Chapter 1

Structure and Nomenclature of Inositol Phosphates, Phosphoinositides, and Glycosylphosphatidylinositols

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1. INTRODUCTION

Inositol is a cyclohexanehexol, a cyclic carbohydrate with six hydroxyl groups one on each of the ring carbons. *myo*-Inositol (hexahydroxycyclohexane) is the oldest known inositol; it was isolated from muscle extracts by Scherer in 1850 who called it inositol from the Greek word for muscle (Posternak, 1965). Since then, interest in inositols and their derivatives has waxed and waned. However, in the last 20 years we have seen a veritable explosion of interest in this area because of the discovery of new inositol derivatives and widespread recognition of the critical roles that phosphoinositides and inositol phosphates play in cellular signal transduction (Irvine and Schell, 2001; Shears, 2004; Toker, 2002; Toker and Cantley, 1997; Vanhaesebroek *et al.*, 2001). *myo*-Inositol occupies a central position in cellular metabolism – the inositol moiety is utilized by nature to biosynthesize a wide variety of compounds including inositol phosphates, phosphatidylinositides, glycosylphosphatidylinositols, inositol esters, and ethers. In addition, *myo*-inositol is converted into other stereoisomers of inositol and uronose and pentose sugars (Loewus, 1990b; Loewus and Murthy, 2000).

Inositol phosphates, phosphatidylinositides, and glycosylphosphatidylinositols encompass a diverse group of inositol-containing compounds with great structural complexity and heterogeneity. They mediate a myriad of biological processes and ongoing research suggests that they may be actively involved in many more cellular processes (Bernfield *et al.*, 1999; Irvine and Schell, 2001;

A. Lahiri Majumder and B. B. Biswas (eds.), Biology of Inositols and Phosphoinositides, 1–20

Low, 2000; Shears, 2001, 2004). Many of these will be detailed in the rest of this book. In this chapter, I will introduce the structural, stereochemical, conformational, and nomenclature aspects of inositol phosphates, phosphatidylinositides, and glycosylphosphatidylinositols.

2. STEREOCHEMISTRY

At first glance inositol appears to be a relatively simple molecule – on closer examination, a host of sophisticated stereochemical issues, chiral, prochiral, and conformational reveal themselves (Parthasarathy and Eisenberg, 1986, 1990; Posternak, 1965). In fact, the complexity of stereochemical issues in cyclitols, namely cyclohexanes with one hydroxyl group on three or more ring atoms, is well recognized (IUPAC, 1976; Posternak, 1965); inositol can serve as a good model for a sophisticated discussion of various forms of isomerism.

2.1 Stereoisomers of inositol

The six secondary hydroxyl groups on the cyclohexane ring can be arranged in one of two orientations, axial or equatorial giving rise to nine stereoisomeric forms (Figure 1). The chair form rather than the Howarth projection

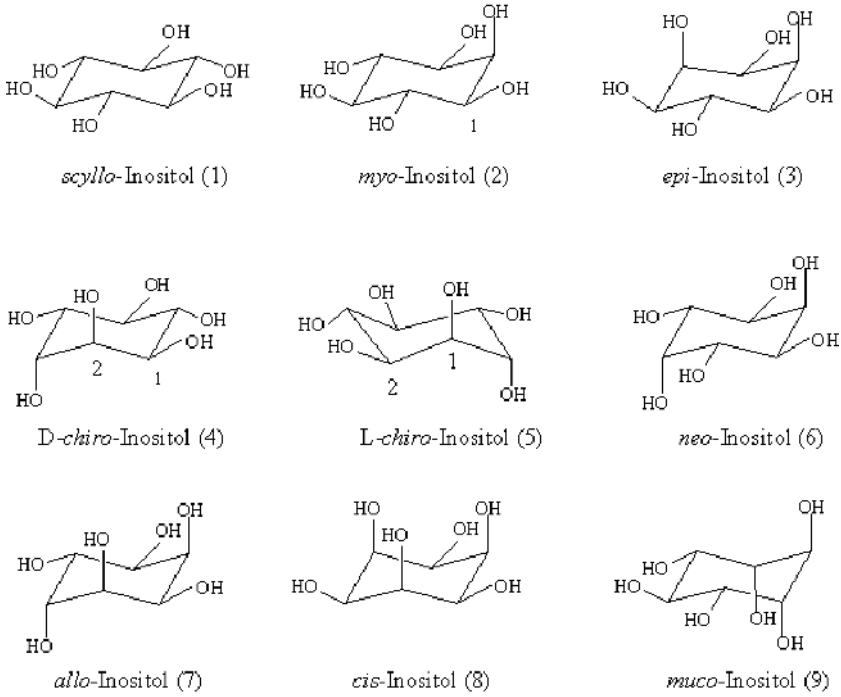


Figure 1. Stereoisomers of inositol.

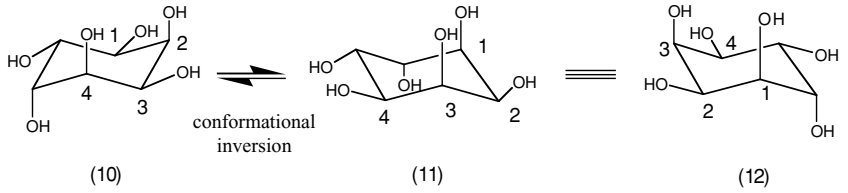


Figure 2. *allo*-Inositol: Conformational isomers and enantiomers.

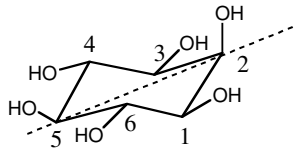
will be employed in this review so that axial/equatorial distinctions of substituents are clearly illustrated and the geometrical and stereochemical consequences of the two orientations are obvious; these structural features are lost in the Howarth projection. Additionally, Howarth projections can sometimes be misleading; for example, it incorrectly indicates that *allo*-inositol has a plane of symmetry in the molecule.

