Interactions between Glycolytic Enzymes and Components of the Cytomatrix

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ABSTRACT Evidence is provided that enzymes absorb to cellular structures in a wide range of tissues. In particular, the interactions between glycolytic enzymes and the microfilaments of the cytoplasm are described. The relevance of these interactions to the compartmentation of carbohydrate metabolism is discussed. Examples are given of the variations in degree of binding during alteration of tissue metabolism and, for individual glycolytic enzymes, during fetal development and differentiation. Overall, these data support the concept that metabolic activities in the cytoplasm have an organized structure. Just as the structural elements of the cytosolic compartment have evolved with the capacity to assemble and disassemble in response to the changing requirements of the organism, so the metabolic elements appear to have evolved a parallel system that provides for the appropriate positioning of an energy-producing sequence in relation to the specific, dynamic requirements of the cytoskeleton.

In other papers in this supplement there are many fascinating examples of the structural role of the cytomatrix and of the phenomena of directed motion in relation to this complex spatial organization. Cell shape, motility, cytoplasmic streaming, organelle distribution, cell division, and differentiation have all been viewed from this perspective, and the data leave little doubt as to the extraordinarily broad involvement of the cytomatrical structure (16). At a functional level, the intricate choreography of structural reorganization during these cellular processes clearly requires an appropriate energy source, one with several special features. In this paper I describe the characteristics of the interactions between the glycolytic enzymes and the components of the cytomatrix, characteristics that may be of particular relevance in this context.

Enzyme Binding and Compartmentation

Recent studies (11, 12, 18) on intermediary metabolism have focused on the importance of compartmentation in the regulation of cellular processes and, to quote a recent treatise on this topic, "... it has become overtly clear that, in biology, order in metabolism is generated by the introduction of inhomogeneity, i.e., by compartmentation" (18).

To most cell biologists, the most familiar examples of the metabolic advantages of the segregation of enzymes and metabolites are associated with compartmentation of cells and subcellular organelles, where a particular metabolic organization is surrounded and separated from other metabolic compartments by a physical permeability barrier, such as a membrane. Although this type of spatial compartmentation is undoubtedly the most intensively studied at this time, it is important to stress that effective metabolic compartmentation may also be achieved within a single membrane-enclosed space by means of the binding of key enzymes and metabolites. Thus, in the case of the cytoplasmic matrix, compartmentation by binding of soluble enzymes to the matrical structures may confer advantages over and above those of the more "classical" forms of compartmentation: such sequestration is flexible and more closely attuned to the changing requirements of the cytoplasm than other kinds of spatial compartmentation.

In a description of the characteristics of the compartmentation of individual glycolytic enzymes by binding to the structure of the cytomatrix, it is useful to consider a general model such as that illustrated in Fig. 1. The several major features of this model (given by the four headings that follow) may then be considered individually.

DEGREE OF BINDING: For the compartmentation of enzymes by binding to be regarded as functionally significant, evidence of an appreciable degree of such binding under cellular conditions is required. The methodological considerations are quite demanding with this type of analysis as a number of artifactual possibilities need to be excluded (e.g. trapping, non-specific binding, binding due to cell lysis, and reassociation in non-physiological buffer systems). Nevertheless, in the case of the individual glycolytic enzymes, a considerable quantity of supportive evidence of the reality of binding

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of these enzymes to structure in cells and tissues has been provided by a variety of techniques (12, 13, 15, 19), and this body of evidence now seems quite compelling. In particular, it has been demonstrated that most of the glycolytic enzymes have a particular affinity for actin and actin-containing structures, such as the thin filaments of muscle and the microfilaments of the cytoskeleton (3, 12, 14), and substantial associations between such structures and individual glycolytic enzymes have been described for both in vivo and in vitro conditions.

One line of experimental support is indicated in Table I. Centrifugation of muscle homogenates leads to the indication of appreciable binding of most glycolytic enzymes as compared with the other enzymes in the system, and certain of the glycolytic components are indicated as more extensively bound than others.

SPECIFICITY OF BINDING: In addition to a requirement for an appreciable degree of binding, application of this form of compartmentation in biological systems would imply several elements of specificity. Once again, substantial evidence from several different sources (9, 10, 13, 17) is now available to substantiate these expectations.

Comparative binding studies with reconstituted thin filaments (F-actin/tropomyosin/troponin) and cytosolic fractions of tissues, for example, indicate the preferential binding of glycolytic enzymes over other enzymic components under these conditions (2). The characteristics of binding of individual glycolytic enzymes to F-actin and F-actin/tropomyosin/troponin have been studied, and the binding characteristics of these interactions have been defined. The adsorption of aldolase to myofibrils indicates binding to specific sites every 10–12 heptameric repeat units with an intrinsic association constant of 410,000 m^{-1}. The binding to microfilaments involves comparatively weaker binding and closer spacing of the specific binding sites (10).

