Experimental and Theoretical Studies of the Regulatory Hierarchy in Glycolysis

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Introduction

Of all known complex biochemical networks the glycolytic system (GS) has received the most extensive study (cf. [1]). During decades it has played the role of an experimental proving ground to test and refine the theories on the regulation of cellular metabolism.

The GS* consists of a great number of reactions in which ATP is synthesized from ADP and inorganic phosphate (P_i) at the expense of anaerobic degradation of glucose or storage polysaccharides (glycogen, trehalose) to the end products (ethanol, CO_2, glycerol, etc.). The relatively simple stoichiometric structure of the GS formed by the individual reactions is entangled in a complex network of allosteric regulations [1]. These regulatory influences are exercised by intermediates of the GS (and species from other metabolic pathways) capable of inhibiting or activating the key enzymes when binding to the specific regulatory sites of the enzyme molecules.

The GS shows a great variety of behaviour patterns due to its strong nonlinearity and multiloop circuitry. It combines incompatible, at first sight, properties such as the ability to generate self-oscillations in metabolite concentrations [2-11], to exhibit trigger behaviour [12,13] and to stabilize the relative concentration of its major product, ATP [11,14-18].

This latter property manifests itself as stability of the so called adenylate energy charge [19,20]. It is determined as

$$\psi = \frac{[ATP]}{A_\Sigma} + \frac{1}{2}\,\frac{[ADP]}{A_\Sigma} \qquad (1)$$

*ABBREVIATIONS: ACA, acetaldehyde, ADH, alcohol dehydrogenase; ADP, adenosine monophosphate; AK, adenylate kinase; ALDH, aldehyde dehydrogenase; AMP, adenosine monophosphate; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; DAP, dihydroxyacetone phosphate; dATP, desoxyadenosine triphosphate; DPG, 1,3-diphosphoglycerate; ENO, enolase; ETOH, ethanol; Fru-6-P, fructose 6-phosphate; Fru-1,6-P_2, fructose 1,6-bisphosphate; Fru-2,6-P_2, fructose 2,6-bisphosphate; GAP, glyceraldehyde 3-phosphate; GAPDH, glyceraldehyde-phosphate dehydrogenase; Glc, glucose; Glc-1-P, glucose 1-phosphate; Glc-6-P, glucose 6-phosphate; GLG, glycogen; GLOH, glycerol; GL3PDH, glycerol 3-phosphate dehydrogenase; GS, glycolytic system; MAL, malate; NAD, nicotinamide dinucleotide (oxydized); NADH, nicotinamide dinucleotide (reduced); P_i, inorganic phosphate; PDC, pyruvate decarboxylase; PEP, phosphoenolpyruvate; PFK, 6-phosphofructokinase; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; PK, pyruvate kinase; PYR, pyruvate; UTP, uridine triphosphate; TIM, triosephosphate isomerase.

where $[ATP]/A_\Sigma$ and $[ADP]/A_\Sigma$ are the relative concentrations of ATP and ADP and $A_\Sigma = [ATP] + [ADP] + [AMP]$ is the total adenylate concentration.

Since the discovery of the mechanisms of allosteric regulation [20,21] and of their widespread occurrence in the metabolism of cells [23,24], it has become natural to consider the numerous manifestations of autoregulation in the cellular metabolism in terms of specific allosteric interactions [20,23-26]. However, theoretical analysis of a variety of stoichiometric schemes of energy metabolism, in particular for the GS, demonstrates the possibility of a stabilization of $[ATP]/A_\Sigma$ and φ without any allosteric interactions [27-30].

The data presented in this paper are the first experimental confirmation of the theory [27-30]. Moreover, they indicate conclusively that the homeostatic properties of the GS are the result of many regulatory mechanisms which provide a stable energy supply for the cell under drastic changes in (a) substrate availability, (b) metabolic load and (c) the very regulatory and stoichiometric structure of the GS.

Methods

The experiments were performed with the cell-free cytosolic system of yeast *Saccharomyces carlsbergensis* strain ATCC 9080 grown on glucose in aerobic or anaerobic 5 liter batch cultures. The cells were harvested in the late logarithmic growth phase at optical density of suspension $OD_{546} < 1.5$. After being washed three times with 0.1 M phosphate buffer pH 6.5, the cells were used for preparation of extracts. The average yield was 60g of wet cells per batch culture. For depletion of endogenous substrates, the cells were resuspended in 0.1 M, phosphate buffer pH 6.5 and aerated vigorously for at least three hours. After washing and centrifugation 20g of wet cells were mixed with 3.3 ml of 0.1 M phosphate buffer pH 6.5 and carefully sonicated keeping the temperature always below 20°C. When microscopic control showed only rare unbroken cells the sonicate was subjected to high speed centrifugation (100.000g for 2 hrs) to remove unbroken cells as well as cell debris and membrane fractions. The resulting supernatant contains all soluble cytosolic components, including the complete GS. It is clear, slightly yellow and opalescent and contains about 50 mg protein per ml.