Of the nine stereoisomers of inositol, the *scyllo*-isomer has no axial hydroxyl, the *myo*-isomer has one, the *epi*-, *chiro*-, and *neo*-isomers have two, and the *allo*-, *cis*-, and *muco*-isomers have three hydroxyl groups (Figure 1). Of these, six isomers (*scyllo*-, *myo*-, *epi*-, *neo*-, *cis*-, and *muco*-isomers) have one or more planes of symmetry in the molecule (*meso* compounds) and are therefore not chiral. D-*chiro*- and L-*chiro*-isomers do not have a plane of symmetry and are chiral molecules; moreover they are enantiomers of each other. The *allo*-isomer is unique – the conformational isomer of (10) (Figure 2) is (11) which is also its enantiomer! Since interconversion between conformational isomers is rapid, *allo*-inositol exists as a 50/50 mixture of the two enantiomers at room temperature. Therefore, although *allo*-inositol is chiral, the compound is optically inactive at room temperature because it is a racemic mixture; a chiral reagent, such as an enzyme, would be expected to preferentially react with one enantiomer and not the other.

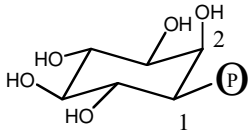
Six isomers of inositol have been found in nature to date; these include *myo*-, *muco*-, *neo*- D-*chiro*-, L-*chiro*-, and *scyllo*-isomers.

2.2 *myo*-Inositol

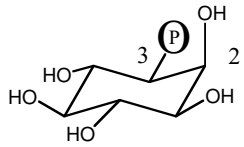
The *myo*-isomer is the most abundant form in nature. It occupies a unique place in inositol metabolism because this is the only isomer synthesized *de novo* from D-glucose-6-phosphate; all other isomers are derived from *myo*-inositol (Loewus, 1990b; Loewus and Murthy, 2000). In early 1900s, *myo*-inositol was called *meso-inositol* (abbreviated ms-inositol) probably because, like mesotartaric acid, it does not exhibit optical activity and cannot be resolved into optical isomers (Posternak, 1965). However, as discussed above, six other isomers also do not exhibit optical activity so the name seemed inappropriate. In 1954, the name *myo*-inositol was introduced – a name not particularly well coined either since it is a pleonasm – both *myo* and *inositol* mean muscle. Nevertheless, the name persists.



myo-Inositol (13) ----- , plane of symmetry



1D-*myo*-inositol-1-monophosphate
(14)



1D-*myo*-inositol-3-monophosphate
(15)

Ⓟ = Phosphate

Figure 3. *myo*-Inositol and phosphorylated derivatives.

myo-Inositol can be divided into two mirror image halves (13) (Figure 3) – a perpendicular plane passes through C-2 and C-5 and splits the molecule into non-superimposable mirror images. Therefore, any modification that will disturb the symmetry of the molecule will render the molecule chiral, thus *myo*-inositol is prochiral. For example, phosphorylation (or any other substitution or reaction) at C-1, C-3, C-4, or C-6 (Figure 3) eliminates the plane of symmetry and leads to a chiral molecule; reaction at C-2 or C-5 preserves the plane of symmetry and the molecule remains achiral. C-1 is enantiopic to C-3 and C-6 is enantiotopic to C-4, thus the product of a substitution at C-1 (14) will be the enantiomer of the same reaction at C-3 (15).

Although the two halves of *myo*-inositol are stereochemically nonequivalent, they are chemically equivalent to an achiral molecule or reagent. In other words, an achiral molecule or reagent does not show preferential selectivity for either side and therefore reacts with both halves at the same rate. However, the biochemical consequence of enantiotopic carbons of most relevance to biochemists is that a chiral molecule, such as an enzyme, can readily distinguish between C-1 and C-3 as well as C-4 and C-6 and preferentially reacts with one enantiotopic carbon or another. For example, *myo*-inositol kinase phosphorylates *myo*-inositol exclusively at the D-3 position and yields the chiral product, 1D-*myo*-inositol-3-monophosphate (15) (Deitz and Albersheim, 1965; Loewus *et al*., 1982). This is the same isomer produced from glucose-6-phosphate by *myo*-inositolphosphate synthase (reviewed in Loewus, 1990a). The only route

by which the enantiomer 1D-*myo*-inositol-1-monophosphate (14) (Figure 3) is biosynthesized is by dephosphorylation of 1D-*myo*-inositol-1,4-bisphosphate by phosphatase. Enantiomers cannot be readily interconverted; conversion of one enantiomer to another involves the cleavage of covalent bonds (about 100 kcal/mol) and reformation with the opposite spatial arrangement. Enzymes react preferentially with one enantiomer or another, so the spatial differences between enantiotopic carbons can have profound biological consequences.

2.3 Nomenclature

The nomenclature of inositol and its derivatives has been a source of confusion for some time. This is partly due to the stereochemical complexity of all cyclitols, including inositols, and partly due to changes in the numbering rules by IUPAC (1976) and IUB (1989). The inositol story is a good illustration of the fact that defining stereochemistry of molecules is a difficult and complex issue and a satisfactory set of rules often evolves after numerous attempts. The underlying rationale behind cyclitol nomenclature and the evolution of the current system of numbering can be found in a number of publications (IUB, 1989; IUPAC, 1976; Murthy, 1996; Parthasarathy and Eisenberg, 1986, 1990). Rules currently followed have been in place since 1986.

myo-Inositol is a *meso* compound with mirror image halves. Therefore, to name the compound two descriptors have to be assigned – the absolute stereochemistry (D or L) and the numbering of the carbon atoms. Consequently, a number of questions arise: which atom should be considered for designating stereochemistry, which atom should be assigned 1, and what is the direction of numbering?