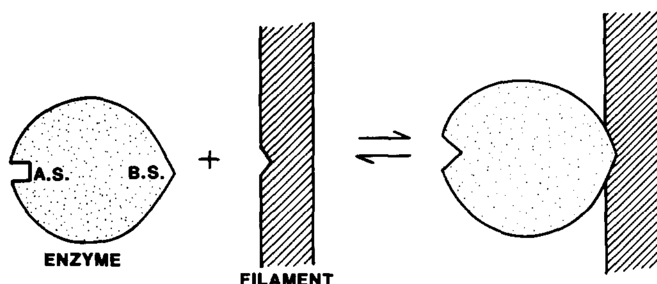

FIGURE 1 Diagrammatic illustration of the interaction between an enzyme and filamentous structure. A binding site (*BS*) on the enzyme interacts with a complementary site on the filament, and this association leads to altered conformational characteristics of the enzyme and its active site (*AS*).

TABLE I

Effect of Tetanic Stimulation on the Extent of Binding of Glycolytic Enzymes in Skeletal Muscle

Enzyme	Percent bound	
	Control	Stimulated
Hexokinase	6.0 ± 1.2	5.1 ± 1.4
Glucose phosphate isomerase	6.2 ± 0.4	7.2 ± 0.6
Phosphofructokinase	12.7 ± 4.2	26.6 ± 5.2*
Aldolase	35.3 ± 4.4	56.8 ± 5.6*
Triosephosphate isomerase	11.2 ± 2.6	10.4 ± 2.4
Glyceraldehyde phosphate dehydrogenase	25.2 ± 2.8	41.7 ± 4.9*
Phosphoglycerokinase	15.6 ± 2.3	17.1 ± 2.6
Phosphoglyceromutase	10.7 ± 3.1	15.2 ± 3.3
Enolase	12.8 ± 4.0	17.2 ± 4.1
Pyruvate kinase	14.4 ± 2.8	16.1 ± 3.0
Lactate dehydrogenase	12.6 ± 2.6	16.2 ± 2.8

* Significant difference between the extent of binding in control and stimulated muscle.

Finally, the results of recent studies on the controlled proteolysis of individual glycolytic enzymes have indicated differential behavior of the binding and enzymatic responses and provided clear indications of a spatial separation of the active site and binding sites on the enzyme (6).

MODIFICATION OF KINETIC PARAMETERS: A further feature of the binding of enzymes to structures in biological systems that is of particular relevance to metabolic compartmentation is the modification of kinetic parameters that may accompany such binding. Comparisons of the kinetic characteristics of aldolase in free solution and when bound to thin filaments (F-actin/tropomyosin/troponin), for example, have shown that binding causes an alteration in the Michaelis constant by an order of magnitude and a substantial increase in the maximum velocity of the enzyme (22).

Also, in allosteric enzymes such as phosphofructokinase, the shift from the soluble phase to a bound form has been noted to be accompanied by a move from sigmoid to Michaelis-Menten kinetics (Fig. 2; 7, 12). It would appear that in such circumstances, the enzyme loses much of its conformational flexibility and is shifted out of metabolite control and into a state of increased activity. Clearly, such alterations of activity may exert a profound influence not only on the immediate contribution of the particular enzyme but also on its metabolic relationships and control.

AMBIQUITY: Having established that the binding of glycolytic enzymes to actin-containing structures is significant in its quantitative aspects and specific in its comparative relationships and that it may involve marked changes in the functional characteristics of the enzymes, I also would like to emphasize that, in a biological context, the distribution of enzymes between bound and soluble states is not invariable but rather may be a dynamic characteristic of tissues (13, 24, 25). That the distribution of a glycolytic enzyme between soluble and particulate phases changes in response to the

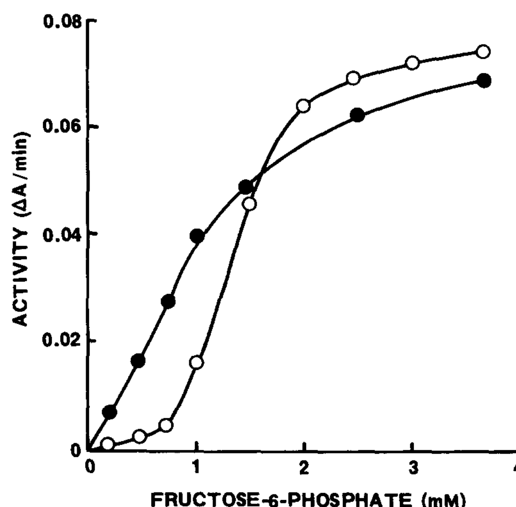

FIGURE 2 Substrate activity curves for free soluble phosphofructokinase (○) and the membrane-bound enzyme (●).

