Average specific activity of glutamine synthetase in invertebrate tissues

Phylum	Animal	Tissue							
		Optic ganglia	Neural retina	Brain	H.P. digestive gland	Digestive gland	Thoracic ganglia	Gut	Skin
Mollusca	*Octopus vulgaris* (2)[a]	11.9	5.3.	5.0	1.24				0.0
	Rossia pacifica (3)	6.9	2.3						
Arthropoda	*Cancer magister* (3)		0.066[b]			0.14	0.66		
			0.21[c]						
Echinodermata	*S. purpuratus* (3)								0.16

[a] Number of specimens; [b] 6 eyes without stalks; [c] 6 eyes with stalks.

shows the presence of very low GS activity in the gut of the sea urchin. Other tissues of this species were not assayed.

We have presented evidence here that glutamine synthetase is present in certain adult tissues of organisms representing 3 major invertebrate phyla. Enzyme specific activity was relatively high in coleoid nervous tissue and less so in digestive tissues. Levels of activity were considerably lower in tissue from functionally comparable crab organs. The high GS specific activity in both the vertebrate and the coleoid nervous systems correlates with the well known convergence concerning their anatomy, electrophysiology, and behavior. It is not possible at present to suggest what functional significance this correlation may have. The role of large amounts of GS in vertebrate nervous systems has been a topic of debate, but may be associated with the peculiar transport problems of nervous tissue. Also, glutamate has recently been implicated as a excitatory neuro-transmitter in certain animals (KRNJEVIC[11]). Consequently, the presence of GS would be functionally indispensable in the synaptic physiology of any system.

Zusammenfassung. Nachweis eines Invertebraten-Enzyms, das bei Vertebraten insbesondere im Nervengewebe eine wichtige Rolle spielt. Diese Glutamin-Synthetase wurde vor allem im optischen Ganglion in der Retina und im Gehirn zweier Tintenfischarten gefunden und überdies in sehr geringer Menge bei Crustaceen und Echinodermen.

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Asymmetrical Activation by Ca^{2+} of the Erythrocyte Membrane K^+-Dependent Phosphatase

It is known that in the presence of Mg^{2+} the ($Na^+ + K^+$)-independent and ouabain-insensitive fraction of the red cell membrane ATPase activity is markedly enhanced by low concentrations of Ca^{2+}, Sr^{2+} or Ba^{2+}[1-3]. We have shown elsewere that in the presence, but not in the absence, of ATP, Ca^{2+}, Sr^{2+} or Ba^{2+} are also able to activate the K^+-dependent fraction of the red cell membrane phosphatase activity, which under these conditions loses its sensitivity to ouabain[4]. Since activation by divalent cations of the membrane phosphatase and ATPase follows very similar curves for each cation, it has been suggested that a single mechanism may be the cause of the activation of both enzymes[4]. If this were the case, membrane ATPase and phosphatase would share the same ATP and Ca^{2+} sites. One of the main predictions that this assumption leads to is that activation of the K^+-dependent phosphatase by Ca^{2+} and ATP should take place, as in the ATPase[5], only when ATP and Ca^{2+} are at the inner surface of the cell membrane.

In this communication we wish to report experiments designed to test this prediction on reconstituted ghosts of human red cells.

Two kinds of ghosts were prepared by a modification of the procedure of SCHATZMANN and VINCENZI[5]. Singly hemolyzed ghosts were prepared lysing 1 volume of packed red cells in 800 volumes of an ice-cold solution containing 5 m*M* $MgCl_2$, 40 m*M* *Tris*HCl, 0.5 m*M* ethyleneglycol-*bis*(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), 0.3 m*M* ATP (*Tris* salt), pH 7.2 at 25 °C. Doubly hemolyzed ghosts were prepared lysing one volume of singly hemolyzed ghosts in 40 volumes of an icec-old solution containing 5 m*M* $MgCl_2$, 10 m*M* *Tris*-HCl, 2 m*M* ATP (*Tris* salt), 0 or 1 m*M* $CaCl_2$, pH 7.2 at 25 °C. For both kinds of ghosts the hemolysate was allowed to stand at 0 °C for 10 min. After this step the ghosts were washed 3 times at 5 °C with about 30 volumes of a solution containing 5 m*M* $MgCl_2$ and 150 m*M* *Tris*-HCl, pH 7.4 at 25 °C. The second hemolysis was found to be necessary because the presence of Ca^{2+} in the lysing solution yielded ghosts retaining a large amount of the intracellular soluble phosphatase normally present in red cells. The volume fraction of ghosts occupied by ghosts sealed to Ca^{2+} or ATP was estimated according to the procedure already described[6]. About 50% of the singly hemolyzed

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Table I. The effect of internal Ca^{2+} on K^+-dependent phosphatase activity in ATP containing ghosts