- According to IUPAC recommendations I-4 (IUPAC, 1976), numbers and the direction of numbering in inositols are assigned with reference to the spatial relations and nature of substituents on the ring. In *myo*-inositol, substituents are assigned to two sets, substituents above the ring are assigned to one set and those below to another. Lowest number is assigned to the set with more substituents. In the case of *myo*-inositol [Figure 4, (18)], there are four hydroxyls (C-1, C-2, C-3, and C-5) in one set and two (C-4 and C-6) in the other.
- Carbon-1 could be assigned to either of two enantiotopic carbons (16) and (17) or (18) and (19) (Figure 4). IUPAC recommendation (1976) is as follows: If the molecule is viewed in the vertical (Fischer – Tollens) projection with C-1 at the top with C-2 and C-3 on the front edge of the ring, the configuration is assigned D if the hydroxyl group or other substituent at the lowest-numbered chiral center projects to the right (16) and L if it projects to the left (17). More commonly a horizontal projection is used and the structure is drawn (18) and (19), so that if the substituent on the lowest-numbered asymmetric carbon is above the plane of the ring and the numbering is counterclockwise, the configuration is assigned D (18), and if clockwise,

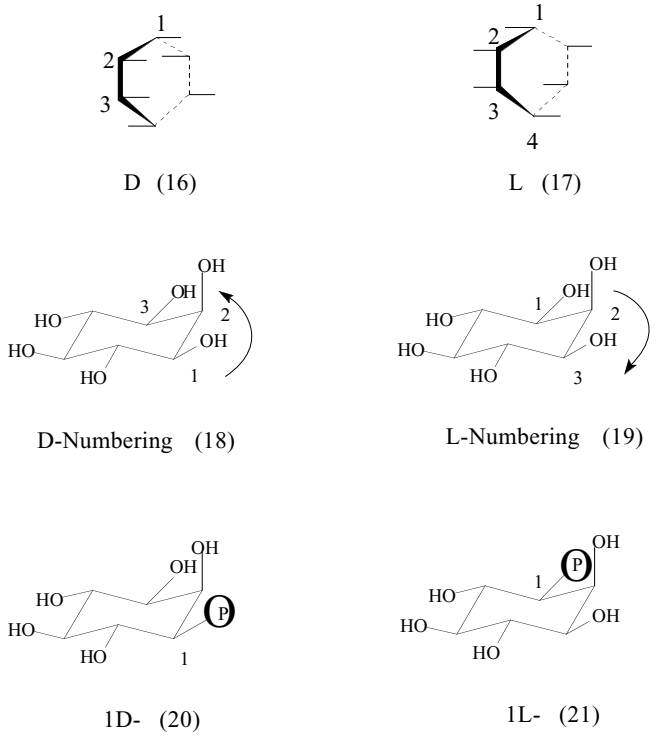


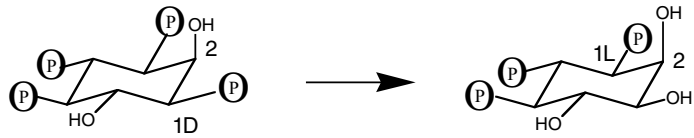
Figure 4. Nomenclature of *myo*-inositol and its derivatives.

the configuration is L (19). Therefore, in substituted inositol phosphates (20) and (21), the starting point could be either of the enantiotopic carbons as shown. The number 1 precedes the D or L to indicate that C-1 is the chiral center considered to define configuration.

- Of the two possibilities, the IUPAC – IUB recommendation of 1976 stipulated that for *meso* compounds, such as *myo*-inositol, the L designation should be applied.

Strict adherence to the lowest-locant rule sometimes obscures straightforward metabolic relationships. For example, consider the dephosphorylation of (22) – (23) (Figure 5). The IUPAC (1976) rules require that compound (22) be labeled 1D-*myo*-inositol-1,3,4,5-tetrakisphosphate rather than 1L-*myo*-inositol-1,3,5,6-tetrakisphosphate, so that substituents are attached to carbons with lower numbers, whereas (23) should be labeled 1L-*myo*-inositol-1,5,6-trisphosphate rather than 1D-*myo*-inositol-3,4,5-trisphosphate. Thus, to describe the dephosphorylation reaction (Figure 5), the necessity to switch between D and L numbering leads to the following statement:

> 1D-*myo*-inositol-1,3,4,5-tetrakisphoshphate is dephosphorylated to 1L-*myo*-inositol-1,5,6-trisphosphate.



1D-*myo*-inositol-1,3,4,5-tetrakisphosphate (22)

1L-*myo*-inositol-1,5,6-trisphosphate (23)

1L-*myo*-inositol-1,3,5,6-tetrakisphosphate

1D-*myo*-inositol-3,4,5-trisphosphate

Figure 5. Hydrolysis of *myo*-inositol phosphates – alternative numbering.

From this statement, it is not immediately obvious which carbon has undergone dephosphorylation. Alternatively, the statement,

> 1D-*myo*-inositol-1,3,4,5-tetrakisphosphate is dephosphorylated to 1D-*myo*-inositol-3,4,5-trisphosphate,

clearly indicates that dephosphorylation has occurred at C-1.

To more easily discern straightforward metabolic relationships, IUB (1989) recommended that the lowest-locant rule be relaxed and either the 1D or the 1L numbering be allowed so long as the prefix 1D or 1L is specified. Thus, the author could use either numbering depending on the relationships that was being stressed.

It was further recommended that the symbol "Ins" be taken to mean *myo*-inositol with numbering proceeding counterclockwise, namely the 1D configuration. These recommendations were in response to the large number of *myo*-inositol phosphates of 1D configuration that were being discovered as hydrolytic products of phosphoinositides in investigations of signal transduction (IUB, 1989).

