PHOSPHOINOSITIDE INTERCONVERSION: A MODEL FOR CONTROL OF Na^+ and K^+ PERMEABILITY IN THE NERVE AXON MEMBRANE

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Summary: Metal complex stability constants and acid association constants of deacylated di- and triphosphoinositide were analyzed to ascertain the effect of phosphoinositide interconversion on the amount of bound Ca^{2+} and ligand charge. These calculations showed that a conversion of triphosphate to diphosphate would result in a release of 70% of bound Ca^{2+} and a 25% decrease in ligand charge. Changes of this magnitude occurring on one side of the nerve axon membrane could conceivably bring about a reorganization of the membrane with a resulting change in Na^+ and K^+ permeability.

Recent evidence suggests that phosphoinositides play an important functional role in excitable nervous membranes. First, polyphosphoinositides (diphosphoinositide, DPI; and triphosphoinositide, TPI) are localized predominately in myelinated nerves (1). Second, of all lipids associated with myelinated nerves, only the phosphoinositides have a significant metabolic turnover (2). Yagihara, _et al_. (3) recently demonstrated a rapid _in vitro_ incorporation of ^{32}P inorganic phosphate into TPI of myelinated peripheral nerve. Third, physical and chemical properties of phosphoinositides indicate that these lipids may be capable of controlling ion permeability in membranes and thus may have an important function in nerve conduction. Phosphoinositides, especially TPI, complex readily with divalent metal ions such as Ca^{2+} and Mg^{2+} (4,5). In the absence of divalent metal ions these lipids are largely water soluble but become water insoluble upon the addition of metal ions. Phosphoinositides complex readily with a variety of proteins (6,7) forming in most cases water-soluble complexes in the absence of divalent metal ions and water-insoluble complexes in the presence of these ions.

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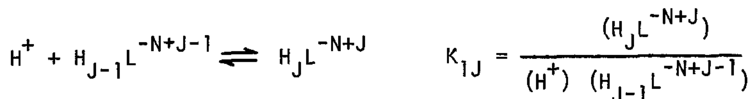
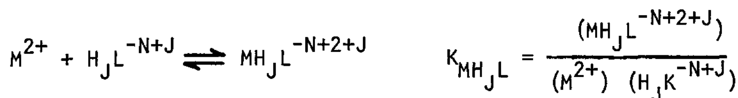
In view of the data accumulating in this laboratory on the ionic and metal-binding properties of phosphoinositides, it was of interest to apply these data to a hypothetical model of ion permeability in membranes. Several workers have suggested that the interconversion of TPI and DPI may be responsible for the changes in Na^+ and/or K^+ permeabilities of the axon membrane which are associated with nerve excitation. Kai and Hawthorne (8) suggest that the hydrolysis of TPI to DPI or MPI in the nerve axonal membrane results in a release of bound Ca^{2+} ions which opens up pores with increased permeability to monovalent ions. Durell and Garland (9) propose a similar mechanism involving the phosphodiesteratic cleavage of phosphoinositides. Selective ion permeability, however, (such as demonstrated during an action potential) is not only a function of pore size. Diamond and Wright (10) indicate that for monovalent ions in biological systems, pore charge is of even greater importance in control of selective ion permeability. A more complete model would include the effect of TPI-DPI interconversion on total ligand charge.

Calculations: Although metal complex stability constants and acid association constants of TPI, deacylated TPI (GIP_3)*, and deacylated DPI (GIP_2)* are known (4,5), it would be difficult to predict, without exact calculations, the extent of change in metal ion binding and ligand charge which would result from the conversion of TPI to DPI on a membrane. Because of the multiple equilibria involved, a computer analysis was undertaken to determine the extent of such changes one might expect to find on a membrane. The following equilibria were considered:



$$H^+ + H_{J-1}L^{-N+J-1} \rightleftharpoons H_JL^{-N+J} \qquad K_{1J} = \frac{(H_JL^{-N+J})}{(H^+)\ (H_{J-1}L^{-N+J-1})}$$



$$M^{2+} + H_JL^{-N+J} \rightleftharpoons MH_JL^{-N+2+J} \qquad K_{MH_JL} = \frac{(MH_JL^{-N+2+J})}{(M^{2+})\ (H_JK^{-N+J})}$$

