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Hypothesis

Remarks on the supramolecular organization of the glycolytic system in vivo

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The great latent catalytic capacity, manifested at the extremely high intracellular concentrations and in large apparent k_{cat}/K_m values, of the glycolytic enzymes on the one hand and their tendency in experiments in vitro to form functionally-specific flux-enhancing (channeling) complexes on the other, is considered and discussed as an apparent discrepancy. A random association of glycolytic enzymes in vivo is probable.

Supramolecular organization; Glycolytic system

1. INTRODUCTION

The occurrence of some glycolytic enzymes at high concentrations (>100 μM) in the cytoplasm of various cells (cf. [1,2]) together with an apparent high k_{cat} (cf. [1–3]) indicates a catalytic efficiency far greater than that required to provide the appropriate glycolytic flux in any extreme need of the cell. An example is the total latent catalytic potency of the glycolytic enzymes in pure glycosomes from *Trypanosoma brucei* [3,4]. Therefore, according to Aman et al. [3], there is no real need to shorten further the diffusion time of intermediates by such mechanisms as metabolite channeling. Other examples also suggest that the glycolytic pathway operates far below its maximal catalytic capacity under most conditions (cf. [5,6]).

On the other hand, as a consequence of the dense packing of the cytoplasm and even the glycosomes with enzymes and various proteins, there is little doubt that protein-protein interactions are the rule rather than the exception (cf. [7,8]). Based on a large amount of, mostly in vitro, experimental data (see [8]), many scientists now accept the specific, functional consequences of these relations, although a limited number of in situ observations [9–11] have disclosed only the existence of protein complexes in the cells: they do not prove, however, that they are functionally specific i.e. that channeling complexes occur in vivo.

One might wonder, however, as to the reason why such *specific* enzyme-interactions would be of advantage when even the individual enzymes can theoretically operate *efficiently* enough. I will show that bowh terms i.e. 'specific' and 'efficient'

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Abbreviations: Ald or aldolase, fructose-1,6-bisphosphate aldolase; CaM, calmodulin; FBP-ase, fructose-1,6-bisphosphatase; GAPD, glyceraldehyde-3-phosphate dehydrogenase; GDH, glycerolphosphate dehydrogenase; LDH, lactate dehydrogenase; PFK, phosphofructokinase; PGK, phosphoglycerate kinase; PK, pyruvate kinase; TPI, triosephosphate isomerase

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need to be qualified, since functionally non-related enzymes are also capable of forming complexes and the k_{cat}, K_m values determined in the classical manner (at low concentrations of the separated, purified enzymes) may often not be relevant in situ.

2. WHICH k_{cat} AND K_m IS THE 'REAL' ONE?

A comparison of the specific activities of individual glycolytic enzymes determined in native glycosomes with the corresponding values for separated, pure forms (all data are from Aman et al. [3]) unambiguously indicates significantly lower specific activities of most of them in situ (table 1). Our earlier observations [12] concerning the characteristic dependence of the specific activities of many glycolytic enzymes on the dilution of the cell-free cytosol (using rabbit muscle, rabbit liver and yeast) confirm this tendency of specific activities to decrease when measured at higher concentrations of the diluted cytosol [12,13]. This indicates that k_{cat} values available in the literature (cf. [1]) - derived from classical activity measurements using isolated, purified enzymes and applying low enzyme concentrations in the tests - are probably misleadingly high. In general, the actual enzyme activity in the cytoplasm is now thought to be a function of the concentrations of many other proteins (cf. [14]). For example, enzymes such as GAPD and aldolase show an affinity for a large variety of enzymes, structural proteins and membranes (cf. [8,15] and fig.1), with the complexed forms being less active in many cases.

On the other hand, K_m values are obviously not relevant if direct, site to site transfer of the intermediates occurs.

