Minireview

Enzyme-enzyme interactions and their metabolic role*

Paul A. Srere[1] and Judit Ovadi[2]

[1]*Veterans Administration Medical Center, 4500 S. Lancaster Rd, and The University of Texas Southwestern Medical Center, Dallas, TX 75216 USA* and [2]*Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences 1518, Budapest PO Box 7, Hungary*

Received 27 April 1990

There are continuing reports on the existence of complexes of sequential metabolic enzymes. New techniques for their detection have been described and include affinity electrophoresis and the use of anti-idiotypic antibodies. Channeling of substrates has been reported for several systems as well as direct substrate transfer through dynamic enzyme associations. Kinetic parameters of metabolic control of organized systems have been formulated and tested in several systems. These recent results are expanding our understanding of metabolic processes and their control.

Enzyme-enzyme interaction; Channeling; Metabolic complex; Metabolic control theory

1. INTRODUCTION

The regulation and integration of the thousands of enzyme reactions in cells are not completely understood but a number of different strategies must be involved. One strategy is compartmentalization of certain activities which has been accomplished in a number of different ways. Intracellular compartmentation can be achieved by the separation of phases in a cell; thus even if a cell were a 'bag' of soluble enzymes one would have the phase of the enclosing membrane (lipid phase) and that of the contents (aqueous phase). In actuality we have many lipid phases surrounding many aqueous phases in cells (organelles). Both the reactions of each lipid phase and each aqueous phase can carry out their reactions autonomously. This may be necessary to prevent futile cycles and to maintain locally separate concentrations of certain intermediates. Additionally, separation of activities and sequestration of intermediates can occur by enzyme interactions and localization by either protein-protein, protein-membrane, protein-nucleic, acid, or protein-polysaccharide interaction.

The fusion of enzyme activities into one polypeptide as in tryptophan synthase [1] or fatty acid synthetase [2] is the most highly evolved example of microenvironment control of intermediate channeling. Similar results are achieved by multienzyme complexes of polypeptides such as in the α-keto acid dehydrogenase complexes [3]. The highly processive syntheses of DNA, RNA, and protein occur by way of highly organized tight complexes of proteins and nucleic acids [4].

The complexes mentioned above are, for the most part, easily isolated from cells by ordinary extraction techniques. Some complexes are easily dissociable so that they cannot be isolated using the usual isolation techniques. If one uses conditions which simulate those that are presumed to exist in cells then they are easily demonstrated. For many different metabolic pathways, specific interactions between sequential enzymes have been demonstrated. It should also be recalled that some complexes are inherently weak with the possibility that both free and complexed enzymes exist within the cells.

A comprehensive review of this area appeared in 1987 [5] and another in 1989 [6]. In this short review we shall limit ourselves to the following areas: (i) new techniques for demonstrating protein-protein interactions; (ii) additional complexes which have been reported; (iii) discussion of the recent controversies on the interpretation of data concerning channeling in the area of glycolysis; and (iv) metabolic control theory and enzyme complexes.

2. ENZYME COMPLEXES

2.1. *Isolating complexes*

A recent article has summarized many of the currently used methods for isolation of complexes [7]. For the purification of the dNTP synthetase complex, a complex of two transformylases of purine synthesis and two enzymes of folate coenzyme metabolism, and a com-

Correspondence address: P.A. Srere, Veterans Administration Medical Center, 4500 S. Lancaster Rd., and the University of Texas Southwestern Medical Center, Dallas, TX 75216 USA

* Dedicated to Tamas Keleti and Sidney Bernhard

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

plex of three enzymes of thymine nucleotide biosynthesis, remarkably similar procedures were used. These included gel filtration, ion exchange chromatography, affinity chromatography, and ammonium sulfate fractionation. Low recoveries were observed probably due to dissociation which accompanies the inevitable dilution which occurs during fractionation.

