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What Process Is Glycolytic Stoichiometry Optimal For?

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Abstract. It has been proposed that the glycolytic stoichiometry of 2 ATP per glucose is the result of an optimization that maximizes the rate of ATP production. However, using a nonequilibrium thermodynamic approach, we show here that glycolysis operates under optimal output power and not at optimal flow of ATP production. Furthermore, it can be proved that the same maximal output power can be achieved with different stoichiometries. However, changes in the glycolytic stoichiometry would dramatically affect the efficiency of all those cellular processes powered by ATP. Our results suggest that the stoichiometric coefficient, as found in most contemporary cells, may be the outcome of an evolutionary process leading to yield an operative quantum energy for the hydrolysis of ATP.

Key words: ATP — Glycolysis — Evolution — Energy converter — Energy dissipation — Optimality

Introduction

A living cell can be considered as a physicochemical system able to transform and use the energy from its surroundings in order to maintain its own identity. The glycolytic pathway is one of the most widely distributed and oldest energy-yielding mechanisms in living beings, with an evolutionary history of billions of years behind it. The idea that contemporary metabolic pathways are the result of an evolutionary optimization process is broadly accepted (Cascante et al. 1996; Heinrich et al. 1997). However, there are uncertainties concerning the target function that is optimized during evolution. Because the main biological function of glycolysis is the production of ATP, it has been assumed that natural selection has led to the optimization of ATP formation (Heinrich et al. 1999; Aledo and Esteban del Valle, 2002).

In the glycolytic pathway, the splitting of glucose to pyruvate (an exergonic process) is tightly coupled to the phosphorylation of ADP (an endergonic reaction). Thus, there are, in evolutionary terms, two ways of increasing the rate of ATP production: i) by maximizing the number of ATPs produced per glucose (energy yield) and ii) by maximizing the flux of material from glucose to pyruvate. However, because of fundamental thermodynamic constraints there is always a trade-off between yield and rate (Waddel et al. 1999; Pfeiffer et al. 2001) and therefore, there must be a compromise between these two factors. Previous studies have concluded that the stoichiometry of glycolysis leading to the production of two moles of ATP per mole of glucose broken down, as found in contemporary glycolysis, has been imposed by evolution in order to optimize the rate of ATP production (Heinrich et al. 1997, 1999; Stephani et al. 1999; Aledo and Esteban del Valle, 2002). However, most of these studies assume implicitly that the phosphate potential (defined as the chemical affinity for the conversion of ATP into ADP and Pi) has remained constant since billions of years ago,

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regardless of the environmental and evolutionary changes. In the present article, we suggest that the current phosphate potential represents an optimal value within the context of global metabolism, and therefore it must be seen as the result of co-evolution of the different metabolic routes where ATP is involved, including glycolysis. We also show that the contemporary glycolytic stoichiometry provides not only the maximum output power (MOP) (energy per unit time that is captured as ATP), but keeps the phosphate potential within an adequate range. As we shall discuss, this is an essential requisite for the efficient use of this nucleotide as an energy-transducing agent.

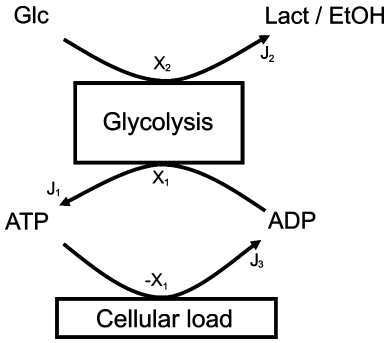

Fig. 1. Phenomenological model of glycolysis. The metabolic pathway known as glycolysis is described in terms of a free-energy converter connected to a cellular load.

Method

A Linear Nonequilibrium Thermodynamic Treatment of Glycolysis

In most living cells, the splitting of glucose is an exergonic process strictly coupled to the phosphorylation of ADP to form ATP, which is an endergonic process. Therefore, glycolysis can be understood as a free-energy transducing process (Fig. 1) (Aledo and Esteban del Valle 2004). In the framework of nonequilibrium thermodynamics, fluxes (J_i) are often linear functions of the forces that drive them (X_i). Although far from equilibrium we lose the mathematical guarantee of linearity (Westerhoff and van Dam 1987), it does not mean that linear flow-force relations cannot be established (Vieira et al. 1972; Stucki 1980; van der Meer et al. 1980; van Rotterdam et al. 2002). In any event, the assumption of linear relations in the case of glycolysis is not without precedent (Waddell et al. 1999; Pfeiffer et al. 2001; Aledo and Esteban del Valle 2004). Therefore, nonequilibrium thermodynamics offers a convenient quantitative description of the energetics of glycolysis. When multiple fluxes and forces are considered, the general equation describing their relationship is