To remember the numbering of *myo*-inositol phosphates, Agranoff (1978) pointed out the resemblance of the chair conformation to a turtle (Figure 4) and suggested a mnemonic. The head of the turtle represents the C-2 axial hydroxyl and the four limbs and tail represent the five equatorial groups. The right hand limb is designated the D-1 position and the proceeding counterclockwise, the head is D-2, etc. The left front limb is D-3. Alternatively, if the L stereospecific numbering is employed, the left front limb is L-1 and the numbering proceeds clockwise as shown in (19) (Figure 4). In phosphoinositides, the right limb carries the diacylglycerol group.

An additional complicating issue should be kept in mind when reading the seminal pre-1968 literature. *myo*-Inositol derivatives that are currently designated D configuration were assigned L configuration and vice versa in the literature published before 1968. The reasons for the pre-1968 nomenclature are as follows: before 1968, rules of carbohydrate nomenclature dictated that the orientation of highest numbered chiral carbon, C-6 in this case, specify the

configuration, D or L. As the hydroxyl groups at C-1 and C-6 are trans to each other, the compound assigned D before circa 1968 are now assigned 1L and vice versa. Additionally, circa 1960, a different nomenclature was employed in the seminal work conducted to determine the chirality of *myo*-inositol-monophosphate, the isomer (15) that resulted from hydrolysis of D-galactinol was designated D-*myo*-inositol-1-monophosphate as it was derived without change of configuration during the reaction (Ballou and Pizer, 1960). The *myo*-inositol monophosphate isomer (14), derived from the hydrolysis of phosphatidylinositides was assigned L as it exhibited optical rotation opposite to that of *myo*-inositol-1-monophosphate (15) assigned D (Ballou and Pizer, 1960). In 1968, as mentioned above, the rule was changed so that the lowest-numbered stereogenic carbon now specifies configuration.

In summary, following are some of the main points regarding inositol nomenclature:

- *myo*-Inositol derivatives that were designed L configuration in the literature before 1968 would be designated D according to current rules.
- Between 1968 and 1986, *myo*-inositol was numbered in L-stereochemistry.
- Currently, both the 1D or 1L designations can be employed.
- The use of the symbol Ins implies *myo*-inositol with numbering counter-clockwise, namely the 1D configuration.

In summary, in the literature before 1968, compound (14) would be labeled L-*myo*-inositol-1-monophosphate in keeping with the then rule that configuration be assigned on the basis of the orientation of substituents on carbon-6. After 1968, it would be designated *myo*-inositol-1-monophosphate (unspecified configuration implies L) or 1D-*myo*-inositol-1-monophosphate. Currently, (14) would be designated Ins(1)P_1.

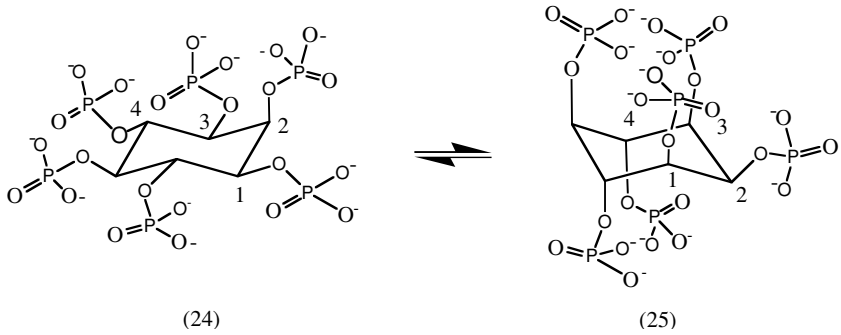
In this article, the current designation will be followed; the symbol Ins will be used to denote *myo*-inositol derivatives. The symbol I will be used to indicate inositol, the unspecified stereoisomerism.

2.4 Conformation of inositol phosphates

Conformational isomers are structural isomers that are interconverted by rotation about single bonds. Properties of conformational isomers including size, shape, energy and chemical reactivity, and biological properties, including binding interactions with other proteins and chelating ability with metal ions can be significantly different. The importance of the effect of conformational changes on biological activity is well illustrated by the dramatic loss of enzyme activity in going from native conformation to denatured conformation, a process woefully familiar to biochemists; the native and denatured structures of enzymes are conformational isomers (except in cases where cleavage of disulfide bonds are involved). Conformational flexibility of biomolecules has a major impact on binding interactions with enzymes and receptors and thus on biological activity. The energy required for rotation

about single (σ) bonds is low, the activation energy for the chair – chair transition of cyclohexane is 45 kJ/mol (10.8 kcal/mol) (Carey and Sundberg, 2000). Thus, in contrast to chiral isomers (Section 2.2), interconversion between conformational isomers can readily occur at room temperature. Because of the facile interconversion of conformational isomers, multiple low-energy conformers exist at room temperature. Therefore, an understanding of the possible low-energy conformations at room temperature is necessary to understand chelating and binding properties as well as ligand – protein interactions at the active site.

Conformational inversion of a chair form leads to an alternative chair form (24) and (25) (Figure 6) through a series of intermediate boat and twisted chair forms (Carey and Sundberg, 2000). During the chair – chair inversion process, the axial and equatorial groups at each of the carbons are interconverted. Generally, of the two alternative chair forms shown below (Figure 6), the conformation in which bulky substituents occupy equatorial positions (24) has less steric hindrance and thus is lower in energy than one in which bulky substituents occupy axial positions (25).



(24) (25)

Figure 6. Conformational interconversion of chair forms of phytate.

