PHYSICAL PROPERTIES AND INTERACTIONS OF PHOSPHOINOSITIDES *

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Introduction

The *myo*-inositol phospholipid complex in the brain consists of monophosphoinositide, diphosphoinositide, and triphosphoinositide. The complete structures of these lipids from beef brain were reported by Brockerhoff and Ballou (1961) from studies on the deacylated products. Intact triphosphoinositide from ox brain was first isolated by Dittmer and Dawson (1961) using a solvent extraction procedure. Subsequently, Hendrickson and Ballou (1964) reported the separation of mono-, di-, and triphosphoinositide by ion-exchange chromatography of intact brain phospholipids on diethylaminoethyl cellulose. Studies on ox brain by Dawson and co-workers have shown that di- and triphosphoinositide are localized predominantly in the myelin and, unlike other myelin lipids, are rapidly metabolized components (Eichberg & Dawson, 1965; Sheltawy & Dawson, 1966).

The high metabolic turnover of phosphoinositides in the brain and their strong affinity for metal ions suggest that these lipids may be important in active membrane transport (Kennedy, 1967). With this in mind, studies were initiated on the metal-binding properties of phosphoinositides (Hendrickson & Fullington, 1965), their interactions with proteins (Fullington & Hendrickson, 1966; Fullington, 1967), and the physical properties and micellar structures of these lipids.

Physical Properties

The phosphoinositides (mono-, di-, and triphosphoinositide) are soluble in water and exist in solution as micelles with the ionic groups oriented towards the bulk aqueous phase. Gel filtration studies of these lipids (TABLE 1) on polyacrylamide (Biogel P-300) and agarose (Biogel A 1.5 m) indicated an apparent micellar weight of about 400,000. Such data, however, are difficult to interpret due to ionic effects of these highly charged lipids and may give anomalous results. Thus, it was decided to determine the micellar weight by ultracentrifugation.

The partial specific volume, intrinsic viscosity, and sedimentation coefficient of triphosphoinositide were determined in 0.1 M N-ethylmorpholine buffer, pH 8, and are shown in TABLE 2. FIGURE 1 shows the viscosity as a function of

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TABLE 1

GEL FILTRATION OF PHOSPHOINOSITIDES *

Sample	Elution Volume/Void Volume	
	Biogel P-300	Biogel A 1.5 m
Triphosphoinositide	1.5	1.2
Diphosphoinositide	—	1.2
Conalbumin (MW 85,000)	2.1	1.5
Human γ-globulin (MW 160,000)	1.9	1.4

* Eluting buffer, 0.1 M N-ethylmorpholine, pH 8.

concentration. Ultracentrifuge sedimentation patterns for di-, and tri-phosphoinositide are shown in FIGURE 2. The sedimentation patterns as well as the gel filtration elution patterns (FIGURE 3) show a rather narrow and symmetrical distribution of particle size. The sedimentation coefficients of triphosphoinositide as a function of concentration are shown in FIGURE 4. When plotted as either $S_{20,w}$ or $1/S_{20,w}$ versus concentration (Tanford, 1961) the sedimentation coefficient extrapolates to 3.6 S at zero concentration. The micellar weight calculated from these data was 78,100, using a value of β equal to 2.16×10^6 (Schachman, 1957). These micelles are small as compared to ganglioside micelles which have a micellar weight, as measured by ultracentrifugation, of 257,000 (Gammack, 1963) and lecithin micelles which have a micellar weight of 1,300,000 (Gammack *et al.*, 1964).