$$J_i = \sum_{j=1}^{n} L_{ij} X_j \tag{1}$$

Hence, when glycolysis is the main ATP-supplying pathway, the process can be described, on purely phenomenological grounds, by the following equations:

$$J_1 = L_{11} X_1 + L_{12} X_2 \tag{2}$$

$$J_2 = L_{21} X_1 + L_{22} X_2 \tag{3}$$

where J_1 and J_2 are the net flows of ATP formation and glucose consumption, respectively, and X_1 and X_2 are the corresponding conjugated forces. It must be noted that the generalized forces X_1 and X_2 can also be referred to as chemical affinities (equal to minus the actual change in free energy) and therefore $X_1 < 0$ and $X_2 > 0$ under the range of intracellular conditions found in living cells. It must be noted that the output force (X_1) is equal, but with opposite sign, to the phosphate potential ($-X_1$). The phenomenological coefficients L_{ij} are the proportionality constants between flows and forces. These phenomenological conductances or coefficients are kinetic in nature because they can incorporate attributes of chemical rate constants. To have a steady state, we shall consider X_2 as a constant, as seems to be the case in many biological systems (Meléndez-Hevia et al. 1997). We also assumed the validity of Onsager's symmetry, that is, $L_{12} = L_{21}$.

Once glycolysis has been described as a free-energy transducing process obeying Equations 2 and 3, we are entitled to make use of the phenomenological theory developed by Kedem and Caplan for this sort of linear converter (Kedem and Caplan 1965). These authors introduced some interesting concepts such as the degree of coupling, $q = L_{12}/(L_{11}L_{22})^{1/2}$, which represents a dimensionless measure of how tightly the driven process is coupled to the driver process. In general, the absolute value of this coupling coefficient will always be between 0 and 1. For glycolysis, where glucose breakdown and ADP phosphorylation are tightly coupled, q takes the maximal value ($q = 1$). Another useful concept is the so-called phenomenological stoichiometry, defined as $Z = (L_{11}/L_{22})^{1/2}$, which is a normalized measure of the straight conductances. Although Z must not be confused with the mechanistic stoichiometry, for values of q close to 1 (as in the case of glycolysis) the phenomenological and the mechanistic stoichiometries coincide.

In our core model (Fig. 1), in addition to the glycolytic free-energy converter, we also consider a load imposed on glycolysis by the cellular ATP-utilizing reactions.

$$J_3 = L_{33} X_3 \tag{4}$$

where L_{33} is an overall phenomenological conductance lumping together the conductances of all ATP-consuming processes in the cell. As pointed by Stucki (Stucki 1980), it is reasonable to assume that the ATP-utilizing process is driven by the phosphate potential ($X_3 = -X_1 > 0$). Thus, Equation 4 can be rewritten as

$$J_3 = -L_{33} X_1 \tag{5}$$

At this point, it may be convenient to normalize the output force (X_1) with respect to the input force (X_2) by defining the force ratio

$$x = ZX_1/X_2 \tag{6}$$

In this way, all the fluxes can now be expressed as explicit functions of the normalized variable x (Appendix A):

$$J_1/(ZL_{22}X_2) = x + 1 \tag{7}$$

$$J_2/(L_{22}X_2) = x + 1 \tag{8}$$

$$J_3/(L_{33}X_2/Z) = -x \tag{9}$$

Under conditions where the overall glycolytic process is spontaneous, the variable x ranges from -1 to 0, what is referred to as the driving region, because process 1 (substrate level ADP phosphorylation) is driven by process 2 (glucose splitting) against its own affinity as a consequence of coupling. Once we have established a glycolytic model that has been described using a nonequilibrium thermodynamic approach, we are in a position to address the aim of this research.

Results and Discussion

Is the Rate of ATP Production the Function Optimized by Evolution?

