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REVIEW ARTICLE

The inositol phospholipids: a stereochemical view of biological activity

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Introduction

With the current upsurge of interest in the inositol phospholipids, now recognized as the source of second messengers in receptor-mediated Ca^{2+} mobilization (Michell *et al.*, 1981; Berridge & Irvine, 1984) and protein kinase C activation (Nishizuka, 1984), many investigators are encountering the subtleties of *myo*-inositol stereochemistry for the first time. Because of the symmetrical structure of *myo*-inositol, certain metabolites of the compound can exist in enantiomeric forms (non-superimposable mirror images). Although there are other instances of the biological formation of enantiomers, the *myo*-inositol example is unique in that the enantiomeric 1-phosphates do not derive from a common precursor. This is illustrated in Fig. 1 by the two major pathways of *myo*-inositol metabolism: the irreversible biosynthesis of free *myo*-inositol from glucose 6-phosphate (Eisenberg, 1967), the source which maintains *de novo* the supply of free *myo*-inositol for the organism, and the cyclic synthesis (Agranoff *et al.*, 1958; Paulus & Kennedy, 1960) and hydrolysis (Hawthorne *et al.*, 1960) of phosphatidylinositol via free *myo*-inositol. Although *myo*-inositol 1-phosphate is the intermediate common to both pathways, two different forms of the compound are produced, the L enantiomer by the synthetic pathway and the D by the cyclic pathway. In each pathway *myo*-inositol 1-phosphate is the immediate precursor of free *myo*-inositol in a reaction catalysed by specific *myo*-inositol 1-phosphatase (Eisenberg, 1967). Both isomers are substrates of the enzyme, liberating *myo*-inositol for incorporation into phosphatidylinositol and its phosphates to maintain receptor function. Since a common product is formed it is virtually impossible to gauge the relative activities of the two pathways *in vivo*.

myo-Inositol 1-phosphatase, however, is inhibited by Li^+ (Fig. 1) both *in vitro* (Naccarato *et al.*, 1974; Hallcher & Sherman, 1980; Parthasarathy & Eisenberg, 1984; Sherman *et al.*, 1984) and *in vivo* (Sherman *et al.*, 1984) and under these conditions *myo*-inositol 1-phosphate remains intact; by determination of the fraction of this pool composed of each isomer, Sherman *et al.* (1984) assessed the relative contributions of the two pathways, an illustration of the importance of stereochemical information to the understanding of a biological process. Hoffmann-Ostenhof *et al.* (1978) have drawn similar

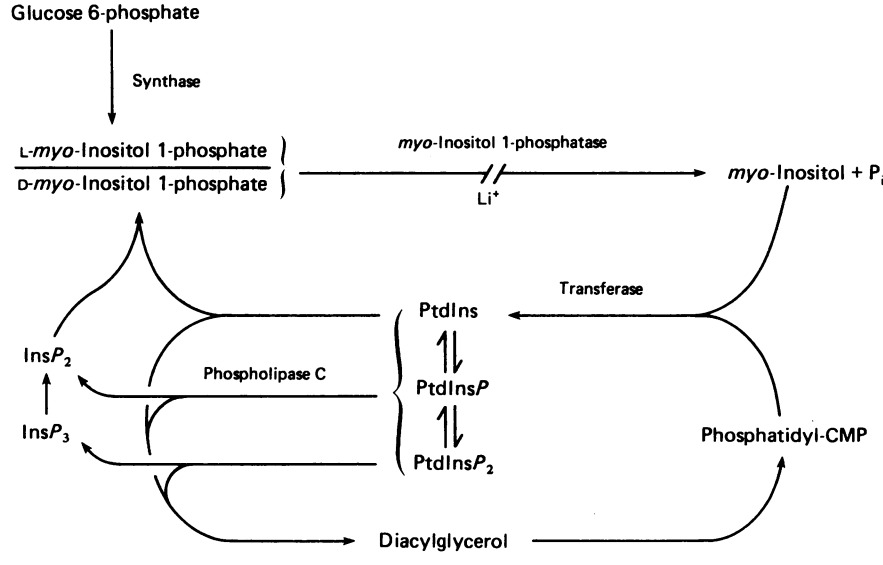

Fig. 1. Biosynthesis *de novo* of *myo*-inositol and the turnover of phosphoinositides

Abbreviations used: Ptd, phosphatidyl; Ins, *myo*-inositol; *P*, phosphate.

