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The Intracellular Equilibrium Thermodynamic and Steady-State Concentrations of Metabolites

SIDNEY A. BERNHARD

Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

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ABSTRACT

A new model for the organization and flow of metabolites through a metabolic pathway is presented. The model is based on four major findings. (1) The intracellular concentrations of enzyme sites exceed the concentrations of intermediary metabolites that bind specifically to these sites. (2) The concentration of the excessive enzyme sites in the cell is sufficiently high so that nearly all the cellular intermediary metabolites are enzyme-bound. (3) Enzyme conformations are perturbed by the interactions with substrates and products; the conformations of enzyme–substrate and enzyme–product complexes are different. (4) Two enzymes, catalyzing reactions that are sequential in a metabolic pathway, transfer the common metabolite back and forth via an enzyme–enzyme complex without the intervention of the solvent environment. The model proposes that the enzyme–enzyme recognition is ligand-induced. Conversion of E_2S and E_2P results in the loss of recognition of E_2 by E_1 and the concomitant recognition of E_2 by E_3. This model substantially alters existent views of the bioenergetics and the kinetics of intracellular metabolism. The rates of direct transfer of metabolite from enzyme to enzyme are comparable to the rates of interconversion between substrate and product within an individual enzyme. Consequently, intermediary metabolites are nearly equipartitioned among their high-affinity enzyme sites within a metabolic pathway. Metabolic flux involves the direct transfer of metabolite from enzyme to enzyme via a set of low and nearly equal energy barriers.

INTRODUCTION

Over the past decade, this laboratory has been interested in some of the enzymes of the glycolytic pathway, and the way the reactions catalyzed by these enzymes are coupled one to another for the organized interconversion of intermediary metabolites (*1,2*). Like many others, we initially sought answers to this problem via the analysis of the molecular properties within individual enzyme-catalyzed reactions. The implicit assumption in such studies is that detailed knowledge of the qualitative and quantitative facts regarding individual enzyme-catalyzed reactions can be assembled into a coherent picture of how a metabolic pathway functions; "a comprehensive collection of K_m's, k_{cat}'s, and the dissociation constants for enzyme-metabolite complexes are adequate to synthesize the behavior of the complete metabolic pathway." Working under this assumption, much useful information has been gained regarding rates and reaction equilibria. Nevertheless, attempts to rationalize overall metabolic flux by the summation of the rates and equilibria of individual enzyme-catalyzed reactions have never led to clear mechanisms for coupled reaction pathways. We have therefore designed experiments to probe mechanisms of coupled enzyme-catalyzed reactions, primarily within the glycolytic pathway (*3*).

In a series of communications, we have indicated that a synthesis of kinetic and equilibrium parameters derived from individual enzyme-catalyzed reactions cannot lead to a reliable prediction of the behavior of the coupled reaction pathway (*3–6*). There are at least two fundamental reasons why the summation of individual properties does not lead to a correct prediction of the properties of the whole system. (1) The mechanistic pathway for the coupled process is not the same as that for each of the two catalyzed reactions studied individually, since the environments of metabolites in the coupled versus the individual uncoupled reaction pathways are different. (2) The coupling process perturbs the individual enzyme-catalyzed processes. In this paper we shall discuss experiments that bear on the relevance of each of these two factors to the metabolic pathways.

Much more is known about the reactions, solution thermodynamics, enzyme structures, and catalytic mechanisms within the glycolytic pathway than elsewhere in metabolism. Therefore, we shall focus our attention on this pathway. This paper deals with two aspects of coupled enzymic reactions relevant to cellular metabolism. These are the kinetics and thermodynamics of the coupled sequences of chemical reactions that constitute a metabolic pathway. A more holistic model of metabolism must ultimately take into account the coupling of all the kinetic parameters with all the equilibrium thermodynamic data concerning the interactions among metabolites and enzymes in order to describe intracellular, or intraorganellar, steady-state metabolism. This paper presents an early progress report toward this goal.

The very sparse state of current theory in these regards is in part because of the secrecy involved in the publication of this volume; had a manuscript been submitted to Terrell's prior scrutiny, a treatise on the irreversible thermodynamics of metabolic reaction pathways would almost certainly have been appended.