To carry out experiments, the extract was thermostated at 25°, supplemented with the required effectors or adenine nucleotides (in the form of MgATP) and adjusted to pH 6.5. At time zero specific substrates for glycolysis were added, either glucose to make the final concentration 40 mM, or glycogen to yield a final concentration of about 40 mM glucose units. After charging the endogenous polysaccharide pools of the cytosolic system for 45 minutes, various amounts of highly purified mitochondrial F_1-ATPase prepared from baker's yeast were added. At different times during charging and decharging samples were taken to be analyzed subsequently for metabolic concentrations according to micro analytical techniques described elsewhere [31].

Results

Fig.1 illustrates the results of a typical experiment with addition of glucose to the extract. It shows changes with time of the concentration of glycolytic intermediates (A), adenine nucleotides (B), glucose,glycogen, ethanol and glycerol (C), and also of the variables

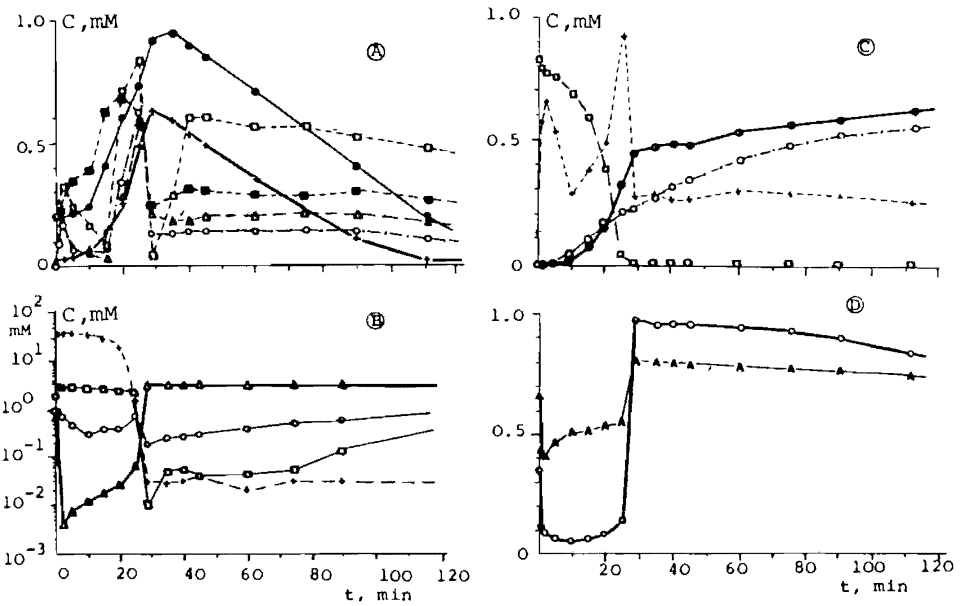


Fig. 1 Changes in time t in the yeast cytosolic cell-free system after addition of glucose (41 mM) at t=0.
A - concentrations of glycolytic intermediates: o-·-o, [Glc-6-P]/10; □ --□ , [Glc-1-P]x4; Δ--Δ, [Fru-6-P]/2; +——+, [Fru-1,6-P_2]/20; ●——●, [DAP]/2; ■ ——■ , [PYR]/2.
B - concentrations of adenine nucleotides (Δ——Δ, [ATP]; o——o, [ADP]; □ ——□ , [AMP]) and glucose (+-+).
C - concentrations of glucose (□ --□ , [Glc]/50), glycogen (+-+, [GLG]/50), ethanol (●——●, [ETOH]/100 and glycerol (o-·-o, [GLOH]/5).
D - the variables characterizing the energy status; o——o, φ; A——A, A_{ATPase}/50 kJ/mol.

characterizing the energy status of the GS: the energy charge φ and the chemical affinity of the ATPase reaction, $A_{ATPase}=\Delta G^{o}_{ATPase}$ (D). The experiment demonstrates the existence of a long-lasting (30'<t<110') quasisteady state during which stable values of φ and A_{ATPase} are maintained at the expense of the endogenous polysaccharides accumulated for 30 min after glucose addition. In this state the concentration of glucose is vanishingly small.

It is of interest to note that during charging of the extract (0'<t<30') free glucose is very rapidly phosphorylated by the highly active hexokinase (HK) at the expense of ATP (Glc + ATP $\xrightarrow{HK}$ Glc-6-P+ +ADP). This results in a dramatic fall of the ATP concentration such that both the deposition of glucose into glycogen, which requires two molecules of ATP per glycosyl bond formed, and the glycolytic phosphorylation of ADP, which is preceded by a two-step hexose phosphorylation, may seem impossible because of limitation by the low ATP level.