3. SPECIFIC OR RANDOM CHARACTER OF THE FORMATION OF COMPLEXES OF GLYCOLYTIC ENZYMES?

If certain enzymes (such as aldolase, GAPD, cf. fig.1 and [8] and references therein) can form complexes with a multitude of other functionally unrelated enzymes (complexes of non-consecutive pairs), cellular proteins and membranes existing in the same compartment of the cell, then their specific roles in the compartmentation of glycolytic intermediates is questionable unless complexes of consecutive enzyme pairs prevail. In order to quantitate the strength of enzyme complexes, their dissociation constants were determined systematically under near identical conditions (of buffer, ionic strength, pH, temperature). The results are summarized in fig.1.

Table 1

The ratio of specific activities of some enzymes in pure forms and in native, intact glycosomes

Enzymes	Ratio of specific activities
Hexokinase	6
Phosphoglucose isomerase	100
Phosphofructose kinase	12
Aldolase	8
Glyceraldehyde-3-phosphate dehydrogenase	5
Triosephosphate isomerase	34
α-Glycerophosphate dehydrogenase	11
Phosphoglycerate kinase	7

Calculated from data of Aman et al. [3]

Although the continuously increasing complexity of this 'interaction matrix' (fig.1) may appear to

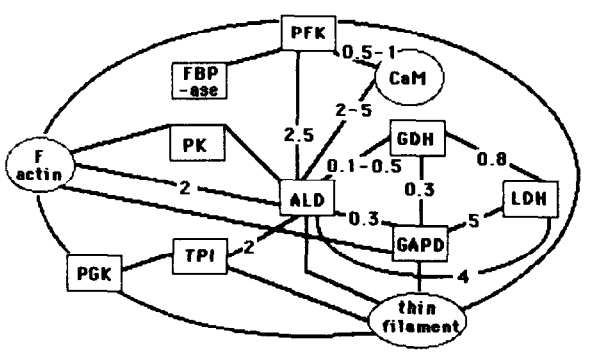

Fig.1. The 'interaction matrix' of pairs of some glycolytic enzymes and structural-proteins prepared from rabbit muscle cytoplasm. Lines between two macromolecules indicate the existence of complexes in vitro, while the numbers show the apparent dissociation constant of the complex (in μM) assuming 1 : 1 stoichiometry in binding. All these constants refer to standard conditions, i.e. 0.05 M Tris-HCl buffer, pH 7.5 or 8.5, at 20°C (cf. [12,13]). *References:* The complexes of Ald with PFK [18], with CaM [18], with GDH [17], with GAPD [20], with thin filament [8], with TPI [8], with F actin [8], with PK and LDH ([21] and Nazaryan and Batke, unpublished); the complexes of GAPD with Ald [20], with GDH and LDH ([1] and Nazaryan and Batke, unpublished), with thin filament and F actin (cf. [8]); other complexes: PFK-CaM [18], PFK-FBP-ase [8], PK-F actin [21], TPI-PGK (cf. [8]), TPI-thin filament, LDH-thin filament, PFK-thin filament, PGK-thin filament, PGK-F actin and PFK-F actin (cf. [8]).

be rather disheartening, other evidence supports the idea of the complex formation in situ. For example translational motions of proteins in the cytoplasm are extremely hindered [9] and the exit of proteins from permeabilized cells is retarded in contrast to their free entry [11]. All these findings strongly suggest, if not prove, that an organized system of proteins exists in the cytoplasm. Gershon et al. [10] have also explained these observations as the consequence of transient interactions of the cellular proteins, i.e. all proteins exist predominantly in a complexed form in the cell. This is also consistent with our data (cf. fig.1), since dissociation constants are almost two orders of magnitude lower than the average concentrations of the respective enzymes in the cell.