The conformation(s) adopted by inositol phosphates, including $InsP_6$, has been the subject of much debate and numerous investigations (Isbrandt and Oertei, 1980; Lasztity and Lasztity, 1990 and references therein; Barrientos and Murthy, 1996; Bauman *et al*., 1999; Volkmann *et al*., 2002). Most of the attention has been focused on phytic acid because of its long history and high endogenous concentration. A full understanding has been complicated by the fact that the energy of conformations is influenced by various factors including,

- number, substitution pattern, and stereochemistry of phosphate groups on the inositol backbone,
- physical state (solid or solution) of the inositol phosphate, and
- factors of the solvating media such as pH and counterions.

The shape, size, and charge distribution of the two possible conformations (24) and (25) are significantly different thus influencing chelating and binding properties.

Generally, as stated above, the conformation in which a maximum number of bulky substituents occupy equatorial positions is energetically more favorable. On this basis, at first glance the 1 ax/5 eq (24) conformation in which the five bulky substituents (hydroxyl or phosphate groups compared to hydrogen, the second substituent) are in equatorial orientations and one is in axial position would be expected to be the sterically favored form (24). This is indeed the case for inositol and the lower inositol phosphates (IP_1 to IP_4). However, counter to this generalization, the higher inositol phosphates (IP_6 and IP_5) undergo ring flip to the sterically hindered (5 ax/1 eq) form (25) at high pH (above 9.0) (Barrientos and Murthy, 1996; Isbrandt and Oertei, 1980; Bauman *et al*., 1999; Volkmann *et al*., 2002).

2.4.1 Inositol hexakisphosphates

Two isomers of inositol hexakisphosphate, *myo*- and *scyllo*-isomers were investigated at different pH (Barientos and Murthy, 1996; Bauman *et al*., 1999). The two isomers differ in the orientation of the phosphate at one of six carbons, however, the impact of this on the proclivity toward ring flip to the sterically hindered form differs greatly.

- $InsP_6$ adopts the 1 ax/5 eq (26) (Figure 7) form at pH $<$ 9.5 and the sterically hindered 5 ax/1 eq form exclusively at pH $>$ 9.5 (27) (Barrientos and

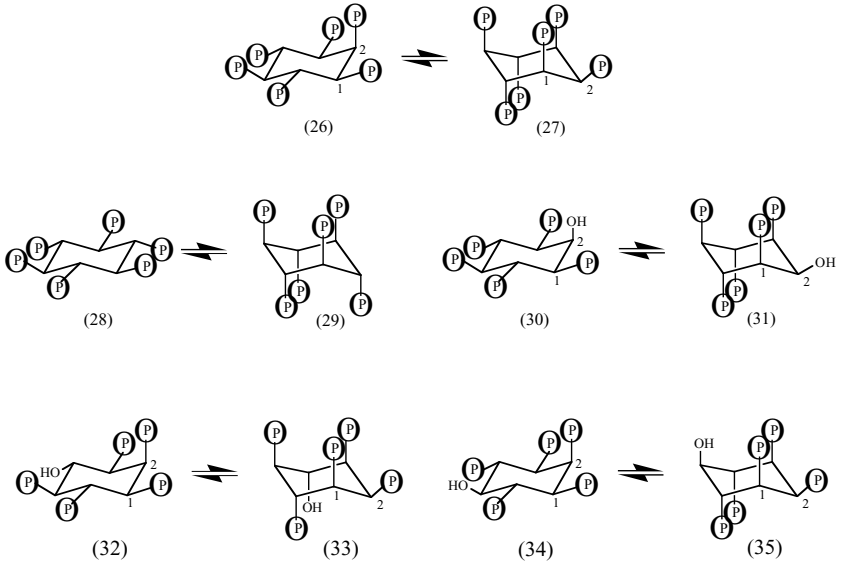


Figure 7. Conformational inversion of inositol phosphates.

Murthy, 1996; Isbrandt and Oertei, 1980). Between pH 9.0 and 9.5 (pK_a range of the three least acidic protons) both conformations are in dynamic equilibrium. Inversion to the sterically hindered form occurs only after the molecule is fully deprotonated to the dodecanionic form, above pH 9.5 (Barrientos and Murthy, 1996; Isbrandt and Oertei, 1980). The driving force for adopting the sterically hindered form (27) may be electrostatic repulsion due to the presence of five contiguous equatorial dianionic phosphates in the 1 ax/5 eq form and reduction of electrostatic repulsion by complexation with counterions in the 5 ax/1 eq form (27). Activation energy for ring flip from the 1 ax/5 eq to 5 ax/1 eq form is 54.8 ± 0.8 kJ/mol. The size of the counterion plays a critical role in stabilizing the 5 ax/1 eq form. In the presence of large counterions such as hydrated Li^+ (radius = 3.4 Å), tetramethyl ammonium or tetrabutyl ammonium cations (radii >5Å), no evidence for the 5 ax/1 eq form was observed suggesting that the formation of a tight chelation cage is critical to stabilizing the 5 ax/1 eq form. On the other hand, small counterions such as Na^+, K^+, Rb^+, and Cs^+ that facilitate the formation of chelation cages stabilize the 5 ax/1 eq form (Barrientos and Murthy, 1996; Bauman *et al*., 1999; Isbrandt and Oertei, 1980) at high pH.

- In the solid state, X-ray crystallography (Blank *et al*., 1971) and Raman spectroscopy (Isbrandt and Oertei, 1980) data suggest that the dodecasodium salt of phytic acid adopts the sterically hindered 5 ax/1 eq conformation. Again, the 5 ax/1 eq form may be adopted to minimize electrostatic repulsion between the five contiguous dianionic phosphates in equatorial positions.
- *scyllo*-Inositol hexakisphosphate [Figure 7, (28) and (29)] undergoes ring inversion from the 6 eq to the 6 ax form more readily than any other inositol phosphate investigated (Volkmann *et al*., 2002). The $\Delta G^{\#}$ is less than 51.2 ± 1.0 kJ/mol; for comparison, the $\Delta G^{\#}$ for ring inversion of unsubstituted cyclohexane is 45 kJ/mol. The low activation energy may be due to destabilization caused by six dianionic equatorial phosphates in the 5 eq form (28) and stabilization of the 6 ax form (29) by the formation of chelation cages on both faces of the cyclohexane ring. The presence of syn-1,3,5-triaxial trisphosphates on both faces (29) facilitates the formation of tight chelation cages with counterions.