2.2. *Demonstrating complexes*

Beeckmans et al. [8] have described a method for detecting protein-protein interactions based on affinity electrophoresis. In this method one protein is immobilized in a portion of an agarose gel and potential interacting partners are electrophoresed through the gel. If interactions occur then the second enzyme will be retained in the region of the gel containing the immobilized enzyme. This method has been used both for Krebs TCA cycle enzymes and glycolytic enzymes.

Somerville et al. [9] have described a method for identifying interacting proteins based on the use of anti-idiotypic antibodies. These workers point out that anti-idiotype antibodies can form complexes with proteins which have surface epitopes that are the internal image of the primary antibody. In other words, the anti-idiotypic antibodies have binding sites similar to the original antigen and thus can interact with proteins which can form complexes with the original antigen. These workers used this technique to identify a group of 5 *E. coli* cytoplasmic proteins which interacted with anti-anti Tyr repressor IgG.

2.3. *New complexes*

There have been reports for many years of the existence of many different multienzyme complexes in plant tissues [10]. Some of the evidence consisted of kinetic and isotope data, and some was concerned with the isolation of complexes. A functional complex of 5 enzymes of the Calvin cycle has been isolated from spinach chloroplasts by conventional techniques of chromatography [11] and a 6 enzyme complex from the same source using partition techniques in an aqueous two phase system [12]. It is interesting to note that when several purified enzymes of the complex were examined with this technique there was no evidence for interactions [13]. This may indicate either the necessity of an additional 'binding' component which is lost on purification or the need for cooperative interactions between all components for complex formation.

Kim et al. [14] have shown by emission anisotropy and affinity chromatography that pyridoxal kinase interacts with aspartate aminotransferase. The pyridoxal phosphate produced by this complex is channeled since it is not available to added alkaline phosphatase.

2.4. *Glycolysis*

An increasing number of studies desribe the interactions between glycolytic enzymes and the structural elements of the cell. Knull and his coworkers have shown that many glycolytic enzymes bind to tubulin and microtubules [15]. Masters and his group have studied the interaction between glycolytic enzymes and actin containing structural elements of the cell [16]. Mejean et al. [17] have located binding sites by immunological methods on the actin monomer for glyceraldehyde phosphate dehydrogenase, aldolase, and phosphofructokinase.

One of the principles that has emerged in the studies of metabolic organization is that organized systems are easier to demonstrate (i.e., there exist stronger interactions between enzymes) in highly processive pathways. These include pathways such as macromolecular biosyntheses, fatty acid synthesis, and oxidation and nucleotide metabolism. Glycolysis, on the other hand, is an example of a pathway that is not very processive since the intermediates are used in pathways in cells. It has been in such 'soluble' systems that the most difficulties seem to exist experimentally. It is not surprising that most of the criticisms of the concept of organizational aspects of metabolism have centered on three experiments, all of which deal mainly with glycolysis. Nonetheless, an impressive literature concerning glycolytic organization exists and continues to accumulate as indicated in this section which indicates: (i) specific interactions between sequential glycolytic enzymes, (ii) specific interactions of glycolytic enzymes with structural elements of the cell, (iii) channeling of glycolytic intermediates, and (vi) specific changes in kinetic behavior of interacting sequential glycolytic enzymes [5,6].

The criticisms of Maretzki et al. [18] rely mainly on the fact that glycolytic enzymes bind poorly to the red cell membrane except under hypotonic conditions or hypotonic hemolysis. This ignores a series of other experiments using a variety of techniques which indicate binding of glycolytic enzymes to the red cell membrane (see [6] for review). It is interesting to note that most Krebs cycle enzymes do not bind to the inner mitochondrial membrane under the usual conditions of disruption [19], but they are firmly bound when disruption is carried out by gentle sonication but not by osmotic shock. The second criticism has to do with the calculation that red cell glycolysis can be modeled using the kinetic constants of the glycolytic enzymes obtained singly in dilute solution and without the necessity of channeling. They assume that the only advantage of organized enzyme systems is an increase in flux in the system. A number of people have emphasized that organization of pathways could have a number of apparently useful cellular functions besides increased flux [20] and, indeed, it has even been suggested that a decrease in flux may serve useful cellular regulatory purposes [21].