Before dealing with the behavior of multiple enzymes and their reaction components, we review some aspects of the behavior of individual enzyme–metabolite systems germane to our considerations of coupled reactions. Relevant conclusions based on studies of individual enzyme-metabolite systems, and some of the experimental bases for these conclusions, are presented in the following sections.

SOME USEFUL GENERALIZATIONS REGARDING THE PROPERTIES OF INDIVIDUAL ENZYME–SUBSTRATE SYSTEMS

In this section, we have deliberately selected out generalizations concerning the properties of (glycolytic) enzyme–substrate systems that seem useful for our discussion of the overall metabolic process. In addition, we present herein some (but by no means comprehensive) experimental verification for these experimentally based generalizations.

Each of the Glycolytic Enzymes Can Exist in at Least Two Alternate Conformational States

A wide variety of experimental evidence, particularly direct X-ray crystallographic structure determinations, have shown that changes in protein structure accompany enzyme–substrate or enzyme–product complexation (*7*). For example, among glycolytic dehydrogenases, two types of enzyme conformational changes have been noted; changes accompanying coenzyme ligation to the apo enzyme and changes accompanying the conversion of oxidized-to-reduced coenzyme (*8*). Changes both in crystal structure and macromolecular structure in solution have also been observed in the interaction of phosphoglycerate kinase (PGK) with the reactant, 1,3-diphosphoglycerate (DPG) versus the product, 3-phosphoglycerate (PGA) (*15–17*). Moreover, changes in protein structure have been detected spectrophotometrically and spectrofluorometrically during the interaction of dihydroxyacetone-PO_4 with aldolase (*9*) and in the interaction of NAD^+ with apo- versus acyl-glyceraldehyde-3-PO_4 dehydrogenase (*10*).

The Enzyme-Bound Substrate-Product Equilibria Can Differ Substantially from the Equilibrium in Aqueous Solution

In discussions of the equilibrium thermodynamics of metabolic pathways, it is usual to consider the equilibrium constants for the *enzyme-*

catalyzed substrate–product interconversions in dilute aqueous solution. Such equilibria are easy to quantitate even when they are displaced far in the direction of either product or substrate formation. Aqueous equilibrium constants for individual glycolytic reactions are conventionally carried out in the presence of minute "catalytic" quantities of enzymes. It is, however, possible to study these same reaction equilibria in the presence of very high concentrations of enzymes, such that the total enzyme concentration exceeds that of the total concentrations of metabolites. Since enzyme concentrations of 0.1–1 m*M* are realizable, particularly with many of the glycolytic enzymes, the reaction equilibria can be studied under more physiologically relevant conditions, such that most of the limiting metabolites are enzyme-bound. A comparison of some enzyme-bound or *internal* equilibrium constants with the corresponding aqueous values for a variety of enzymes are contained in Table 1. The results are noteworthy in two ways: (1) The *internal* equilibrium constants generally differ quite markedly from the aqueous equilibrium constants; and (2) The *internal* equilibrium constants are all close to unity.

We presume, as did Albery and Knowles in a much earlier communication, (*11*), that the nearly equal free-energy levels for enzyme-bound reactant and product is a consequence of enzyme evolution. Presumably, specific ligand-induced conformational states of differing energy offset the intrinsic free-energy difference between reactant and product.

The Concentrations of Individual Enzymes and Intermediary Metabolites

The concentrations of the constitutive glycolytic enzymes in muscle are listed in Table 2. Although these concentrations are approximate, and dependent on the activity estimates for each enzyme in the complex mixture, some straightforward conclusions are derivable from the data. First

Table 1
Equilibrium Constants (Aqueous vs Enzyme Bound)
for Some Glycolytic Enzyme-Catalyzed Reactions[a]

Enzyme	Keq	
	Aqueous	Enzyme bound
Hexokinase	2×10^{3}	~ 1.0
Pyruvate kinase	3×10^{-4}	1.0 –2.0
Phosphoglycerate kinase (yeast)	3×10^{-4}	0.8
Phosphoglycerate kinase (muscle)	8×10^{-4}	0.5 –1.5
Triosephosphate isomerase	2.2×10^{1}	0.6
Phosphoglucomutase	1.7×10^{1}	0.4
Lactate dehydrogenase	1×10^{4}	1.0 –2.0
Yeast alcohol dehydrogenase	1.4×10^{4}	0.15–0.25
Horse liver alcohol dehydrogenase	2×10^{4}	0.10–0.20

[a]For details, *see* ref. (1).