2.4.2 Inositol pentakisphosphates

All isomers of *myo*-inositol pentakisphosphate investigated, Ins(1,3,4,5,6)P_5, (30); Ins(1,2,3,5,6)P_5, (32); and Ins(1,2,3,4,6)P_5, (34), adopt the 1 ax/5 eq forms at low pH and the 5 ax/1 eq chair forms [(31), (33), and (35), respectively] at high pH. However, the activation energy for ring flip is influenced by the substitution pattern of phosphates.

- Ins(1,3,4,5,6)P_5 exists in the 1 ax/5 eq form (30) up to pH 10.5 and converts to the 5 ax/1 eq form (31) above pH 10.7. Between pH 10.5 and 10.7 both forms are in dynamic equilibrium. Of the different isomers of IP_5, Ins(1,3,4,5,6)P_5

undergoes chair – chair interconversion to the sterically hindered form most readily The activation energy for ring flip is $\Delta G^{\#}$ is 59.6 ± 0.5 kJ/mol. Stabilization of the 5 ax/1 eq form may be due to the five contiguous equatorial phosphates in (30) and the ability of the *syn*-1,3,5-triaxial trisphosphate arrangement (31) to form chelation cages with counterions (Volkmann *et al*., 2002; Bauman *et al*., 1999).

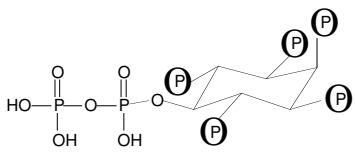
- In the case of Ins(1,2,3,5,6)P_5 (32) and Ins(1,2,3,4,6)P_5 (34), the situation is different. Ins(1,2,3,5,6)P_5 exists in the 1 ax/5 eq form (32) up to pH 8.0 and Ins(1,2,3,4,6)P_5 (34) up to pH 9.5. At higher pH, an interconverting mixture of the 1 ax/5 eq and the 5 ax/1 eq forms exist. The exclusive presence of the 5 ax/1 eq form was not observed. NMR spectra indicate that the $\Delta G^{\#}$ for Ins(1,2,3,5,6)P_5 is lower than that of Ins(1,2,3,4,6)P_5 ($\Delta G^{\#}$ = 73.9 ± 0.8 kJ/mol). This may be due to the destabilizing effect of three contiguous equatorial phosphates in the 1 ax/5 eq form of Ins(1,2,3,5,6)P_5 compared to two sets of two in Ins(1,2,3,4,6)P_5, as well as stabilization of the 5 ax/1 eq form by the formation of a tight chelation cage due to the presence of a *syn*-1,3,5-triaxial trisphosphate arrangement on one face of (33) and the lack of such an arrangement in (35) (Barrientos and Murthy, 1996; Volkmann *et al*., 2002).

The results summarized above were obtained from dynamic nuclear magnetic resonance spectroscopy techniques. Conformational predictions using molecular modeling calculations (Gaussian) agree with experimental results in aqueous solution. Moreover, calculations in gas phase indicated that the sterically hindered forms of charged IP_6s and IP_5s are indeed more stable even in the gas phase (Bauman *et al*., 1999; Volkmann *et al*., 2002).

In summary, the electrostatic repulsion due to four or more equatorially oriented dianionic phosphates disfavors the 1 ax/5 eq orientation and induces ring flip to the 5 ax/1 eq form. The proclivity toward ring flip is influenced by the number, position, and orientation of dianionic phosphate substituents on the inositol ring; the facility for ring flip is as follows: (29) > (27) > (31) > (33) > (35).

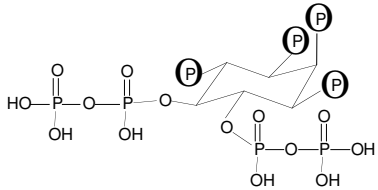
3. DIPHOSPHORYLATED INOSITOL PHOSPHATES

myo-Inositol phosphates with one or more diphosphate or pyrophosphate groups have been detected in plant and animal cells (Figure 8) (Shears, 1998, 2001, 2004). Compound (36) with diphospho-substituent at the 5-position (5-diphosphoinositol pentakisphosphate) and compound (37) with two diphospho-substituents at 5- and 6-positions (5,6-bisdiphosphoinositol-tetrakisphosphate) have been identified. Two structural features are noteworthy: with the possibility of 12 negative charges on a relatively small cyclohexane ring, IP_6 is probably the most negatively charged small molecule in living cells; heparin also has a high negative charge density but it is a much larger molecule. The fact that



5-diphospho-*myo*-inositol pentakisphosphate

5-[PP]-InsP$_5$ (36)



5,6-bisdiphospho-*myo*-inositol tetrakisphosphate

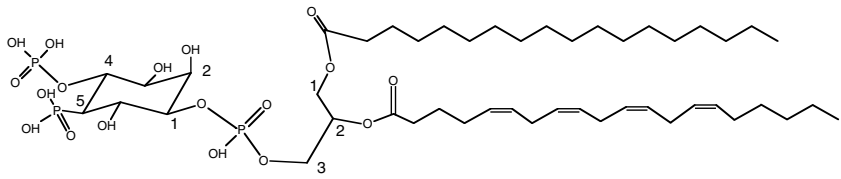
5,6-[PP]$_2$-InsP$_4$ (37)

Figure 8. Diphosphorylated inositol phosphates.

diphosphorylated inositol phosphates can carry higher negative charge density than IP_6 puts it in an unique class. Second, diphosphorylated inositol phosphates contain high-energy phosphoric anhydride bond(s) (ΔG hydrolysis = ~7.5 kcal/mol). Thus, they have the ability to function as phosphate donors and phosphorylate hydroxyl groups in inositol phosphates, proteins, or other molecules. The biological roles of diphosphorylated compounds as well as the enzymes that are involved in their metabolism are being actively investigated (Shears, 1998, 2001, 2004).