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*GIP_3: glycerylphosphorylinositol diphosphate; GIP_2: glycerylphosphorylinositol monophosphate.

where K_{1J} is the acid association constant and K_{MH_JL} is the metal complex stability constant. These equilibrium constants for GIP_3 and GIP_2 are listed in Table 1. These data were used in a computer program (11) to calculate the mole-fractions of all ionic species present in solutions of GIP_3 and GIP_2 at a specified pH.

TABLE 1. EQUILIBRIUM CONSTANTS[a]

	GIP_3		GIP_2
Cation	Log K_{ML}	Log K_{MHL}	Log K_{ML}
H^+	8.05[b]	5.70[b]	5.99[b]
Ca^{2+}	3.27	2.22	2.04
Mg^{2+}	3.45	2.37	2.29

a. T = 20.0°, u = 0.1 M tetrapropylammonium iodide (Hendrickson and Reinertsen (5)).

b. These values are acid association constants (log K_{11} and log K_{12}).

Results and Discussions: The results of these calculations at pH 7.0 are shown in Table 2. At this pH, the conversion of GIP_3 to GIP_2 results in a release of 70% of bound Ca^{2+} and a 25% decrease in average ligand charge. The distribution of ionic species and charge as a function of pH is shown in Figure 1. Although the difference in ligand charge does not change appreciably between pH 6-8, there is considerable change in release of bound Ca^{2+} as GIP_3 becomes more deprotonated and binds more Ca^{2+} with increasing pH.

There is some question of validity in extrapolating from a solution of monomeric ligands to polymeric aggregates in a membrane system. The analysis of ionic species presented does not take into effect differences in activity coefficient or the electrostatic surface potential of a charged membrane surface. However, it does seem reasonable to expect the same direction and mag-

TABLE 2. MOLE-FRACTIONS OF IONIC SPECIES IN SOLUTION

GIP_3[a]		GIP_2[b]	
Ionic Species	Mole-Fraction	Ionic Species	Mole-Fraction
L^{-5}	0.055	L^{-3}	0.830
HL^{-4}	0.613	HL^{-2}	0.080
H_2L^{-3}	0.031		
ML^{-3}	0.241 } 0.301	ML^{-1}	0.089
MHL^{-2}	0.060 }		
Ca^{2+}	0.698×10^{-3} M	Ca^{2+}	0.911×10^{-3} M
Ligand Charge	-3.66	Ligand Charge	-2.74

a. Calculated for a solution of GIP_3 (total conc. 1×10^{-3} M) and Ca^{2+} (total conc. 1×10^{-3} M) at pH = 7.

b. Calculated for a solution of GIP_2 (total conc. 1×10^{-3} M) and Ca^{2+} (total conc. 1×10^{-3} M) at pH = 7.

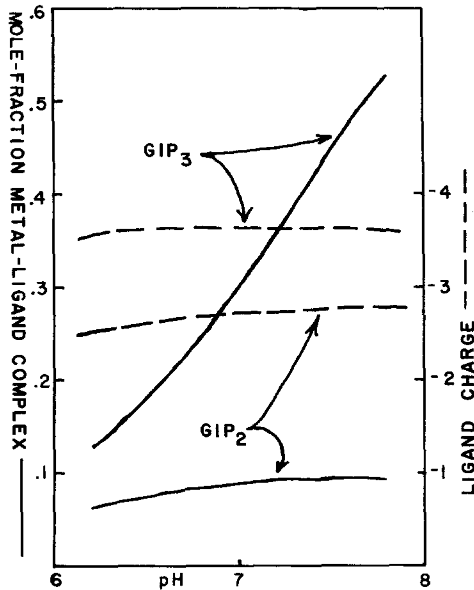
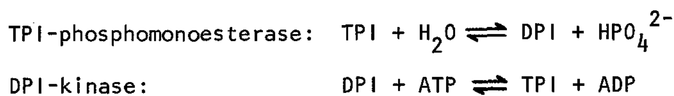


