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Design of glycolysis

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The design of the glycolytic pathway resulting from the continuous refinement of evolution is discussed with regard to three aspects.

1. Functional and structural properties of individual enzymes. The catalytic constants of the glycolytic enzymes are remarkably optimized; the turnover numbers are within one order of magnitude. The same is true for the molarities of catalytic centres in the cytosol, as is noted for yeast. Functional properties of the enzymes are reflected in their tertiary and quaternary structures.

2. Regulatory mechanisms of single enzymes. A classification of the various types of enzymic control mechanisms operating in the glycolytic pathway is given. In addition to the usual Michaelis–Menten saturation kinetics and the various types of inhibition, there is control by positive and negative effectors based on oligomeric structures (fast acting, fine control) as well as regulation by chemical interconversion based on enzyme cascades (slow acting, very effective).

3. Functional and regulatory mechanisms of the whole glycolytic reaction pathway. A prominent feature is the high enzyme:substrate ratio, which guarantees fast response times. However, a quantitative treatment of the overall kinetics is limited by an incomplete knowledge of the enzymes' dynamic and chemical compartmentation as well as some of their control properties. From an analysis of the oscillatory state, certain control points in the glycolytic chain can be located that coincide with major branching points to other metabolic pathways. These points are controlled by fast-acting cooperative enzymes that operate in a flip-flop mechanism together with the respective antagonistic enzymes, preventing futile cycles. The gating enzymes leading to the glycogen store and the citric acid cycle are of the slow-acting but very effective interconvertible type.

The combination of all the complex and intricate features of design yields a glycolytic network that enables the cell to respond to its various metabolic needs quickly, effectively and economically.

1. General properties of glycolytic reactions and enzymes

Glycolysis is one of the major metabolic pathways that supplies the cell with energy and at the same time with the material for syntheses. Enzymically catalysed glycolytic reactions take place in every cell, from microorganisms to man, and, despite their functional and regulatory complexity found today, have evolved during the long timespan of biological evolution from uncatalysed chemical reactions of simple carbohydrates in the presence of phosphate, salt and water. The basis of energy conversion from the C–O bonds of carbohydrates to the energy-rich pyrophosphate bond has not changed in millions of years; the uncatalysed primordial reaction and today's complex enzymic conversion rely on a few basic chemical reactions like aldolization, isomerization, phosphorylation, transphosphorylation, oxidation and reduction.

Although all glycolytic reactions can proceed spontaneously, their conversion times are many orders of magnitude shorter in the enzymically catalysed reactions (Koshland & Neet 1968). Owing to the constant refinement of evolution for most glycolytic enzymes, turnover numbers as high as 10^2 and 10^3/s are reported (Eigen & Hammes 1963). With regard to the evolutionary optimization of the enzymic turnover numbers to roughly the same order of

magnitude, it is interesting to see whether this optimization process is also reflected in the structural features. In fact, specific patterns in the tertiary structure of glycolytic enzymes have been recognized, and almost identical structures are found at the catalytic sites of analogous enzymes like phosphoryl transferases or isomerases (see other papers in this symposium). Further progress of X-ray studies may eventually lead to a structural classification of active site domains.

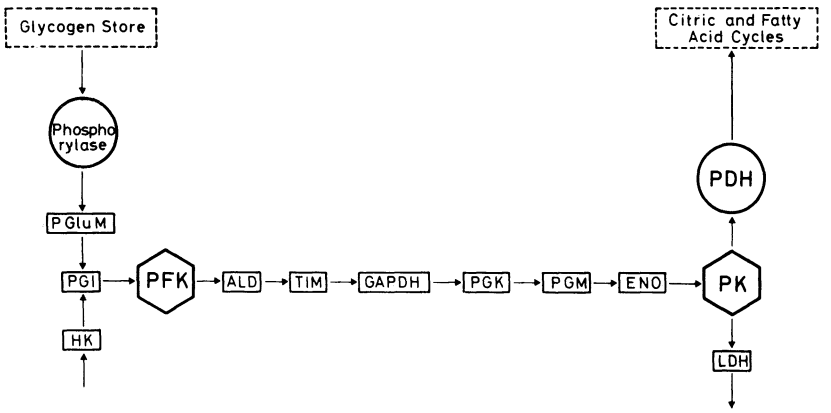

FIGURE 1. Schematic diagram of the glycolytic pathway. Symbols used: circles, interconvertible enzymes; hexagons, cooperative enzymes; rectangles, Michaelis–Menten enzymes. Although cooperative in many species, for the sake of simplicity GAPDH is represented as a Michaelis–Menten enzyme.

However, enzymes are more than catalysts that function with an optimized reaction rate of fast elementary reaction steps. Their essential feature, which makes higher organized life possible, is their ability to *control* their function in response to their own substrates and products as well as to other controlling factors: thus, the intricate interconnected regulation and kinetic fitting of all participating enzymes relative to each other enable a cell to control the rate of glycolytic flux according to its actual need.

Different organisms or different cells (e.g. muscle or liver) of one organism, as well as different microorganisms, have developed different ways of exerting control, and it seems appropriate to speak of a cell-specific design of enzymic reactivity and control of glycolytic metabolism. Unfortunately, only for *Escherichia coli* and, to a lesser extent, for *Saccharomyces cerevisiae* and *S. carlsbergensis* are mutant studies available to specify the genetic maps for glycolytic enzymes, and to help us to understand the mechanism of phenotype expression (Clifton *et al.* 1978; Fraenkel & Banerjee 1972).

Thus, summarizing, we understand three levels of design of glycolysis which will be discussed here: (*a*) functional and structural properties of individual glycolytic enzymes, (*b*) regulatory mechanisms of single enzymes, and (*c*) functional and regulatory mechanism of the whole glycolytic reaction pathway.