4. PHOSPHATIDYLINOSITIDES

Phosphatidylinositides include a group of compounds in which inositol phosphates are esterified to diacylglycerol by a phosphodiester linkage [Figure 9 (38)]. The involvement of phosphoinositides in signal transduction was first observed in the late 1950s (Hokin and Holin, 1953) and the critical roles they play in signal transduction were established in the 1980s (Agranoff *et al*., 1983, Streb *et al*., 1983). Since then, phosphoinositides have been intensively investigated and their involvement in a myriad of biological processes has been identified. A number of reviews detail our current understanding of the metabolism and roles of phosphoinositides in biological processes (Irvine and Schell, 2001; Shears, 2004; Stephens *et al*., 2000; Toker, 2002; Toker and Cantley, 1997; Vanhaesebroek *et al*., 2001).



(38)

Figure 9. sn-1-stearoyl-2-arachidonyl-phosphatidylinositol-4,5-bisphosphophosphate.

A great deal of structural heterogeneity is observed in phosphoinositides (38). These include (a) variation in the fatty acids esterified at the *sn*-1 and *sn*-2 positions of the glycerol unit, (b) the presence of isomeric inositols in the head group, and (c) variation in the position and degree of phosphorylation on the inositol ring. Phosphatidyl-*myo*-inositol (PtdIns) is the parent compound of most of the phosphoinositides; others are derived from it. The five free hydroxyls on PtdIns can be phosphorylated to yield isomeric phosphatidylinositol phosphates. Three isomers of $PtdInsP_1$ with phosphates at the C-3, C-4, or C-5 positions [$PtdIns(3)P_1$, $PtdIns(4)P_1$, or $PtdIns(5)P_1$] have been identified in a wide variety of living cells, however, interestingly, phosphatidylinositol monophosphate isomers with phosphates at C-2 or C-6 positions have not been discovered in natural sources to date. Three isomers of phosphatidylinositol bisphosphates [$PtdIns(3,4)P_2$, $PtdIns(3,5)P_2$, and $PtdIns(4,5)P_2$] and one isomer of $PtdInsP_3$ [$PtdIns(1,3,4)P_3$] have been characterized in cells. Although the presence of 4- and 5-phosphorylated derivatives has been known for many years (Folch and Woolley, 1942), the discovery of 3-phosphorylated derivatives is relatively recent (Whitman *et al*., 1988).

myo-Inositol is the predominant isomer in most of the inositol-containing lipids. *scyllo*-Inositol-containing phosphoinositide lipids have been found in plant cells (Narasimhan *et al*., 1997) and *chiro*-inositol-containing lipids have been characterized in plant and animal cells (Larner *et al*., 1988; Mato *et al*., 1987; Pak and Larner, 1992).

PtdIns is the most abundant phosphatidylinositide in cells; it constitutes about 90% of the inositol lipids in cell membranes. The other six mono- and bis-phosphorylated PtdIns make up the remaining 10% with $PtdIns(4)P_1$ and $PtdIns(4,5)P_2$ constituting up about 9% of the lipid pool, each contributing different amounts depending on the tissue. The remaining five lipids make up 1%. $PtdIns(3)P_1$ or $PtdIns(5)P_1$ contribute about 0.4%, and the 3-lipids jointly constitute about 0.1%. These numbers vary slightly from cell to cell (Stephens *et al*., 2000; Vanhaesebroek *et al*., 2001), however, it is clear that the quantitatively minor inositol lipids $Ptd(4,5)P_2$ and the 3-phosphorylated lipids play critical roles in signal transduction (Stephens *et al*., 2000; Toker and Cantley, 1997; Vanhaesebroek *et al*., 2001).

5. GLYCOSYLPHOSPHATIDYLINOSITOL

Glycosylated forms of PtdIns (GPI) have been found to anchor a variety of cell surface proteins to cell membranes by forming a covalent linkage to the C-terminal end of proteins (Low, 1989, 2000; Low and Saltiel, 1988; Saltiel, 1996). GPI anchors are found in diverse organisms from primitive eukaryotes to mammals and plant cells. GPI molecules are structurally complex, they are made up of variable and non-variable components; the non-variable structural features have been conserved through evolution from protozoa to mammals