Some relations (for example the complexes of aldolase-GAPD, aldolase-GDH and GAPD-GDH) are somewhat stronger (with dissociation constants approx. 0.1–0.5 μM) than others (with K_d values of several micromolar). Accepting the principle of Alberts et al. [16] that a knowledge "of the strength of the bonding between two macromolecules is a useful index of the specificity of the recognition process", a possible physiological importance might be attributed to the stronger complexes. (Of course, increased affinity between functional pairs of enzymes alone is only a necessary condition for the channeling of intermediates: the sufficient one is the appropriate constellation of active centers in the complex.) This is why we have assumed that aldolase-GDH and aldolase-GAPD complexes play a role in the compartmentation of triosephosphates (glyceraldehyde-3-phosphate and dihydroxyacetonephosphate) in the metabolic 'crossroad' ([12,13,17,18] and references therein) where these intermediates have a chance to be metabolized by alternative pathways such as glycolysis, pentosephosphate pathway and lipid synthesis. Recently, similar functional complexes have also been recognized between dehydrogenases at the crossroads of NAD/NADH transformation (cf. [1]).

However, there are also some puzzling observations. For example, complexes of functionally unrelated (non-consecutive pairs) enzymes can occur (cf. fig.1, and [20,21]), as well as the formation in vitro of hybrid complexes of proteins originating from evolutionary-distant species [12,13] and from different tissues such as muscle and brain ([19] and Nazaryan and Batke, unpublished).

In the following section I attempt to assemble these experimental findings into a coherent picture in the context of the possible appearance, evolution and specificity of these macromolecular interactions.

4. THE POSSIBLE ORIGIN AND EVOLUTION OF ENZYME INTERACTIONS IN THE GLYCOLYTIC SYSTEM

It is quite possible that the enzymes of ancient cells were inferior biocatalysts to the present ones with much lower k_{cat}/K_m values. To provide adequate metabolic fluxes, which undoubtedly would have been needed at an early stage of evolution, an increase in the amount of enzymes would then have been necessary. However, increasing the concentrations of enzymes in the cytoplasm would then have produced complexes between them due to the nature of their building elements (i.e. the amino acids). In this context a new, functionally extremely favorable relation could then have appeared, i.e. the 'neighboring arrangement' of the functionally related active centers. It is very likely that this 'fitting together' of active centers happened by chance and therefore other complexes, unimportant or even disadvantageous from the viewpoint of metabolite compartmentation or flux-enhancement, could also have formed. Some of these exist today either as a consequence of the very conservative evolution of the glycolytic enzymes [22] or because of other functions which are not related directly to metabolite flux and compartmentation. For example, some of these complexes are now thought to be involved in the network of cytoplasmic structural proteins (cf. [21]), which indirectly implies a role in the regulation of growth, gene expression and cell differentiation (cf. [23]).

When discussing the general 'usefulness' of enzyme-enzyme interactions in the cell all the above facts should be considered together, since an evolutionary imperfection of the glycolytic process is rather improbable. In contrast, if we consider that only a fraction of the glycolytic enzymes is involved directly in glycolysis, then the remainder of these enzymes in the cytoplasm could be organized in functionally specific complexes (i.e. channeling complexes). As stated previously, the diffusion limitations of macromolecules in 'dense' cytoplasm (cf. [9,10]) and the recent experiments of Clegg and

Jackson [11] with permeabilized L-929 cells indicate that a complexing of glycolytic enzymes in vivo is very likely. However, despite the large number of channeling complexes observed in vitro, the continuously accruing examples of apparently 'non-specific' interactions (at least from the viewpoint of compartmentation or flux-enhancement) suggest the need for caution in the interpretation of the importance of such complexes in metabolic regulation in vivo. The random nature of enzyme-complex formation (so that non-channeling complexes also form) also suggests a 'loose' organization of the glycolytic system which allows some free diffusion of metabolites. The relative importance of channeling and free diffusion of the various intermediates in the whole system depends on the metabolic status of the cell. In some cases (in ascites cells [24] and erythrocytes [25]) free diffusion seems to be predominant and therefore 'classical' models of metabolic regulation apply. In these cases, the excess enzyme activities could be explained as providing simple mass-action reversibility and high intrinsic sensitivity of the pathway (cf. [26]).

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