However, this is far from being so: the rapid accumulation of storage polysaccharides and ethanol (Fig.1C) give clear-cut evidence that both of the ATP-dependent functions are fulfilled even at such low concentration of ATP.

The surprisingly high flux rates driven by a very low ATP level can be explained by the fact that the chemical affinity of the ATPase reaction A_{ATPase} changes only slightly during the phase of charging (from 40 to 20 kJ/mol, Fig.1D), whereas ATP drops from 3.0 to $4 \cdot 10^{-3}$ mM. The high level of A_{ATPase} is maintained by the activities of ADP-phosphorylating reactions in the lower part of the GS, which keep the net ATPase reaction far from thermodynamical equilibrium.

In order to establish the relative role of allosteric and stoichiometric mechanisms in the stabilization of $[ATP]/A_{\Sigma}$, φ and A_{ATPase} in the quasisteady state, several independent series of experiments were carried out measuring a number of glycolytic substrates, intermediates and products. In these experiments additions of different effectors were made blocking the allosteric properties of the key enzymes and changing the structure of the GS to a partially non-allosteric network. The major results of the experiments are summarized in Fig.2.

Fig.2A illustrates the case where the ATP pool is substituted by the pool of dATP. This results in elimination of the allosteric inhibition by ATP of phosphofructokinase (PFK) and the corresponding accumulation of its product fructose 1,6-bisphosphate (Fru-1,6-P_2) at high levels (up to 20 mM). It is well known that Fru-1,6-P_2 is a specific allosteric activator (the activation constant $K_a \approx 2$ mM) of pyruvate kinase (PK), which is the key allosteric enzyme of the lower part of the GS. At high Fru-1,6-P_2 levels PK is completely activated

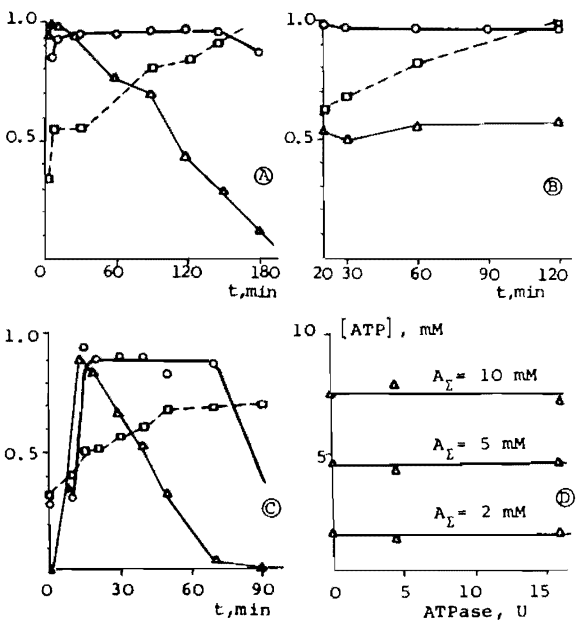


Fig. 2 Stabilization of the energy charge φ (A,B,C) and absolute concentrations of ATP (D) in the extracts deprived of the allosteric regulations of their key glycolytic enzymes. Allostericity was suppressed in different ways: A - substitution of adenylates by deoxyadenylates (the extract with a very low initial adenylate pool was loaded with 7.0 mM dATP, 4 units of F_1-ATPase and 5.3 mM $MgCl_2$). o——o, φ ; Δ——Δ, [Fru-1,6-P_2]/20 mM; □--□ , [ETOH]/200 mM. B - addition of 100 mM NH_4Cl and 1.5 mM MgATP. The energy charge $\varphi \approx 0,96$ in the time interval from 20 to 120 min characterized by a nearly constant concentration of Fru-1,6-P_2 ([Fru-1,6-P_2]/10, Δ——Δ) and a linear accumulation of ethanol ([ETOH]/200, □--□). C - addition of 1.0 mM Fru-2,6-P_2 to the extract precharged with 44 mM Glc. A_{Σ}=1,6 mM and $P_i \approx 80$ mM (o——o, φ; Δ-- Δ, Fru-1,6-P_2/20; □ --□ , [ETOH]/200). D - the quasisteady state load characteristics (Δ—Δ) of the GS loaded with 40 mM GLG at three different levels of the adenylate pool A_{Σ}.

and hence insensitive to Fru-1,6-P itself and other allosteric effectors. The linear fall of Fru-1,6-P_2 and the conjugated stoichiometric accumulation of ethanol at a practically constant value of $\varphi \approx 1$ prove the fact that the lower part of the GS does stabilize φ stoichiometrically. This stabilization cannot be mediated by the key allosteric enzymes of the upper part of the GS (the phosphorylase complex and PFK) because of the significant nonstationarity of Fru-1,6-P_2 and hexose monophosphates (not shown).