2. CONFIGURATIONAL SPECIFICITY OF GLYCOLYTIC ENZYMES

Exclusively, the D-configuration of sugars and their phosphorylated derivatives down to 2-phospho-D-glycerate is metabolized by glycolytic enzymes, and only the very last step of

Table 1. List of abbreviations

(*a*) *Enzymes*

abbreviation	trivial name	number	systematic name
ADH	alcohol dehydrogenase	EC 1.1.1.1	alcohol: NAD oxidoreductase
AK	adenylate kinase	EC 2.7.4.3	ATP: AMP phosphotransferase
ALD	aldolase	EC 4.1.2.13	fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphatelyase
ENO	enolase	EC 4.2.1.11	2-phospho-D-glycerate hydrolyase
epimerase	–	EC 5.1.3.15	glucose-6-phosphate 1-epimerase
FBPase	fructosebisphosphatase	EC 3.1.3.11	D-fructose-1,6-bisphosphate 1-phosphohydrolase
GAPDH	glyceraldehyde phosphate dehydrogenase	EC 1.2.1.12	D-glyceraldehyde-3-phosphate: NAD oxidoreductase (phosphorylating)
G6PDH	glucose-6-phosphate dehydrogenase	EC 1.1.1.49	D-glucose-6-phosphate: NADP oxidoreductase
HK	hexokinase	EC 2.7.1.1	ATP: D-hexose-6-phosphotransferase
LDH	lactate dehydrogenase	EC 1.1.1.27	L-lactate: NAD oxidoreductase
PDC	pyruvate decarboxylase	EC 4.1.1.1	2-oxoacid carboxylyase
PDH	pyruvate dehydrogenase complex:		
	pyruvate dehydrogenase	EC 1.2.4.1	pyruvate: lipoate oxidoreductase
	lipoate acetyltransferase	EC 2.3.1.12	acetyl-CoA: dihydrolipoate *S*-acetyltransferase
	lipoamide dehydrogenase	EC 1.6.4.3	NADH: lipoamide oxidoreductase
PFK	phosphofructokinase	EC 2.7.1.11	ATP: D-fructose-6-phosphate 1-phosphotransferase
PGI	phosphoglucose isomerase	EC 5.3.1.9	D-glucose-6-phosphate ketol-isomerase
PGK	phosphoglycerate kinase	EC 2.7.2.3	ATP: 3-phospho-D-glycerate 1-phosphotransferase
PGluM	phosphoglucomutase	EC 2.7.5.1	α-D-glucose-1,6-bisphosphate: α-D-glucose-1-phosphate phosphotransferase
PGM	phosphoglycerate mutase	EC 2.7.5.3	2,3-diphospho-D-glycerate: 2-phospho-D-glycerate phosphotransferase
PK	pyruvate kinase	EC 2.7.1.40	ATP: pyruvate phosphotransferase
PPC	phosphopyruvate carboxylase	EC 4.1.1.32	GTP: oxaloacetate carboxylyase (transphosphorylating)
		EC 4.1.1.38	PP: oxaloacetate carboxylyase (phosphorylating)
		EC 4.1.1.49	ATP: oxaloacetate carboxylyase (transphosphorylating)
TIM	triose phosphate isomerase	EC 5.3.1.1	D-glyceraldehyde-3-phosphate ketol-isomerase

(*b*) *Metabolites*

ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
DAP	dihydroxyacetone phosphate
FBP	D-fructose-1,6-bisphosphate
F6P	D-fructose-6-phosphate
GAP	D-glyceraldehyde-3-phosphate
NAD(H)	nicotinamide adenine dinucleotide oxidized (reduced)
PEP	phosphoenolpyruvate

glycolytic degradation in higher organisms leads to the L-configuration of lactate. Only a few microorganisms are adapted to metabolize the opposite configuration (Alworth 1972; Hanson & Rose 1975). In addition to the D,L-stereospecificity of the open-chain carbon skeleton of sugars, upon formation of the pyranose or furanose ring, further asymmetry appears: the α- and β-anomeric forms of the sugars and their derivatives, which are reflected in the anomeric specificity of glycolytic enzymes, as summarized in figure 2.

Hexokinase phosphorylates both anomeric configurations of glucose (Wurster & Hess 1974).

Phosphoglucomutase reacts with the α-anomeric configuration (Gadian *et al.* 1974) and glucose-6-phosphate dehydrogenase with the β-anomer of the substrate. In yeast, *E. coli*, *Rhodotrula gracilis* and potato tubers, glucose-6-phosphate 1-epimerase equilibrates the anomeric configurations of glucose-6-phosphate (Chance *et al.* 1975; Wurster & Hess 1974). Glucose phosphate isomerase has the unique property of simultaneous isomerization and anomerization of the various configurations of glucose-6-phosphate and fructose-6-phosphate (Plesser *et al.* 1979; Schray *et al.* 1973; Wurster & Hess 1974).

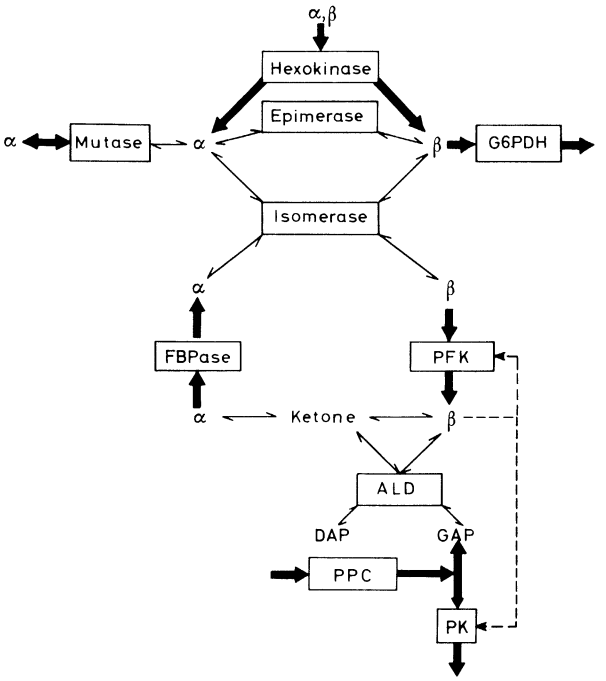

FIGURE 2. Diagram of anomeric specificities in the glycolytic pathway. The respective configuration of the anomer (α or β) is indicated where an enzyme shows anomeric specificity. Heavy arrows indicate the directions of glycolytic or glycogenetic fluxes, respectively. The dashed line shows the allosteric control exerted by β-FBP.

This type of *chemical compartmentation* is also found further along the glycolytic pathway: the β-anomer of fructose-6-phosphate reacts with fructose-6-phosphate kinase (Wurster & Hess 1974), and the β-anomer of fructose-1,6-bisphosphate takes part in the reaction of fructose-bisphosphate aldolase and binds allosterically to fructose-6-phosphate kinase and allosteric pyruvate kinase (Midelfort *et al.* 1976; Rose & O'Connell 1977; Wurster & Hess 1974; Wurster *et al.* 1976). One cannot conclude, however, that analogous enzymes from different biological sources would in general exhibit the same anomeric specificity. This is especially true if the corresponding enzymes possess different properties as, for example, the two types of fructose-bisphosphate aldolase. Nevertheless, for hexokinase, glucose-6-phosphate dehydrogenase and glucose-phosphate isomerase, the anomeric specificity of analogous enzymes from various biological sources has been found to be identical (Wurster & Hess 1974).