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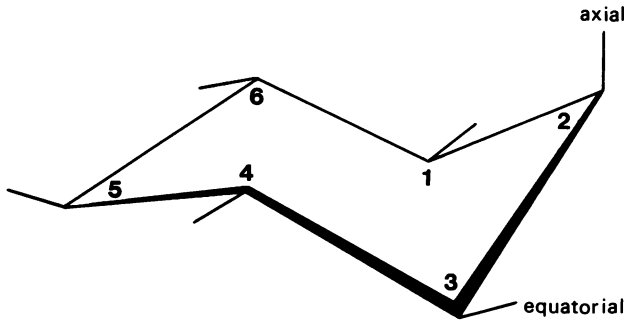

Fig. 2. Conformational diagram of *myo*-inositol with axial OH at C-2; all other OH groups are equatorial

Hydrogen atoms are not shown.

inferences from studies of stereospecific enzymatic methylation of *myo*-inositol.

Although the subject of stereochemistry is vast, complex, and marked by controversy even among experts (O'Loane, 1980), the stereochemistry and the nomenclature of *myo*-inositol and its congeners are governed by a few simple principles and rules. Unfortunately, textbooks and other publications codifying these rules have often been wrong (IUPAC–IUB Commission on Biochemical Nomenclature, 1977/1978) and many of the errors in structure and nomenclature found in the current literature, relating the *myo*-inositol phosphates to mobilization of Ca^{2+}, stem from these misleading sources. Hawthorne (1983) has similarly commented on the confusion experienced by many authors in this field.

With a view toward simplification of these rules, Klyashchitskii *et al.* (1969) have proposed a system of nomenclature for asymmetric inositol derivatives, based on stereospecific numbering similar to the system used for glycerol derivatives. We concur in their proposal but until their system is given serious consideration, we shall adhere to the system presently in use. We hope in this Review to explain in a systematic way the origin and meaning of the configurational notation used in designating lipid-bound and water-soluble derivatives of *myo*-inositol and to illustrate these concepts with further biochemical examples.

Conformation

Basic to stereochemistry is the solid geometry of the particular molecules under consideration, as illustrated by Fig. 2, which shows in perspective the conformation (three-dimensional structure) of *myo*-inositol. Most readers will recognize in this Figure the bonds linking carbon atoms, but many, in our experience, will not understand the spatial relationships among the carbons and the basis for placement of bonds projecting from the ring. As far as we are aware, no textbook or other published source presents a clear exposition of the origin of this construct. On the contrary, Fig. 2 is rather more often the starting point for illustration of stereochemical principles.

To explain these concepts we must refer to the more fundamental structure shown in Fig. 3, which is a diagram of a thermodynamically favorable arrangement of a ring of six tetrahedral carbon atoms, the basic structure of the cyclohexanehexols, commonly known as inositols. Barnett (1950) has presented an identical model

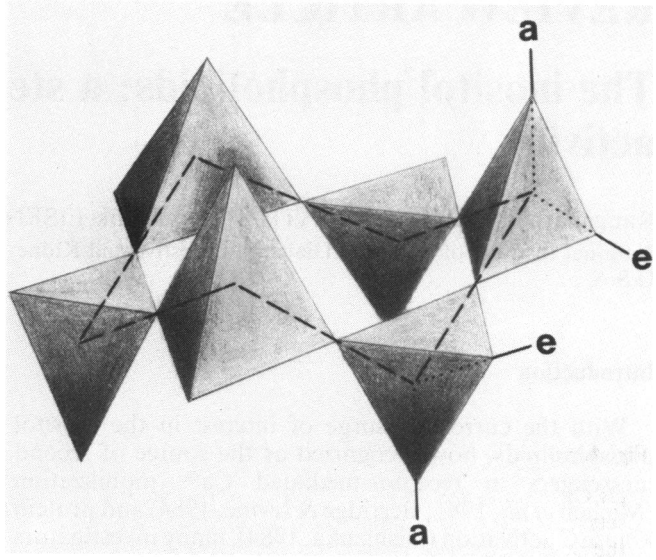

Fig. 3. Chair form of the six-carbon ring with axial (a) and equatorial (e) bonds

of cyclohexane but omitted any discussion of carbon–carbon bonds or carbon–substituent bonds.