Table 2
Concentrations of Enzymes and Metabolites of the Glycolytic Pathway[a]

Enzymes	Concentration, μ*M*	Metabolites	Concentration, μ*M*
		Precursors and products	
Phosphoglucomutase	31.9	Glucose-6-P	3,900
Aldolase	809.3	Fructose 6-P	1,500
α-glycerol-P-dehydrogenase	61.4	Lactate	3,700
Triose-P-isomerase	223.8	ATP	8,050
Glyceraldehyde-3-P-dehydrogenase	1,398.6	Inorganic-P	8,000
		Intermediates	
Phosphoglycerate kinase	133.6	Fructose 1,6-di P	80
		Dihydroxyacetone-P	160
Phosphoglycerate mutase	235.9	Glyceraldehyde-3-P	80
Enolase	540.7	1,3-di-P-glycerate	50
Pyruvate kinase	172.9	3-P-glycerate	200
Lactate dehydrogenase	296.0	2-P glycerate	20
		P-enol pyruvate	65
		Pyruvate	380
		NAD^+	541
		NADH	50

[a]Values adapted or recalculated from the data available in the literature [for detail, *see* ref. (*1*)].

of all, the concentration of each of the enzymes is high. High glycolytic enzyme concentrations are not unique to muscle cells. Equally high or even higher concentrations are found in yeast and prokaryotic microorganisms (*1*). The concentrations of intermediary metabolites of glycolysis are low or comparable to the concentration of high-affinity enzyme sites (Table 2). This relationship between enzyme and metabolite concentration was pointed out over two decades ago by Srere (*12*). Shortly thereafter, this laboratory demonstrated that the thiolester of 3-phosphoglyceric acid ligated to the enzyme glyceraldehyde-3-PO_4 dehydrogenase (GPDH) is the most abundant 3-carbon intermediate in the glycolytic pathway in muscle; approximately half of the GPDH sites are so acylated (*13*).

Since the concentrations of the high-affinity sites exceed or are comparable to the metabolite concentrations, their absolute concentrations in the cell largely determine the extent of enzyme-bound versus aqueous intermediary metabolite; these concentrations are, in general, sufficiently high to ensure nearly total sequestering of the intermediary metabolites at their high-affinity enzyme sites (*1,2*). Note, however (Table 2), that the intracellular concentrations of precursors (hexose monophosphates) and final product (lactate) do exceed the concentration of their high-affinity

enzyme sites. In muscle, phosphocreatine is an additional, high-concentration precursor.

Bessman has suggested that this high concentration metabolite acts as a shunt for directing mitochondrion-generated energy to the actomyosin complex. Likewise, the hexose phosphates may direct mitochondrion-generated energy to the pentose phosphate pathway and to the initiation of glycolysis. The stoichiometric relationship between ATP and high-affinity ATP binding sites in muscle is uncertain. In addition to the well-characterized actin-myosin and kinase-binding sites for ATP, other important ATP binding sites, such as the active site of 3-phosphoglyceroyl-GPDH, have been largely overlooked, and perhaps unnoticed. Estimates of the intracellular "free" ATP concentration are variable, dependent both on the metabolic state and the method of assay. Assays of free aqueous ATP by NMR techniques give lower estimates than do assays of total ATP extracted from the freeze-quenched cellular milieu. No other adenine nucleotide or dinucleotide attains a concentration comparable to that of its high-affinity enzyme sites.

The high concentration of protein in muscle cytosol (200–300 mg/mL) is not substantially different from the protein concentration in other cells (*1*). These concentrations are almost as high as the concentration of proteins in wet crystals.