Taking the α-anomer of fructose-1,6-bisphosphate to be the substrate of fructose-bisphosphatase, and the binding of glucose-6-phosphate to phosphoglucomutase (Gadian *et al.* 1974) and that of glucose-1-phosphate to phosphorylase to be stereospecific for the α-anomer

(Battersby & Radda 1976), it has been suggested that the key enzymes of the glucogenetic pathway are stereospecific for the α-anomers of their carbohydrate substrates, whereas those of the glycolytic process are specific for the β-anomers (Hess & Plesser 1977; Wurster & Hess 1974). This conclusion, drawn from a kinetic analysis of the stereospecificity of glycolytic enzymes, is strongly supported by recent studies of the distribution of tautomeric and anomeric configurations in intact cells as well as organs with the use of n.m.r. techniques under glucogenetic and glycolytic conditions (Brown *et al.* 1978; Cohen *et al.* 1978; Evans & Kaplan 1977; den Hollander *et al.* 1979; Koerner *et al.* 1977; Navon *et al.* 1977).

3. Functional and structural properties of glycolytic enzymes

In addition to the optimization of catalysis, evolution has led to a remarkable balance of the relative molarities of glycolytic enzymes and of their saturation with substrates inside the cell. This can readily be demonstrated for yeast.

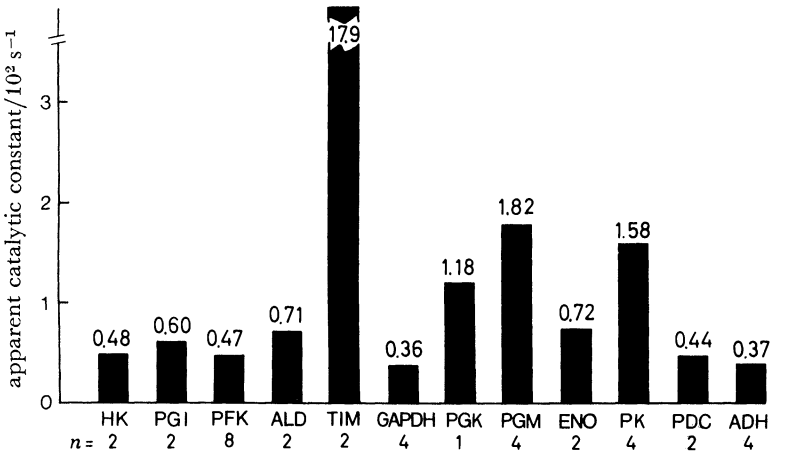

Figure 3. Apparent catalytic constants of glycolytic enzymes in *S. carlsbergensis*. The turnover numbers per catalytic site were calculated from the steady-state enzymic activities at medium glycolytic flux, and the measured molarities of the respective enzymes and the number of catalytic sites per enzyme molecule taken from the literature.

Figure 3 shows the apparent turnover numbers of glycolytic enzymes in *S. carlsbergensis* calculated for a medium glycolytic flux from the steady-state enzymic activities and the molarities of the respective enzymes. Although the apparent turnover numbers are smaller by a factor of 0.1–0.2 than the maximal values known from literature, they are all within one order of magnitude, with only the exception of triosephosphate isomerase, which again illustrates the kinetic design of the glycolytic process (Hess 1973; Hess *et al.* 1969).

A comparison of the normalized molarities of glycolytic enzymes in yeast based on the number of catalytic centres or monomeric subunits is given in figure 4. The data have been calculated from antibody titrations as well as from kinetic measurements, and show a remarkably high and uniform level of the order of 10^{-4} M in the cytosol (Hess *et al.* 1969). Catalytic centres and the respective substrates vary obviously within the same molarity range, which is especially interesting with respect to the dynamics of the whole system (see §5*a*). The apparent kinetic

constants are of the order of 1 mM, well above the steady-state concentrations of most glycolytic intermediates. These intrinsic properties of the glycolytic system enable the cell to respond quickly to various metabolic needs by changing the overall flux (see §5).

In addition to the simple first-order kinetic response of most glycolytic enzymes, a fine and complex regulation of the glycolytic flux is exerted by some key enzymes, such as phosphorylase, phosphofructokinase or pyruvate kinase. Their kinetic properties are allosteric and are commonly described with the concerted structural transition theory or the induced fit model on the basis of an oligomeric quaternary structure. However, it should be mentioned that not all oligomeric enzymes show this property.

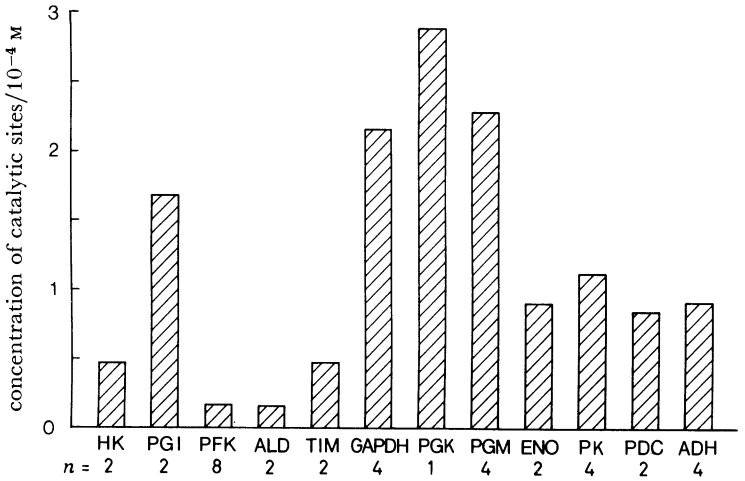

FIGURE 4. Molarity of catalytic sites of glycolytic enzymes in *S. carlsbergensis*. The molarities of the enzymes were determined by antibody titration and by kinetic measurements. The numbers of catalytic sites per enzyme molecule were taken from the literature.