These six tetrahedra reside on a common horizontal plane defined by the bases of alternating up and down members. Fig. 2 can be seen to emerge from this diagram as the broken line connecting the centres of each tetrahedron through the joined apexes; it is this line of valence bonds between adjacent carbon atoms that traces the familiar, but often vaguely described, puckered ring or stable chair form characteristic of the inositols. Valence bonds to the 12 substituent positions likewise emanate from the centre of each tetrahedron. These bonds are designated singly according to their absolute spatial relationship to the horizontal plane. A line from the centre of a tetrahedron to an apex vertically above or below the plane represents an axial bond (a); there are six of these, three pointing up from the plane and three pointing down. A line from the centre to the remaining apex represents an equatorial bond (e). There are likewise six equatorial bonds, three pointing up from the plane at about 20° and three pointing down at the same angle. Fig. 2 is thus a simplified three-dimensional version of the structure of *myo*-inositol based on the model of Fig. 3. For simplicity hydrogen atoms (equatorial at C-2, all others axial) are omitted from Fig. 2. *myo*-Inositol, the principal inositol in nature, is uniquely characterized among nine possible isomeric cyclohexanehexols by a single axial OH (C-2); all other OH groups of *myo*-inositol are equatorial (Posternak, 1965a; Anderson, 1971; Angyal, 1971).

[Fig. 3 can be converted into another thermodynamically favoured structure by rotating each tetrahedron about the line connecting it to its neighbours, so that those pointing up now point down and conversely. Axial and equatorial bonds are now interchanged. Because there is no rupture of carbon–carbon bonds in changing from one form to the other, this interconversion is referred to as a conformational change and the two forms are conformational isomers or conformers. But in the free cyclohexanehexols the most stable forms are those containing the least number of axial OH groups; the only conformer, then, of *myo*-inositol is depicted in Fig. 2.]

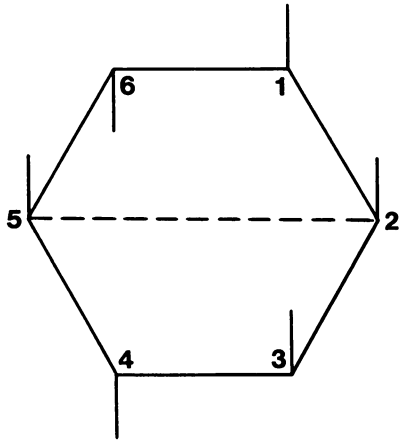

Fig. 4. Haworth diagram of *myo*-inositol

cis OH groups are at C-1, -2, -3, -5 and C-4, -6; the two sets are *trans* to each other. The broken line is the trace of the plane of symmetry, dividing the molecule into chiral halves.

Although providing the groundwork for an understanding of conformational principles, Fig. 2 is cumbersome and can be replaced for an explanation of stereochemistry by Fig. 4, the Haworth convention, in which the puckered ring of Fig. 2 is projected on to a plane, equivalent to the plane of Fig. 3. In Fig. 4 the axial and equatorial distinction is lost since all carbon–oxygen bonds are perpendicular to the ring. Substituents are now oriented in relation to each other and designated *cis* for those pointing in the same direction from the plane and *trans* for those in the opposite direction. Thus there are two sets of *cis* OH groups, those at C-1, -2, -3, -5 and at C-4, -6; the two sets in turn are *trans* to each other.

The lack of conformational information provided by Fig. 4 must not obscure the feature that sets *myo*-inositol apart from its isomers, the single axial OH at C-2. Is there an easy way to discern this structure from a diagram that makes no obvious distinction between axial and equatorial groups? We can formalize a relationship between the Haworth convention (Fig. 4) and the conformational diagram (Fig. 2) through a general rule which states that vicinal (adjacent) *cis* groups cannot be both axial or both equatorial, but must be one of each. In a series of three vicinal *cis* OH groups, as C-1, -2 and -3 of *myo*-inositol, then, according to the rule, there must be a single axial OH in the middle or one at each end. But since *myo*-inositol has only one axial OH, it is the middle one (C-2) that is axial. A corollary to the rule states that vicinal *trans* groups must be both axial or both equatorial. Obviously the remaining OH groups of *myo*-inositol must be equatorial.

