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An excess of glycolytic enzymes under glucose-limited conditions may enable *Saccharomyces cerevisiae* to adapt to nutrient availability

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Microorganisms, including the budding yeast *Saccharomyces cerevisiae*, express glycolytic proteins to a maximal capacity that (largely) exceeds the actual flux through the enzymes, especially at low growth rates. An open question is if this apparent expression level is really an overcapacity, or maintains the (optimal) enzyme capacity needed to carry flux at (very) low substrate availability. Here, we use computational modelling to suggest that yeast maintains a genuine excess of glycolytic enzymes at low specific growth rates. During fast fermentative growth at high glucose levels, the observed expression of the glycolytic enzymes matched the predicted optimal levels. We suggest that the excess glycolytic capacity at low glucose levels is a preparatory strategy in the adaptation to sugar fluctuations in the environment.

Keywords: budding yeast; enzyme capacity; glycolysis; resource allocation

It is a common observation that microorganisms, growing in carbon-limited conditions, possess reserve glycolytic capacity, which means that the abundances, and, subsequently, the maximal reaction rates of glycolytic enzymes largely exceed *in vivo* fluxes. Such a phenomenon was observed in various microbes, such as *Escherichia coli* [1], *Bacillus subtilis* [2] and *Lactococcus lactis* [3]. Among them, the budding yeast *Saccharomyces cerevisiae* is renowned for its ability to consume glucose at high rates [4].

Although the fluxes through glycolytic enzymes are very low at strong glucose limitation in *S. cerevisiae*, various studies have reported that enzyme activities [5] and mRNA levels [6] barely change for most glycolytic enzymes across a broad span of dilution rates in glucose-limited chemostats [5,7]. We recently reported a (high) stable fraction of total *S. cerevisiae* proteome occupied by glycolytic enzymes in glucose-limited chemostats spanning $D = 0.20$ to $0.34 \text{ h}^{-1}$ (8, Fig. 1A), a cumulative effect of the expression of individual glycolytic enzymes at high levels (Fig. 1B). Studies [7,9–11] combined, these are remarkable observations, considered that in some of these studies the specific glucose uptake rate varied up to ca. 15-fold across conditions. Overall, there seems to be a profound reserve capacity of glycolytic enzymes in *S. cerevisiae* growth in glucose-scarce environments.

However, whether the glycolytic capacity is genuinely in excess under these conditions is not as trivial as the difference between maximal rate ($v_{\text{max}}$) and actual flux may suggest. One option is that the overcapacity is no excess at all: in glucose-limited chemostats the extracellular glucose concentration is very low and consequently (i) there is a low thermodynamic driving force for all subsequent enzymes, and (ii) the enzymes tend to be strongly undersaturated. Both consequences affect the rate of enzyme-catalysed reactions: thermodynamic driving forces are dominant for reactions close to equilibrium, while saturation effects are dominant for reactions far from equilibrium [12]. To maintain the...
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maximal possible flux under a low substrate regime may require high expression of the enzymes. So perhaps the observed high glycolytic capacity at substrate saturation simply reflects the (high) optimal enzyme levels needed to accommodate the maximal attainable flux at very low substrate levels.

Alternatively, the glycolytic proteins are indeed over-expressed, and the overabundance may be rationalised as a short-term adaptation to fluctuations in glucose levels, for example, sudden availability (glucose pulse), or feast/famine cycles, which are fairly frequent in natural environments. The increased consumption of glucose straight after a glucose pulse was shown to be protein synthesis-independent [13], and is driven by metabolic regulation, reflected in increased concentrations of glycolytic intermediates [14–16]. Only tens of minutes post-pulse, increased mRNA levels of glycolytic enzymes [15,17] and synthesis of low-affinity glucose transporters [18] are observed. Being prepared for quick growth acceleration at high glucose levels even when they are not present, may provide a fitness advantage under dynamic environments.