TABLE 2. NUMBER OF SUBUNITS AND HILL COEFFICIENTS OF GLYCOLYTIC ENZYMES IN YEAST

enzyme	number of subunits	Hill coefficient	references
HK	2	1	Steitz *et al.* (this symposium)
PGI	2	1	Achari *et al.* (this symposium)
PFK	8	2–6	Hess *et al.* (1975), Tamaki & Hess (1975*a*, *b*)
ALD	2	1	Horecker *et al.* (1972)
TIM	2	1	Alber *et al.* (this symposium)
GAPDH	4	1–2	Boiteux & Hess (1974), Ovadi *et al.* (1979)
PGK	1	1	Blake & Rice (this symposium)
PGM	4	1	Winn *et al.* (this symposium)
ENO	2	1	Brewer & Weber (1968)
PK	4	1–3	Markus *et al.* (1980), Johannes *et al.* (1973), Wieker & Hess (1971)
PDC	2	2	Boiteux & Hess (1970), Ullrich & Kempfle (1969)
ADH	4	1	Brändén *et al.* (1975)

Table 2 summarizes data on the quaternary structure of the glycolytic enzymes from yeast. As in most other organisms (Ruth & Wold 1976), most glycolytic enzymes show a variety of different oligomeric structures; one namely phosphoglycerate kinase, is monomeric. In general, a larger number of enzyme subunits indicates a greater complexity of structural changes upon binding of substrates and effectors, and thus a more intricate kinetic control (see §5).

The latter property is represented by the Hill coefficient, which varies from 2 to 6 in regulatory enzymes.

The structural stability of some oligomeric enzymes is remarkable and results in a catalytic function stable over a large concentration range. Figure 5 demonstrates the stability of the activity of phosphofructokinase, an octameric enzyme of relative molecular mass 720000, over five orders of magnitude of enzyme concentration (Goldhammer & Paradies 1979; Tamaki & Hess 1975*a*, *b*; Uyeda 1979). Similar ranges have been measured for pyruvate

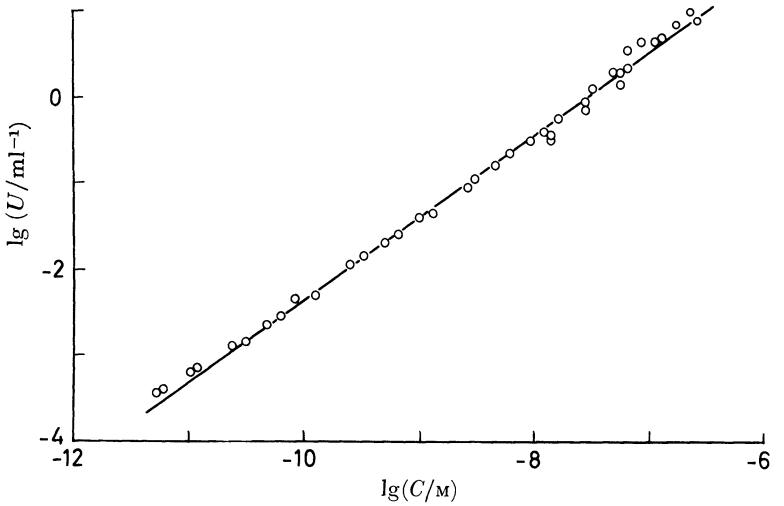

FIGURE 5. Activity plotted against molarity of phosphofructokinase from *S. carlsbergensis*. The kinetic measurements were performed in a coupled assay with pyruvate kinase and lactate dehydrogenase.

kinase, lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, aldolase and pyruvate decarboxylase (Hess & Boiteux 1972; Hess & Wurster 1970; Wurster & Hess 1970). Kinetic stability usually indicates a high stability of the oligomeric structure. Indeed, for muscle pyruvate kinase the stability of the tetrameric enzyme against dissociation is of the order of 67 kJ/mol, comparable with that of deoxyhaemoglobin (Doster & Hess 1981).

This high degree of stability, however, should not be generalized for enzymes with oligomeric structures. It is known that, for example, pyruvate kinase from *E. coli* (unpublished observation) or glyceraldehyde-3-phosphate dehydrogenase from muscle (Ovadi *et al.* 1979) reversibly dissociate into subunits upon changes of temperature. Furthermore, it has been reported that phosphofructokinase from muscle aggregates to large complexes under physiological conditions (Goldhammer & Paradies 1979; Uyeda 1979). It has been suggested that this aggregation is an effective measure for regulating the concentration of the active enzyme in addition to the allosteric control features of this enzyme (see §4).

4. REGULATORY MECHANISMS OF SINGLE ENZYMES

Today, Nature displays a multiplicity of different mechanisms to control the catalytic activity of individual glycolytic enzymes; some of these mechanisms are widely distributed, some are found only in enzymes from certain species or organs, few are the sole control of an

enzyme, many exert – and usually in various combinations – their control in cooperation with others. Most often the following seven types of control are found:

(1) substrate control by saturation kinetics of the classical Michaelis–Menten type;
(2) control by availability of cofactor(s) or metal ions;
(3) control by inhibitors (competitive, non-competitive, uncompetitive or mixed-type);
(4) control by positive and negative effectors (isosteric and allosteric);
(5) regulation by dissociation, association and aggregation;
(6) regulation by chemical interconversion;
(7) control of enzyme concentration and isozyme ratio by synthesis and degradation.

The mechanisms of types 1 and 2 are basic features of enzymic catalysis and can be found in structurally relatively simple enzymes like triose phosphate isomerase, 3-phosphoglycerate kinase, phosphoglycerate mutase or alcohol dehydrogenase, while the regulation of the other glycolytic enzymes is more complex and can be attributed to combinations of types 1 and 3, 2 and 4, or 4 and 6, for example. Control by positive or negative effectors is usually based on conformational changes of the quaternary structure of an enzyme (see §3). Allosteric binding and concomitant concerted transition of all subunits of the enzyme to an active R-form or an inhibited T-form is often found with the key enzymes of the glycolytic chain, namely phosphofructokinase and pyruvate kinase. This type of regulation allows fast and effective changes of the enzyme activities, almost like an on–off switch.

There is, however, great variability of regulatory mechanisms of one enzyme from species to species. Although only allosteric phosphofructokinase is found in general from bacterial to mammalian cells, the slime mould *Dictyostelium discoideum* uses a simple Michaelis–Menten phosphofructokinase (Baumann & Wright 1968).

