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"AMBIQUITOUS" BEHAVIOR OF BRAIN HEXOKINASE

RAPID AND REVERSIBLE INTERACTION OF HEXOKINASE WITH THE OUTER MITOCHONDRIAL MEMBRANE

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Certain enzymes exhibit rapidly reversible associations with subcellular structures. These enzymes have been called "ambiquitous" (both places; cf., "ubiquitous," all places) to emphasize that their intracellular location may be variable, being a function of metabolic status in the cell (1). The most thoroughly studied ambiquitous enzyme is brain hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) (2). Hexokinase binds reversibly and specifically to the outer mitochondrial membrane, with the binding equilibrium being sensitive to physiologically relevant metabolites such as ATP and glucose-6-P. Variations in distribution of the enzyme between bound and solubilized forms are believed to be involved in regulation of catalytic activity, the bound form of the enzyme being more active. Variation in the soluble:particulate distribution of hexokinase has been reported to occur in response to in vivo perturbations of normal cerebral energy metabolism with increased proportion of mitochondrially bound enzyme found during periods of increased glycolytic rate (e.g., 3, 4).

Ambiquitous behavior has two fundamental requirements: there must be complementary recognition signals on enzyme and membrane which permit specific interaction; and there must be some mechanism by which the strength of that interaction can be modulated by parameters reflecting metabolic status of the cell. Current efforts

