The Metabolic Productivity of the Cell Factory*

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It is widely accepted that some performance function has been optimized during the evolution of metabolic pathways. One can study the nature of such a function by analogy with the industrial manufacturing world, in which there have been efforts over recent decades to optimize production chains, and in which it is now accepted that fluxes are not the only important system variables that determine process efficiency, because inventory turnover must also be considered.

Inspired by the parallels between living cells and manufacturing factories, we propose that fluxes and transit time may have simultaneously been major targets of natural selection in the optimization of the design, structure and kinetic parameters of metabolic pathways. Accordingly we define the ratio of flux to transit time as a performance index of productivity in metabolic systems: it measures the efficiency with which stocks are administered, and facilitates comparison of a pathway in different steady states or in different tissues or organisms.

For a linear chain of two enzymes, at a fixed total equilibrium constant, we have analysed the variation of flux, transit time and productivity index as functions of the equilibrium constants of the two steps. The results show that only the productivity index has a maximum, which represents a good compromise in optimizing flux and transit time. We have extended control analysis to the productivity index and derived the summation theorem that applies to it. For linear chains of different length with maximum productivity index values, the distribution of control coefficients with regard to the three parameters has a characteristic profile independent of the length of the chain. Finally, this control profile changes when other variables are optimized, and we compare the theoretical results with the control profile of the first steps of glycolysis in rat liver.

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Introduction

Metabolic flux J and the metabolite concentrations X_j have traditionally been considered the system variables of interest in metabolic pathways, and so the summation and connectivity theorems for flux and concentration are taken to be a sufficient subset of relationships for fully characterizing the steady state of a system (Kacser & Burns, 1973; Heinrich & Rapoport, 1974; Cascante *et al.*, 1989a, b; Heinrich *et al.*, 1991; Fell, 1992). The summation theorem for flux control coefficients supplies valuable information about the degree of control that any enzyme exercises over the flux through a pathway; in contrast the summation theorem for concentration control coefficients do not provide information about the distribution of control among the enzymes.

From the point of view of production efficiency, however, flux control cannot be considered independently of the control of metabolite concentrations. As Hofmeyr & Cornish-Bowden (1991) have pointed out,

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"an efficiently regulated metabolic system must likewise allow the flux of matter to vary with the least possible variation in metabolic concentrations. . . . We shall consider both aspects of metabolic regulation and their relationships with one another". To take account of these concentrations, it may be informative to consider control coefficients for their sum, $\sigma = \Sigma X_j$, as a weighted average of individual metabolic control coefficients, in addition to control coefficients of fluxes.

It is often said that the main problem in analysing metabolic systems is their great complexity. This complexity is due to the level of interactions of the parts rather than to the number of forces and laws to which they are subjected (Bergethon & Simons, 1990). Trying to understand and measure their behaviour by exploring each one of these interactions may lead us to a labyrinth, but as a first approximation well characterized macroscopic systems may provide a starting point. For example, exploring similarities between industrial manufacturing and biological processes will give us important clues for understanding and measuring metabolism. Like a cell, a factory can be outstandingly complex, with a large number of machines, products and control systems, but in the end the number of really important variables is very small.

Consider, for example, a car assembly plant in which the average stock of wheels during a year is 100 units. Is this too high? Is the factory using the inventory efficiently? For a factory selling 100 cars per year, the inventory is clearly too high, but if it is selling 100 000 cars per year it has attained excellence. Thus, the absolute level of stocks of components is not by itself informative about system fitness, and it is useful to relate inventory to sales. Dividing sales by inventory (stocks) for a given period provides the number of times that the inventory has turned over or been replaced during that period. Inventory turnover has been widely used as a fundamental business measure of productivity and it is easy to understand; it can be measured and monitored, and it reacts with minimum lag to changed conditions (Fogarty *et al.*, 1991). Moreover, the inverse of the turnover gives us the average time that an item remains in store. This time must be minimized in order to have the maximum production flux with the minimum accumulation of intermediate products inside the system.