The variable complexity of the regulatory property of one enzyme isolated from different sources is illustrated in figure 6. Pyruvate kinases from muscle, yeast and *E. coli* catalyse the same reaction with comparable turnover numbers, but they differ widely with respect to their affinity constants. In the enzymes from all three organisms the active complex is formed by binding phosphoenolpyruvate, adenosine diphosphate and magnesium to the enzyme. However, whereas the muscle enzyme is controlled by Michaelis–Menten kinetics (types 1 and 3), the other two enzymes show concerted transitions to different (and inactive) quaternary structures (types 2 and 4) upon binding of adenosine triphosphate or magnesium, respectively. The ATP-liganded inactive states are reversibly transformed to the active R-states by dissociation of the ATP or by binding of fructose-1,6-bisphosphate. The magnesium-liganded inactive state of the *E. coli* enzyme is converted into the active configuration upon dissociation of the magnesium. Thus, the inactive conformation of the enzyme represents a readily accessible storage form that can be mobilized in a few seconds (Boiteux *et al.* 1979; Markus *et al.* 1980).

Whether a regulation by dissociation–association (type 5) plays any significant role under physiological conditions is an open question. Animal phosphofructokinase has been isolated in various polymeric forms, enzymatically active as well as inactive. However, a significant function of these states for glycolytic regulation *in vivo* has not been established. Prokaryotic phosphofructokinase does not aggregate (Evans & Hudson 1979; Goldhammer & Paradies 1979; Uyeda 1979). Studies *in vitro* of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase demonstrated an intricate monomer–dimer–tetramer equilibrium with the tetrameric form

being enzymatically inactive. However, again, its physiological significance is not known (Ovadi *et al.* 1979).

A more complex form of regulation is the reversible interconversion of an enzyme between two chemically different species (type 6). Usually, this interconversion is coupled to dissociation–association equilibria (type 5). The best known example of this type is the regulation of the catalytic activity of phosphorylase, which is converted from the active phosphorylated *a* form by the enzyme phosphorylase phosphatase to the inactive dephosphorylated *b* form. The reverse reaction is catalysed by phosphorylase kinase upon electrical stimulation or the addition

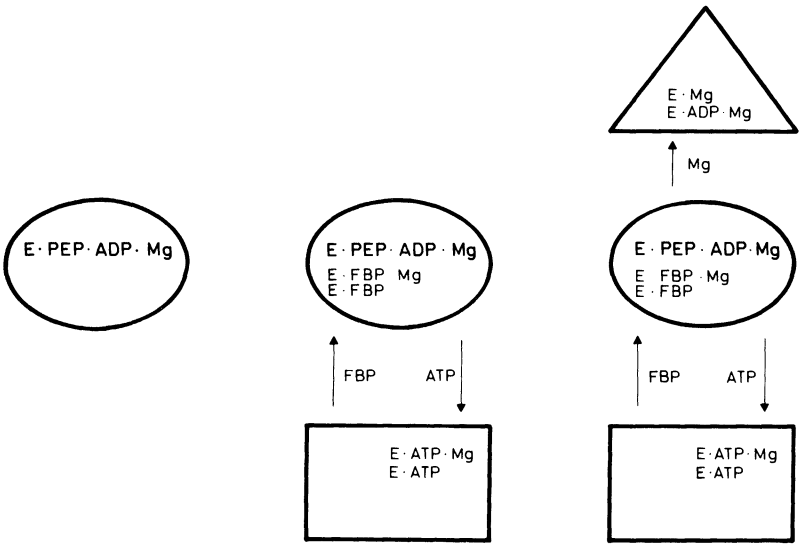

FIGURE 6. Effect of conformational changes of pyruvate kinases from different sources on binding of effectors. The catalytically active conformations are indicated by ovals, the inactive ones by rectangular or triangular figures. The various enzyme–ligand complexes found with the respective conformations are listed inside the figures.

of adrenalin. This enzyme itself is in turn converted from its active form by phosphoprotein phosphohydrolase to its inactive form and, of course, the reverse reaction is again performed by a fourth interconverting enzyme, called phosphorylase kinase kinase. Obviously, this rather complex type of enzyme cascade represents an extremely effective measure for regulation of enzymic activity and is used by Nature only at certain critical and specifically located switching points in the interconnected network of cellular metabolism (see §5). Such an enzyme cascade allows the conversion of a chemical signal appearing in the concentration range 10^{-9} to 10^{-8} M into the turnover of glycolytic metabolites with concentrations of the order of 10^{-4} M (Busby & Radda 1976; Cohen 1980; Stadtman & Chock 1978).

The regulation of enzyme concentration by synthesis (type 7) has been studied extensively, and the mechanisms of repression–derepression of certain operons to lead to the controlled production of an enzymic species is well understood, at least for prokaryotic microorganisms. The picture is not so clear for eukaryotic cells and for the control of the manifold processes leading to the degradation of enzymes. It is apparent, however, that for a proper function of cellular metabolism, proteolytic reactions must be controlled as effectively as synthetic ones (Holzer *et al.* 1975; Lenney 1980; Maurizi & Switzer 1980).

5. Regulatory mechanisms of the enzyme chain

(a) *Quantitative aspects*

The dynamics of the glycolytic flux in the intact cell results from the complex interplay of the regulatory properties of the individual enzymes of the glycolytic chain, and – given all the necessary data – it should be possible to calculate correct values for overall flux and individual metabolite concentrations. Today, however, we still meet obstacles in such a quantitative treatment of the glycolytic network, due mainly to a number of partly unsolved problems: Do cytosolic compartmentations or glycolytic enzyme complexes exist? Are data obtained from experiments *in vitro* applicable to conditions *in vivo*? Are available kinetic data sufficient for an extensive mathematical treatment of the glycolytic network?

The question of compartmentation of the cytosolic space inside cells has been raised by different authors (Friedrich 1974; Hess & Boiteux 1972) and has since been approached by different methods. There is now evidence that at least some type of *dynamic compartmentation* may exist, that is a metabolic flux-dependent inhomogeneous distribution of enzymes and metabolites in the cytosol (Boiteux & Hess 1978, 1980; Boiteux *et al.* 1980; Hess *et al.* 1980). The inhomogeneous distribution of various configurations of carbohydrate metabolites due to the anomeric specificity of enzymes has already been described as *chemical compartmentation* in §2. Despite a number of efforts to demonstrate glycolytic particles or at least functional aggregates of different glycolytic enzymes (Busby & Radda 1976; Friedrich *et al.* 1977; Mowbray & Moses 1976; Ottaway & Mowbray 1977; Ovadi *et al.* 1978), the evidence for this type of polymeric anisotropy is still weak, and it seems reasonable to neglect in the first approach these possible sources of error in a quantitative treatment.