Fig.2B presents the results of the experiment on activation of both PFK and PK by high concentrations of NH_4^+, a powerful effector for these enzymes. In this experiment the stability of φ is maintained unaltered even with a non-allosterically operating PFK and an only partially allosteric PK.

Fig.2C demonstrates the outcome of experiments with a third method to block the allosteric properties of PFK and PK. A highly effective activator of PFK, fructose 2,6-bisphosphate (Fru-2,6-P_2) was used at a concentration of 1 mM to obtain completely activated PFK. Evidence for the strong activation of PFK in this condition are the rapid decrease of the hexose monophosphate pool to a very low level (not shown) and the accumulation of high concentrations of Fru-1,6-P_2 (up to 20 mM).

Fig.2D plots the absolute concentration of ATP as a function of the ATPase load with three different sizes of the adenylate pool, A_Σ. In these experiments, the initial substrate for glycolysis was glycogen added to the extracts. It is remarkable that the breakdown of exogenous glycogen proceeds in uncontrolled reactions [11], possibly, due to lack of the specific spatial organization of the respective enzymes which exists in the case of endogenously synthesized glycogen. In any case, the rate of glycogen breakdown was independent of ATPase activity. In the absence of such feedback, the upper part of the GS becomes overloaded with metabolites and PFK is activated by high concentrations of Fru-6-P and Fru-1,6-P_2. PK is also activated by a high level of Fru-1,6-P_2. Nevertheless, $[ATP]/A_\Sigma$ is stabilized in spite of the change in the activity of added ATPase, as seen from the load characteristics.

It is important to note that only the relative concentration ($[ATP]/A_\Sigma$) and not the absolute concentration [ATP] is stabilized by the GS, contrary to what might be expected from the allosteric principle of regulation. The principle of stoichiometric regulation is very simple: the phosphorylating pathway of the GS tends to maintain the least possible concentration of ADP. Actually this principle is realized in glycolysis as is evident from the insignificant decrease in A_{ATPase} at a very strong overloading (Fig.1D) and from the dramatic decrease in the rate of glycolysis V_{ETOH} for $\varphi \to 1$ (Fig.3).

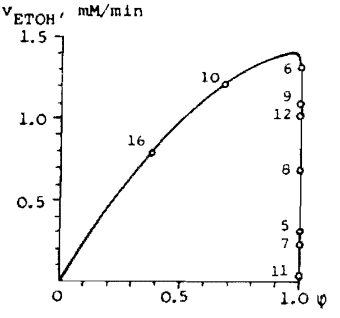


Fig. 3 Quasisteady state output characteristics of the GS. Circles are the quasisteady state values of the rate of ethanol production (V_{ETOH}) averaged for the time during which [Fru-1,6-P_2] ≈ const. Different amounts of ATPase were added to the untreated extract charged with 40 mM glucose and containing ≈3.0 mM MgATP

Fig.3 plots V_{ETOH} as a function of ψ for experiments with extract which underwent no changes other than the addition of ATPase. The values of ψ and V_{ETOH} correspond to the average quasisteady state values of these veriables at $[Fru\text{-}1,6\text{-}P_2] \approx$ const.

The quasisteady state (Fig.4) is maintained by the slow expenditure of metabolic Fru-1,6-P_2 precursors, which counterbalances the expenditure of Fru-1,6-P_2 in the lower part of the GS ($v_{PFK} \approx v_{ALD}$). Depletion of the precursors makes the formation of Fru-1,6-P_2 in the PFK reaction impossible and $[Fru\text{-}1,6\text{-}P_2]$ decreases at a rate $v_{ALD} \approx 4 v_{ATPase}$. As seen from Fig.5, ψ remains constant even when Fru-1,6-P_2 decreases steadily. This proves the existence of at least one more quasisteady state in the lower part of the GS. The energy to maintain this state is derived from the Fru-1,6-P_2 breakdown.