A sedimentation coefficient, $S_{20,w}$, equal to 3.2 S was obtained for diphos-

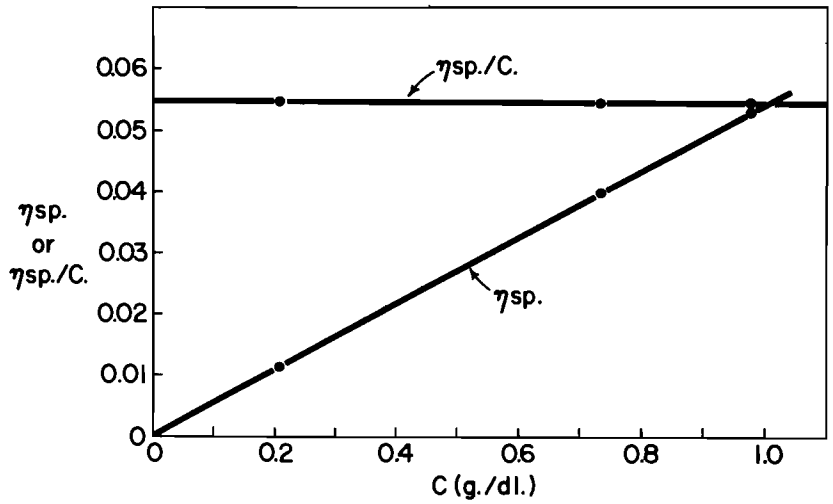


FIGURE 1. Viscosity of triphosphoinositide as a function of concentration. Determined with a Hewlett Packard (Ubbelohde) autoviscometer in 0.1 M N-ethylmorpholine, pH 8, at 29.1° C.

TABLE 2

PHYSICAL PROPERTIES OF TRIPHOSPHOINOSITIDE *

Partial specific volume †	$\bar{v}$ (ml/g)	0.795
Intrinsic viscosity	$[\eta]$ $(g/100ml)^{-1}$	0.0548
Sedimentation coefficient	$S_{20,w}$ (S)	3.6
Calculated micellar weight		78,100

* All physical measurements made in 0.1 M N-ethylmorpholine buffer, pH 8.
† Determined by standard pycnometric techniques.

phoinositide under similar conditions. This indicates a micellar weight for diphosphoinositide similar to that of triphosphoinositide. This is confirmed by the similar retention of these two lipids on agarose gel (TABLE 1). Monophosphoinositide dissolves in aqueous buffer, but the solutions are slightly cloudy. Such solutions were completely sedimented before the ultracentrifuge reached full speed (56,100 rpm). Thus, monophosphoinositide, dissolved directly into buffer, forms micellar aggregates of much larger size. The much greater charge on di- and triphosphoinositide (about -2.5 and -4.5 respectively at pH 8) undoubtedly accounts for this difference in behavior.

Interactions

Stability constants were determined for Ca(II), Mg(II), and Ni(II) complexes of triphosphoinositide and its deacylated product, glycerylphosphorylinositol diphosphate (Hendrickson & Fullington, 1965). These constants are

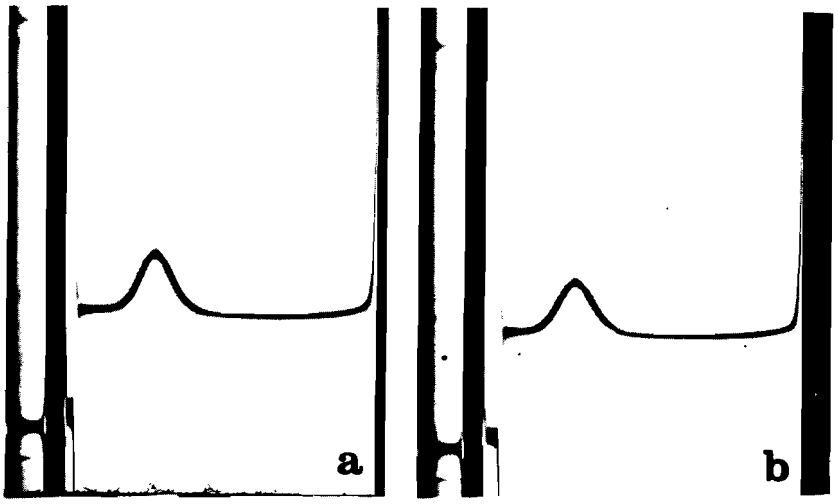


FIGURE 2. Sedimentation patterns for triphosphoinositide (a) and diphosphoinositide (b). Determined in a Spinco model E ultracentrifuge equipped with schlieren optics. Pictures taken 84 minutes after rotor reached full speed (56,100 rpm). Concentration, .2% in 0.1 M N-ethylmorpholine, pH 8.