A high flux does not necessarily indicate a efficient production chain; if high flux is accompanied by excessive accumulation of stocks, the turnover is low and the production process is unlikely to be optimal. An important parameter to be minimized in a production process is the average manufacturing throughput time (the flow time required for a part to move from the start to the completion of a process), which is directly proportional to the stocks of components and inversely proportional to the production flux.

Cellular concentrations of metabolite pools are generally given as absolute values, with no reference to the flux through each pool. But if we tell the production manager of a car company that the concentration of ATP in the human cell is around 1–3 mM, he will immediately object that this number provides no information about the performance of energy metabolism, and will want to know the annual turnover of ATP in a human being? If we answer around 12 million per human being, he will realize that car factories are far away from the productivity of a living cell.

How Can Metabolite Pools be Used to Describe the Performance of Metabolic Pathways?

The values of internal metabolite pool levels in the pathway ($\sigma = \Sigma X_j$) provide little information about pathway performance. A high value of σ may correspond to a very well designed system, or a very badly designed one, depending on the value of the corresponding steady-state flux.

In this paper we propose that the performance of a metabolic pathway with regard to pool metabolite levels (X_j or σ) must be evaluated as a value relative to the flux along it, as is generally done in production process design and inventory management in manufacturing companies. The value of X_j/J or σ/J provides a measure of metabolite levels referred to a temporal scale. In many cases, this quotient has the additional physical meaning of the average time that a molecule remains in the metabolite pool before being transformed, which is known as the transit time, τ, (Easterby, 1981; Meléndez-Hevia *et al.*, 1990; Cascante *et al.*, 1995).

Thus, τ must be seen not only as a transit time through the pathway, but also as a relative measure of metabolite stocks with regard to the flux, allowing us to compare a metabolic system in different situations or two similar systems, as for instance the same metabolic pathway in different tissues. It is not very useful to compare metabolite concentrations directly without first dividing by the flux. For instance, under the appropriate stimulus, a metabolic pathway may move to another steady state, and we can observe a high flux increase that satisfies the demand, accompanied by a moderate increase in internal meatabolite levels. If we directly compare σ

in the two steady states we reach the erroneous conclusion that the quality of the new steady state with regard to σ is worse! However, if we compare τ, the relative stocks of internal metabolite with regard the flux, we will see that it is lower in the second steady state, as it corresponds to a system better adapted to work in the steady state, resulting from the given stimulus. It should be noted that it is not correct to compare τ in systems with very different features. So, for instance, the minimum τ that a system with large number of steps can achieve will always be greater than that for a shorter system.

We can conclude that τ is a good function for comparing internal metabolite pool levels under different cell conditions, in different tissue or in different organisms. It should also be noted here that τ as defined above does not describe the rate of transition between steady states; its temporal meaning refers the average time that a molecule spends in the intermediate pool of a given metabolic pathway.

Evolutionary Optimization of Metabolic Performance Functions

If it is assumed that some performance function was optimized during evolution (Cornish-Bowden, 1976; Heinrich & Holzhütter, 1985; Heinrich et al., 1987; Pettersson, 1992), then obviously such a function should depend on the physical properties characterizing the metabolic systems, such as pool concentrations and fluxes. The essential is to define a relevant performance function based on systemic properties of the metabolic pathway. Minimization of response times (Heinrich & Sonntag, 1982; Meléndez-Hevia et al., 1994; Heinrich & Schuster, 1991; Torres et al., 1991), and stoichiometric simplicity (Meléndez-Hevia & Isidoro, 1985; Meléndez-Hevia & Torres, 1988; Meléndez-Hevia, 1990) have been proposed as targets of evolutionary optimization. Fluxes leading to important metabolic end products have also been proposed (Waley, 1964; Atkinson, 1969; Cornish-Bowden, 1976; Kacser & Beeby, 1984; Reich, 1985; Dykhuizen et al., 1987; Heinrich et al., 1987, 1991; Heinrich & Hoffmann, 1991; Meléndez-Hevia & Montero, 1991). However, fluxes are not the only relevant performance functions; transit time, the sum of intermediate metabolite levels referred to a temporal scale ($\tau = \sigma/J$), could also be a target of natural selection. There are many different soluble metabolic intermediates in cells, and their total level is close to the solubility limit (Atkinson, 1969). Several authors (Atkinson, 1969; Savageau, 1976; Schuster & Heinrich, 1987; Ovádi, 1991; Brown, 1991) have proposed that there is likely to be selective