The current opinion that the contemporary glycolytic stoichiometry of two ATP per glucose is the result of an optimization process leading to maximize the rate of ATP production is broadly accepted (Heinrich et al. 1997, 1999; Stephani et al. 1999; Aledo and Esteban del Valle 2002). Nevertheless, in this section we would like to raise some caveats.

According to Equation 7, it becomes obvious that the flow of ATP production is maximal when $x = 0$, that is, when the phosphate potential vanishes. The maximal rate of ATP formation would therefore lead to the production of high quantities of ATP, which would be useless for the cell. Then why have most authors argued that $Z = 2$ is the outcome of an evolutionary process maximizing the rate of ATP production? In our opinion, that conclusion may be a tautological consequence of the premise used: the phosphate potential has remained constant, at the value found in modern cells, throughout all the evolutionary history. Only under such condition, natural selection could have settled the current stoichiometry as an optimal output maximizing the flux of ATP formation. Hitherto, this premise has been taken for granted in most studies, without questioning its validity. However, if we do not impose constraints on the phosphate potential, a further conclusion can be reached: the current phosphate potential itself may be the output of an evolutionary optimization process. Now, we will present these arguments in a formal way.

As pointed out in the Introduction, there is an inevitable trade-off between rate and yield in ATP-producing pathways. For glycolysis, the relationship between rate and yield can be formally analysed by means of the notation introduced in the previous section. For this purpose, the phenomenological coefficient can be considered as a measure of the yield: the number of ATP molecules produced per glucose. If the maximal yield thermodynamically feasible is denoted by Z_{Ymax}, then it follows that

$$Z_{Ymax} = -X_2/X_1 \tag{10}$$

Because we consider X_2 as a constant, Z_{Ymax} is a lineal function of the inverse of the phosphate potential ($-X_1$). Introducing Equation 10 into Equation 7 and reorganizing, we show that the normalized flow of ATP formation is a bivariate function depending on Z and Z_{Ymax}:

$$J_1/(L_{22}X_2) = (1 - Z/Z_{Ymax}) \tag{11}$$

The trade-off between rate and yield arises because the function given by Equation 11 shows a maximum at $Z = \frac{1}{2}\, Z_{Ymax}$. A yield higher than $\frac{1}{2}\, Z_{Ymax}$ can only be obtained at the cost of a lower rate (Fig. 2A). It must be noted that in accordance with Equation 11, there are two ways of affecting the normalized J_1 flow: through the modulation of Z and/or Z_{Ymax} (Fig. 2B). If, as in most previous studies, we assume that the current value for the maximal yield thermodynamically feasible ($Z_{Ymax} = 4$) is an unchangeable parameter, then we can conclude, as the aforementioned studies do, that the current stoichiometry optimize the flux of ATP production (Fig. 2B, thick line). However, if we do not impose constraints on the phosphate potential, and therefore the flux of ATP formation can be regarded as a bivariate function, depending on Z and Z_{Ymax}, then we can argue that an increase in the yield would always lead to higher J_1 (Fig. 2B, see discontinuous line). In other words, for a given stoichiometry, the rate of ATP production can increase by decreasing the phosphate potential (note that Z_{Ymax} is inversely proportional to the phosphate potential). Thus, if $Z = 2$ represents an optimal solution, then J_1 cannot be, by itself, the only target function of the optimization process. Because $Z = 2$ is optimal only when $Z_{Ymax} = 4$, we should focus our attention on Z_{Ymax}. Then, what is $Z_{Ymax} = 4$ optimal for ?

Having thrown doubts on the rate of ATP production as the main target of natural selection, one may raise the question: what is then the best working regime for glycolysis? This question will lead us to the optimization problems presented in the next section.

Stationary Working Regimes

In the foregoing section, we have advanced the idea that besides the flow of ATP formation, there are other output functions (Ω) that may be targets of optimization (Table 1). With the description of glycolysis as a free-energy converter connected to a cellular load, it should be possible to identify and analyse these functions. Because we have also noted that the phosphate potential ($-X_1$) can play a major role in determining the optimal working regime, the force ratio will be a convenient variable for these thermodynamic functions: $\Omega = f(x)$. The remainder of this section will be devoted to analyse the restrictions imposed on x to satisfy diverse working regimes. Afterwards, the comparison of these theoretical values with the *in vivo* measured force ratio will enable us to decide which one of these working regimes is performing under optimal conditions in modern cells. Finally, we will answer the driving question of this work: what are the advantages of setting $Z = 2$?