Stereochemistry

Despite its limitations, the Haworth diagram is sufficiently informative for presentation of stereochemical principles. By convention the lower edge of the ring (Fig. 4) is assumed to project toward the viewer and the upper edge away from the viewer, a rule often ignored but one that must be applied consistently to avoid the ambiguity implicit in a two-dimensional figure. Although *myo*-inositol is a symmetrical molecule, and therefore not optically active, it is composed of chemically identical but geometrically non-identical halves. A plane passed perpendicularly through the plane of Fig. 4 on a line between C-2 and C-5 divides the molecule into segments which are non-superimposable mirror images, containing optically active or chiral (right- or left-handed) secondary alcohols at C-1, -3, -4 and -6. C-1 and C-3 are of equal and opposite chirality; C-4 and C-6 are likewise paired but different from C-1 and C-3. No other plane can divide *myo*-inositol symmetrically.

[In free *myo*-inositol the number 1 is assigned to the carbon nearest C-2 (axial) which on substitution gives a derivative of L configuration. Numbering proceeds clockwise on the axial OH side of the ring (IUPAC Commission on the Nomenclature of Organic Chemistry and IUPAC–IUB Commission on Biochemical Nomenclature, 1976). Carbons which on substitution with achiral (symmetrical) groups, e.g. phosphate, give optically active derivatives are assigned chirality (*R* or *S*) on the basis of sequence rules (Cahn *et al.*, 1966). Accordingly, C-1, C-3, C-4 and C-6 of *myo*-inositol are assigned the following absolute configurations: *R*, *S*, *R* and *S*, respectively, amending erroneous assignments made earlier (Cahn *et al.*, 1956).]

A molecule composed of compensating chiral halves is known as a *meso* molecule. *myo*-Inositol is such a *meso* species; it is neither D nor L and derivatives of *myo*-inositol in which OH is replaced at the plane of symmetry are likewise *meso* and neither D nor L. *myo*-Inositol 2-phosphate is a *meso* derivative of *myo*-inositol. The use of D and L symbols with such compounds, often seen in the current literature, is meaningless. By contrast, replacement of OH at any of the other four positions, C-1, -3, -4 or -6, will disturb the symmetry; the product must then be designated D or L according to which half of the molecule is involved. *myo*-Inositol 1-phosphate is a compound that can exist in enantiomeric (D and L) forms because it contains no plane of symmetry (Fig. 5). From this Figure it can be seen that C-2 and C-5 are now chiral while in *myo*-inositol (Fig. 4) they were achiral. Carbons that can be changed from achiral to chiral by a single substitution are called prochiral. C-2 and C-5 retain their numbering regardless of the configuration of the derivative, but other carbons are numbered according to configuration. How configuration is assigned to a particular enantiomeric form will be explained in what follows.

Configuration

Some 26 years ago Ballou & Pizer (1960) prepared both isomers of *myo*-inositol 1-phosphate and determined their absolute configuration. By absolute configuration is meant the relationship of component parts of a structure in three-dimensional space. Starting with the naturally occurring disaccharide, galactinol (1-galactosyl-*myo*-inositol), which had been shown independently to be of D configuration, they prepared an optically active *myo*-inositol 1-phosphate; since there was no possibility of optical inversion in this synthesis, the product was assigned D configuration. By hydrolysis of soybean phosphatidylinositol they prepared *myo*-inositol 1-phosphate of equal but opposite optical rotation and this isomer was assigned L configuration, confirmed by comparison with synthetic model compounds (Brown & Clark, 1963).

These configurational assignments were based on the convention in use for carbohydrates, in which the highest

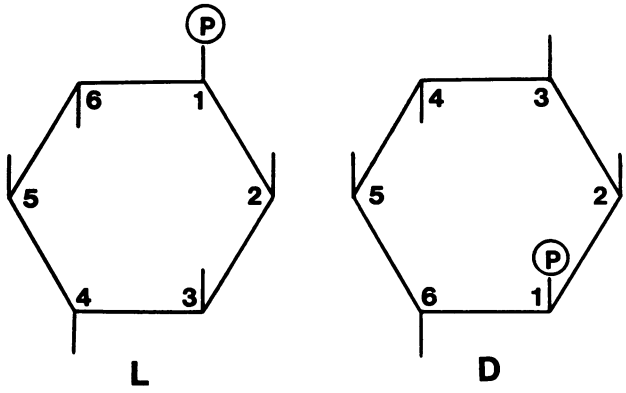

Fig. 5. Enantiomeric *myo*-inositol 1-phosphates

Abbreviation used: (P), phosphate.