Summary of Conclusions from Background Information on Individual Enzyme–Metabolite Systems

(1) The concentration of enzyme sites in the cytosol are generally high, exceeding the concentrations of their high-affinity metabolites.
(2) The affinity of the enzyme for the metabolite is sufficiently high to sequester most of the metabolite within the enzyme site.
(3) Each enzyme interacts differently with reactant versus product so as to overcome the intrinsic difference in chemical potential between reactant and product. This results in a net free-energy change near zero for the *internal* reaction.
(4) The conformation of the enzyme–product complex differs from that of the unliganded enzyme and from the conformation of the enzyme–substrate complex.

THE DIRECT TRANSFER OF METABOLITE VIA ENZYME–ENZYME COMPLEXES

Elsewhere, we have presented evidence that the transfer of metabolite from one enzyme (its site of synthesis) to another (its site of utilization) can proceed by the direct transfer via an enzyme–metabolite–enzyme complex (*1–5,14*). A list of enzyme pairs for which this direct transfer phenomenon has been demonstrated to be the predominant

Table 3
A Short Compilation of
Enzyme Pairs Known to Specifically Interact[a]

Enzyme pairs	
E_1	E_2
Aldolase	Glyceraldehyde-3-P dehydrogenase
Aldolase	Triose-P-isomerase
Aldolase	αGlycerol-P dehydrogenase
Aldolase	Fructose-*bis*-phosphatase
Phosphofructo kinase	Fructose-*bis*-phosphatase
Glyceraldehyde-3-phosphate dehydrogenase	Phosphoglycerate kinase
Citrate synthase	*m*-Malate dehydrogenase
Citrate synthase	Pyruvate dehydrogenase complex
Citrate synthase	Thiolase
Fumarase	*m*-Malate dehydrogenase
α-ketoglutarate dehydrogenase	Succinate thiokinase
m-Malate dehydrogenase	*m*-Aspartate aminotransferase
m-Malate dehydrogenase	*m*-Glutamate dehydrogenase
m-Glutamate dehydrogenase	*m*-Aspartate aminotransferase

[a]For details, *see* ref. (2).

route (at or near physiological concentrations) is contained in Table 3. Admittedly, these examples represent a miniscule fraction of enzymes and metabolites involved in coupled reactions in the cell.

The most convincing results demonstrating direct transfer are derived from experiments in which one of the two enzymes (E_1) is in excess over total metabolite, and because of its high ligand affinity, greatly reduces the aqueous metabolite concentration (3). In such situations, a slow rate of E_2-catalyzed conversion to M_2 can be calculated provided that aqueous M_1 is the only competent substrate. The calculation is based on the known dissociation constant for the E_1–M_1 complex, and the Michaelian parameters (K_M and V_{max}) for the E_2-catalyzed reaction of M_1. If the actual rate of E_2-catalyzed reaction greatly exceeds this prediction, the direct transfer mechanism must obtain, or else formation of E_1–E_2 complex must greatly perturb the E_2-catalyzed reaction rate (3). Experiments involving high enzyme concentrations are difficult to carry out. E_1 must be available in high concentrations. Also, E_1 must be very substantially free from contamination by small quantities of E_2.

Several generalizations regarding the experimentally demonstrated direct transfer processes are worth restating. (1) The rate of transfer of metabolite, and/or its rate of reaction at the E_2 site shows a hyperbolic dependence on the E_1–M concentration. The rate of E_1–M reaction is *in*effectively inhibited by an excess of unliganded E_1; in general, the recognition of E_1–*M* by E_2 is enhanced by metabolite ligation. (2) The apparent affinity of E_2 for the E_1–*M* complex can be estimated from the K_m for the