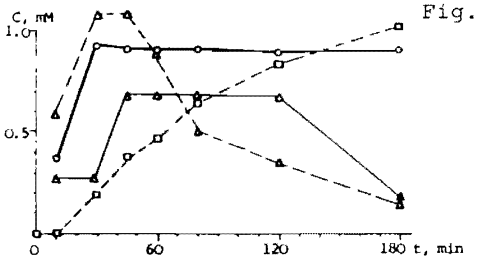


Fig.4

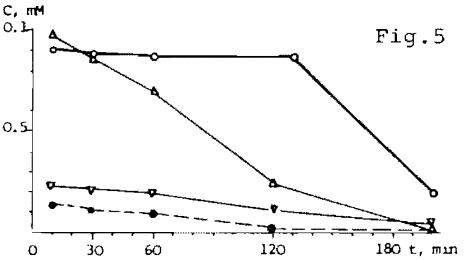


Fig.5

Fig. 4 Quasisteady state in Fru-1,6-P_2 concentration (45'<t<120') in the untreated extract with the initial pool of endogenously synthesized polysaccharides equal to about 38.5 mM glucose equivalents. Note that ψ remains stable even during the phase of Fru-1,6-P_2 breakdown(t>120 min). Δ—Δ, $[Fru\text{-}1,6\text{-}P_2]/10$; Δ--Δ, $[Fru\text{-}6\text{-}P]$; □--□ $[ETOH]/200$; o—o, ψ

Fig. 5 Stabilization of ψ by the lower part of the GS at the expense of the Fru-1,6-P_2 + GAP pool in conditions of $v_{PFK} \approx 0$. The extract was precharged with polysaccharides synthesized endogenously from 40 mM glucose and was then loaded with 4 units of ATPase at t=0. Total adenylate cocentration A_Σ=6.0 mM. Δ—Δ, $[Fru\text{-}1,6\text{-}P_2]/10$; ▽—▽, $[DAP]/10$; ●—●, $[Fru\text{-}6\text{-}p]$; o—o, ψ

In the upper part of the GS the quasisteady state may become transient and even non-existent because of high load imposed on the system by the added ATPase, while in the lower part it persists long enough to provide the stabilization of ψ for an extended period of time

$$t = 2(2[Fru\text{-}1,6\text{-}P_2]_o + [DAP]_o)/(L \cdot [ATP]_{st}) \qquad (2)$$

determined by the initial concentrations of Fru-1,6-P_2 and DAP ($[Fru\text{-}1,6\text{-}P_2]_o$ and $[DAP]_o$), the rate constant of the net ATPase (endogenous + added), L, and the concentration of ATP being stabilized, $[ATP]_{st}$.

Discussion

The experiments demonstrate unequivocally that allosteric regulation is not necessary to make the GS a stable energy source. Even when the enzymes PFK and PK are deprived of their allosteric properties, the GS stabilizes ψ and $[ATP]/A_\Sigma$ (Fig.2) as effectively as does the untreated GS (Fig.1).

This result is consistent with the theory [27-30], according to which the stabilization of φ is accomplished stoichiometrically by the lower part of the GS (below the PFK reaction).

The idea that the lower part might be the only stabilizer in glycolysis is confirmed by the experiments in which Fru-1,6-P_2 is produced in great excess by different ways. The resulting prolonged transient state (Fig.2A,C; Fig.5) is characterized by a linear fall of [Fru-1,6-P_2] coupled stoichiometrically with the accumulation of ethanol and with the quasisteady state in the lower part, in which φ and metabolite concentrations between GAPDH and alcohol dehydrogenase (ADH) are constant.

Jonnalagadda and co-authors [11] tried to evoke such a state adding to yeast extracts either glycogen or large amounts of Fru-1,6-P_2 (up to 60 mM). In these conditions a linear decrease in the concentrations of (Fru-1,6-P_2) and DAP and the conjugated linear accumulation of ethanol were observed, suggesting the existence of a rapidly attainable quasisteady state in the lower part of the GS. Based on these findings, these authors [11] first proposed that the stabilization of φ should be accomplished stoichiometrically by the lower part of glycolysis. However, in their experiments the value of φ did not remain constant on addition of excess Fru-1,6-P_2. And when the mean value of $\varphi \approx 0.84$ was maintained (linear accumulation of ethanol at the expense of added glycogen) oscillations of [Glc-6-P] and [Fru-1,6-P_2] were observed with a phase shift of 180°. This indicated that the PFK reaction continued to be allosterically regulated.

In our experiments the stabilization of φ was next to ideal in all conditions in which the contribution of PFK was excluded either because of the exhaustion of the metabolic precursors of Fru-1,6-P_2 ($v_{PFK} \ll v_{ALD}$) or because of the complete activation of PFK (fig.2).