To distinguish between these two options, we set out to quantify the enzyme levels minimally needed to run central carbon metabolism (CCM) at the observed growth rates and corresponding external glucose concentrations (Fig. 1C). For this we made use of the Enzyme Cost Minimisation (ECM) method, and compared these predictions with published quantitative proteomics data. We find that the computed enzyme

Fig. 1. Observed expression levels of enzymes of CCM in S. cerevisiae and our computational approach. (A) Proteome mass fractions of glycolytic and TCA cycle enzymes as a function of specific glucose uptake rate. (B) The breakdown of the proteome mass fractions for different glycolytic (left) and TCA cycle (right) enzymes (isozymes lumped together) as a function of specific growth rate. (C) The modelling approach used in this study. We computed the steady-state fluxes for the glucose-limited and glucose-excess conditions described in [8] in the pcYeast8 proteome-constrained model of S. cerevisiae metabolism (P. Grigaitis & B. Teusink, unpublished results) and used that as an input for the ECM models (see Methods for details of model construction and Fig. S1 for the distribution of kinetic values). Quantitative proteomics data for (a, b) from [8].
demands match the experimental measurements in the high flux, respiro-fermentative regime, while at the low flux, fully respiratory growth state, the observed expression levels are higher than needed. We thus conclude that excess capacity of glycolytic enzymes exists but is condition dependent.

Methods

Data collection

Label-free quantitative proteomics data from yeast cultures, grown in glucose-limited chemostats at six different dilution rates, as well as batch cultures with excess glucose, were acquired from [8]. Kinetic data ($k_{cat}$ and $K_M$ values) for the wild-type enzymes of *S. cerevisiae* at conditions closest to the physiological ones (temperature 30 °C, pH 7, etc.) were gathered from BRENDA database [19] (Fig. S1). Information on protein masses, stoichiometry of complexes were collected from UniProt [20].

Enzyme Cost Minimisation model

*Enzyme Cost Minimisation* modelling framework [21] was used. The models of yeast CCM were reconstructed using of the model of *E. coli* CCM as a template, provided in the method paper [21]. The model was curated as follows: reactions *ppc* and *fbp* from the template model were removed, and we added the ethanol fermentation branch (reactions *pdc* and *adhl*, as well as the external- and internal NADH oxidases (*nde* and *nidi*, cytosolic and mitochondrial respectively). To allow metabolites to acquire different concentration values in different compartments, the reconstructed CCM model was split into a cytosolic (glycolysis, pentose phosphate pathway, ethanol fermentation) and mitochondrial (TCA cycle) model. When there was no experimentally determined $K_M$ value, a default $K_M = 0.1$ mM was assumed. The parameters of pH, pMg, and ionic strength were retained as in the *E. coli* model (7, 3 and 250 mM respectively).

Concentrations of intracellular glucose and ethanol were fixed as follows: for glucose, the intracellular [glucose] was estimated from the data of glucose transport capacity at different dilution rates in glucose-limited chemostats/glucose batch cultures from [22] using the computation provided in [23] (Table S1). For ethanol, the intracellular concentration of 0.001 mM was assumed. Concentrations of all other metabolites were allowed to vary.

For every condition (as described in the Results, ‘Overview of the modelling approach’), a set of cytosolic and mitochondrial models was generated. Intracellular steady-state fluxes were computed by the pc*Yeast8* model ([8], P. Grigaitis & B. Teusink, unpublished results) and used as input to the ECM models. Reactions, carrying zero flux in the pc*Yeast8* model were removed altogether from the ECM models. The predicted enzyme concentrations in mM were converted to proteome fractions by assuming the fixed relationship of $V_{cell} = 1.7 \text{ mL-gDW}^{-1}$ and bulk protein composition in biomass: $f_p = 0.42436 \mu + 0.35364$ (g protein (g biomass)$^{-1}$).