E_2-catalyzed reaction utilizing E_1–M as a substrate (*1*). Under cellular glycolytic enzyme concentration conditions, a major fraction of the E_1–M complex would be anticipated to be complexed to the cognate E_2 enzyme. (3) Effectors of the direct transfer process exist, whose roles are not revealed from studies of individual enzyme-catalyzed reactions (*4*). (4) The rate of transfer of NADH between cognate dehydrogenases can be faster than or slower than the rate at which the E–coenzyme complex dissociates into the aqueous environment; the enzyme–enzyme transfer rates are nearly independent of the specific enzyme pair for which direct transfer of metabolite is possible (*14*). The rates of the E_2-catalyzed reactions that occur via direct transfer of metabolite from E_1 are comparable in magnitude to the rates of metabolite transfer between the two enzymes. (5) In the cases of direct transfer of NADH among NAD-dependent dehydrogenases, the equilibrium distribution of limiting NADH between E_1 and E_2 in the E_1–E_2 complex is near unity. The affinity of the E_1–E_2 complex for NADH far exceeds the affinity for NADH by either enzyme alone (*6*).

THE EVOLUTION OF ENZYMES IN METABOLIC PATHWAYS

The evolution of enzyme efficiency has been considered in detail by Albery and Knowles (*11*). These authors differentiate between *internal* unimolecular transformation rates and the bimolecular rates of association of metabolic reactants and products with the enzyme. The dissociation of the enzyme–reactant and enzyme–product complexes into aqueous solution are among the unimolecular transformations. According to the Albery-Knowles theory, maximal enzyme efficiency will be achieved when the bimolecular association rates of reactant and product are comparable to the unimolecular rates of transformation and dissociation. As a consequence, the free energies of formation of enzyme–reactant and enzyme–product complexes must be nearly equal; hence, the free-energy change near zero for the internal chemical reaction (Table 1).

Although seemingly plausible, the quantitative predictions of the Albery-Knowles model of enzyme evolution are in conflict with the data presented on cellular concentrations of metabolites vis-à-vis their high-affinity enzyme sites. This is because the concentrations of *free* metabolites are, in general, far lower than the total metabolite concentrations, as determined from chemical analysis of whole cell extracts. For example, in muscle cytosol the concentration of aldolase and glyceraldehyde-3-PO_4 dehydrogenase are each far in excess over the total concentration of the two triose phosphates; the affinities of these two enzymes for dihydroxyacetone phosphate and glyceraldehyde-3-PO_4, respectively, are quite high (the K_d's are much smaller than the molar concentrations of enzymes). Thus, even ignoring the relatively high concentration of triose phosphate isomerase, very little free triose phosphate can be anticipated in the aqueous cytosol (*2,14*).

Since most of the metabolite is sequestered at enzyme sites, the diffusion controlled "on"-rates are limited primarily by concentration of the excessive free enzyme sites. Under such conditions, the balance between diffusion controlled "on"-rates and unimolecular interconversions among enzyme–metabolite complexes would involve much slower internal rates to balance the slower diffusion rate. The overall enzyme-catalyzed efficiency characterized by the steady-state constant, k_{cat}, has now evolved to a magnitude far in excess of the intracellular diffusion limit. Since this implies evolution beyond the "limit of perfection," some mechanism, which is more efficient than the limits set by cytosolic diffusion, must be operative in vivo. We postulate that the mechanism involves the transfer of metabolite from enzyme to enzyme via the formation of enzyme–enzyme complexes. The consequences of such a proposal are considered below.

THE EQUILIBRIUM THERMODYNAMICS OF DIRECT TRANSFER

The most generally verified aspect of our proposal for metabolic flow is that intermediary metabolite transfer involves enzyme–metabolite complexes as virtually the only stoichiometrically significant metabolite species. Regardless of the mechanism of transfer, the experimental data demand an equilibrium free-energy diagram for the stoichiometrically significant species, as is illustrated in Fig. 1. Note in this free-energy diagram for a metabolic pathway the assumptions of equipartition of free