To answer the second question, one has to consider that kinetic data are generally collected *in vitro* with purified enzymes, which might be kinetically altered by preparation techniques. Also, the enzyme concentration used in these studies is usually several orders of magnitude lower than the actual enzyme concentration in living cells. The problem has been approached by two methods. Kinetic data from measurements *in vitro* have been used to calculate fluxes *in vivo*, through enolase in rabbit muscle (Bücher & Sies 1969), and through glucosephosphate isomerase as well as pyruvate kinase in yeast (Barwell & Hess 1972; Hess 1973; Wurster & Schneider 1970). In all three cases the relations between calculated and experimentally measured fluxes were satisfactory. Furthermore, the kinetic properties of phosphofructokinase and pyruvate kinase from yeast did not show significant alterations when measured *in vitro* or *in situ* in toluene-treated cells (Banuelos *et al.* 1977; Sols *et al.* 1973). From these experiments it seems justifiable to use kinetic parameters obtained from purified enzymes for calculations of intracellular fluxes.

All calculations, however, have to take into account the high concentration of glycolytic enzymes inside the cell. As shown in figure 4, the concentrations of single catalytic centres in the cytosolic space of the yeast cell are around 10^{-4} M, about equimolar with the concentration of some of the glycolytic intermediates (Hess *et al.* 1969). This low substrate:enzyme ratio, of course, is not consistent with the usual steady-state treatment of enzyme kinetics. Furthermore, the rather high dissociation constants of glycolytic substrate–enzyme complexes in the cytosol result in low degrees of saturation of enzymes with their respective substrates. Figure 7 shows the saturation of glycolytic enzymes in *S. carlsbergensis* measured under steady-state

conditions at medium glycolytic flux (Hess *et al.* 1969). The ratio of steady-state activity to maximum activity was between 0.01 and 0.2, corresponding to 1 and 20% saturation; in other words, the enzymes operate at substrate levels far below their half-saturation constants, in a range that is especially sensitive to mediation by position effectors.

The third question is easier to answer. Although glycolytic enzymes have been studied quite extensively over many years, we still lack dependable kinetic data for a number of enzymes, e.g. glyceraldehyde-3-phosphate dehydrogenase or aldolase. Furthermore, it seems clear that we are far from knowing all metabolic effectors mediating enzymic activities, or all feed-forward and feedback loops involved. Despite these restrictions, some partly successful quantitative approaches have been made with tumour cells (Garfinkel & Hess 1964) and heart extract (Garfinkel *et al.* 1968), including a number of simplifying assumptions. This gives room for hope that a quantitative treatment of the dynamics of the glycolytic network is really at hand.

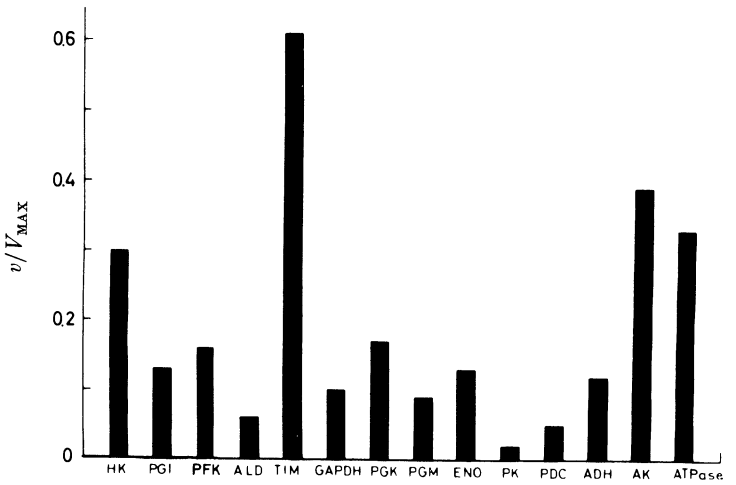

FIGURE 7. Saturation of glycolytic enzymes at medium glycolytic flux in *S. carlsbergensis*. The kinetic measurements for v (steady-state concentrations of metabolites) and V_{max} (saturation with substrates) were performed with yeast extracts obtained from yeast cells by sonication and high-speed centrifugation.

(*b*) *The oscillatory state*

An effective tool for studying and eventually understanding the principles and mechanisms involved in the quantitative aspect of metabolic control is the analysis of the oscillatory state of glycolysis. As observed in the early 1960s, cellular extracts from sources as different as yeast or heart, and intact cells from various organisms, maintain under certain experimental conditions steady self-induced temporal oscillations of concentrations of glycolytic intermediates (Boiteux & Hess 1974*a*, *b*, 1980; Hess & Boiteux 1971, 1980).

This phenomenon has been studied most extensively in yeast. Here the analysis revealed that the feature of glycolytic self-excitation is based on the cooperative properties (type 4 of §4) of phosphofructokinase, the so-called oscillophore, and an intricate network of feed-forward and feedback loops of various metabolic activations and inhibitions, involving the enzymes pyruvate kinase, adenylate kinase, glyceraldehyde-3-phosphate dehydrogenase and alcohol dehydrogenase (Boiteux & Hess 1974). In short, phosphofructokinase is allosterically inhibited

by its substrate ATP (and citrate), synergistically activated on the other hand by its second substrate F6P, its product FBP and the effector AMP. As shown in experimental and model studies (Boiteux *et al.* 1975; Hess & Plesser 1979), the combined features of substrate inhibition and product activation give rise to self-sustained oscillations of the enzymic activity, modulated by the relative velocities of substrate supply and product removal, respectively.

A detailed study on the propagation of the metabolic pulses generated by the oscillophore produced interesting results: two more control points of the complex phenomenon were located (Boiteux & Hess 1973), namely the enzymes pyruvate kinase, and glyceraldehyde-3-phosphate dehydrogenase together with phosphoglycerate kinase. The latter enzyme couple operates obviously under twin control from the metabolic pools of nicotinamide adenine dinucleotides and adenosine phosphates, in addition to substrate activation by phosphate and glyceraldehyde-3-phosphate. Pyruvate kinase, on the other hand, is controlled by feed-forward activation by ADP and FBP as well as feed-forward inhibition by ATP (type 4, §4).