2.5. *Transport systems*

Transport activity may depend on the physical cou-

pling of an enzyme of a metabolite's utilization to the metabolite's transport protein. Yeast has 3 hexokinases and high K_m and low K_m uptake systems for glucose and fructose. Bisson and Fraenkel [22,23] have shown that in a mutant yeast cell lacking two hexokinases (but not a glucokinase), fructose showed only the high K_m uptake while glucose still exhibited both uptake systems. In a mutant yeast cell lacking all 3 kinases glucose was taken up only by the high K_m system. Reinsertion of the missing kinases on a plasmid restored the low K_m hexose uptake system. This effect cannot be attributed to further metabolism of the hexoses since a non-metabolizable analogue, 6-deoxyglucose, shows a similar pattern of low and high K_m uptake in the wild type and mutant cells. Fraenkel and Bisson speculate that the three kinases have a role other than their enzymatic function such as a direct role in hexose uptake by binding to the carriers.

Another system in which the coupling of transport and utilization seems to occur is ornithine uptake by mitochondria. Raijman [24] and her associates loaded mitochondria with unlabeled ornithine. When these mitochondria were incubated with labeled ornithine then the specific activity of the biosynthesized citrulline was the same as that for the exogenous labeled ornithine. The internal unlabeled ornithine did not mix with the exogenous ornithine. It is possible that the transport of ornithine is coupled to the matrix enzyme for its utilization.

There has been speculation for some years that phosphoglycerokinase or some other glycolytic enzyme may be coupled to the Na-K transporter of red blood cells. Although some evidence exists for such an interaction it is not possible at the present time to say the hypothesis is proven [25,26]. A well known mitochondrial system that links transport with metabolism is the protein driven ATP synthase (ATPase) of the inner membrane.

2.6. *Molecular biological approaches*

Recently attempts have been made to engineer cells in a way that would disturb putative enzyme-enzyme interactions. In one such study Kispal et al. [27] showed that yeast cells containing cytosolic citrate synthase but lacking mitochondrial citrate synthase regained their ability to grow on acetate if an inactive but structurally unchanged citrate synthase were reinserted into the mitochondrion. This experiment was interpreted by hypothesizing that the inactive protein restored a disrupted TCA cycle complex and thus allowed the cycle to operate more efficiently on the cytosolically produced citrate. Similar experiments by McAllister-Henn and her group with other Krebs cycle enzymes show phenotypic changes in yeast in accord with these results [28].

One interesting molecular biological method has been described for testing for protein-protein interactions [29]. The Gal-4 protein of yeast is a transcriptional activator required for expression of genes required for galactose utilization. It consists of two discrete and separable domains, one for DNA binding and one for transcription activation. If the cDNA coding for each domain is subcloned adjacent to genes coding for separate proteins and introduced on plasmids into yeast cells then galactosidase will be produced only if the two proteins interact in such a way that the two domains of the activator are brought together. This method could be used to screen for proteins that interact with a known protein. Using this method interaction was shown between a protein kinase and a protein needed for its maximal activity.

A new development has been the discovery of a class of proteins called chaperonins [30]. These proteins are apparently involved in formation of complexes and in the folding of proteins. It is possible that these proteins may be involved in complex formation in the systems we are discussing in this review. The assembly of complexes within the mitochondrion requires the presence of hsp60 (a conserved heat shock protein) and ATP. The assembly of Rubisco [31] is shown to require the presence of two proteins which also are required for assembly of phage particles.

3. DYNAMIC ENZYME ASSOCIATIONS AND CHANNELING

The area of channeling of glycolytic intermediates and dynamic coupling of glycolytic enzymes has raised the greatest controversies in this field of research.

It is not our intention to repeat these arguments and counter arguments since they are detailed and have been recently published [6]. We will instead take a wider view of these problems in an attempt to give a 'non-expert' a broader perspective.