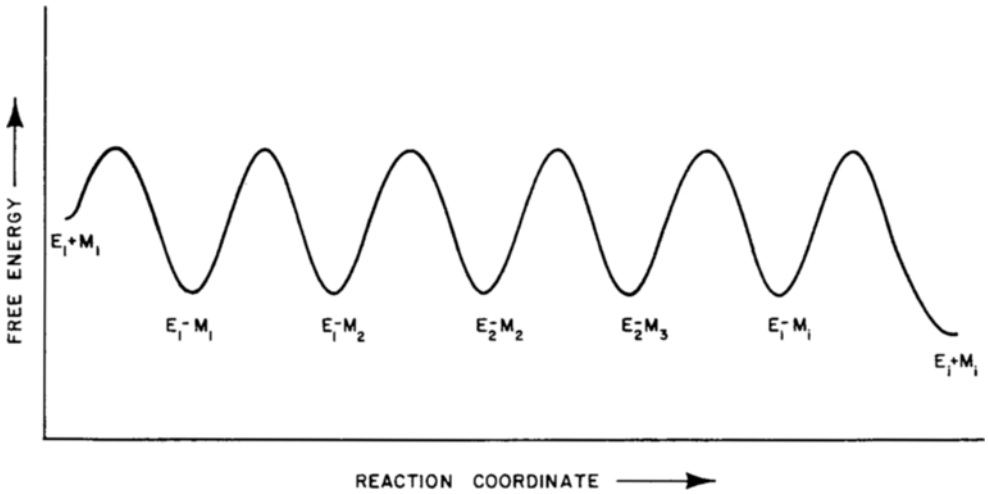

Fig. 1. The energetics of the metabolite transfer. A hypothetical free-energy diagram is depicted for the sequence of enzyme-catalyzed reactions, operating via the direct transfer of metabolites, throughout the pathway. Note equipartitions of substrate and product at individual enzyme sites, as well as the product and substrate of adjacent enzyme sites.

energy between ES and EP for individual enzyme-catalyzed reactions and the equipartition of free energy between E_1–M, and E_2–M. The equipartition between ES and EP is now well established (*2*). The equipartition of M_1 between E_1 and E_2 is less well established; whenever we have measured the partition between E_1–M and E_2–M, the equipartition is as striking as it is for the internal enzyme–reactant versus enzyme–product complex (*6*). The free energy diagram (Fig. 1) implies that rather than allow the free energy to rise or fall precipitously between aqueous reactants and aqueous products, the metabolic intermediates are equipartitioned among the stoichiometrically substantial sites of the pathway.

The intermediary metabolites that have moderately high free energies in aqueous solution are stabilized by their sequestered interaction with the various enzyme proteins of high affinity. It is interesting to note that the equilibrium constants near unity for bound reactants and products extends to reactions that are intrinsically driven toward product formation in the pathway of glycolysis (Table 1). For example, both the reactions catalyzed by phosphoglycerate kinase and phosphoenol pyruvate kinase strongly favor the formation of glycolytic products in aqueous solution. Nevertheless, in muscle where glycolysis is unidirectional, the internal equilibrium constants are still close to unity. If direct transfer were the exclusive mechanism for metabolite transfer in glycolysis, or if (as appears almost certain) nearly all of the glycolytic intermediates were sequestered as enzyme–metabolite complexes, metabolite interactions within these two kinases present a strong thermodynamic drive *against* the formation of final product (lactate). Glycolysis is not driven toward completion (i.e., the formation of pyruvate or lactate), as would be predicted in aqueous solution; rather, all of the intermediary metabolites attain substantial concentrations, even at equilibrium. The complete conversion of sugar to lactate is driven by the export and utilization of the final product by other organelles or cells. In muscle metabolism these latter pathways include the export and conversion of pyruvate to acetyl CoA in the mitochondrion or the export of lactate across the cell membrane and its intake by liver cells for gluconeogenesis.

It is worth noting that the precursors of glycolysis (glucose-6-PO_4 and fructose-6-PO_4) and the products of glycolysis (pyruvate and lactate) attain maximal concentrations comparable to the total concentration of glycolytic enzyme sites, consistent with the reversibility of the entire internal metabolic sequence. The free-energy change for the conversion of one equivalent of hexose phosphate to two equivalents of lactate must be compensated for by the sum of the free-energy wells for the sequestering interactions between intermediary metabolites and the various enzymes of the pathway. Any process that increases the concentration of entering hexose phosphate or increases the rate of export of lactate out of the system will affect the flux of metabolites through the direct transfer pathway, and may, in the limit, be responsible for the kinetic regulation

of metabolism. Such mechanisms can include the increased pumping of metabolic energy from the mitochondrion into the formation of hexose phosphate and any of the processes that alter the permeability of the muscle membrane to lactate. Ultimately, present-day evolved muscle glycolytic metabolism may be limited by physiological processes external to the glycolytic pathway, rather than by the catalytic efficiency of particular glycolytic enzymes.