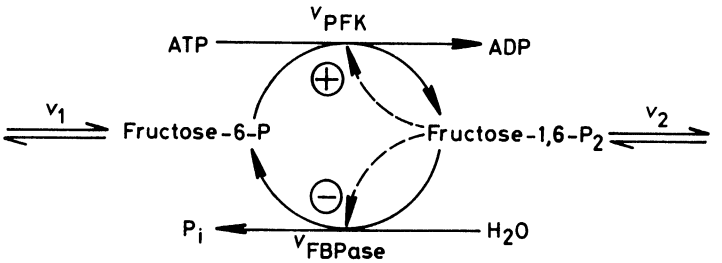

FIGURE 8. Schematic diagram of the concerted regulation of the antagonistic enzyme couple phosphofructokinase–fructosebisphosphatase. The scheme represents an open system with v_1 and v_2, the velocities of source and sink reactions, and v_{PFK} and v_{FBPase}, the velocities of the enzymic fluxes through phosphofructokinase and fructosebisphosphatase, respectively. Fructose-1,6-bisphosphate exerts cooperative product activation and substrate inhibition on the enzyme couple.

There is no doubt that the enzymic control features of the glycolytic pathway that show up from the analysis of the oscillatory state are also effective, if only partly or temporarily latent, in the non-oscillatory state. This is certainly true for the location of the control points and for the intricate interconnected network of the various control mechanisms. Actually, it turns out that every single control point identified by the analysis of the oscillatory state is situated at a major branching point of the glycolytic and related metabolic pathways (see §5*d*).

(*c*) *The glycolysis–glycogenesis flip-flop*

In contrast to the rather complex quantification of enzymic cooperation in the glycolytic network, it is relatively easy to understand the most important regulation of catabolic and anabolic reactions of the carbohydrate metabolism in its quantitative aspects. It is obvious that degradation and synthesis of glycosyl units cannot proceed simultaneously without waste of energy in futile cycles.

The situation is shown schematically in figure 8 in a simplified form for the enzyme couple phosphofructokinase–fructose-1,6-bisphosphatase, which in contrast to the model of Newsholme & Start (1973) is treated as an open system. These enzymes catalyse reactions in opposite

directions with the velocities v_{PFK} and v_{FBPase}, respectively, and simultaneous operation results according to (1) in the net flux

$$v = v_{PFK} - v_{FBPase}. \quad (1)$$

The smaller of the opposite fluxes acts as an apparent ATPase, wastefully splitting ATP, and if $v_{PFK} = v_{FBPase} = v_{ATPase}$ the net flux, v, will be zero and all energy lost. The simplest way to suppress the futile cycling is to include in a model calculation cooperative product activation in one direction and cooperative substrate inhibition in the other, as indicated in figure 8 (Boiteux *et al.* 1980).

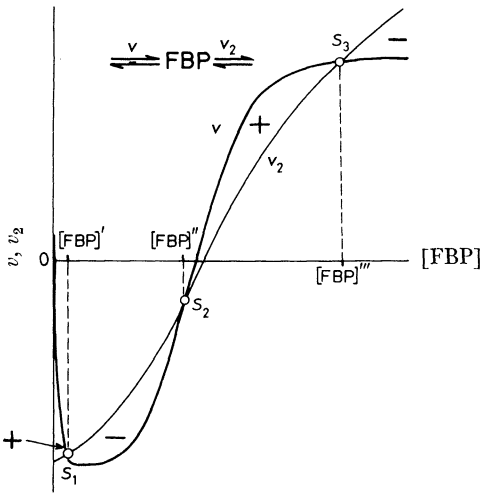

FIGURE 9. Flip-flop system of the antagonistic enzyme couple phosphofructokinase–fructosebisphosphatase. According to (1), the net flux of fructosebisphosphate from the enzyme couple is v (heavy curve), whereas the sink reaction v_2 is represented by the light curve. The system has two stable quasi-stationary states, S_1 at negative values of v and v_2 (glycogenetic state) and S_3 at positive fluxes (glycolytic state). The quasi-stationary state S_2 is unstable; any change of the concentration [FBP]″ causes an imbalance of the condition $v - v_2 = 0$, leading to S_3 for $v - v_2 > 0$ or to S_1 for $v - v_2 < 0$. The symbols + and − in the areas between the two curves indicate unstable regions of the system where [FBP] increases or decreases, respectively.

This antagonistic modulation of enzyme activities results in the metabolic flip-flop system of figure 9, where v and the flux via aldolase, v_2, are plotted against the concentration of FBP. The system can attain only two quasi-stationary values, S_1 at high glycogenetic flux and S_3 at high glycolytic flux. The intermediate point S_2 is unstable and any small fluctuation of the FBP level will shift the system towards the glycolytic or the glycogenetic states. Actually, this type of antagonistic regulation is widely found in Nature, often supplemented with additional reciprocal effectors, like AMP or citrate in our example, that reversibly activate and inhibit the respective enzyme couple. The situation is similar with the enzyme couples pyruvate kinase – phosphopyruvate carboxylase and phosphorylase – glycogen synthetase. Whereas the PFK–FBPase and PK–PPC couples are controlled by cooperative transitions in quaternary structure caused by the allosteric effector fructose-1,6-bisphosphate, the phosphorylase step is regulated by an enzyme cascade (see §4). In every case, Nature has developed effective mechanisms to prevent the operation of futile cycles and to realize successfully antagonistic on–off switches for anabolic and catabolic reactions (Boiteux *et al.* 1980).

Two examples for the operation of the FBP-actuated flip-flop mechanism, switching from

glycolysis to glycogenesis and vice versa, are given by the experimental data in table 3. The cytosolic concentrations of the indicated metabolites were measured in steady-state glycolysis or glycogenesis in cells of *S. cerevisiae* (Barwell & Hess 1971; Hess 1973) and *E. coli* (unpublished results), respectively. As can be seen, the concentrations of the three adenosine phosphates vary very little on switching from one state to the other, whereas there is a great change in the F6P and an extreme change in the FBP levels. The saturations of PFK and PK in the last

TABLE 3. GLYCOLYSIS–GLYCOGENESIS: METABOLITE LEVELS AND ENZYME SATURATION

(The metabolites were measured in cells of *S. cerevisiae* and *E. coli* during steady-state glycolysis and glycogenesis The ratios v/V_{max} for the FBP-sensitive enzymes were calculated from the concentrations of respective metabolites and the known kinetic constants of the enzymes. For details see text.)

metabolic state	concentration/mM						v/V_{max}	
	ATP	ADP	AMP	PEP	F6P	FBP	PFK	PK
				S. cerevisiae				
glycolytic	3.12	2.00	0.37	0.26	0.88	**4.31**	0.24	0.29
glycogenetic	3.36	2.08	0.37	0.45	0.37	**0.04**	<0.001	<0.001
				E. coli				
glycolytic	2.46	1.40	0.36	0.29	0.71	**3.64**	0.15	0.08
glycogenetic	2.24	1.76	0.40	0.96	0.23	**0.52**	<0.001	<0.01