Maximum Output Power. As we have already explained, J_1 must not take extreme values. Alternatively, one could expect that it is more convenient to maximize the phosphate potential. However, from

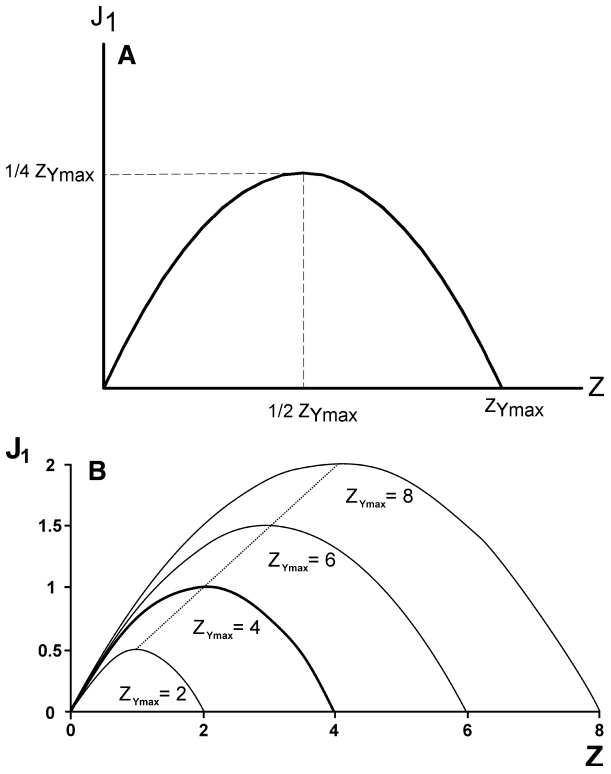

Fig. 2. Dependence of ATP production flow on the phenomenological stoichiometry. When the maximal yield thermodynamically feasible, Z_{Ymax}, is taken as a parameter, the flow of ATP formation, J_1, is a parabolic function of the variable Z. (**A**) The thermodynamic trade-off between rate (J_1) and yield (Z) is graphically illustrated. A yield higher than ½ Z_{Ymax} can only be obtained at the cost of a lower rate. (**B**) Plot of J_1 for different values of Z_{Ymax} from 2 to 8, as indicated in the figure. The thick line is the curve obtained with $X_1 = -50$ kJ/mol and $X_2 = 205$ kJ/mol, which are sound values for these variables in mammalian cells. For each curve, the flow vanishes when the yield is maximal and exhibits a maximum at half maximal yield. Loci of maximal flows (discontinuous line) are given by a lineal function of Z_{Ymax}. Flows are normalized using $L_{22}X_2$ as unit.

Equation 7 it becomes obvious that this potential is highest ($x = -1$) when the rate of ATP synthesis is zero ($J_1 = 0$). This again lacks biological sense. In contrast, the product of both the phosphate potential and the ATP production flux defines a new function referred to as output power (P_o), whose optimization by natural selection may be of biological interest (Appendix B).

$$P_o = -J_1X_1 = -L_{22}X_2^2\left(x^2 + x\right) \tag{12}$$

It is important to note that although for a fixed force ratio the rate of ATP synthesis depends on Z, an important emerging property of the output power is its independence from the phenomenological stoichiometry, in the sense that for a given value of x, the output power will always be the same regardless of the value taken by Z. Now, the value of x can be calculated for which a MOP regime is achieved:

$$dP_o/dx = -L_{22}X_2^2\,(2x + 1) = 0 \tag{13}$$

Thus, the output power exhibits an extremum at $x = -1/2 = x_{MOP}$. If the system is to operate under a MOP regime installed in a steady state, there is a second condition that needs to be satisfied: the force ratio must minimize the entropy production (Kondepudi and Prigogine 1999). The double-condition, referred to as conductance matching, takes the form of $L_{33} = L_{11}$ (Aledo and Esteban del Valle 2004).

In the foregoing discussion, we have maximized J_1 and P_o without considering the energy costs to obtain optimal working regimes. However, as pointed out by Maddox, for biological systems a high degree of fitness implies not only high flows or output powers, but also high efficiencies, low entropy productions and low energy consumption rates (Maddox 1991). We now consider these requirements. In this context, Stucki (1980) has proposed that the appropriate parameter to introduce the constraints of minimal energy costs into the previous optimization problems is the efficiency, η, defined by Kedem and Caplan (1965) as the ratio between the output power of the driven process and the input power of the driver process:

$$\eta = -J_1X_1/J_2X_2 \tag{14}$$

Herein, the efficiency can be considered as a ponderation factor affecting the function of interest.