pressure to keep individual metabolite intermediate levels low, in order to avoid "salting out" of other metabolites and proteins, and to reduce the occurrence of unintended side reactions and interference between different pathways. Unnecessarily high stocks of metabolites also result in increased transit times, which, in general, correspond to systems that are slow to switch from one steady state to another.

In our macroscopic system, a high inventory is viewed as a index of inadequate management, a method of hiding inefficiencies and problems (Hall, 1983) and does not add value but instead incurs costs, and thus is a waste (Fogarty et al., 1991). The real goal is to have no stocks anywhere that are not actively in process.

We can conclude, therefore, that not only fluxes but also transit times may have been simultaneously important targets of natural selection in the evolutionary optimization of the design structure and kinetic parameters of the pathway.

Which System Properties Must be Considered to Construct the Most Suitable Performance Function to Optimize the Production in a Metabolic System?

Let us consider two-enzyme variant of the system shown in Fig. 1, where an initial substrate S is converted into the end product P through a linear sequence of two enzyme-catalysed steps. The net equilibrium constant, $q_T = (P/S)_{eqm}$, between S and P is taken as 100 by definition, but the individual equilibrium constants of steps 1 and 2 (q_1 and q_2
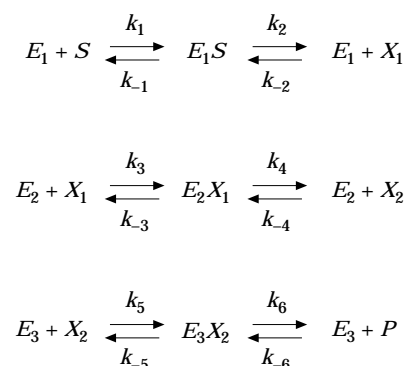
$$E_1 + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_1S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E_1 + X_1$$

$$E_2 + X_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E_2X_1 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E_2 + X_2$$

$$E_3 + X_2 \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} E_3X_2 \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} E_3 + P$$