FIGURE 1. Variation of ligand charge (------) and mole fraction metal-ligand complex (————) with pH for deacylated triphosphoinositide (GIP_3) and deacylated diphosphoinositide (GIP_2).

nitude of changes on the membrane surface as is found in solutions even though the absolute values of metal binding and ionic charge might be different. Given this assumption, the magnitude of changes of bound Ca^{2+} and ligand charge resulting from a conversion of TPI to DPI on a membrane surface could result in significant changes in membrane charge and structure with resultant changes in Na^+ and/or K^+ permeabilities.

The net increase in positivity of the membrane would favor an increase in K^+ over Na^+ permeability (10) implicating this mechanism in the falling phase of the action potential. On the other hand, changes in membrane structure might lead to an increased negativity at the channel sites despite an overall increase in positivity of the membrane. Such a mechanism would favor an increase in Na^+ over K^+ permeability, such as occurs during the rising phase of the action potential. Since in the absence of divalent metal ions, phosphoinositides or phosphoinositide-protein complexes are largely water soluble, hydrophilic pores of increased local negativity could be formed under these conditions.

Several experimental observations are favorable to this model. First, the enzymes necessary for the interconversion of TPI and DPI are known to exist at high activities in nervous tissues. For example, TPI-phosphomonoesterase (12) is present in fresh brain at 2/3 the activity of acetylcholinesterase (8). TPI-phosphomonoesterase and DPI-kinase (13) catalyze the following reactions:



TPI-phosphomonoesterase: $TPI + H_2O \rightleftharpoons DPI + HPO_4^{2-}$

DPI-kinase: $DPI + ATP \rightleftharpoons TPI + ADP$

Second, Kai and Hawthrone (8) consider both enzymes to be soluble enzymes and indicate that they may be localized in the axoplasm. Third, Hodgkin and Keynes (14) showed a discharge of Ca^{2+} into the squid axoplasm during stimulation of the squid axon. Such a phenomenon would be consistent with the hydrolysis of TPI and the loss of calcium phosphate from the interior surface of the axonal membrane. Fourth, Keynes (15) has demonstrated changes in

birefringence and light scattering during passage of an action potential, consistent with structural changes in the membrane. Fifth, Papahadjopoulos and Ohki (16) recently reported on the stability of asymmetric phospholipid membranes. They prepared bilayers of phosphatidyl serine and showed that these membranes were unstable under conditions of asymmetric distribution of Ca^{2+} or H^{+} ions. The action of TPI-phosphomoneosterase on one side of a membrane containing TPI would result in a similar asymmetric distribution of Ca^{2+} and ligand charge. This asymmetry could then result in the breakdown or reorganization of membrane components leading to changes in Na^{+} and/or K^{+} permeabilities. Resynthesis of TPI by action of DPI-kinase and ATP would result in the binding of Ca^{2+} to the newly formed TPI. The resulting TPI-Ca^{2+} complexes would be more hydrophobic and could reorganize the membrane into its original state.

It is important to recognize that these charge and structural alterations would probably be localized at scattered pores. Moore, *et al.* (17) estimate that there are only 13 Na^{+} channels per square micron of axon membrane. TPI turnover would therefore not need to be very large to affect membrane permeability considerably, especially if the enzymes and/or the polyphosphoinositides were localized near such channels. This might explain the inability of Larrabee (18) to detect an increased incorporation of ^{32}P into phosphoinositides in activated versus resting nerves. More sensitive methods or better experimental systems might be able to detect such an increase.

This model for the function of phosphoinositides in myelinated nerves is, of course, hypothetical. Other ionic phospholipids could also function in this manner and this general model might be applicable to the function of phosphoinositides in other membrane processes, since phosphoinositides are also found in small but significant levels in non-neural tissues, especially kidney (19). This model does, however, invite many experiments with model membrane systems and with intact axons.

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