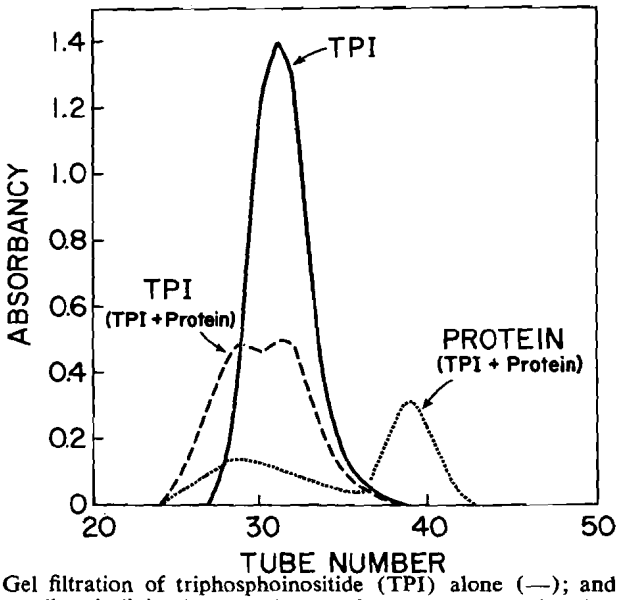
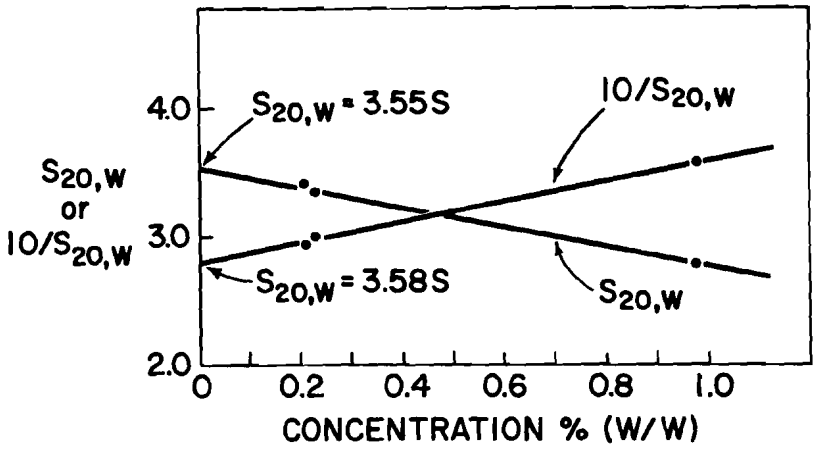


FIGURE 3. Gel filtration of triphosphoinositide (TPI) alone (—); and a mixture of lipid and ovalbumin-lipid (— — —), protein (- - - - - - - - -). Conditions described in TABLE 5.

shown in TABLE 3. The apparent stability constants, K_{ML}, for the metal-lipid complexes include an electrostatic term due to the high concentration of negative charge on the micelle surface. Thus, they cannot be directly compared with those for the monomeric deacylated product. Displacement constants, B, which are defined by the equilibrium $M^{2+}+H_2L \rightleftharpoons ML+2H^+$, are more valid measures of intrinsic binding since there is no net change in charge on the



ligand. Displacement constants for the lipid and its deacylated product are similar and indicate similar intrinsic metal binding for the polymeric and monomeric ligand groups.

Interactions of triphosphoinositide-metal complexes with several coligands were studied by liquid-liquid partition and gel filtration (Fullington & Hendrickson, 1966. When ovalbumin, poly-L-lysine, polyaspartate, or ethylenediamine was added to a biphasic chloroform-methanol-water system containing the Mg(II), Ca(II), or Ni(II) complex of triphosphoinositide, ternary complexes were formed as evidenced by the formation of an interfacial precipitate containing phospholipid, metal ion, and protein or polyamino acid, or the presence of ethylenediamine in the chloroform-rich phase where it is ordinarily insoluble.‡ These results were not obtained in the absence of divalent metal ions. Stable mixed complex formation was shown by gel filtration. The triphosphoinositide-metal ion-ethylenediamine complexes were analyzed after formation in the liquid-liquid partition system and after gel filtration (TABLE 4). The stoichiometry of two bound ethylenediamines, one of which is still bound