Though the theory of the stoichiometric stabilization of φ by the cellular energy metabolism, in particular by the GS, has been primarily developed for the case of steady state [27-30], it can be easily extended to the nonstationary case [32]. Fig.6 presents a kinetic model of the lower part of the GS. If we take the simplest approximations for the rates

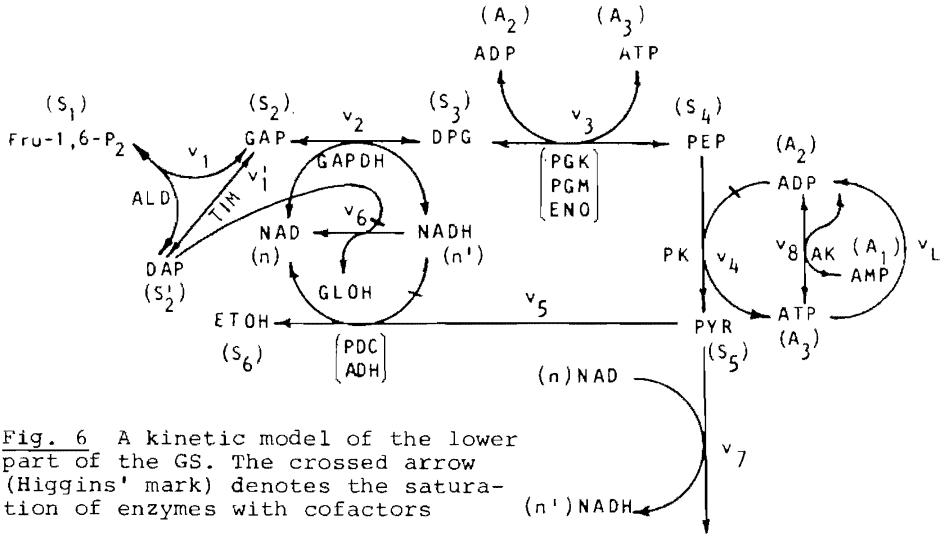


Fig. 6 A kinetic model of the lower part of the GS. The crossed arrow (Higgins' mark) denotes the saturation of enzymes with cofactors

$$v_1 = k_{+1}S_1 - k_{-1}S_2S_2', \quad v_1' = k_{+1}'S_2 - k_{-1}'S_2',$$
$$v_2 = k_{+2}S_2N - k_{-2}S_3N', \quad v_3 = k_{+3}S_3A_2 - k_{-3}S_4A_3, \tag{3}$$
$$v_4 = k_4S_4A_2/(M_4 + A_2), \quad v_5 = k_5S_5N'/(M_5 + N'),$$
$$v_6 = k_6N'S_2'/(M_6 + S_2'), \quad v_7 = k_7S_5N,$$
$$v_8 = k_{+8}A_1A_3 - k_{-8}A_2^2,$$

$v_L = LA_3$ (for numbering of reactions, see the kinetic scheme in Fig.6), then with constant nucleotide pools

$$A_\Sigma = A_1 + A_2 + A_3, \quad N_\Sigma = N + N', \tag{4}$$

the behaviour of the lower part of the GS in time t can be described by the following equation system:

$$\frac{dS_1}{dt} = -v_1, \quad \frac{dS_2}{dt} = v_1 - v_1' - v_2, \quad \frac{dS_2'}{dt} = v_1 + v_1' - v_6,$$
$$\frac{dS_3}{dt} = v_2 - v_3, \quad \frac{dS_4}{dt} = (v_3 - v_4)e_4, \quad \frac{dS_5}{dt} = v_4 - v_5 - v_7, \tag{5}$$
$$\frac{dN'}{dt} = v_2 - v_5 - v_6 + v_7, \quad \frac{dA_3}{dt} = v_3 + v_4 - v_8 - v_L,$$
$$\frac{dA_2}{dt} = -v_3 - v_4 + 2v_8 + v_L.$$

In this system the following notation is used:

$A_1 = [AMP]$, $A_2 = [ADP]$, $A_3 = [ATP]$, $N = [NAD]$, $N' = [NADH]$;

$S_1 = [Fru\text{-}1,6\text{-}P_2]$, $S_2 = [GAP]$, $S_2' = [DAP]$, $S_3 = [DPG]$, $S_4 = [PEP]$, $S_5 = [PYR]$;

$v_1 = v_{ALD}$, $v_1' = v_{TIM}$, $v_2 = v_{GAPDH}$, $v_3 = v_{PGK} = v_{PGM} = v_{ENO}$, $v_4 = v_{PK}$, $v_5 = v_{PDC} = v_{ADH}$, $v_6 = v_{GL3PDH}$, v_7 stands for the net NAD-dependent leakage of PYR or ACA from the GS, $v_8 = v_{AK}$, $v_L = v_{ATPase}$;

$$e_4 = \left[1 + K_{PGM}\,(1 + K_{ENO})\right]/(K_{ENO}K_{PGM}) \approx 3 \tag{6}$$

where K_{PGK}, K_{ENO} and K_{PGM} are the equilibrium constants for the corresponding reaction; the K's are the rate constants and the M's are the Michaelis constants.