FIG. 1. Pathways analysed. A series of enzymes E_1, E_2... catalyse conversion of a starting substrate S into a final product P, via intermediates X_1, X_2.... The particular case of three enzymes is illustrated, but other cases are also considered, with an obvious extension of the symbolism. For example, in the case of a two enzyme pathway the last reaction is absent and P replaces X_2 in the second.
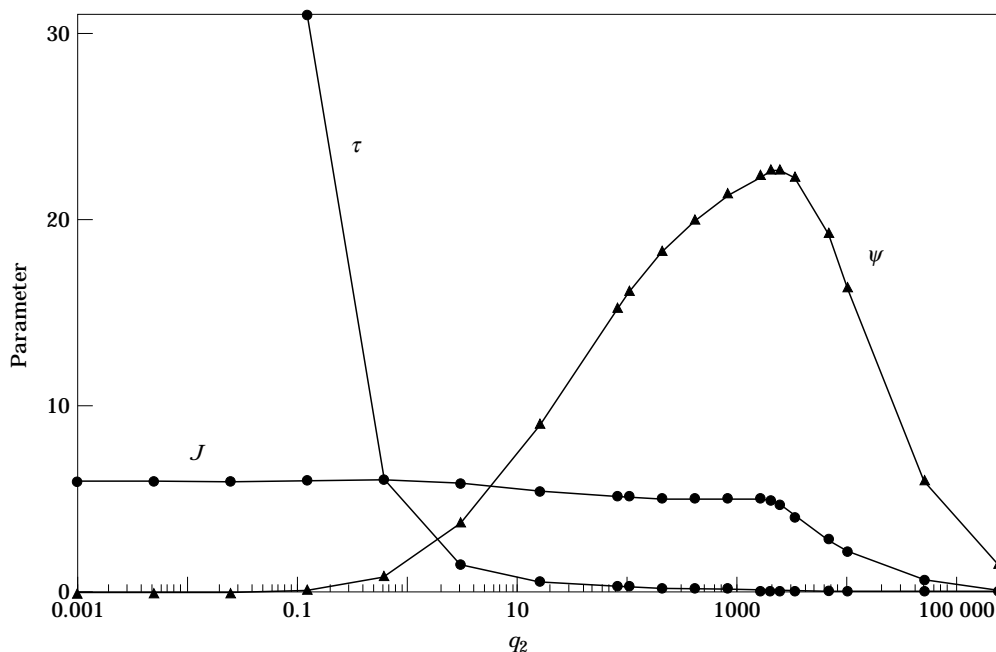
Fig. 2. Variation of J, τ and ψ with respect to the equilibrium constant q_2 for the second reaction of Fig. 1 with a total of two reactions (i.e. without the third reaction shown in Fig. 1). At each value of q_2 the equilibrium constant q_1 for the first step was constrained to maintain the overall equilibrium constant $q_1 q_2$ constant at 100, and the concentrations of S and P were held at 100 and 5 respectively. In each case the kinetic constants k_1, k_{-1}, k_2 and k_{-2} were adjusted to find the maximum value possible for J, or ψ, or the minimum value of τ, the three system variables being separately optimized in three separate adjustments of the kinetic constants.

respectively) are allowed to vary, subject to the following constraint:

$$q_T = q_1 q_2 = 100$$

In addition, the concentrations of S and P are fixed at $S = 100$ and $P = 5$, so that the overall driving force is constant.

In analysing the design of any pathway, one can distinguish between the *kinetic design* (expressed in the kinetic constants k_i, k_{-i}) and the *thermodynamic design* (expressed in the equilibrium constants q_1, q_2). Different thermodynamic designs for a metabolic pathway converting S into P can result from different choices of internal metabolites (X_j). It is also possible to couple ATP synthesis and degradation to particular steps in the pathway, thereby changing the apparent q_1 and q_2 values. For any particular thermodynamic design (fixed q_1 and q_2) the values of the kinetic constant can be optimized. However, out of all the possible thermodynamic designs with the kinetic constants optimized, there may exist a unique optimum design with regard to a given performance function.

In this paper we have used a gradient method to optimize several performance functions by adjusting the values of the kinetic constants, for various values of q_1 and q_2. Note that there is only one degree of

freedom for varying the thermodynamic design, because q_1 is automatically fixed by any choice of q_2. With J as the performance function and q_2 greater than about 1000, the optimum J decreases monotonically as q_2 increases, with a limit of zero (Fig. 2). With q_2 in the range 1–1000, the optimum J is approximately constant, independent of q_2. The range of q_2 values in which the optimum J is approximately independent of q_2 depends on the degree of saturation, the overall equilibrium constant and the overall driving force. However, when q_2 is less than one the optimum J is more strictly constant, and in this range the property is independent of the degree of saturation, the overall equilibrium constant and the overall driving force.

Figure 2 also includes the results of similar analysis of the variation of optimum τ, done by searching for the values of k_i and k_{-i} that minimize τ at different values of q_2 and q_1. There is a monotonic decrease in τ when q_2 increases above 100, whereas it increases sharply towards when q_2 decreases below zero.