TABLE 3

EQUILIBRIUM STABILITY CONSTANTS *

Cation	Log K_{ML} (M^{-1})		Log B (M)	
	TPI †	$GPIP_2$‡	TPI †	$GPIP_2$
H^+	8.45	8.05	—	—
Mg(II)	4.85	3.45	— 10.0	— 10.3
Ca(II)	5.04	3.27	— 9.8	— 10.3
Ni(II)	5.92	—	— 8.9	—

* Data from Hendrickson and Fullington (1965).
† Triphosphoinositide.
‡ Glycerylphosphorylinositol diphosphate.

after gel filtration, to one phospholipid and metal ion indicated that binding occurs through cochelation of the phospholipid and coligand about a central metal ion. It is not clear, however, whether this same type of interaction occurs in the lipid-metal ion-protein complexes. It is interesting to note that although there is little or no difference in Ca(II) and Mg(II) binding with triphosphoinositide or ethylenediamine alone, there is considerable difference in the abilities of the Ca(II) and Mg(II) complexes of triphosphoinositide to bind ethylenediamine. This illustrates a significant effect of tertiary structure on metal ion specificity which could be quite important in membrane selectivity.

Gel filtration of mixtures of triphosphoinositide and protein shows that weak to moderate complex formation, depending on the particular protein, occurs in the absence of divalent metal ions; upon the addition of metal ions, however, strong complexes are formed which are largely insoluble in aqueous

‡ Similar results were reported by Dawson (1965) for triphosphoinositide, Ca(II) or Mg(II), and serum albumin.

TABLE 4

TRIPHOSPHOINOSITIDE-M(II)-ETHYLENEDIAMINE INTERACTIONS *

Metal	Complex (Mole Coligand/Mole Lipid)	
	Before gel filtration	After gel filtration
—	0.0	0.0
Ni(II)	1.99	0.97
Mg(II)	1.58	1.03
Ca(II)	0.88	0.44

* Data from Fullington and Hendrickson (1966).

or organic solvents. FIGURE 3 shows the gel filtration elution pattern for a mixture of triphosphoinositide and ovalbumin. The amount of complexing with this and other proteins is shown in TABLE 5. Clearly, there is some difference in protein specificity, but the nature of these interactions is not obvious.

Triphosphoinositide-histone binding was studied because of the similarity of histones to the basic myelin proteins (Tomasi & Kornguth, 1967). The migration of calf thymus histones during polyacrylamide gel electrophoresis is shown in FIGURE 5. It consists of two major bands (1 and 3) and at least two minor bands (2 and 4). When triphosphoinositide is added to the histone sample, a new slower-moving band (5) appears at the expense of band 3 and some of the minor bands. Similar results occur when Mg(II) is added, precipitating a lipid-protein complex, and the soluble portion is electrophoresed. It appears that triphosphoinositide complexes preferentially with the histone in band 3, giving a new complex (band 5) which is much less basic. Divalent metal ions precipitate this lipid-protein complex.

Maas and Colburn (1965) have presented indirect evidence for the formation of a phospholipid-metal ion-adenosine triphosphate (ATP) ternary complex, and have discussed a possible role for this complex in membrane transport.

TABLE 5

TRIPHOSPHOINOSITIDE-PROTEIN INTERACTIONS *

Protein	Protein Complexed to Lipid (%)	Free Protein (%)
Ovalbumin	50	50
Human γ-globulin	65	35
Bovine serum albumin	84	16
Transferrin	86	14
Conalbumin (Fe free)	100	0
Histone (calf thymus)	100	0

* Determined by gel filtration on a Biogel A 1.5 m column (1.0 × 75 cm) using 2.5 mg lipid + 2.5 mg protein/0.5 ml buffer (0.1 M N-ethylmorpholine, pH 8).