With consideration for the typical enzyme activity distribution in the extract [4], and also for the initial conditions $S_{10} \approx 2 \cdot 10^{-2}$M, $A_\Sigma = 2 \cdot 10^{-3}$M, $N_\Sigma = 1 \cdot 10^{-3}$M (experiment of the type shown in Fig.2D), model (5) can be reduced to a simpler one

$$\dot{S}_1 = -v_1, \quad \dot{S}_2 = v_1 - v_1' - \tilde{v}_2, \quad \dot{S}_2' = v_1 + v_1' - v_6,$$
$$\dot{S}_4 = (\tilde{v}_2 - \tilde{v}_4)e_4, \quad \dot{S}_5 = \tilde{v}_4 - v_5 - v_7, \quad \dot{A}_3 = \tilde{v}_2 + \tilde{v}_4 - v_L, \tag{7}$$

where

$$\tilde{v}_2 = k_{+2}\frac{N\tilde{A}_2S_2 - N'A_3S_4Q_2}{m_2N' + A_2}, \qquad \tilde{v}_4 = k_4S_4\frac{\tilde{A}_2}{M_4 + \tilde{A}_2} \tag{8,9}$$

$$\tilde{A}_2 = 0.5K_{AK}A_3\left\{\sqrt{1 + \frac{4}{K_{AK}}\left(\frac{A_\Sigma}{A_3} - 1\right)} - 1\right\} \tag{10}$$

$$\tilde{A}_1 = A_\Sigma - \tilde{A}_2 - A_3, \quad \tilde{\psi} = (A_3 + 0.5\tilde{A}_2)/A_\Sigma \tag{11,12}$$

are the quasistationary variable values attainable at

$$v_2 = v_3, \; v_8 = 0 \tag{13,14}$$

Computer fitting of the solution of model (7) (Fig.7) to the behaviour patterns of intermediates and nucleotides observed in the experiment with an almost linearly decreasing Fru-1,6-P_2 (Fig.2C) yields the following parameter values:

$$\begin{aligned} &A_\Sigma = 1.66 \text{ mM}, \; N_\Sigma = 1.0 \text{ mM}, \; L = 0.8, \; k_{+1} = 0.13, \; k_{-1} = 3.85, \\ &k'_{+1} = 3.34, \; k'_{-1} = 0.45, \; k_{+2} = 300, \; m_2 = 0.04, \; Q_2 = 0.2, \\ &k_4 = 5.83, \; M_4 = 0.2, \; k_5 = 1.15, \; M_5 = 0.01, \; k_6 = 0.12, \; M_6 = 0.1, \\ &k_7 = 0.019, \; K_{AK} = k_{+8}/k_{-8} = 5. \end{aligned} \tag{15}$$

With these parameter values, model (7) describes quantitatively (within the experimental error) the change with time in the concentrations of Fru-1,6-P_2, DAP, GAP (Fig.7) and also of PYR, ETOH, GLOH, and NADH. The model predicts, however, a somewhat higher value of ψ as compared to the experiment (Fig.7).. The discrepancy may be explained by the fact that the kinetic model (Fig.6) takes no account of inhibition of GAPDH by DPG and NADH and the inhibition of PK by ATP.

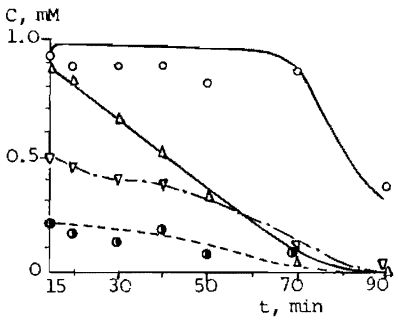


Fig. 7 Solution of the mathematical model (7) (curves) fitted to the experimental data (points): Δ——Δ, [Fru-1,6-P_2]/20; ▽-·-▽, [DAP]/5; ●---●, [GAP]; o——o, ψ

Fig.8 presents the solution to model (7) for the numerical data (15), which demonstrates the ability of the lower part of the GS to stabilize ψ during the phase of Fru-1,6-P_2 breakdown. The high stability of ψ in this phase (Fig.8B) is due to strong apparent saturation of GAPDH with GAP. Because of the saturation, the decrease in [Fru-1,6-P_2] and the resulting decrease in the quasisteady state value of [GAP] do not affect the reactions below GAPDH, unless [Fru-1,6-P_2] drops to extremely low values (Fig.8A). It should be noted that the high degree of saturation and the high apparent affi-