One controversy involved the work of Srivastava and Bernhard [32] who provided kinetic evidence for dynamic channeling of NADH between glycerol-3-phosphate dehydrogenase and lactate dehydrogenase. In their criticism of the work Chock and Gutfreund [33] presented kinetic data which seemed to disagree with the work of Srivastava and Bernhard. However, the work of Chock and Gutfreund included an assumed value for a K_D upon which their argument was based. When the experimentally determined K_D is used the discrepancy with Srivastava and Bernhard disappears [34].

Even more to the point is the fact that even if one assumes that some special kinetic scheme can be devised so that the kinetic data of Srivastava and Bernhard could be interpreted as free diffusion rather than direct transfer one must explain their observations that the kinetic evidence for the direct transfer mechanism operates only between dehydrogenases with opposite chiral specificity for NADH. When transfer was

measured between dehydrogenases of the same chiral specificity then the kinetics could be interpreted simply and straightforwardly as transfer occurring through the solution.

Another disagreement exists between Ovadi and her coworkers [35] and Kvassman et al. [36] concerning the mechanism of intermediate transfer in the aldolase/glyceraldehyde 3-phosphate dehydrogenase system. The decrease of the apparent K_m of the intermediate, glyceraldehyde 3-phosphate for the dehydrogenase was observed when it was generated in the coupled reaction through the action of aldolase from hexose bisphosphate [35]. This effect could be attributed to the direct transfer of the aldehyde form of glyceraldehyde 3-P within the heterologous enzyme complex. Recently, Kvassman et al. [36] reanalyzed the system and the result was compatible with a free diffusion mechanism. In fact, for detection of channeling complex formation the concentration of dehydrogenase should be chosen so that the rate of the enzymatic reaction catalyzed by the dehydrogenase is slower than that of the aldehyde-diol interconversion. Then the unfavorable aldehyde-diol conversion of glyceraldehyde 3-P, at least partly, would occur if the substrate were to mix with the bulk medium. This sine qua non condition was not fulfilled in the experiment of Kvassman et al. Therefore, their system is not capable of differentiating between the two mechanisms. The direct transfer mechanism in this system has been demonstrated by an isotope dilution approach as well [37].

In permeabilized cells it has been shown that added cold glycolytic intermediates did not mix freely with the [^{14}C]glucose being metabolized by the cells [38]. The authors conclude that leaky channeling is occurring in these cells.

4. METABOLIC CONTROL THEORY AND MULTIENZYME SYSTEMS

The kinetic parameters of metabolic control for organized multienenzyme systems were formulated and the control analysis for organized heterologous enzyme systems was presented ([16] and references therein). For description of control of heterologous enzyme systems an extrinsic factor arising in the organized state is introduced [39]. Recently Kacser and his coworkers [40,41] reported how the association of enzyme pairs inducing changes in the kinetic parameters of the individual enzyme affects the formulations and theorems of control analysis. The data of the steady-state analysis of the coupled reactions of the aspartate aminotransferase/glutamate dehydrogenase system [42] was used for calculation of the interacting mechanism. Since in these experiments the ratios of the enzymes were chosen so that the steady-state flux rate would be directly proportional to the activity of asparatate aminotransferase (control coefficients for aspartate aminotransferase and glutamate dehydrogenase are close to 1 and 0, respectively), no information about the existence of the channeling mechanism could be obtained from the steady-state velocity vs enzyme concentrations plot. The existence of the direct transfer mechanism was suggested by the reduction of the transient time which occurred at high enzyme concentrations [42]. Therefore, the channeling must be described in terms oter than protein elasticity.

A new description of the channeling effect has been elaborated based on inherent parameters such as channel efficiency and intermediate life time [43,44]. For the determination of these inherent parameters the steady-state flux rate of the consecutive reactions at various enzyme concentration ensuring different degrees of complexation should be measured. The data of the analysis of the aspartate aminotransferase/glutamate dehydrogenase system has been applied to illustrate the applicability of this approach [43].

4. CONCLUSION

The few and very specific challenges against the erythrocyte evidence and dynamic channeling (shown themselves to be vulnerable to criticism) are usually used to reflect adversely on other well established metabolic organization data and the danger exists that the 'baby will be thrown out with the bath water'.