Although we are at this time unsure of the general validity of the direct transfer pathway *everywhere* in metabolism, it is clear that nearly all of the metabolites and coenzymes of the tricarboxylic acid cycle in mitochondria, as well as the redox coenzymes, are almost entirely sequestered within the excessive protein sites. Arguments for both enzyme–enzyme interactions and for metabolite channeling within the tricarboxylic acid cycle have been previously summarized (*1*).

THE LIGAND-INDUCED INTERACTIVE FUNCTIONAL ENZYME MODEL OF METABOLISM

We have formulated a kinetic-structural model for the coupling of chemical reactions into a metabolic pathway. The model combines three features discussed above. (1) The free-energy diagram of Fig. 1. (2) The ligand-dependent conformations of individual enzymes. (3) The ligand-dependent nature of enzyme–enzyme recognition for direct transfer of metabolite.

The model is illustrated schematically in Fig. 2. The model implies that enzyme–enzyme interactions are dynamically signalled by the formation of specific enzyme-bound ligands. The chemical transformation, $S \rightleftarrows P$, is the effector of enzyme conformational change. Thus, E_1–M_1 is the cognate structure for E_2 recognition, and direct transfer of M_1 can occur thereby. Chemical transformation of M_1 and E_2, however, yields E_2–M_2, which is no longer the cognate structure to E_1. Rather, E_2–M_2 is the cognate structure for recognition of E_3, and the formation of complex once again permits the transfer of metabolite. In this way, metabolite is transferred directly through the pathway, or through a segment of the metabolic pathway, by recognitions coupled to dynamic chemical transformations. According to this model, metabolic flux is coupled to rapid and repetitive changes in enzyme–enzyme interaction.

The model precludes the formation of a rigid complex of interactive enzymes, as might conceivably be envisaged for a structurally intact multienzyme system. However, the most extensively studied multienzyme complex, viz tryptophan synthase, provides ample evidence for ligand-dependent dynamic structural changes for metabolite channeling (K. Kirschner and A. Lane, personal communication; M. F. Dunn, personal communication). Structurally defined channels linking the two dif-

