27 Supramolecular organization-dependent responses to stimuli

G. Wágner, K. Liliom, J. Oláh, F. Orosz and J. Ovádi

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, H-1518, P.O.B. 7, Hungary

Microtubule dynamics and glycolysis

The multifunctional cytoskeletal network of eukaryotic cells is complex; its dynamic structure is decorated by many different macromolecules. Our recent experimental data have showed that the assembly of microtubule (MT) is facilitated by not-yet-identified neuronal protein(s) which exists in brain and neuroblastoma extracts but not in muscle extract [1]. On the other hand, the glucose metabolism (the upper part of glycolysis) in neuronal cell-free extract is also specifically stimulated by microtubular proteins isolated from brain [1]. Therefore, the combined effect appears to be specific for neuronal systems where MTs, the major component of axon, play crucial role in several physiological events. The segment of energy producing pathway investigated is controlled by the two glycolytic kinases, hexokinase (HK) and phosphofructokinase (PFK). While the PFK activity is not changed, HK is activated by tubulin/MT in brain extract (unpublished results). However, the contributions of these kinases to the control are slightly dependent on the presence of microtubular proteins (unpublished results) probably because Control Coefficient of HK is much higher respect that of PFK.

Response coefficient in non-ideal system

Bivalent cations (e.g., copper, cadmium, mercury) inhibit the steady-state flux of the upper part of glycolysis [1]. However, the parameters for characterization of the system, IC_{50} and cooperativity, are highly dependent on the incubation time (unpublished data). The determination of the response coefficient, R, for the inhibitory cations from the double logarithmic plots of flux *vs.* effector concentration is not possible as suggested since the dependence is not linear. In

order to quantify the sensitivity of the system to stimuli we suggest IC50 values of dose-response curves to be the operational point for calculation of R. Thus an apparent R, R^{app}, can be defined and calculated from the pseudo Hill coefficient evaluated from the direct fitting of dose-response curves by the Hill-like equation: $J = J_o(1 - X^n/(IC50^n + X^n))$. By deriving $R_X^J = (X/J) \cdot dJ/dX$, and substituting $J = J_o/2$ and $X =$ IC50, a special formula for R^{app}, namely $R^J IC50^{app} = -n/2$, can be obtained. This formula has been applied to quantify and to compare the sensitivity of glycolytic flux to various cations.

Toxic effects of bivalent ions and bisindols

Bivalent ions at toxic concentrations effectively inhibit the microtubule assembly as well as the glycolysis in the separated systems, but not necessarily in the combined system [1]. The addition of microtubular proteins to the neuronal cytosolic fraction not only enhanced the glycolytic flux, but diminished the inhibitory effect of the cations and, in addition, the flux-stimulating effect of tubulin/MT was completely preserved even in the presence of the ions [1,3].

Microtubules decorated by cytosolic proteins including glycolytic enzymes as we demonstrated by immunoblotting were visualized by a means of electron microscopy [4]. This superstructure exhibits less sensitivity against toxic compounds such as pollutants or anti-microtubular drugs. Indeed, antimitotic drugs (vinblastine or a new potent bisindol, KAR-2 that we developed recently) are also able to differentiate between single and bundled/decorated microtubules [5]. The finding that drugs could specifically target superstructure-dependent microtubular network give us a hint to develop drugs for killing cancer cells which are characterized by high glycolytic flux and extensive cell division mediated by microtubular system [6].

Toxic metabolite versus enzyme microcompartmentation

Another related issue is proposed connection of toxic concentration of a glycolytic intermediate, dihydroxyacetonphosphate (DHAP) and the microcompartmentation of the relevant enzyme, triosephosphate isomerase (TPI). DHAP occurs at extremely high concentration in the TPI deficient cells which apparently induces extensive toxic effect. It has been suggested that the high level of DHAP could be responsible for the neurological disorder which is a well-known clinical symptom for the patients suffering in isomerase deficiency. In red blood cell of affected patients 3 per cent TPI activity of healthy controls produces about 30-fold higher DHAP level compared to the control normal cells. We have suggested on