numbered chiral carbon (C-6 in *myo*-inositol) specifies configuration. By viewing the C-6–C-1 edge of each form in Fig. 5 from outside the ring and placing C-6 at the top, the viewer will find that the OH at C-6 points right in the left-hand structure and left in the right-hand structure. Accordingly, the compounds were named D- and L-*myo*-inositol 1-phosphate, respectively. From the biogenetic standpoint this was the logical assignment, since the structure on the left was subsequently shown to originate biologically from D-glucose 6-phosphate (Eisenberg, 1967). At about this time, however, the IUPAC Commission on the Nomenclature of Organic Chemistry and the IUPAC–IUB Commission on Biochemical Nomenclature (1968) placed the inositol nomenclature on a more rational basis by changing the convention for cyclitols to the lowest numbered chiral carbon (C-1), resulting in a reversal of configuration. For the *myo*-inositol 1-phosphates this can be seen in Fig. 5 by the same viewing manoeuvre as above: the phosphate group faces left in the left-hand structure and right in the right-hand structure. The compounds were now named L- and D-*myo*-inositol 1-phosphate, respectively. From these studies with the enantiomeric *myo*-inositol 1-phosphates Ballou & Pizer (1960) inferred the structure of phosphatidylinositol, which since 1968 has been assigned D configuration.

A consequence of designating newly introduced substituents by the lowest number is that each enantiomer is numbered in the opposite direction, clockwise for L isomers and anticlockwise for D, *referred to the side of the ring bearing the axial OH*. The numbering of carbons 2 and 5, prochiral in *myo*-inositol, remains invariant in all derivatives of *myo*-inositol; C-2 identifies the position of the axial OH in these compounds.

[The configuration of chiral derivatives of *myo*-inositol is specified by the direction of numbering, which is found after assigning the newly substituted carbon the lowest possible number. Substitution at C-1 and C-4 (Fig. 4) produces L isomers, e.g. L-*myo*-inositol 4-phosphate, all numbered clockwise; substitution at C-3 and C-6 produces D isomers, e.g. D-*myo*-inositol 1-phosphate, phosphatidyl-1-D-*myo*-inositol (phosphatidylinositol), and D-*myo*-inositol 4-phosphate, all numbered anticlockwise. D-*myo*-Inositol 1-phosphate is the same as L-*myo*-inositol 3-phosphate (and D-3 as L-1) but convention dictates the use of the lower available number.]

Stereospecificity

The *myo*-inositol derivatives discussed thus far (except L-*myo*-inositol 4-phosphate) arise as products of enzymically catalysed reactions of synthesis or breakdown. Interesting stereochemical subtleties can be discerned in these reactions in the light of principles already set out. Although *myo*-inositol is symmetrical, as stated earlier it is a *meso* compound, containing within the molecule geometrically distinct halves. Just as D and L isomers (enantiomers) are selectively attacked by asymmetric agents (e.g. enzymes) the two chirally distinct but chemically identical edges of the *myo*-inositol ring are in effect intramolecular D and L isomers and can similarly be recognized selectively by enzymes or other chiral agents, resulting in the formation of one or the other of a pair of enantiomeric products. Paired sites within a molecule that give rise to enantiomeric products bear an enantiotopic relationship to each other. We illustrate these points in the following discussion.

In the phosphorylation of *myo*-inositol by ATP, catalysed by a plant kinase, the product is L-*myo*-inositol 1-phosphate (Fig. 5), clearly an attack at an enantiotopic site (Loewus *et al.*, 1982). This is the only system known for the direct phosphorylation of *myo*-inositol; in animal systems the enantiomeric *myo*-inositol 1-phosphates are formed by indirect routes, the L isomer from glucose 6-phosphate (Eisenberg, 1967) and the D isomer from the hydrolysis of phosphatidylinositol (Sherman *et al.*, 1981). In the glycosylation of *myo*-inositol there is similarly stereoselective attack; the enzymic transfer of galactose from UDP-galactose to C-1 of *myo*-inositol produces an optically active compound, L-1-*O*-galactosyl-*myo*-inositol, or galactinol (Frydman & Neufeld, 1963). In the transfer of galactose from lactose to C-6 of *myo*-inositol by β-galactosidase to give D-4-galactosyl-*myo*-inositol (Kuo & Wells, 1978), an isomer of galactinol, the site of attack is again geometrically determined in the substrate *myo*-inositol, since the paired C-4 is chemically equivalent.

