate correlated most strongly \( (r^2 = 0.98) \) with glucose consumption. The rate of glucose consumption correlated best with PPI-PFK of all metabolic enzymes measured \( (r^2 = 0.95) \). As was to be expected, PPI-PFK also correlated better with the rate of lactate production than did any other enzyme \((r^2 = 0.93)\). Therefore lactate production seems to be controlled primarily by the rate of glucose consumption rather than by LDH, the only enzyme exclusively involved in lactate formation.

The specific activities of most metabolic enzymes were strongly correlated to PGI \((r^2 > 0.9)\) and only slightly less to PPI-PFK. Exceptions were G-3-Pase, PK, MDH and, most notably, LDH. Therefore PGI was selected as the reference enzyme for comparing relative specific activities (Fig. 5). These high correlation coefficients were due to almost proportional change in activities of the metabolic enzymes observed in excess-glucose-grown cells. LDH was the only enzyme that changed in its specific activity independently from the other enzymes measured. The specific activities increased markedly with increasing growth rates (Fig. 3). Hence increasing activities correspond to increasing flux of metabolites. The variation in the specific activities often exceeded an order of magnitude. In contrast, the range of specific activities measured in cells grown under glucose limitation was usually limited to less than a factor of two. The relationships between the specific activities of the different enzymes was basically random (data not shown). This striking difference between the changes in the specific activities in cells grown with limiting or excess concentrations of glucose indicates that in *T. vaginalis* the flux of metabolites only influences the enzyme levels if the carbon source is present in excess.

**Ratio of the end products**

The ratio of the end products changed as a function of the growth rate both in glucose-limited and in excess-glucose-grown cultures of *T. vaginalis*. In Fig. 6 the actual concentrations of the end products measured at each steady state are given as a function of the growth rate. At steady state the rates of removal and formation of the end products are equal. Therefore the concentration of the end products is the result of their production by the culture, not normalized to biomass. The rates of end product formation by the culture are in turn affected by the metabolic changes occurring in the cell. When glucose was limiting, growth rate, lactate and acetate were the primary metabolic end products. Glycerol was a major product only at the higher growth rates. When glucose was present in excess, the amount of lactate produced increased three- to fourfold. Glycerol also represented a substantial proportion of the end products at lower growth rates and the quantities of acetate produced remained roughly equal to those of glucose-limited cultures. When glucose was rate-limiting, the carbon consumed equalled the carbon produced as lactate, acetate and glycerol within the precision of the measurements. This was unexpected because one molecule of \( CO_2 \) is formed for every molecule of acetate, indicating that fixation of this \( CO_2 \) in an as yet not understood process has taken place. When glucose was present in excess, almost twice as much of it was consumed than recovered in the end products at the low growth rates. The production of \( CO_2 \) is coupled to acetate production and cannot account for the missing carbon. The formation of storage carbohydrates is a more likely explanation for this discrepancy. In an earlier study excess-glucose-grown cells contained roughly 0.6 mg total carbohydrates (mg

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**Fig. 3.** Specific activities of PPI-PFK (a) and LDH (b) as a function of the growth rate \((D; \text{measured in doubling times per day})\) for *T. vaginalis* grown on glucose as rate-limiting substrate \((\bigcirc)\) or in the presence of excess glucose \((\square)\).
Fig. 4. (a)–(c) Rates of formation of the end products of glucose metabolism of *T. vaginalis* as a function of the specific activities of the enzymes catalysing the final step of their formation. (d) The consumption of glucose as a function of the specific activity of PPI-PFK. The enzyme abbreviations are given in Table 1. The variation in the specific activities was induced by the changing culture conditions, i.e. growth rate and glucose availability.

*Discussion*

**End products formed**

This study revealed that in *T. vaginalis* both the overall formation of non-gaseous end products and their ratio can change within broad limits. Such changes occur when the glucose concentration and/or the growth rate is changed. The overall rate of metabolism of slowly multiplying cells is slower than that of fast-growing cells. The end products recovered accounted for the glucose consumed in most cases. The rates of production of various end products changed in different manners. The influence of culture conditions on the ratios of the end products of *T. vaginalis* has been noted before (Müller, 1989; Paget & Lloyd, 1990), but was not documented as a function of growth rate and substrate concentration. Only the rate of formation of lactate seems to be controlled by the flux of carbon through the initial steps of the pathway. The formation of glycerol and acetate may be rate-controlled by the enzymes catalysing the final steps of their formation, G-3-Pase and ASCT, respectively. This indicates that the production of acetate and glycerol is...
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![Graph showing specific activities of enzymes](image)

**Fig. 5.** Specific activities of PPI-PFK (■), aldolase (●), G-3-PDH (○), GAPDH (▲), G-3-Pase (■), PK (○), malic enzyme (●) and LDH (◇) as a function of the specific activity of PGI for excess-glucose-grown cultures. The same relationships in glucose-limited organisms were random (data not shown). The enzyme abbreviations are given in Table 1.