These results show that maximization of flux does not guarantee the optimal performance of the system, because maximal J can be accompanied by high values of τ, which means that the system is working with unnecessarily high stocks, to the detriment of cellular osmolarity and possibilities to stock

other metabolites necessary for other pathways. In addition, a system with high τ will be slow to switch from one steady state to another. Although τ may seem attractive as an alternative choice of performance function, as its minimization results in minimization of the stocks (σ), this is achieved with flux tending to zero, as it is tacitly assumed that σ can be zero. So, internal metabolite concentrations must be minimized within the constraint that they are kept above a minimum level that ensures that enzymes are working. In the same way, stock levels in the factory must not be so low that the process comes to a halt. The nature of this inventory may be diverse: minimum quantities of material required to keep the machinery running, inventory to fill the transportation network, etc. The living cell similarly has indispensible inventories, which we could call physiological inventories.

Consequently, in choosing the most suitable performance function to optimize the production of the end product in a metabolic system we need not only to minimize τ, but also to maximize flux simultaneously. We therefore define a new function $\psi = J/\tau$, which we regard as a good performance index for measuring the optimization of productivity of the metabolic system. It facilitates the comparisons of a pathway in different steady states, or the same pathway in different tissues or organisms, because it measures the efficiency with which stocks are administered. Optimal administration of stocks means the ability to produce what is needed and convey it to where it is needed precisely when required; this requires high efficiency and substrate specificity of the enzyme machinery, good structural design of the pathway, adequate control patterns, a good quality control, etc. A decrease in ψ is an indicator of the loss of efficiency that may follow from failure of any of these. We can expect that the more evolved a living organism is, the higher ψ is for the same pathway with the same purpose. A high value of ψ indicates flexibility, quality and responsiveness in a metabolic system.

In the context of business, significantly improved inventory turnover typically requires process improvements (Clay, 1994). Companies with high inventory turnover usually prove to have focused their attention on reducing set-up times, improving control and scheduling systems, reducing lead times, increasing flexibility, improving relationships with suppliers, achieving good quality control and reliable processes, etc. These general objectives also apply to the living cell, and ψ provides an overall measure of them.

Properties of the Index of Metabolic Productivity Performance

The behaviour of optimum ψ when the kinetic constants k_i and k_{-i} are varied to maximize ψ for different values of q_1 and q_2 is shown in Fig. 2. In the particular case illustrated ψ shows a maximum at $q_2 = 2273$ and decreases monotonically to a limit of zero on either side of this. In general, for any pathway of two enyzmes, the value of q_2 that maximizes ψ always satisfies the inequality $q_2 \geqslant q_T$.

The absolute maximum of the performance index, $\psi = 22.65$, corresponds to a value of J of 4.66, close to the maximum possible, and to a low value of τ of 0.206, values that illustrate the fact that ψ represents a good compromise in optimizing J and τ simultaneously in the metabolic system, and that it is a suitable performance index, a high value indicating high productivity of the system. Thus, it is reasonable to expect that optimization of productivity may have been the target of natural selection, with flux and transit time optimized simultaneously.

Metabolic Control Analysis of ψ: the Summation Theorem for its Control Coefficients of ψ

Control analysis has mainly been focused on the distribution of flux control among the enzymes on a pathway (Kacser & Burns, 1973; Heinrich & Rapoport, 1974; Fell, 1992), but in recent years the control analysis of τ has also been developed (Meléndez-Hevia *et al.*, 1990; Cascante *et al.*, 1995). As we have shown the suitability of the systemic variable ψ as a performance index of a metabolic system, we now extend control analysis to it and derive the relevant summation theorem.

We define the *productivity performance index control coefficient* as the ability of an enzyme to control ψ:

$$C_{e_i}^{\psi} = \frac{\partial \psi}{\partial e_i} \frac{e_i}{\psi}.$$

As ψ was defined as J/τ, it is evident that the differentiation with respect to e_i must yeild the following expression:

$$C_{e_i}^{\psi} = C_{e_i}^{J} - C_{e_i}^{\tau}$$

where $C_{e_i}^{J}$ and $C_{e_i}^{\tau}$ are the familiar flux and transit time control coefficients respectively:

$$C_{e_i}^{J} = \frac{\partial J}{\partial e_i} \frac{e_i}{J}$$

$$C_{e_i}^{\tau} = \frac{\partial \tau}{\partial e_i} \frac{e_i}{\tau}.$$

TABLE 1

Values of the equilibrium constants q_i *that give maximum* ψ, *for fixed overall equilibrium constant* $q_T = 100$, *and fixed concentrations* $S = 100$ *and* $P = 5$ *of the external metabolites*