Results

Overview of the modelling approach

The ECM method computes the minimal enzyme demand required to support given fluxes in metabolic networks, based on the kinetic properties of the enzymes and thermodynamic properties of the reactions (Gibbs free energy change) [24]. The method takes steady-state flux values as input. Then, the weighted sum of enzyme demands is minimised, with the enzyme concentrations and natural logarithms of metabolite concentrations as decision variables. The overall enzyme demand is defined as a combination of the minimal demand, that is, the ratio between the flux through the enzyme and its turnover value ($k_{cat}$), and two aspects of enzyme kinetics: reaction reversibility (thermodynamic driving force) and enzyme (under-)saturation (low substrate availability). Note that throughout the text, we discriminate between excess enzyme capacity (enzyme levels higher than the optimal demand) and enzyme undersaturation (the saturation function $f < 1$).

We adapted the ECM model of *E. coli* CCM to reconstruct two pathway models of *S. cerevisiae*: the cytosolic CCM (glycolysis and pentose phosphate pathway), and mitochondrial CCM (tricarboxylic acid [TCA] cycle) (Methods). In this study, we considered the seven conditions of growth on glucose reported in [8]: six glucose-limited chemostats (0.20, 0.23, 0.27, 0.30, 0.32 and 0.34 h$^{-1}$) and a culture in excess of glucose ($\mu = 0.37$ to 0.40 h$^{-1}$). We used the reported fluxes and glucose concentration as constraints, and used the metabolite concentrations as optimisation variables to minimise the enzyme levels (Fig. 1C).

Glycolytic proteins are expressed in excess in respiratory growth

We asked what the predicted enzyme levels are at different growth rates and compared them to the experimental data (Fig. 2A,B). We first considered the hypothetical 'minimal' enzyme demand assuming operation of all enzymes at maximal capacity and observed a large discrepancy between the measured enzyme levels versus the minimal demand, even at glucose-excess conditions. Inclusion of the effect of substrate availability with enzyme kinetics resulted in a much
higher demand for enzyme, as expected. The key finding is that at low, fully respiratory, growth ($D < D_{\text{crit}} = 0.28 \text{ h}^{-1}$), the measured abundance of pooled glycolytic enzymes was higher than the computed levels (Fig. 2A), indicating suboptimal excess enzyme expression. At high glucose levels where respiro-fermentative growth is observed, however, the predicted demand seemed to correspond well with the experimental measurements. In contrast to glycolytic enzymes, the predicted demand of the total of TCA cycle enzymes (Fig. 2B) matched the experimental measurements well at all growth rates.

We then looked at the demands at the individual enzyme level (Fig. 2C,D), where we observed more discrepancies. As the fluxes increased (=increasing growth rate), we observed that the predicted demand of glycolytic enzymes generally increased (Fig. 2C), but their demand was almost universally underpredicted by a large margin at lower growth rates. The latter conclusion is even more evident comparing the minimal predicted versus observed enzyme abundance (Fig. S2), where we observed the underprediction of minimal levels to be generally in the range of 5- to 50-fold for many of the glycolytic enzymes. As expected, the major contribution to enzyme demands comes from the undersaturation of glycolytic enzymes (Fig. S3), with a substantial contributions of enzyme reversibility for the isomerases (glucose 6-phosphate isomerase, and triose phosphate isomerase), as well as fructose 1,6-bisphosphate aldolase.