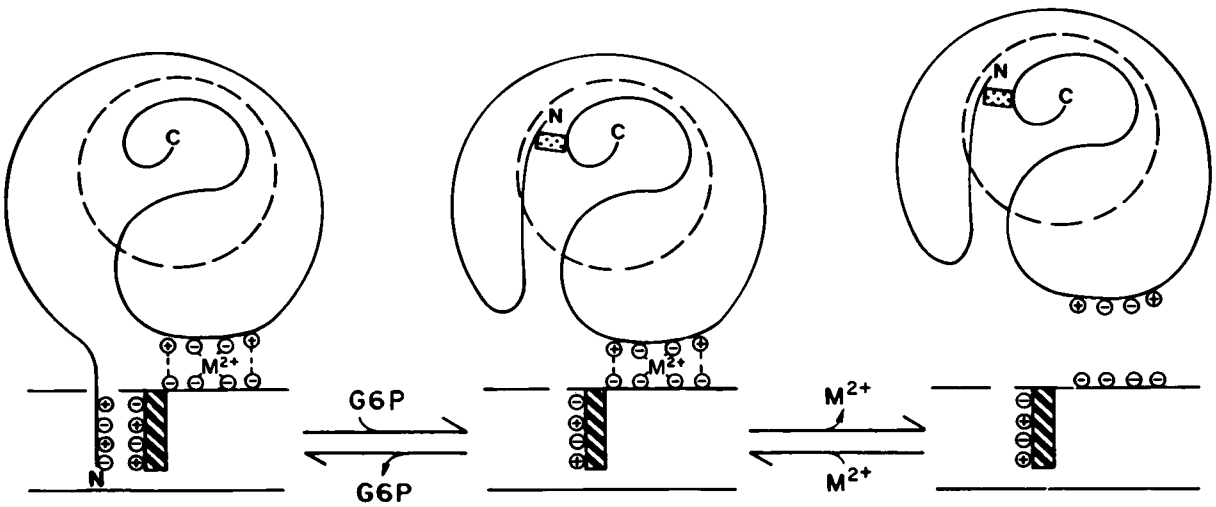

FIGURE 1 Speculative model representing interaction of brain hexokinase with the outer mitochondrial membrane. The enzyme is pictured as having two functional domains: one, enclosed by dashed circle, includes the C-terminus (arbitrarily) and the catalytic site; the other includes the N-terminal region and is responsible for interaction with the membrane. The N-terminal segment, led by the hydrophobic triplet, Tyr-Tyr-Phe, is inserted into the membrane and interacts with the hexokinase binding protein (crosshatched rectangle) in the membrane. Additional divalent cation-mediated interactions also occur (6). As a result of binding glucose-6-P (small stippled rectangle), the enzyme undergoes a conformational change which results in retraction of the N-terminal segment from the membrane (and also causes inhibition of catalytic activity). The enzyme is now bound less tightly, with only the divalent cation-mediated forces operational, i.e., the binding equilibrium is shifted toward increased amounts of "solubilized" enzyme. In the presence of 1 mM Mg^{++} the association of enzyme with the membrane can largely be maintained even in the presence of glucose-6-P. Since the intracellular concentration of Mg^{++} has been estimated at ~ 1 mM (7), it may be that, in vivo, the reversible interactions between hexokinase and the outer mitochondrial membrane are primarily restricted to the glucose-6-P sensitive states shown at the left of the figure, i.e., between tightly bound (N-terminal inserted) and loosely bound (N-terminal retracted) states. (Reprinted, with permission of Academic Press, from reference 2).

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in our laboratory are directed at determining how these fundamental requirements are fulfilled in the hexokinase–outer mitochondrial membrane system.

RESULTS

A "hexokinase binding protein" (HBP) has been highly purified from outer mitochondrial membranes by a procedure involving dissociation of the membrane with octyl glucoside followed by reconstitution of lipid vesicles into which the HBP is selectively incorporated (5). Based on its inaccessibility to exogenous proteases, the HBP (subunit mol wt, 31,000) is thought to be an integral protein of the outer mitochondrial membrane. This protein appears to be the sole membrane component responsible for specific interaction with hexokinase. Indirect evidence suggested involvement of the N-terminal region of the enzyme in the binding. The N-terminal amino acid sequence has now been determined. The first three residues are a triplet of aromatic amino acids, Tyr-Tyr-Phe. This unusually hydrophobic sequence may provide a "wedge" by which the N-terminal portion of the enzyme secures access to the HBP buried in the membrane. A speculative model for the hexokinase-membrane interaction is shown in the accompanying figure.

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THE MEMBRANE ENZYME COMPLEX REQUIRED FOR L-PROLINE TRANSPORT AND UTILIZATION IN *ESCHERICHIA COLI* K12

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L-proline is actively accumulated by *Escherichia coli* K12 and can be oxidized to L-glutamate, serving as sole carbon and/or nitrogen source for bacterial growth. Two specific transport systems, denoted proline porters I and II (PP-I and PP-II), catalyze proline uptake (1).[1] Proline oxidation to Δ^1-pyrroline carboxylate is coupled via the respiratory chain with the reduction of O_2 (2). The membrane-associated enzyme proline dehydrogenase catalyzes that reaction and probably also the NAD^+-linked oxidation of Δ^1-pyrroline carboxylate to glutamate (2, 3). Proline dehydrogenase is encoded in the *putA* gene and mutations at the adjacent locus, *putP*, inactivate PP-I (1). Although *putP* and *putA* are transcribed separately, their expression is controlled coordinately by catabolite repression and specific induction (1, 3). In addition to its enzymatic

TABLE I
ESCHERICHIA COLI K12 STRAINS

Strain*	Genotype*	Gene dosage‡	
		putP	*putA*
CSH4	F⁻ *trp lacZ rpsL thi*	1	1
RM2	CSH4 Δ*putPA100*	0	0
JT31	CSH4 *putA1*::Tn*5*	1	0
JT34	CSH4 *putP3*::Tn*5*	0	1
WG2400	RM2 *recA srl*::Tn*10*	0	0
WG2404	WG2400/pLC35-38	>1§	0
WG2405	WG2400/pLC43-41	>1	>1
WG2406	WG2400/ColE1	0	0

*The derivation of these strains and genetic nomenclature have been described (1, 4).

‡Gene dosages are given as numbers of copies of the intact gene per chromosome.

§Although pLC35-38 complements *putP* defects, the plasmid insert has not been shown conclusively to include *putP* (4).

[1]Stalmach, M. and J. M. Wood. Unpublished results.