Equilibrium constant	Number of enzymes			
	2	3	4	5
q_1	0.0439	0.0332	0.0293	0.0289
q_2	2273	21.98	9.771	4.231
q_3		136.8	6.387	5.269
q_4			54.63	5.275
q_5				29.41

The values refer to the set of schemes represented by Fig. 1, with different numbers of enzymes from 2 to 5.

As it is established that $\Sigma C_{e_i}^J = 1$ (Kacser & Burns, 1973; Heinrich & Rapoport, 1974), and $\Sigma C_{e_i}^\tau = -1$ (Meléndez Hevia *et al.*, 1986) it follows that:

$$\Sigma C_{e_i}^\psi = 2$$

where in each case the summation is over all enzymes. This is the summation theorem for control coefficients of ψ.

If an enzyme has a high control coefficient for ψ it has a high degree of control over the performance of the pathway, so that increasing its activity results in a productivity improvement, with better administration of the system stocks.

Characteristics of a Pathway with Maximized Productivity Performance Index (ψ)

For any metabolic sequence with a fixed overall equilibrium constant (q_T) there is one (and only one) distribution of kinetic constants of the individual steps that maximizes ψ. This optimal set of kinetic constants determine the thermodynamic profile of the pathway (Table 1).

There is no general solution for this distribution of kinetic constants, because it depends on the overall equilibrium constant, the values of the fixed external variables of the system and the number of steps in the pathway. However, there are some general characteristics of the control distribution in this state of maximum ψ. If we consider a sequence of two, three, four or five enzymes (Fig. 1) and we analyse the control distribution of J, τ and ψ among the enzymes

TABLE 2

Values of the performance indices (J, σ, τ, ψ), *intermediate concentrations of* (X_1 *to* X_4) *and control coefficients* (C) *in the optimized schemes represented by Fig. 1, with different numbers of enzymes from 2 to 5*

Parameter	Optimal cases				Sub-optimal cases		
	Number of enzymes				Three enzymes		
	2	3	4	5	3a	3b	3c
J	4.66	3.81	3.28	2.85	5.41	0.188	3.59
σ	0.962	1.84	2.99	4.10	258.5	0.042	25.66
τ	0.206	0.48	0.91	1.43	47.7	0.223	7.1
ψ	22.65	7.80	3.59	1.98	0.11	0.840	0.50
X_1	0.962	0.93	0.98	1.126	66.8	0.020	25.02
X_2		0.91	1.09	1.013	191.6	0.022	0.64
X_3			0.92	1.000			
X_4				0.966			
$C_{e_1}^J$	0.565	0.497	0.440	0.381	0.958	0.572	0.42
$C_{e_2}^J$	0.434	0.416	0.390	0.345	0.020	0.411	0.33
$C_{e_3}^J$		0.080	0.110	0.165	0.020	0.015	0.24
$C_{e_4}^J$			0.030	0.073			
$C_{e_5}^J$				0.033			
$C_{e_1}^\tau$	0.492	0.35	0.28	0.259	0.959	0.00	0.42
$C_{e_2}^\tau$	−1.492	−0.48	−0.17	−0.067	−0.381	−0.486	−0.79
$C_{e_3}^\tau$		−0.86	−0.52	−0.313	−1.57	−0.513	−0.62
$C_{e_4}^\tau$			−0.59	−0.416			
$C_{e_5}^\tau$				−0.461			
$C_{e_1}^\psi$	0.072	0.14	0.150	0.122	−0.001	0.572	0.0004
$C_{e_2}^\psi$	1.927	0.90	0.570	0.413	0.402	0.897	1.12
$C_{e_3}^\psi$		0.95	0.638	0.479	1.59	0.529	0.87
$C_{e_4}^\psi$			0.630	0.490			
$C_{e_5}^\psi$				0.490			

In the sub-optimal cases the values of the equilibrium constants differed from those that maximized ψ (see text).