Economical Maximum Flow. Fast but inefficient production of ATP may be a good individual strategy under certain circumstances where the cooperative behaviour is not rewarded (Pfeiffer et al. 2001), because the benefits resulting from high rates of ATP formation are confined to the individual, whereas the cost in terms of glucose depletion and energy dissipation are shared among all users of the resource. However, cooperative use of external energy resources may evolve in spatially structured environments (Pfeiffer and Bonhoeffer 2003). In this scenario, an economical maximum flow (EMF) regime could represent an advantageous *modus operandi*. The economical flow function is given by the product of the net flow of ATP production, J_1, and the thermodynamic efficiency, η (Appendix C):

$$J_1\eta = -ZL_{22}X_2\left(x^2 + x\right) \tag{15}$$

Plots of this function versus the force ratio for various stoichiometric coefficients are depicted in Fig. 3. In all cases, the force ratio maximizing Equation 15 is $x_{EMF} = -1/2$. Hence, it can be concluded that MOP and EMF regimes require the same conductance matching ($L_{33} = L_{11}$). However, as has been already noted, the value of the maximal economic flux that can be achieved depends on the stoichiometric coefficient (Fig. 3). Then, if the evolutionary goal was to provide an economical flux as

Table 1. Putative target functions for the evolutionary optimization of glycolytic bioenergetics[a]

Biophysical meaning	Function $\Omega = f(x)$	Force ratio making $_{\Omega max}$	Conductance matching
Flow	$J_1 = ZL_{22}X_2\,(x + 1)$	0	—
Output power	$P_o = -L_{22}X_2^2\,(x^2 + x)$	−1/2	$L_{33} = L_{11}$
Economical flow	$J_1\eta = -ZL_{22}X_2\,(x^2 + x)$	−1/2	$L_{33} = L_{11}$
Economical power	$P_o\eta = L_{22}X_2^2\,(x^3 + x^2)$	−2/3	$L_{33} = ½\ L_{11}$

[a]The description of glycolysis as a free-energy converter allows identification and analysis of diverse functions, Ω, which may have been the target of optimizing processes through evolution.

high as possible, there would be two approaches: one, imposing an EMF by setting the phosphate potential in order to yield x_{EMF}, and the other by increasing Z. However, both aims could be achieved simultaneously only by decreasing the chemical potential of ATP. If x_{EMF} and X_2 are constants, then any increase in Z would imply an equivalent decrease in the phosphate potential:

$$x_{EMF} = \text{constant} = X_1 \downarrow \frac{Z \uparrow}{X_2} \tag{16}$$

Economical Maximum Output Power. Finally, we consider the output power of glycolysis as constrained by minimal energy costs. The value of force ratio permitting an economical maximum output power (EMOP) can be found by maximization of the function (Appendix C)

$$P_o\eta = L_{22}X_2^2\left(x^3 + x^2\right) \tag{17}$$

which occurs at $x = -2/3 = x_{EMOP}$. Now, the conductance matching allowing the system to perform at EMOP in steady state, is given by the expression $L_{33} = ½\ L_{11}$ (Aledo and Esteban del Valle 2004).

What Is the Glycolytic Stoichiometry of Two ATP per Glucose Optimal for?

In the foregoing section, we have noticed different working regimes, and we have also explicitly expressed the conditions necessary and sufficient to allow glycolysis to operate in steady state under these regimes. Thus, we are now able to address the question: Which regime operates for glycolysis in living cells? In contemporary glycolysis, where Z = 2, and the phosphate potential in most cells is around 50 kJ/mol whereas the affinity for glucose splitting is about 205 kJ/mol, the force ratio turns out to be approximately $x = -1/2$. This means that glycolysis must be performing at MOP as well as at EMF (Table 1). Next, we have calculated Ω (−1/2) for different values of Z (Table 2). It is important to stress that although the MOP is not correlated with Z, in contrast, the EMF increases with an increase in Z. Thus, if