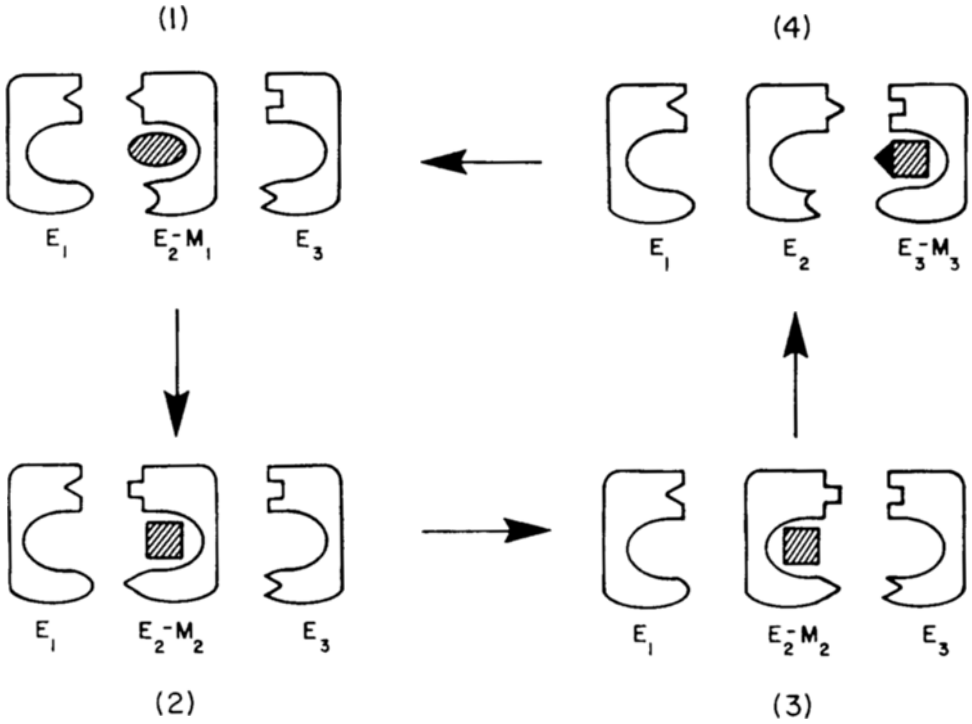

Fig. 2. Schematic representation for the ligand interactive functional enzyme model. Metabolite-induced protein conformational change, and its effect on cognate protein recognition, is schematized. (1) The cleft-to-cleft oriented E_1 and E_2 recognize each other to allow the transfer of M_1 from E_1 to E_2 site. (2) The transformation of M_1 to M_2 at the E_2 site results in a concomitant change in the E_2 conformation, which is no longer recognized by E_1. (3) The E_2 rotates for complementary interaction with E_3. (4) The E_2-catalyzed transformation of M_2 to M_3 results in a change of conformation of E_3 so that E_3 is no longer complementary to E_2.

ferent enzyme sites have recently been noted in the electron density map (C. Hyde and D. R. Davies, personal communication).

The ligand-induced dynamic model of interactions we propose has kinetic, as well as equilibrium thermodynamic, consequences. Given a direct transfer pathway, as we have proposed for glycolysis, "do the metabolite transfers from enzyme site to cognate site, or covalent transformations within a site, determine the flux through the pathway?" To answer this question we must assess the microscopic rates of metabolite transfer within enzyme–enzyme complexes and the extent to which enzyme–enzyme interactions alter covalent catalysis rates at individual sites. Our studies up to this point (*2,6*) indicate that metabolite transfer is partially rate-limiting for the subsequent covalent transformation; catalysis and transfer rates are similar. Moreover, the unimolecular rates of metabolite transfer within enzyme–enzyme complexes are remarkably similar in magnitude, one to another, in the case of NADH transfer among

the hydrogenases. This transfer can be observed directly because of the alternate conformational states in which the NADH is bound at each site in the enzyme–enzyme complex. The alternate conformational states are spectrophotometrically distinguishable. From such studies it is apparent that the transfer rates are modulated toward a merely uniform magnitude, regardless of either the NADH-dissociation rate from individual enzyme sites or the maximal turnover velocity for the reaction at individual sites (*6*). Although we have accumulated some quantitative data regarding metabolite transfer and reaction, we cannot as yet determine the extent to which such kinetic factors affect the distribution of metabolite among the various high-affinity sites. It is interesting that in the transient interactions and reactions we have studied, the "turnover numbers" are similar to, but usually somewhat slower than, the "turnover numbers" for individual enzymes. This is in contrast to some of the static enzyme–enzyme complexes, such as tryptophan synthase, in which the cognate enzymes are strong positive effectors of the complementary enzyme activities. The overall rate of flux in glycolysis is considerably slower than the individual rates that we have determined in the direct transfer of metabolite between cognate enzyme pairs. Thus, either steps in the glycolytic pathway that we have not examined (e.g., the reaction catalyzed by phosphofructo kinase) are almost totally rate-limiting, or processes outside the glycolytic pathway limit flux through the pathway.

The enzyme–intermediary metabolite complexes are poised for rapid catalytic interconversions via direct transfer to succeeding and preceding enzyme sites. These storehouses of potential energy need not necessarily have equivalent concentrations within the cell; the concentrations of 3-phosphoglyceroyl-GPDH and dihydroxy acetonyl-aldolase are both high relative to the total enzyme site concentrations of many other glycolytic enzymes (*1,2*). Metabolites are rapidly transferred and transformed because of the facile direct transfer mechanism among the enzyme metabolite complexes.

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