It should be noted here that the predicted enzyme demands are particularly sensitive to the $k_{\text{cat}}$ values: the enzyme demand, accounted for kinetic effects, are multipliers of the minimal enzyme demand, rather than additive increments. The predicted demands of individual TCA cycle enzymes (Fig. 2D) seem to be influenced by the uncertainty of the $k_{\text{cat}}$ values. Yet, unlike the glycolytic enzymes, there was no systemic underprediction of the enzyme demands for TCA enzymes. This thus suggests that the ‘combined’ prediction of the proteome fractions (Fig. 2B) is a robust outcome, although for individual enzymes we observed under- and overpredicted demands. Moreover, a distinct property of the predicted TCA cycle enzyme demands is that their minimal predicted demand was already a good approximation of their expression levels (Fig. 2B, Fig. S4), with a substantially smaller contribution to the overall demand coming from enzyme
undersaturation (Fig. S4) due to generally lower $K_M$ values (Fig. S1). With this, we conclude that the demand of the TCA cycle enzymes seems to be set by the flux through these enzymes.

Prediction of metabolite levels suggests optimal growth at glucose batch conditions

The key feature of the ECM method, compared to other optimisation-based metabolic modelling approaches, is that both the enzyme demands and the natural logarithms of the concentrations of metabolites are optimisation variables. Thus, alongside enzyme demands, we can describe the predicted metabolic states in terms of the steady-state concentrations of metabolites (Fig. 3).

The predicted metabolite concentrations (Fig. 3A–C) were, as for the enzyme demands, in best alignment with experimental data [7] for the glucose-limited respiro-fermentative (Fig. 3B) and glucose-excess conditions (Fig. 3A). We also compared the predicted cytosolic [NAD]/[NADH] ratio, and found a good qualitative agreement with experimental data at all growth rates (Fig. 3D). Eventually we observed that the predicted cytosolic [NAD]/[NADH] ratio and the concentration of cytosolic phosphate were in a good quantitative agreement with experimental data for the batch conditions (Fig. 3D,E). Based on this, we suggest that at glucose-excess conditions, most of the enzymes in yeast CCM are expressed and operate optimally.

Discussion

In this study, we sought to elucidate the nature of the perceived overcapacity of glycolytic enzymes, which is a trait observed in many microorganisms. Here, we focused on *S. cerevisiae* for our analysis, and compared enzyme demands, predicted by computational models, with experimental measurements. Note that the ECM models we used neglect allosteric regulation. This may be one reason for the deviation of individual enzymes from the predicted optimality. This layer of information would require more detailed mechanistic models of enzyme-catalysed reactions, as used in ordinary differential equation-based models. Another potential source of deviation is the uncertainty of the kinetic parameters used. We expect, however, that positive and negative interactions cancel each other out, and that the conclusion on the pathway-level expression of enzymes is robust and remains valid.

We found that, at low growth rates, the kinetic factors of enzyme catalysis (enzyme reversibility and undersaturation) explain only a fraction of the

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**Fig. 3.** Comparison of predicted metabolite levels at different specific growth rates. (A–C) Predicted metabolite concentrations at the glucose-excess (A) and glucose-limited (B, C) conditions. The green, yellow and orange shaded regions represent the agreement of predicted versus measured levels in the 2-, 5- and 10-fold range respectively. (D, E) Predicted cytosolic [NAD]/[NADH] ratio (D) and cytosolic phosphate concentration (E). Shaded regions in (D, E) represent the third degree polynomial fit, or experimentally determined range of the experimental measurements, in (D) and (E) respectively. Metabolite concentration data from [7].
observed protein pool, pointing at a genuine excess capacity of enzymes, that is, above the optimal level needed to support the steady-state flux under that condition (Fig. 2A). One could reason that the allocation of additional resources to glycolysis is a preparatory (dynamic) resource allocation strategy in glucose-scarce environments [24], as was also suggested for ribosomal proteins [25].

Under glucose-limited conditions, glucose availability seems to be the constraint which actively limits growth [8] as the response of prolonged cultivation in glucose-limited conditions is the duplication of glucose transporter loci [26], only later to be accompanied by a decrease of fermentative capacity [27] and activity of glycolytic enzymes [28]. Thus, at dynamically changing conditions, the selection pressure is rather on scavenging the limiting nutrients, and suboptimal (at that condition) allocation of resources is unlikely to be outcompeted by individuals whose expression of glycolytic enzymes matches the demand set by the glycolytic flux. This hypothesis is supported by computational modelling, as we observed that increased expression of gratuitous protein does not affect the predicted growth rate in nutrient-limited conditions ([8], P. Grigaitis & B. Teusink, unpublished results). It was shown that perturbations to the proteome composition have no influence on the physiology of glucose-limited chemostat cultures, for example, when mitochondrial biosynthesis was increased by overexpression of transcription factor Hap4 [29].