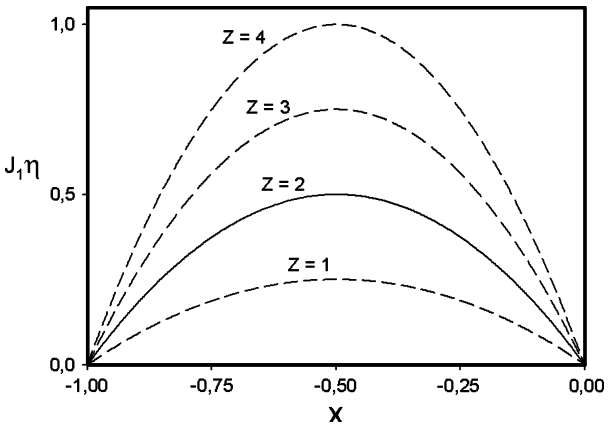

Fig. 3. Economical flow as a function of the force ratio. Plots of the economical flow function described by Equation 16 are shown for different phenomenological stoichiometries as indicated in the figure. In all of the cases, the force ratio maximizing the economical flow is $x_{EMF} = -1/2$. Flows are normalized using $L_{22}X_2$ as unit.

achieving an economical flow as high as possible was the main goal of glycolytic evolution, optimally one would expect to find Z > 2. Hence, we conclude that optimization of the output power most probably has been an important driving force leading to the actual design of the glycolytic pathway. However, because under optimal conditions ($x = -1/2$) the output power is always the same, regardless of the stoichiometric coefficient, one question still remains open: why Z = 2? One may feel tempted to conclude that this value is evolutionarily neutral and that the current glycolytic stoichiometry is a frozen accident. Although this possibility cannot be completely ruled out, there are some considerations suggesting that Z = 2 may have conferred a selective advantage. Hitherto, we have only been concerned with the evolutionary optimization of glycolytic as a device for extracting energy from glucose under anaerobic conditions. However, glycolysis must perform in the context of global cellular metabolism. Therefore, in the remaining discussion we shall consider the effects of the glycolytic stoichiometry on other metabolic processes.

From Table 2, it can be noted that the phosphate potential is negatively correlated with Z. At this point, it is important to realize that because the output power is the same in all the cases, a lower

Table 2. What is Z = 2 optimal for?[a]

Z	$-X_1$	J_1	P_o	$J_1\eta$	η	Quantum energy (J)
1	1/2	0.5	0.25	0.125	0.5	$16.6\ 10^{-20}$
2	1/4	1.0	0.25	0.500	0.5	$8.30\ 10^{-20}$
3	1/6	1.5	0.25	0.750	0.5	$5.53\ 10^{-20}$
4	1/8	2.0	0.25	1.000	0.5	$4.15\ 10^{-20}$

[a]The force ratio found in most cell is around $x = -1/2$ allowing glycolysis to perform at MOP and EMF. The values shown are the outcome of the indicated functions when $x = -1/2$. Phosphate potential is expressed as the fraction of the input force. The flow of ATP formation and the economical flow are normalized using $L_{22}X_2$ as unit. The output power is normalized with $L_{22}X_2^2 = 1$. The quantum energies are calculated assuming an input force of 200 kJ/mol. The quantum energy is defined as the energy released by the conversion of a single molecule of ATP to ADP + P_i.

phosphate potential in this case does not mean a reduced energetic budget. Although the glucose-derived energy used by the cell is the same independently of the phosphate potential, low phosphate potential implies low ATP and high ADP concentrations, and vice versa. In both cases, the kinetic and regulatory implications would be deep (Hardie and Hawley 2001). Because ATP and ADP are involved, either as substrates or as allosteric modulators, in most catabolic and anabolic pathways, their concentrations will affect the rates of many cellular reactions. Furthermore, these adenine nucleotides are also substrate and product of protein kinases that regulate most aspects of cell life. Not surprisingly, one of the most fundamental parameters that any healthy cell must maintain is a suitable ratio of ATP to ADP, even if the energetic demand is properly met.