![Graph showing metabolic end products](image)

**Fig. 6.** Metabolic end products of *T. vaginalis* growing on glucose in chemostats on growth-rate-limiting (a) or excess concentrations (b) of glucose, as a function of the growth rate (D; measured in doubling times per day). The units for the end products in this graph are mM because in a steady state situation the rates of removal and production are equal. Hence the concentration is an appropriate measure for the rate of formation by the culture, not normalized to biomass. ○, Acetate; □, glycerol; ◇, lactate.

limited by internal factors, whereas the pathway for the formation of lactate has overcapacity. This overcapacity and the dominance of lactate as end product during growth on excess glucose are in agreement with the suggestion of Müller (1989) that lactate is a product of overflow metabolism. The previously observed 1:1 molar ratio for the formation of glycerol and acetate (Chapman *et al.*, 1985; Steinbüchel & Müller, 1986a) was found only during growth on excess glucose. The equimolar formation of acetate and lactate in the virtual absence of glycerol production found at medium growth rates under glucose limitation suggest that *T. vaginalis* maintains its redox balance in a manner not understood at present, but which might involve H₂ production. The cultures were grown under an N₂ atmosphere. Calculations based on the O₂ contaminating the N₂, the volume of the culture and the gas phase above it, and the flow rate of the gas suggest that the O₂ levels remained well below 0.25 μM. This is the minimal O₂ concentration found to influence the carbon metabolism of *T. vaginalis* (Paget & Lloyd, 1990). O₂ is thus unlikely to have influenced the ratio of the end products under the conditions applied.
The capacity of the glycolytic pathway

The highest rates of glucose consumption by \textit{T. vaginalis} measured in this study were very similar to the maximum rates measured during short term incubations in buffered salt solution (Mack & Müller, 1980) [105 and 905 nmol min\(^{-1}\) (mg protein\(^{-1}\)]\(^{-1}\), respectively]. The rate of end product formation increased with increasing growth rate, both in glucose-limited and excess-glucose-grown cells. This indicates that the capacity of the metabolic pathway for glucose of \textit{T. vaginalis} is strongly influenced by the growth rate. In contrast, the consumption of glucose by \textit{S. cerevisiae} growing on excess glucose was independent of the growth rate (Sierkstra et al., 1994). In an earlier study (Ter Kuile, 1994b) on the adaptation of glucose uptake to growth rate and carbon regimes it was found that cells growing on excess glucose took up less \[^{3}H\]glucose in intermediate length incubations (20 min) than glucose-limited cells. These data, combined with the present study, suggest that the higher the specific activities of the metabolic enzymes, the less label will be recovered inside the organism. The most probable explanation for this observation is that the turnover of the label is more rapid when the metabolic activity is high, and that more label has exited from the cell already at the end of the incubation. The earlier conclusion that \textit{T. vaginalis} adapts its carbon metabolism by increasing the capacity of the metabolic pathway when glucose is scarce (Ter Kuile, 1994b) must be reconsidered in the light of the present findings. The latter mode of adaptation would counteract the environmental challenges. In fact the opposite was observed; the increased specific activities of the metabolic enzymes when glucose was present in excess allow an increase in the flow of metabolites. This increased turnover of glucose caused the reduced yield (cell mass produced per mole glucose consumed) observed during growth on excess glucose (Ter Kuile, 1994a).

Flux control by adapting specific activities

The two most striking observations about the adaptation of the metabolic enzymes of cells cultured on excess glucose to the growth rate are that the specific activity of not even one enzyme remains constant and that the specific activities of most of them change proportionally. The first observation is in agreement with the central theorem of the metabolic control analysis theory that the flux through a metabolic pathway at any one time is never controlled by a single enzyme only (Kacser et al., 1995). A similar conclusion was reached experimentally for glycolysis in yeasts (Schaaff et al., 1989). In contrast to the often-made assumption that the initial step in a metabolic pathway controls its rate, GK is less correlated to the metabolic flux than PGI and PPI-PFK and most other enzymes. This can explain the results of experiments measuring the uptake of radiolabelled glucose, which showed that GK specific activity is not always correlated to glucose uptake (Ter Kuile, 1994b). The high degree of correlation between PPI-PFK and the consumption of glucose does not prove with certainty that this enzyme catalyses a rate-determining step. The pyrophosphate-consuming reaction leading to the formation of fructose 1,6-bisphosphate in \textit{T. vaginalis} is reversible and is thus unlikely to exert a major part of the rate control over the glycolytic pathway (Mertens, 1991, 1993). Probably it reflects the fact that the extent of its adaptation is the average of that of the other enzymes of the pathway. The ATP-dependent PKF of \textit{S. cerevisiae} also has a low control coefficient for glycolysis (Davies & Brindle, 1992), but this comparison may not be valid because the reaction it catalyses is not reversible.

The proportional adaptation of most metabolic enzymes of \textit{T. vaginalis} during growth on excess glucose can be explained in two different ways: (1) only the exception, LDH, has overcapacity or (2) the expression of the enzymes' proportionally changing activity is regulated by the same mechanism. The first explanation is unlikely because of the wide variation in specific activities even of those enzymes that change proportionally. In \textit{S. cerevisiae} growing in chemostats on excess glucose the specific activities of four enzymes were constant: hexokinase, PGI, glucose-6-phosphate dehydrogenase and pyruvate decarboxylase. This indicates that these enzymes exercised little rate control over the entire flux and may have had overcapacity. Three, phosphoglucomutase and alcohol dehydrogenase I and II, adapted by a factor of up to 10, however the highest specific activities were found at lower D values (Sierkstra et al., 1994). In \textit{S. cerevisiae} various internal metabolites regulate the expression of different genes for glycolytic enzymes (Müller et al., 1995). This suggests that the various metabolic steps are regulated independently from each other. Comparison of the metabolism of \textit{T. vaginalis} with that of \textit{S. cerevisiae} or other eukaryotes may not always be valid because they have far greater possibilities for changes in the mode of energy metabolism. One possible explanation for the groupwise adaptation of most glycolytic enzymes of \textit{T. vaginalis} is that a single metabolite controls the expression of most of the genes coding for core metabolic enzymes. This, however, remains to be investigated.

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