(Table 2), under the constraints of $q_T = 100$, $S = 100$, $P = 5$ and $e_i = 10$, we observe that despite the variation in number of enzymes the control of flux is mainly shared between the first two steps. The control coefficient for τ is always positive for the first enzyme and negative for the others, increasing in absolute value for the second enzyme to the last. Finally, the control coefficient for ψ is always low for the first enzyme and it is approximately equally distributed among the other enzymes in the pathway. All control coefficients for ψ are positive in these examples, though negative values are possible.

On the other hand, the absolute maximum of ψ in each system decreases as the number of steps increases, as does the optimum flux, whereas the corresponding τ and σ at the point of optimum ψ both increase. Selective advantages of short pathways have been described by Meléndez-Hevia *et al.* (1994) [see also Heinrich *et al.* (1987) and Savageau (1975)]: the maximum value of the flux possible in a metabolic system decreases as the number of reactions increases. Our results amplify this result by demonstrating that increasing the length of the chain also decreases the maximum possible value of the productivity performance index (ψ), and increases the minimum possible value of τ and σ.

Characteristics of a Pathway With Low Productivity Performance Index (ψ)

We have now examined a system of three reactions in conditions where q_3 is less than its optimum value, and ψ and τ are far from their optima ($q_3 = 0.1$, $q_2 = 10$, $q_1 = 100$), with the kinetic constants k_i then adjusted to maximize the flux. The results (case 3a of Table 2) show a control distribution of flux, transit time and productivity performance index completely different from that obtained in the same system with optimized ψ (case 3). Flux control is now exerted primarily by the first enzyme, and the control coefficients for τ and ψ are higher in absolute value than those in systems with optimized ψ. This means that τ is more sensitive to small changes in total enzyme concentrations than in a system with optimized ψ. Control of τ is shared mainly by the first and third enzymes, whereas control of ψ is mainly exerted by the last.

We have also analysed a system in which the kinetic constants k_i are adjusted to minimize τ (case 3b of Table 2), with q_3 is higher than the value for maximal $\psi (q_3 = 4640.3$, $q_2 = 43.1$, $q_1 = 0.0005$); in this system ψ and J are far from their optimal values. Flux control is mainly shared between the first and second enzymes, and control of transit time is shared roughly

TABLE 3
Control coefficients for flux, transition time and productivity in the early steps of glycolysis in rat liver

Enzyme	C^J	C^τ	C^ψ
Hexokinase D	0.76	1.73	−0.97
Glucose-6-phosphate isomerase	0.00	−0.12	0.12
Phosphofructokinase	0.25	−2.60	2.85
Aldolase	0.00	−0.26	0.26

All values refer to a 5 mM glucose concentration. The flux and transition time control coefficients are taken from earlier work (Torres *et al.*, 1986; Cascante *et al.* 1991; Meléndez-Hevia *et al.*, 1992).

equally between the two last enzymes. Control coefficients of ψ are all positive; all the enzymes share the control, with the highest proportion for the second enzyme.

Finally (case 3c of Table 2), we have analysed a system with fixed q_i values far from those that maximize ψ ($q_3 = 1000$, $q_2 = 0.1$, $q_1 = 1$), with kinetic constants then adjusted to maximize ψ. Even though ψ has a value far less than the maximum, the distribution of control coefficients for ψ is very similar to that obtained in the three-enzyme system with ψ at its maximum (case 3 of Table 2). So we can conclude that irrespective of the thermodynamic profile of the system, for a given total driving force and overall equilibrium constant adjustment of kinetic constants to optimize ψ leads to a similar distribution of control of ψ. Control of J is shared among all the enzymes, decreasing slightly from the first to the last. Control of τ is positive for the first enzyme and negative for the others, decreasing in absolute value from the second to the last.