In addition to the preceding kinetic-regulatory reasoning, there are also energetic arguments supporting the convenience of an intermediate phosphate potential, that is of Z = 2. Again we must stress that these energetic arguments are independent of the amount of energy that the cell can withdraw, and use, from glucose. As long as glycolysis performs at MOP, the cell will be provided with a fixed amount of energy per time, regardless of Z. However, the quantum energy, defined as the maximal work that the conversion of a single molecule of ATP to ADP plus P_i could do, is strongly influenced by Z (Table 2). Consequently, changes in Z might dramatically affect the efficiencies and/or the stoichiometries of all those cellular processes powered by ATP. In this sense, a value of Z = 1, implying high quantum energies, would lead to highly inefficient ATP-driven reactions. On the other hand, Z > 2 lead to low quantum energies that may compromise the exergonity of those reactions coupled to the hydrolysis of ATP (see Appendix D for a concrete example illustrating these points). Even if the change in actual Gibbs free-energy of the overall reaction is made favourable by coupling the endergonic process to the hydrolysis of two or more ATP molecules, a low quantum energy can represent a handicap in evolutionary terms. Using more than one ATP to impulse a enzyme-catalyzed transformation imply, in mechanistic terms, an increase of the number of elementary reactions involved in the global process. It must be noted that for a spontaneous multistep reaction, a negative value for the free energy change of the global process is a necessary requisite, but it is not sufficient. The overall reaction will proceed spontaneously if, and only if, all and every one of the elementary reactions are spontaneous by themselves (Aledo et al. 2003), which still may be hampered by low ATP quantum energies. On the other hand, ATP forms a hub within the metabolic network (Pfeiffer et al. 2005). That means that this molecule is involved in far more reactions than the average. Under physiological conditions, the number of reactions using ATP as reactant is overwhelming, in sharp contrast with the low number of reactions where ATP is a product (Keseler et al. 2005). Therefore, in the considered scenario, where the ATP stoichiometries of the anabolic processes are allowed to increase, cells would face the formidable necessity to find a tremendous number of suitable elementary reactions, further constrained by low endergonicity as imposed by low ATP quantum energies.

In the previous section, we have reached the conclusion that, in terms of output power, the value of Z may be evolutionarily neutral. However, as has been already pointed out, glycolysis certainly did not evolve as an isolated entity. Additional arguments against Z > 2 can be found when the chemical and biological feasibility of such alternatives is analysed. Any Z > 2 would mean the necessity of additional reactions able to transfer phosphoryl groups to ADP (Manchester 2000). Even if we assume that these additional reactions are feasible, an assumption that seems to be unlikely (Meléndez-Hevia et al. 1997), the consequences of increasing both the number of enzyme-catalyzed reactions and metabolite intermediates (Pfeiffer and Bonhoeffer 2004) would be selectively disadvantageous with respect to lower values of Z.

Conclusions

A substantial number of studies addressing the optimal stoichiometric design of glycolysis have

been published. Hitherto, most of these studies focus their attention on the flow of ATP formation as a target of natural selection, and they conclude that the stoichiometry of two ATP per glucose is the outcome of evolution in order to optimize the rate of ATP formation. However, because the main biological function of glycolysis is to supply energy to the cell, it seems obvious that our interest should be focused on the output power, instead of the ATP formation flow, as a target of natural selection. Herein, using the formalisms introduced by Kedem and Caplan, we have shown that in most cells, glycolysis may perform under optimal output power and not at optimal flow of ATP production. Furthermore, we argue that the contemporary stoichiometry is not a necessary condition to yield an optimal output power, because different stoichiometric coefficients could be compatible with the same optimal output power. Thus, we conclude that the current glycolytic stoichiometry is the output of an evolutionary process optimizing not only the energy transformation from glucose to ATP, but also the ATP-derived energy utilization. Consequently, we hypothesize that changes in the glycolytic stoichiometry might dramatically affect the efficiency of all those cellular processes powered by ATP. In summary, the current stoichiometry of glycolysis can only be fully understood if we are aware that metabolism is a small-world type network wherein ATP is one of the most prominent hubs.