	K^+-dependent phosphatase activity (mmole *p*-NP/l sealed ghosts/h)
Ca^{2+}-free ghosts	0.24
Ca^{2+}-containing ghosts	0.82
Ca^{2+}-free ghosts + ouabain	0.00
Ca^{2+}-containing ghosts + ouabain	0.67

For each experiment doubly hemolyzed ghosts (see text) were prepared from the same batch of cells and assayed simultaneously. The final incubation medium contained (m*M*): EGTA, 0.5; *p*-NPP (*Tris* salt), 10; KCl, 50; *Tris*-HCl, 100 (pH 7.8 at 37 °C). K^+-dependent phosphatase activity is the difference between the rate in the above-mentioned medium and in a medium in which all the KCl was replaced by an equivalent amount of *Tris*-HCl. Ouabain concentration was 10^{-3}*M*.

Table II. The effect of external Ca^{2+} and ATP on K^+-dependent phosphatase activity of ATP-containing ghosts

	Additions to the external medium (m*M*)		K^+-dependent phosphatase activity (mmole *p*-NP/l orig. cells/h)
	Ca^{2+}	ATP	
Intact ghosts	–	–	0.30
	0.75	–	0.32
Intact ghosts	–	0.25	0.22
	0.75	0.25	0.47
Disrupted ghosts	–	0.25	0.26
	0.75	0.25	0.79

Singly hemolyzed ghosts (see text) were prepared from the same batch of cells and assayed simultaneously. Disrupted ghosts were obtained by freezing and thawing a suspension of intact ghosts. The final incubation medium was similar to that in experiments in Table I except that 5 m*M* $MgCl_2$ was present. Ca^{2+} was added as $CaCl_2$. For other conditions see Table I.

and about 20% of the doubly hemolyzed ghosts sealed to ATP and Ca^{2+}. Phosphatase activity was measured estimating the release of *p*-nitrophenol (*p*-NP) from *p*-nitrophenylphosphate (*p*-NPP) by the ghosts after a 15 min long incubation at 37 °C, following the procedure already described[7]. In all experiments the amount of ghosts present was that which gave a hematocrit of 10% calculated on the original volume of cells.

The effects of intracellular Ca^{2+} on K^+-dependent phosphatase activity are shown in Table I. It is clear that addition of intracellular Ca^{2+} to ATP-containing ghosts results in a more than 3-fold increase in K^+-dependent phosphatase activity. If this increase were due to the ATP-dependent effect of Ca^{2+}, it should be associated with the loss of ouabain sensitivity of the K^+-dependent phosphatase activity. This prediction was tested in the second experiment of Table I in which the effect of internal Ca^{2+} on K^+-dependent phosphatase activity was assayed in media containing ouabain. Data clearly show that, in contrast with the lack of effect of K^+ in Ca^{2+}-free ghosts, a large K^+-dependent phosphatase activity is measurable when Ca^{2+}-containing ghosts are assayed in the presence of ouabain.

Although results in Table I show that internal Ca^{2+} and ATP are sufficient for phosphatase activation, they do not rule out the possibility that Ca^{2+} and ATP are also effective from the outer surface of the cell membrane. To test this point, Ca^{+2}-free reconstituted ghosts were assayed for K^+-dependent phosphatase activity in media with and without Ca^{2+} and ATP. Results in Table II show that: 1. addition of Ca^{2+} to the suspending media of ATP-containing ghosts has very little effect on K^+-dependent phosphatase activity, and 2. if external Ca^{2+} is added together with ATP K^+-dependent phosphatase activity is increased, but this increase is almost doubled after disruption of the permeability barrier of the ghosts which allows Ca^{2+} to have access to both sides of the membrane. The activating effect of external ATP and Ca^{2+} can therefore be fully accounted for by the penetration of ATP and Ca^{2+} into the population of ghosts which do not regain their low permeability after hemolysis.

Asymmetrical activation of the membrane phosphatase by internal ATP and Ca^{2+} suggests that the system responsible for this effect has a definite orientation within the membrane. Presumably as a result of this orientation, ATP and Ca^{2+} can only have access to their specific sites from the inner surface of the cell membrane. The similarity of these asymmetrical requirements with those of the Ca^{2+}-activated ATPase activity of the red cell membrane[5] is consistent with the idea that activation by Ca^{2+} of the ATPase and of the K^+-dependent phosphatase of red cell membranes may be related phenomena.

Resumen. Los sitios con los cuales se combinan el ion Ca y el ATP para activar la fracción dependiente ce K^+ de la fosfatasa de la membrana del glóbulo rojo, están ubicados en la superficie interna de dicha membrana. Esta característica de asimetría, favorece la idea de la relación de esta enzima con la ATPase activada por Ca^{2+} de la membrana del glóbulo rojo.

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