Yet the situation changes in respiro-fermentative growth and/or glucose-excess conditions (Fig. 2A,C). There, the predicted enzyme demand matches experimentally determined expression, and the ca. two-fold increase in glycolytic flux between the last chemostat point ($\mu = 0.34$ h$^{-1}$) and the glucose excess ($\mu = 0.39$ h$^{-1}$) is followed by a similar increase in the proteome fraction, allocated to the glycolytic enzymes – predicted and measured alike. Previously we proposed that the growth in glucose-excess conditions is proteome-limited [8] and is thus defined by optimal proteome allocation. A body of experimental data points at optimal expression of glycolytic enzymes in glucose excess: both overexpression of glycolytic enzymes [30] or construction of a ‘glycolysis-minimal’ strain [31] (i.e. cumulative deletion mutant of minor isoforms of glycolytic enzymes) have no influence on the maximal growth rate $\mu_{\text{max}}$ and the glycolytic flux. Glycolytic flux can be increased in the overexpression strains only when selecting for higher glucose-consuming mutants [32]. The quantitative agreement of the predicted metabolite concentrations (Fig. 3A,E) in glucose-excess conditions further supports this conclusion.

Unlike the glycolytic enzymes, we observed that the demand of the TCA cycle enzymes, in most of the cases, followed the demand set by flux (Fig. 2B,D). Our findings support the previous observations, such as the idea that metabolically active organelles (in this case, cytosol versus mitochondria) compete for resources in yeast. As an example, a major rearrangement of cell composition happens during the glucose-ethanol diauxic shift: [33] reported a ca. seven-fold increase in the cell volume occupied by mitochondria, between the glucose- (pre-shift) and ethanol-excess (post-shift) conditions. As ethanol enters the cell metabolism primarily through the TCA cycle, and growth on ethanol requires high fluxes through the TCA cycle to assimilate enough ethanol, such a transition points to a demand-driven allocation of resources to mitochondria.

To conclude, we argue that the excess capacity of glycolytic proteins in glucose-scarce conditions is an adaptation to fluctuations of nutrient availability in the environment. Being able to swiftly consume as much glucose as possible provides an advantage over competitors and happens without new protein synthesis, as an increase in uptake of glucose happens in the time-scale of seconds and minutes post-pulse [15]. We here considered only one microbe as an example, S. cerevisiae, yet expect this excess capacity of enzymes to be a unifying trait of nutrient-limited growth of microbes.

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**Author contributions**

PG involved in data curation, formal analysis, investigation, methodology, resources, software, validation, visualisation, writing—original draft, and writing—review and editing; BT involved in conceptualisation, formal analysis, funding acquisition, project administration, supervision, validation, and writing—review and editing.

**Data accessibility**

Models, data and code to reproduce the results are provided on Zenodo (10.5281/zenodo.6801406) [34].
References


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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Comparison of the kinetic values in the cytosolic and mitochondrial Enzyme Cost Minimization models.

Fig. S2. Predicted proteome fractions of individual glycolytic (blue points) and TCA cycle (orange points) enzymes at different specific growth rates.

Fig. S3. The contribution of different kinetic factors to the computed glycolytic enzyme demand (Fig. 2) at different specific growth rates.

Fig. S4. The contribution of different kinetic factors to the computed TCA cycle enzyme demand (Fig. 2) at different specific growth rates.

Table S1. The computation of the internal glucose levels across conditions.