Relationship Between the Distribution of the Control of ψ and Classical Ideas of a Regulatory Enzyme: Control Analysis of the Early Steps of Rat Liver Glycolysis

We have analysed the control distribution of ψ in first steps of rat liver glycolysis using experimental data of flux control coefficients (Torres *et al.*, 1986) and calculated data of transit time control coefficients (Cascante *et al.*, 1991). The results (Table 3) show that 76% of flux control of exerted by hexokinase D (often but misleadingly called "glucokinase"), and 24% by phosphofructokinase. Control of transit time is mainly shared among hexokinase D (positive coefficient) and phosphofructokinase (negative coefficient), which thus exerted effects in opposite directions. Control of ψ resides mainly in phosphofructokinase: slight increases in the activity of this enzyme generated a great increase in ψ, with a concomitant improvement of the productivity of the

system. Hexokinase D also exerts a substantial effect on ψ, in the opposite direction.

Thus, although phosphofructokinase (classically regarded as the main regulatory enzyme in glycolysis) is not the main enzyme controlling the flux, it is indeed the main point for controlling τ and ψ. We may therefore suggest that the concept of a regulatory enzyme better matches with the concept of an enzyme with a high control coefficient for ψ than of an enzyme that only has a high flux control coefficient. In studying metabolic regulation, therefore, we need to focus not only on flux control but also on the control distribution of τ and mainly the control of ψ.

Optimization of Productivity Performance Index (ψ) Versus Optimization of Distribution of Control of ψ Among the Different Enzymes

In addition to flux maximization, and minimization of osmolarity and transition time, as already mentioned, several authors have suggested controllability of the system as a target of optimization in the evolutionary process (Atkinson, 1969; Heinrich & Holzhütter, 1985; Heinrich *et al*., 1987; Schuster & Heinrich, 1987), leading to the conclusion that the optimal distribution of control is to concentrate all in the first step of the pathway. However, as Brown (1991) pointed out, the extensive experimental evidence that metabolic control is distributed among several steps (see Fell, 1992) suggests that the evolutionary constraints are not exactly those listed above. Brown (1991) suggested protein concentration as a strong evolutionary constraint that allows all the enzymes to have significant control coefficients, and pointed out that several selection pressures may operate in evolution to determine control distributions and those found in real systems must reflect a compromise between these. Until now it has not been clear which is the optimal distribution of control of flux and transit time. In this paper we have shown that different control profiles are obtained according to which system variables are considered to be the primary target of optimization, and we propose ψ as a productivity performance index that has been maximized during evolution, as a compromise between maximization of fluxes and minimization of transition time. Nonetheless, even if ψ is maximized this does not imply that the distribution of control of ψ among the different steps is optimized. Several regulatory mechanisms have appeared during evolution, and interactions of enzymes with components of the system other than their immediate substrates and products are found in most pathways; such interactions redistribute control among the enzymes.

According to the experimental evidence of regulatory enzymes, and given that allosteric enzymes and other regulatory strategies represent a high evolutionary effort in order to optimize the system performance, we can expect the classical regulatory enzymes must show a high control coefficients for ψ. The results presented here for rat liver glycolysis showing higher control coefficient for phosphofructokinase accord with this prediction.

Further work will be needed to analyse theoretically the most suitable distribution profile of control coefficients of J, τ and ψ for optimizing performance and achieving maximum productivity at the lowest cost. Many factors must be taken into account, such as the chemical and thermodynamic contraints that condition the design of the pathway, the osmolarity of the system, the need to coexist with other pathway in the same compartment, etc.

In addition to this, we are carrying out a more exhaustive analysis of ψ in different systems under different circumstances, to study the optimization of metabolic performance and the control of pathway productivity, and we hope to develop a complete theory of metabolic system productivity in the near future. We expect that this will be a useful step toward realizing a major dream of Kacser and of the coordinator of this issue: to develop the theory of productivity of metabolic system, one of the frontiers of biochemistry.

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