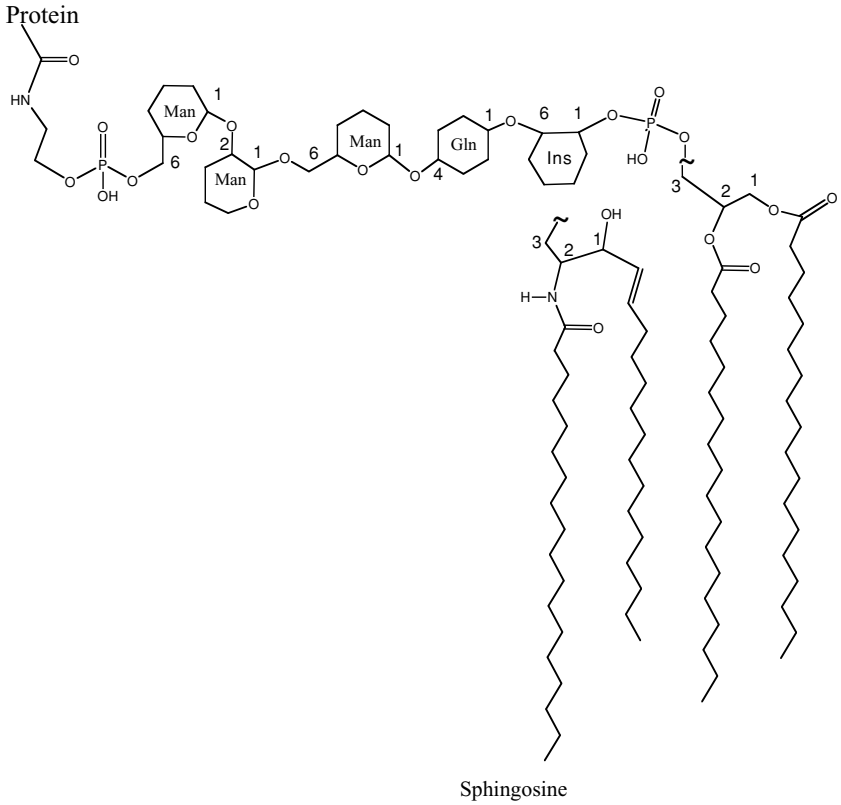


Figure 10. Glycosylphosphatidylinositol. Man, mannose; Gln, glucosamine; Ins, inositol. Sphingosine lipid portion in glycophosphosphingolipid is also shown.

and flowering plants suggesting that it may fulfill a critical role. GPI consists of three distinct units (Figure 10):

- an inositolphospholipid with variable hydrophobic groups,
- a short conserved glycan linking the inositol lipid to the protein, and
- variable substituents on the conserved mannose sugars.

The lipid portion of the molecule has been found to be 1,2-diacylglycerol, 1-alkyl-2-acylglycerol, or a ceramide-based lipid (glycosylinositolphosphorylceramide or glycophosphosphingolipid in which the lipid is a long chain N-acylated amino diol with a single double bond) (Figure 10). In fact, the 1-alkyl, 2-acylglycerol, and ceramide-based unit are more prevalent than the 1,2-diacylglycerol species. Thus, although this group of compounds is referred to as glycosylphosphatidylinositols for convenience, it should be kept in mind that the lipid portion may not be a 1,2-diacylglycerol moiety (Low, 2000).

An unusual structural feature from the inositol perspective is the derivatization of the 2-hydroxyl group of inositol by a long chain fatty acid (palmitic acid). In many mature GPI-proteins, the palmatoyl side chain is not present; the palmatoyl chain is added during the biosynthesis of GPI and removed after the GPI anchor is attached to the protein during posttranslational modification. Although the *myo*-isomer of inositol is the most prevalent form in GPI molecules, the presence of *chiro*-inositol has also been detected.

The conserved core consists of a short glycan chain of four sugar residues glycosidically linked to 6-hydroxyl of the Ins moiety of PtdIns (Figure 10). The four-sugar glycan consists of one glucosamine and three mannose units. The nonreducing end of the mannose moiety is attached to a phosphodiester-linked ethanolamine. The protein is attached to GPI by an amide bond between the amino group of the ethanolamine and the carboxy terminal amino acid of the protein during posttranslational modification in the lumen of endoplasmic reticulum. The lipid part of the molecule is inserted into the lipid bilayer and anchors the protein in the membrane (Low, 2000).

Great variability is observed in the substituents that are attached to the three mannose residues. Substituents include simple units such as ethanolamine, glucose, or mannose or oligoglycans of variable size, structure, and complexity (Low, 2000).

GPI anchors are biosynthesized in the endoplasmic reticulum and added to proteins during posttranslational modification. The biosynthesis of complex GPI molecules is a substantial commitment of resources by the cell and the fact that GPI molecules and the biosynthetic machinery have been conserved through evolution has lead to speculation that these molecules may confer unique advantages to the cell and also fulfill additional functions. GPI anchors provide stable binding of proteins to the lipid bilayer as well as localize proteins in particular regions of membranes. In addition to the role of anchoring cell surface proteins, a number of other functions, including its role in signal transduction, are being pursued by investigators (Low, 2000). The roles of GPI in insulin action and nutrient uptake (nitrate and phosphate uptake) in plants are also being investigated (Kunze *et al*., 1997; Low, 2000; Saltiel, 1996; Stöhr *et al*., 1995).

GPI molecules have been found to anchor another important class of proteins, the heparin sulfate proteoglycans (HSPG), at the cell surface (Bernfield *et al*., 1999). Heparin sulfate, a strongly anionic linear polysaccharide, is covalently attached to proteins; these proteins are called glypicans because of the use of GPI to attach proteins to the cell surface. By way of the heparin sulfate chains, glypicans bind a variety of extracellular signals, form signaling complexes with receptors, and modulate receptor – ligand interactions. Glypicans mediate many cellular processes including cell growth, cell division, cell – cell adhesion, and cell defense (Bernfield *et al*., 1999). Extracelluar ligands that bind to glypicans include growth factors, cytokines, cell-adhesion molecules, and coagulation factors among others.

6. CONCLUSIONS

Inositol is a deceptively simple molecule. On closer study, a number of sophisticated stereochemical, prochiral, chiral, and conformational issues associated with inositols and their derivatives become evident. Inositols, in particular *myo*-inositol, play a central role in cellular metabolism. An array of complicated molecules that incorporate the inositol moiety are found in nature. Structural heterogeneity of inositol derivatives is compounded by the presence of stereo- and regioisomers of the inositol unit. Because of the large number of isomeric inositols and their derivatives present in nature, a detailed understanding of the structural, stereochemical, and nomenclature issues involving inositol and its derivatives is essential to investigate biological aspects. A discussion of the stereochemical, conformational, prochiral, chiral, and nomenclature issues associated with inositols and the structural variety of insoitol derivatives is presented in this chapter.

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