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metabolic status of the cell (a phenomenon referred to as ambiquity [25]) was first demonstrated when the subcellular distribution of hexokinase was shown to be markedly influenced by insulin treatment and anoxia (8). Many of the other glycolytic enzymes, particularly aldolase, glyceraldehyde-3-phosphate dehydrogenase, and phosphofructokinase, have also been shown to vary substantially in their distribution under a variety of physiologic perturbations, and there seems little doubt that these ambiquitous aspects of binding broaden the metabolic significance of the phenomenon (Table I).

Tissue Differentiation

Another aspect of the binding of glycolytic enzymes to subcellular structure that has been studied in some detail in recent years, and one that pertains to the subject matter of this section, is the extent of association during development and tissue differentiation. An intensive study of the localization of glycolytic enzymes in mouse tissues during development (6), for example, has led to the conclusion that a significant degree of binding occurs in all major tissues, with extensive variations in the degree of binding being evident during all stages of ontogeny.

Space does not permit a detailed discussion of these results and their physiological correlations, but the fact that the degree of binding alters along with changes in the metabolic emphasis of the tissues during development may again be said to strengthen the hypothesis that this widespread form of compartmentation has biological significance. The data also provide interesting insights into both the extensive degree of binding in the early embryonic stages and the rationale and optimum localization of a particulate glycolytic capacity.

Assemblies of Glycolytic Enzymes and the Cytomatrix

Now that we have considered the nature of the available data on the binding of individual glycolytic enzymes to actin-containing structures, it is appropriate to shift the focus of attention to the level of a metabolic pathway, i.e., to consider the possibility of the compartmentation of glycolysis as a whole by this mechanism. A number of workers (5, 12, 15) over many years have sought evidence for the existence of a glycolytic complex. Most of these investigations were concerned with the cytosolic fraction of cells, however, and it is now well known that homogenization and centrifugal fractionation are deeply disruptive to the complex structure and organization of the cytomatrix.

Our group first obtained evidence for an assembly of glycolytic enzymes in liver cytoplasm some years ago (1) but noted that this complexing occurred only in the presence of contractile components. Taken together with evidence of the type that has been presented for the individual glycolytic enzymes, this has led to the concept of an active complex being achieved not by interactions between the individual soluble components but by the plating of the glycolytic enzymes onto the actin-containing components of the cell, e.g., thin filaments of muscle and microfilaments of the cytomatrix (Fig. 3). Such a model includes many of the features previously discussed for the binding of individual glycolytic enzymes, such as an appreciable degree of binding, specificity, modification of kinetic parameters, and ambiquitous response. In addition, the existence of a glycolytic complex or assembly would imply the further characteristics of preferential binding of certain glycolytic enzymes to structures such as the cytomatrix, an incremental effect of these "anchor" enzymes on the binding affinity of other components of the pathway, and a distinction of functional characteristics of the pathway between soluble and particular phases.

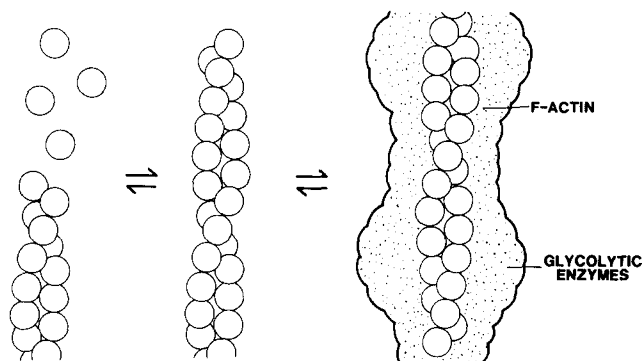

FIGURE 3 Diagrammatic representation of the dynamic equilibrium between actin monomers, microfilaments (F-actin), and glycolytic enzymes.

Evidence is now available for each of these aspects. The same enzymes that were described in previous sections as binding with special affinity (i.e., aldolase, glyceraldehyde-3-phosphate dehydrogenase, and phosphofructokinase) appear to play an especially important role in anchoring the glycolytic complex to actin-containing fibers, and the initial binding of these enzymes provides a basis for the increased cooperative binding of other components (12, 20). This latter aspect, the so-called piggy-back response, is not greatly surprising when viewed in relation to the previously considered data on the modulation of the activity of enzymes by binding. Just as catalytic activity is a general characteristic of enzymes, so the ability to bind and recognize other molecules is a fundamental property of all biologically active proteins—hence, a modification in the binding characteristics of anchor enzymes by association may be viewed as not dissimilar, it may even be closely analogous, to the activity changes that occur with association.