the bases of experimental flux analysis and simulation of glycolysis that the reduced isomerase activity due to the mutations of TPI at positions of 145 and 240, cannot be responsible itself for the high metabolite level [7]. First of all, because from the two affected brothers carrying the same mutations only the youngest one (propositus) suffer from neurological disorder [8]. Secondly, comparative binding studies with normal control and deficient cells indicated that the mutant enzyme exhibited higher affinity to the red cell membrane than the normal (wild) type and, in addition, the hetero-association induces further decrease of the activity of the mutant enzyme. Significant differences in the associative properties of isomerase from the hemolysates of the two brothers were detected. The distinct behaviour of the deficient cells could cause, on one hand, dramatic enhancement of the DHAP level, on the other hand, may contribute to the formation of the different clinical symptoms [4]. Therefore, the high metabolite level is a consequence not only the mutation of isomerase but its enhanced association to the red cell membrane plus a not-yet-identified factor present in the propositus erythrocytes. Application of purified recombinant human wild type and mutant enzymes will allow us to establish the role of different factor(s). To mimic the situation which may occur in brain (neuronal) tissue of the patients, the binding of isomerase to microtubular proteins, as potential target in neuronal cells, was investigated. Indeed, we have found that TPI co-polymerizes with tubulins into MT, and the enzyme both from brain and red cell extracts able to associate to MTs.

Physiological and pathological relevance

In conclusion, we suggest that the supramolecular organization of structural proteins/enzymes and responses of the components to external as well as internal stimuli are highly coupled events. We have revealed that MTs decorated by glycolytic enzymes and other cytosolic proteins are less sensitive to the toxic effects of bivalent ions and drugs. Therefore, an organized structure could provide protecting mechanism against undesired effects. The protecting effect appeared to be specific, depending on the elements of the system, the complexity of the system as well as on the chemical nature of the effectors. In an organized structure the availability of binding sites targeted by toxic compounds could be entirely different than in non-organized systems, the reactive compounds could be unbound or sequestered by other, less crucial sites within the superstructure. This issue might be important in the understanding, on one hand, how cells function even at relatively high concentration of toxic compounds; on the other hand, the pathological features of diseases caused by the dysregulated or elevated concentration of effectors in cells, specifically in neuronal cells. The distinct microcompartmentation of mutant proteins may be relevant in the development of the neurodegenerative process in the isomerase deficiency and in other more common neurological diseases.

References

1. Liliom, K., Wágner, G., Kovács, J., Comin, B., Cascante, M., Orosz, F. and Ovádi, J. (1999) Combined enhancement of microtubule assembly and glucose metabolism in neuronal systems *in vitro*. Decreased sensitivity to copper toxicity. *Biochem. Biophys. Res. Comm.* **264**, 605–610.

2. Vértessy, G.B., Orosz, F., Kovács, J. and Ovádi, J. (1997) Alternative binding of two sequential glycolytic enzymes to microtubules: Molecular studies in the phosphofructokinase/aldolase/microtubule system. *J. Biol. Chem.* **272**, 25542–25546.

3. Liliom, K., Wágner, G., Orosz, F., Kovács, J. and Ovádi, J. (2000) Implications of cytoarchitectural analysis in *Technological and Medical Implications of Metabolic Control Analysis* (Eds. A. Cornish-Bowden and M.L. Cárdenas) pp. 183–190, Kluwer Academic Publishers, Dordrecht, The Netherlands.

4. Orosz, F., Wágner, G., Liliom, K., Kovács, J. Baróti, K., Horányi, M., Farkas, T., Hollán, S. and Ovádi, J. (2000) Enhanced association of mutant triosephosphate isomerases to red cell membranes and to brain microtubules. *Proc. Natl. Acad. Sci. USA* **97**, 1026–1031.

5. Orosz, F., Kovács, J., Löw, P., Vértessy, G.B., Urbányi, Z., Ács, T., Keve, T. and Ovádi, J. (1997) Interaction of a new bis-indol derivative, KAR-2 with tubulin and its antimitotic activity. *British J. Pharmacol.* **121**, 947–954.

6. Orosz, F., Comin, B., Rais, B., Puigjaner, J., Cascante, M., Kovács, J., Tárkányi, G., Ács, T., Keve, T. and Ovádi, J. (1999) New semisynthetic vinca alkaloids: chemical, biochemical and cellular studies. *British J. Cancer* **79**, 1356–1365.

7. Orosz, F., Vértessy, G.B., Hollán, S., Horányi, M. and Ovádi, J. (1996) Triosephosphate isomerase deficiency: predictions and facts. *J. Theor. Biol.* **182**, 437–447.

8. Hollán, S., Fujii, H., Hirono, A., Hirono, K., Karro, H., Miwa, S., Harsányi, V., Gy-di, E. and Inselt-Kovács, M. (1993) Hereditary triosephosphate isomerase (TPI) deficiency: two severely affected brothers one with and one without neurological symptoms. *Hum. Genet.* **92**, 486–490.