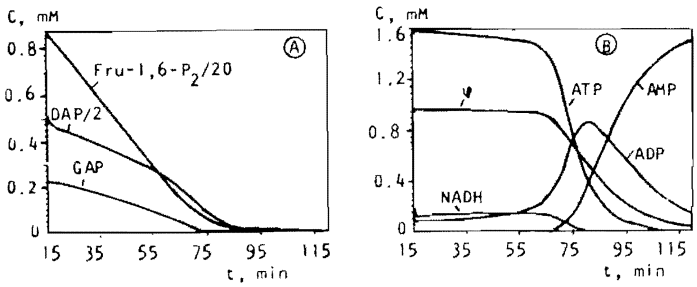
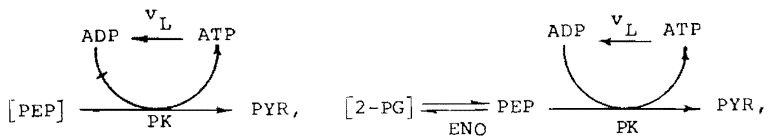


Fig. 8 Extended solution of model (7)

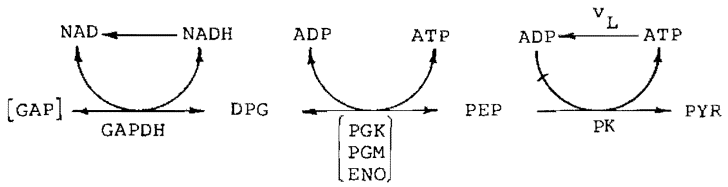
nity of GAPDH for GAP are purely system effects which manifest themselves only in the quasisteady state when $\dot{S}_3=\dot{S}_4=\dot{S}_5=\dot{A}_1=\dot{N}_1=0$. Indeed, in our model v_2 is a linear function of S_2 at constant values of N and S_3.

As follows from [33], the lower part of the GS can be decomposed to a number of less effective stabilizers of ψ, interacting synergistically with each other. Some of them are very simple, such as



(16,17)

others are much more complicated



(18)

Saturation of the enzymes is shown in the schemes (16-18) by Higgins' mark. Concentrations of the initial substrate (square brackets) must be either excessive or kept constant.

Because of its consequences it seems noteworthy to stress the high stability of A_{ATPase}. Even in the strongly overloaded GS which shows a dramatic decrease of [ATP] (Fig.1), the affinity of the ATPase reaction, A_{ATPase}, changes very little and is able to drive effectively glycogenesis at a high flux rate. In fact, this stability serves as the basis for a hierarchical priority among the many different ATP consumers in the cell.

When the concentration of ATP is low, it becomes accessible only to those consumers which have significantly greater affinity for ATP than others. This hierarchy immediately results in the temporal organization of the consumers. This statement is illustrated by Fig.1: after the addition of glucose, it is first deposited by the "starved" GS in the form of storage polysaccharides, and it is not until the free glucose is completely exhausted that the stable supply of the consumers with ATP from the endogenous energy sources begins.

This intrinsic selection of metabolic priority is obviously the consequence of the very design [1] of the whole metabolic network: those pathways which are absolutely necessary for survival of the cell (in this case the requisition of food by phosphorylation) do have the highest affinity for substrates, i.e. the lowest values for half saturation of the respective enzymes. In due order follow all less important pathways according to their priority rating which automatically translates to the respective temporal sequence.

In our case not only a quick requisition of the offered glucose and the temporally delayed expenditure of the gained energy takes place but a major part of the phosphorylated hexose is reversibly converted to storage polysaccharides. This adds a new dimension of regulatory complexity to the system: the balance between katabolic and anabolic reactions. In either pathway the GS operates with different enzymes and coenzymes. In yeast the deposition of storage polysaccharides proceeds via uridine diphosphoglucose pyrophosphorylase, cofactor is UTP, whereas the opposing katabolic reactions are catalyzed by phosphorolytic or hydrolytic enzymes with the general use of adenine nucleotide coenzymes throughout the glycolytic reaction sequence.

Such diversity of enzymes, coenzymes (and eventually effectors) constitutes a higher order of regulatory hierarchy [1] than the allosteric mechanisms of enzyme reaction or the basic stoichiometric regulatory principles of the metabolic network outlined in this study. This class of the regulatory hierarchy is designed to switch on or off major reaction pathways avoiding effectively futile cycles by temporal separation of opposite or competitive reaction sequences [33,34].

The basis, however, for all regulatory principles of higher order is a stable energy source. For the glycolytic system it has been shown here that a stable energy output is maintained by a number of equivalent regulatory mechanisms. Their common principle is that pools of various glycolytic intermediates separately or in concerted action do serve as buffering reservoirs to maintain a quasisteady state in the ATP generating part of the system. Concomitant with the stability of this state, the energy charge, φ, is very stable and well protected against experimental perturbations which impose on the GS drastic changes in a) metabolic load, b) substrate availability, c) the length of its operating reaction sequence, and d) the very regulatory structure of higher hierarchical order. The simple stoichiometric nature of these surprisingly effective mechanisms of energy stabilization suggest that they are a very early product of evolution, much older than the allosteric regulation of enzymes or the sophisticated switching of reaction pathways.

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