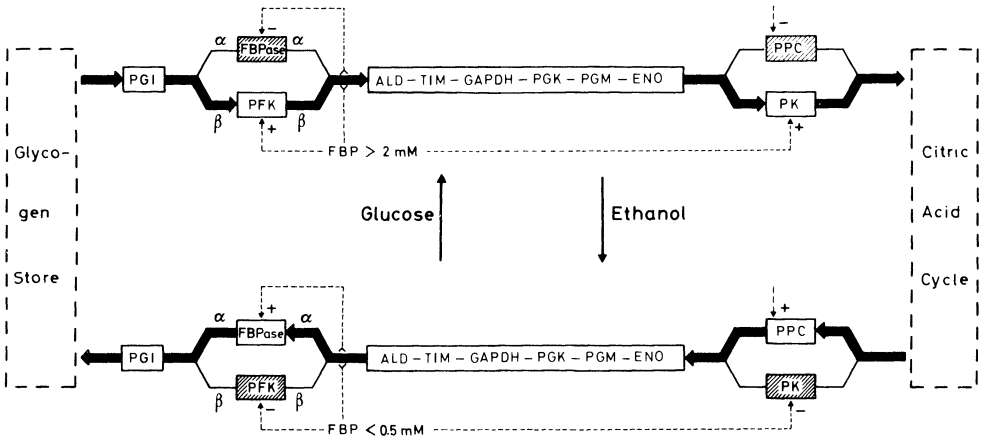

FIGURE 10. Diagram of the glycolysis–glycogenesis flip-flop. Heavy arrows indicate the direction of the metabolic fluxes for the glycolytic and the glycogenetic states, respectively. The switched-off enzymic bypasses are shown by thin lines. Broken lines indicate the threshold action of fructosebisphosphate and the allosteric actions of various effectors on phosphopyruvate carboxylase. The transitions between the two states are shown here for yeast, for other types of cells the triggering compounds glucose and ethanol must be chosen accordingly. For further information see text.

columns were calculated from the known kinetic parameters of the enzymes. The fluxes through both enzymes are switched on for the glycolytic and switched off for the glycogenetic state in both types of cells. The PFK activity is controlled by the cooperative actions of the F6P and FBP levels, whereas PEP and FBP exert anti-cooperative effects on PK. The relatively incomplete switch-off of PK in *E. coli* is probably improved in the cells by a yet unknown effector that also controls the FBP-insensitive PK always found with the FBP-sensitive enzyme (unpublished results).

From the known data on metabolic concentrations and enzyme kinetics, one can construct a rather general scheme of the flip-flop mechanism between the metabolic states of glycolysis and glycogenesis. Figure 10 shows that the main effector is the FBP concentration, which exerts a threshold function: below a certain concentration, which differs somewhat from organism to organism, it inactivates in a combined feedback and feed-forward action the catabolic enzymes PFK and PK and relieves antagonistically the inhibition from the anabolic FBPase. Above the threshold level, FBP activates the glycolytic enzymes and inhibits the glycogenetic activity of FBPase. This basic mechanism is supported by similar effects of AMP, citrate and other metabolites acting synergistically on the above-mentioned key enzymes and the various anabolic phosphopyruvate carboxylases. In any case, the physiological transition inside a cell from glycolysis to glycogenesis and vice versa is definitely a quantized all-or-none effect, quite different from the subtle and intricate regulation of fluxes in either direction.

(*d*) *Location of control points*

It is rewarding to study the enzymes of the glycolytic chain with respect to their control properties. As shown in figure 1, we observe a special pattern of Michaelis–Menten enzymes, cooperative enzymes and interconvertible enzymes. This design of the glycolytic pathway obviously serves regulatory functions.

At the entrance and exit of the glycolytic chain we find two enzymes that are very effectively controlled by enzymic interconversion to chemically different species (see §4, type 6), namely phosphorylase and pyruvate dehydrogenase. Phosphorylase opens access to the glycogen store of the cell which is a store both for metabolic energy and for simple carbon chains needed in synthesis. As pointed out in §4, the enzyme cascade regulating the activity of phosphorylase is extremely effective, so that here the glycolytic flux can be switched on and off directly at the gate of the store. The same is true for pyruvate dehydrogenase, located at the end of the glycolytic chain and channeling its product acetylcoenzyme A to either the catabolic citric acid cycle or various anabolic chains like the fatty acid cycle. Obviously, the strategically most prominent positions of the glycolytic pathway, namely the entrance and the exit, are taken by two enzymes with interconvertible forms that operate as slow-acting but very effective on–off switches.

Two further important control points are located in the upper and lower parts of the glycolytic pathway: phosphofructokinase and pyruvate kinase, respectively. Both enzymes are cooperative enzymes (see §4, type 4) with glycolytic intermediates as homotropic and heterotropic effectors. The lack of long feedback loops or slowly acting enzyme cascades make the control here extremely rapid in response, which is especially important with respect to the suppression of futile cycles (see §5*c*). Regulation of the glycolytic flux at the position of phosphofructokinase controls not only glycolysis but also, via the concentration of dihydroxyacetone phosphate, indirectly the synthesis of glycerol, lipids and phospholipids. In the same manner, regulation of the glycolytic flux at the position of pyruvate kinase influences the synthesis of alanine, serine, ethanolamine and related compounds. It is clearly evident that the two cooperative and fast-responding enzymes control major branching points of the glycolytic chain to non-glycolytic pathways.

The rest of the glycolytic enzymes in figure 1 are represented as less tightly controlled (types 1–3 of §4). This does not mean, however, that these enzymes are only catalysts with no control properties. Multiple functions of substrate, cofactor and inhibitor concentrations

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definitely modulate the activities of these enzymes as well. An example of this type of regulation is the tri-substrate two-product enzyme glyceraldehyde phosphate dehydrogenase. This enzyme, located at the intersection of the glycolytic pathway and the pentose phosphate shunt, is obviously controlled not only by its substrates, D-glyceraldehyde-3-phosphate and phosphate, but in addition by the redox potential of its cofactor nicotinamide adenine dinucleotide.

One should bear in mind that the short outline given above is rather general, and is subject to variations in different cell types. It must be emphasized, however, that the widely differing needs of cells for regulation of the glycolytic pathway are actually met in every case by a meticulous design of enzymic control properties and a strategic location of the respective control points.

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