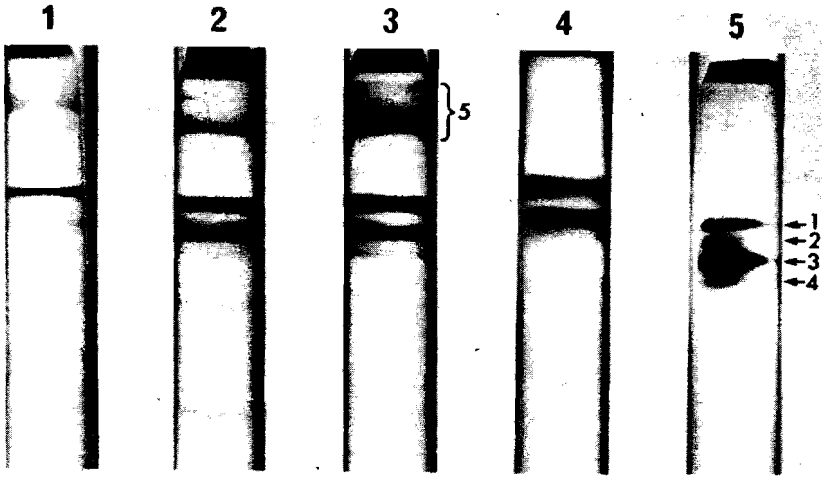


FIGURE 5. Polyacrylamide gel electrophoresis. (1) Soluble portion (20 μl) of a mixture of 1 mg triphosphoinositide (TPI) + 3 mg histone (calf thymus) + 1 μmole Mg(II)/0.5 ml. (2) Same as (1), but with 4 mg of histone. (3) 200 μg histone + 100 μg TPI. (4) 100 μg histone + 0.05 μmole Mg(II). (5) 50 μg histone. Conditions for electrophoresis similar to those described by Tomasi and Kornguth (1967).

The demonstration of such a complex by direct means was thus of interest. Gel filtration showed that triphosphoinositide-Mg(II)-ATP ternary complexes are indeed formed and contain up to 0.3 mole ATP/ mole lipid (TABLE 6). In the absence of divalent metal ions, however, there is still a significant binding of ATP, on the order of 0.1 mole ATP/ mole lipid. This complexing is surprising for two very negatively charged molecules, but perhaps occurs through hydrophobic interactions with the adenine ring or hydrogen bonding with the adenine amino group. The complex formed in the presence of metal ions may not involve cochelation. The metal ions may only act to inhance those interactions that occur in the absence of metal ions by reducing the negative charge on the micelle surface.

TABLE 6

TRIPHOSPHOINOSITIDE-MG(II)-ATP INTERACTIONS *

Applied to Column *			Complexed
Lipid	ATP·2Na$^+$	Mg(II)	Mole ATP/Mole Lipid
2.7 mg	1.85 μmole	—	0.1
2.7 mg	1.76 μmole	2.5μ mole	0.24
2.5 mg	3.21 μmole	2.5 μmole	0.29

* Sample applied to column (Biogel A 1.5 m, 1.0 $\times$ 75 cm) in 0.5 ml of 0.1 M N-ethylmorpholine buffer, pH 8.

Conclusions

Phosphoinositides, in the presence of divalent metal ions, can bind small molecules such as ethylenediamine by a process of cochelation about a central metal ion. The phosphoinositide-protein complexing that has been studied here apparently does not involve this type of interaction since complexing occurs in the absence of divalent metal ions and with the same protein specificity (at least for histone complexing) as is found in the presence of metal ions. Complexing of these highly anionic lipids with anionic serum proteins or ATP in the absence of divalent metal ions suggests that hydrophobic interactions may be important in these complexes. Electrostatic interactions must also be important with the very basic histone proteins. These studies show that there is indeed a wide range of complexing possible between phosphoinositides and proteins, metal ions, or even small molecules such as ATP or ethylenediamine. An understanding of these interactions would certainly be helpful in understanding membrane structure and function. Further work will be directed toward understanding these interactions, particularly as they apply to the myelin sheath, using techniques such as magnetic resonance spectroscopy (proton relaxation rate studies) in which paramagnetic metal ions such as Mn(II) or Ni(II) can be used as magnetic probes on the micelle surface.

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