It should be remembered that evidence that most proteins in Euglena [45] and Neurospora [46] are bound and not free has been firmly established by ultracentrifugal techniques 20 years ago. Recent experiments using fluorescence technology to measure diffusion coefficients of enzymes in tissue culture cells have confirmed this for one glycolytic enzyme, aldolase [47], but not for enolase [48]. Since those early experiments a large number of experiments using a variety of techniques have demonstrated specific interactions between sequential enzymes in a given pathway [5,6]. The bulk of the experiments have been reproduced in many laboratories and go unchallenged by word or deed. In some of these cases kinetic consequences of the specific interaction have been clearly demonstrated.

The channeling of the indole intermediate of the tryptophan synthase complex has been known for years based upon kinetic, isotope dilution, and molecular biological data. Each of the earlier claims for channeling could be criticized on some basis or another. However, recent X-ray crystallography of this complex has shown unequivocally the existence of a physical channel for indole between active sites [1]. It also seems likely that in many other systems similar unequivocal evidence about the role of complexes in metabolism will continue to accrue.

Acknowledgements: We are grateful to our colleagues who have contributed to the ideas in this review. We thank Penny Kerby for her ex-

cellent secretarial assistance. Research support of P.A.S. is from the Veterans Administration, USPHS, and NSF and of J.O. from Grant OTKA 315/86.