Kinetic assessments have established the distinctive nature of metabolism in the quantitative aspects of glycolysis in soluble and particulate phases. In general, the soluble system displays the classic characteristics of a controlled, maintenance-oriented role in cells and tissues, whereas the bound complex exhibits a comparatively high, "emergency" level of metabolism. The physiologic significance of these differences is referred to below.

Conclusions

The data in this paper provide a substantial basis for the concept of an organization of the metabolic system in the cytoplasm and the attendant role of compartmentation of these functions by binding to cytomatrical elements. Just as the structural elements of the cytoplasm have evolved the capacity to assemble and disassemble in response to the changing, multifarious requirements of the organism, so the metabolic elements appear to have developed a parallel system that can assemble and disassemble a major energy-producing complex where and when required in relation to these structural changes. Both the structural and metabolic systems have made central use of the versatile and highly conserved building

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block, actin, which occurs in a variety of cell types and is centrally involved in the cytomatrical alterations implicated in changes of cell shape, motility, and metabolism.

Consideration of the comparative data on the binding of glycolytic enzymes to the thin filaments of muscle and to microfilaments in nonmuscle cells provides insight into the respective roles of the complexes in relation to the fine structure of these different cellular situations. The anchor enzymes of glycolysis, for example, attach less avidly to microfilaments than to thin filaments. This difference, it would appear, confers a greater flexibility on the microfilament system, allowing the use of a greater number of sites and a more sensitive response to metabolite signals. In other words, the binding characteristics of microfilaments seem more appropriate to the dynamic characteristics of the cytomatrix, allowing a more rapid variation in the positioning of functional elements than is required in the relatively fixed architectural environment of myofibrils and thin filaments.

During fetal growth and differentiation in vertebrates, most major tissues exhibit rapid cell division and morphogenesis—conditions under which the cytomatrix may need to be frequently reorganized. During fetal development, the major energy source available in these tissues is glycolysis. Hence, there is a mutuality of requirements that appears to have favored the parallel evolution of structural and metabolic controls. At later stages of development, alternative sources of energy develop (e.g., mitochondria), and the extent of morphogenetic change diminishes. Hence, in the adult there is in general a lessened requirement for interaction between the cytomatrix and glycolysis, and the degree of binding tends to diminish as compared with that of the early fetal stages.

Nevertheless, this strategy of energy provision by binding compartmentation, so evident in early morphogenesis, does appear to persist and carry over to several specialized processes in the adult, where the energy requirement is rapid and intermittent rather than continuous. There are indications, for example, that even the subtle cellular modulations that involve memory may be associated with the presence of such interactions. Francis Crick (4) was the first to postulate that changes in shape of the dendritic spines may be the means by which long-term memory is laid down. He postulated that the twitching of the spines might be effected by the presence of actin filaments; this localization, along with presence of glycolytic enzymes in the spines, has since been confirmed. Hence, in this situation, conditions exist for control of glycolysis by compartmentation. They may also exist in skeletal muscle and other special structures in the adult.

It is also necessary to stress that the organization of glycolysis in many cells and tissues may be far more complicated than was indicated in the sequence of catalytic components of the usual textbook representations. Most of the glycolytic enzymes exist as multiple forms of activity (isozymes), for example, and attention has been drawn to the fact that the possibility may allow a tailoring of individual sequences to suit the requirements of different areas of carbohydrate metabolism. Ureta (21) postulates that there may be as many as ten different combinations of the glycolytic isozymes forming pathways associated with distinct aspects of carbohydrate metabolism.

Again, in relation to the binding and function of glycolytic enzymes in association with microfilaments of the cytomatrix, it is clear that individual multiple forms of single enzymes possess strikingly different capacities for binding with actin (12). Hence, the glycolytic enzymes associated with filamentous structures in the cell often represent a particular spectrum of those isozymes present. "Muscle-type" isozymes, in particular, tend to predominate in those locations and processes employing this type of compartmental control.

The entire array of glycolytic enzymes may not necessarily be required for the rapid provision of energy by compartmentation in specified locations of the cell. Rather, it is the glycolytic tail that is most relevant in this regard, i.e., the sequence from aldolase to pyruvate kinase. It is also relevant that much of the fructose-1-6-diphosphate in the cell is normally bound to aldolase; thus, the important binding characteristics of aldolase and glyceraldehyde-3-phosphate dehydrogenase mentioned above may apply not only to their function as anchor enzymes, but also to their role as initiators of energy production and even the transport of necessary substrates.

Finally, it may also be noted that glycolysis is not the only major pathway that is localized in the cytoplasm of vertebrate cells. The pentose phosphate pathway, amino acid activation, and pyrimidine catabolism are some of the other processes occurring in this compartment, and evidence is accumulating that the coordination of structure and function of the cytomatrix found for cytoskeleton-glycolytic associations may also extend to other "soluble" pathways.

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