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Appendix A: Derivation of the Flows as Functions of the Force Ratio

Starting from Equation 2 in the main text,

$$J_1 = L_{11} X_1 + L_{12} X_2 \tag{2}$$

After extracting common factor X_2, we have

$$J_1 = (L_{11} X_1/X_2 + L_{12}) \cdot X_2 \tag{A1}$$

From the definitions of coupling degree ($q = L_{12}/(L_{11}L_{22})^{1/2}$), phenomenological stoichiometry ($Z = (L_{11}/L_{22})^{1/2}$), and force ratio ($x = ZX_1/X_2$), it follows

$$L_{12} = q(L_{11}L_{22})^{1/2} \tag{A2}$$

$$L_{11} = Z^2 L_{22} \tag{A3}$$

$$X_1/X_2 = x/Z \tag{A4}$$

Substituting A2, A3, and A4 into Equation A1

$$J_1 = X_2 \left(Z^2 L_{22} x \Big/ Z + q(L_{11}L_{22})^{1/2}\right) \tag{A5}$$

Taking out common factor L_{22} from Equation A5

$$J_1 = L_{22} X_2 \left(Zx + (q/L_{22})\,(L_{11}L_{22})^{1/2}\right) \tag{A6}$$

Again from the definition of phenomenological stoichiometry, it can be established

$$(1/L_{22})\,(L_{11}L_{22})^{1/2} = (L_{11}L_{22})^{1/2} = Z \tag{A7}$$

Thus, Equation A6 can now be rewritten as

$$J_1 = ZL_{22}X_2\,(x + q) \tag{A8}$$

Because for glycolysis, where glucose consumption and ATP production are tightly coupled, we can take $q = 1$. Then it follows

$$J_1/ZL_{22}X_2 = x + 1 \tag{7}$$

it can be noted that Equation 7 from the main text is a particular case of the more general Equation A8. Similar reasoning leads to Equations 8 and 9.

Appendix B: Output Power as a Function of the Force Ratio

From Equation 7 and the definition of output power as the product $-J_1X_1$, it follows

$$P_o = -ZL_{22}X_2\,(x + 1)\,X_1 \tag{B1}$$

According to the force ratio definition: $ZX_1 = xX_2$. Thus, substitution into Equation B1 yields Equation 12:

$$P_o = -L_{22}X_2^2\,(x^2 + x) \tag{12}$$

Appendix C: Economical Flow and Economical Output Power

We have defined the thermodynamic efficiency as the ratio between the output power of the driven process and the input power of the driver process:

$$\eta = -J_1X_1/J_2X_2 \tag{14}$$

Introducing Equations 7 and 8 into Equation 14, we have

$$\eta = -ZL_{22}X_2\,(x + 1)X_1/L_{22}X_2\,(x + 1)X_2 \tag{C1}$$

Bearing in mind the definition of the force ratio, Equation C1 can be simplified to

$$\eta = -x \tag{C2}$$

Therefore, the economical flow can be obtained multiplying Equations C2 and 7:

$$J_1\eta = -ZL_{22}X_2\left(x^2 + x\right) \quad (15)$$

Similarly, the product of the efficiency (Equation C2) and the output power (Equation 12) gives the function we have denoted as economical output power (Equation 17).

Appendix D: Influence of the Glycolytic Stoichiometry on the Efficiency and/or Structure of the Cellular Processes Driven by ATP

From Table 2 we can see that, whenever glycolysis is performing under MOP, different glycolytic stoichiometries imply different quantum energies. How may these quanta affect those processes driven by ATP? Although to answer this question we will focus on one concrete example, the sodium pump, the conclusions can, in fact must, be extrapolated to other processes propelled by ATP. The (Na^+-K^+)-ATPase pumps three Na^+ out of the cell and two K^+ into the cell, against their respective electrochemical gradients at the expense of one molecule of ATP. Considering transmembrane electrochemical potential differences of −12.7 and +0.9 kJ/mol for sodium and potassium, respectively, which are sound values for these variables in mammalian cells (Aledo 2004), the energy required to carry out a pumping cycle is $(3 \times 12.7 + 2 \times 0.9)/6.02 \cdot 10^{23} = 6.6 \cdot 10^{-20}$ J. There are two points to be noted. First, with the known stoichiometry of the (Na^+-K^+)-ATPase of 3 Na^+: 2 K^+: 1 ATP, only when $Z < 3$ the (Na^+-K^+)-ATPase can be powered by ATP (see the quantum energy column in Table 2). Second, the efficiency of the pumping process is drastically reduced from $\eta = 6.6 \cdot 10^{-20}/8.6 \cdot 10^{-20} = 0.76$ when $Z = 2$ to $\eta = 6.6 \cdot 10^{-20}/16.6 \cdot 10^{-20} = 0.39$ when $Z = 1$. This simple example illustrates how a change of the structural design of a metabolic pathway such as glycolysis can deeply influence all those cellular processes that are powered by ATP.

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