REFERENCES

[1] Hyde, C.C., Ahmed, S.A., Padlan, E.A., Miles, E.W. and Davies, D.R. (1988) J. Biol. Chem. 263, 17857–17871.
[2] Wakil, S.J. (1989) Biochemistry 28, 4523–4530.
[3] Roche, T.E. and Patel, M.S. (eds), α-Keto Acid Dehydrogenase Complexes, Ann. NY Acad. Sci. 573, 1989.
[4] Alberts, B.M. (1985) Trends Genet. 1, 26–30.
[5] Srere, P.A. (1987) Annu. Rev. Biochem. 56, 89–124.
[6] Keleti, T., Ovadi, J. and Batke, J. (1989) Prog. Biophys. Molec. Biol. 53, 105–152.
[7] Srere, P.A. and Mathews, C.K. (1990) Methods Enzymol. 182, 539–551.
[8] Beeckmans, S., Van Driessche, E. and Kanarek, L. (1989) Eur. J. Biochem. 183, 449–454.
[9] Somerville, R.L., Zeilstra-Ryalls, J.H. and Shieh, T.-L. (1990) in: Structural and Organizational Aspects of Metabolic Regulation (Srere, P.A., Jones, M.E. and Mathews, C.K., eds), Liss, New York, pp. 181–197.
[10] Hrazdina, G. and Jensen, R.A. (1989) in: Structural and Organizational Aspects of Metabolic Regulation (Srere, P.A., Jones, M.E. and Mathews, C.K., eds), Liss, New York, pp. 27–42.
[11] Gontero, B., Cardenas, M.L. and Ricard, J. (1988) Eur. J. Biochem. 173, 437–443.
[12] Persson, L.-O. and Johansson, G. (1989) Biochem. J. 259, 863–870.
[13] Persson, L.-O. (1989) Phase Partitioning in Analysis of Weak Heterogeneous Enzyme-Enzyme Interactions and in Protein Purification, Lund, Sweden.
[14] Kim, Y.T., Kwok, F. and Churchich, J.E. (1988) J. Biol. Chem. 263, 13712–13717.
[15] Knull, H.R. (1990) in: Structural and Organizational Aspects of Metabolic Regulation (Srere, P.A., Jones, M.E., and Mathews, C.K., eds), Liss, New York, pp. 215–228.
[16] Masters, C. (1989) Trends Biochem. Sci. 14, 361.
[17] Mejean, C., Pons, F., Benyamin, Y. and Roustan, C. (1989) Biochem. J. 264, 671 677.
[18] Maretzki, D., Reimann, B., and Rapoport, S.M. (1989) Trends Biochem. Sci. 14, 93–96.
[19] Robinson, J.B. and Srere, P.A. (1985) J. Biol. Chem. 260, 10800–10705.
[20] Spivey, H.O. and Merz, J.M. (1989) BioEssays 10, 127–130.
[21] Swezey, R.R. and Epel, D. (1990) BioEssays 12, 98–99.
[22] Bisson, L.F. and Fraenkel, D.F. (1983) Proc. Natl. Acad. Sci. USA 80, 1730–1734.
[23] Bisson, L.F. and Fraenkel, D.G. (1983) J. Bacteriol. 155, 995–1000.
[24] Cohen, N.S., Cheung, C.-W. and Raijman, L. (1987) J. Biol. Chem. 262, 203–208.
[25] Parker, J.C., Orringer, E.P. and McManus, T.J. (1978) in: Physiology of Membrane Disorders, (Andreoli, T.E., Hoffman, J.F. and Fanestil, D.D., eds.), Plenum, New York, pp. 783–786.
[26] Schrier, S.L. (1966) Am. J. Physiol. 210, 139–145.
[27] Kispal, G., Evans, C.T., Malloy, C. and Srere, P.A. (1989) J. Biol. Chem. 264, 11204–11210.
[28] Steffan, J.S., Minard, K., and McAlister-Henn, L. (1990) in: Structural and Organizational Aspects of Metabolic Regulation (Srere, P.A., Jones, M.E. and Mathews, C.K., eds), Liss, New York, pp. 375–387.
[29] Fields, S. and Song, O.-K. (1989) Nature 340, 245–246.
[30] Hemmingsen, S.M., Woolford, C., Van der Vies, S.M., Tilly, K., Dennis, D.T., Georgopoulos, C.P., Hendrix, R.W. and Ellis, R.J. (1988) Nature 333, 330–334.
[31] Ellis, R.J. and Van der Vies, S.M. (1988) Photosynthesis Res. 16, 101–115.
[32] Srivastava, D.K. and Bernhard, S.A. (1986) Science 234, 1081–1086.
[33] Chock, P.B. and Gutfreund, H. (1988) Proc. Natl. Acad. Sci. USA 85, 8870–8874.
[34] Srivastava, D.K., Smolen, P., Betts, G.F., Fukushima, T., Spivey, O.H. and Bernhard, S.A. (1989) Proc. Natl. Acad. Sci. USA 86, 6464–6468.
[35] Ovadi, J. and Keleti, T. (1978) Eur. J. Biochem. 85, 157–161.
[36] Kvassman, J., Petterson, G. and Ryde-Petterson, U. (1988) Eur. J. Biochem. 172, 427–431.
[37] Orosz, F. and Ovadi, J. (1987) Biochim. Biophys. Acta 915, 53–59.
[38] Clegg, J.S. and Jackson, S.A. (1989) Biochem. Biophys. Res. Commun. 160, 1409–1414.
[39] Welch, G.R., Keleti, T. and Vertassy, B. (1988) J. Theor. Biol. 130, 407–422.
[40] Kacser, H., Sauro, H.M., and Acerenza, L. (1990) Eur. J. Biochem. 187, 481–491.
[41] Sauro, H.M. and Kacser, H. (1990) Eur. J. Biochem. 187, 493–500.
[42] Salerno, C., Ovadi, J., Keleti, T. and Fasella, P. (1982) Eur. J. Biochem. 121, 511–517.
[43] Tompa, P., Batke, J. and Ovadi, J. (1987) FEBS Lett. 214, 244–248.
[44] Ovadi, J., Tompa, P., Vertessy, B., Orosz, F., Keleti, T. and Welch, G.R. (1989) Biochem. J. 257, 187–190.
[45] Kempner, E.S. and Miller, J.H. (1968) Exp. Cell Res. 51, 141–149.
[46] Zalokar, M. (1960) Exp. Cell Res. 19, 114–132.
[47] Pagliaro, L. and Taylor, D.L. (1988) J. Cell Biol. 107, 981–991.
[48] Pagliaro, L., Kerr, K. and Taylor, D.L. (1989) J. Cell Sci. 94, 333–342.