In contrast with the stereospecificity displayed by each galactose transferase, the positional specificities of the two enzymes are derived from chemical differences between adjacent, non-paired, carbon atoms. The two galactosyl-*myo*-inositols are diastereomers, i.e. stereoisomers that are not mirror images. The two positions of substitution, C-1 and C-6, bear a diastereotopic relationship to each other.

The ability of chiral agents to distinguish enantiomers in general is in fact based on the formation of diastereomeric products. The complexes formed between an enzyme and enantiomeric substrates are diastereomers; they might either be of unequal stability or unequal capability to participate in further reaction to the final product. Similarly, the attack of an enzyme on a single substrate having enantiotopic halves (e.g. *myo*-inositol) involves intermediate complexes that are different chemically; only one of these diastereomers is capable of further reaction, the principle underlying enzymic selection from like groups.

Phytic acid, the completely phosphorylated ester of *myo*-inositol, undergoes hydrolysis to diastereomeric *myo*-inositol pentakisphosphates by phytases (Cosgrove, 1980) from wheat bran and *Pseudomonas*. Like *myo*-

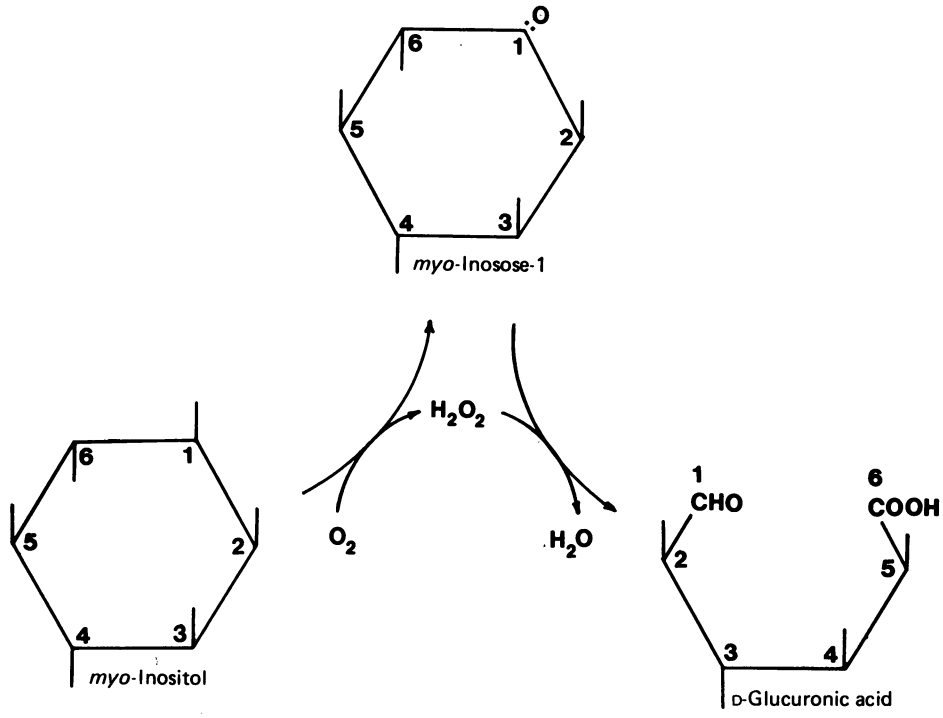

Fig. 6. Oxidation of *myo*-inositol to D-glucuronic acid catalysed by *myo*-inositol oxygenase

inositol this compound is symmetrical about C-2 and C-5 and is numbered clockwise from C-1. Both phytases produce optically active pentakisphosphates, showing stereoselective attack on the molecule, but further enzyme-specific selection is determined by chemical differences between adjacent diastereotopically related groups at C-6 and C-1. The *Pseudomonas* enzyme is a 1-phytase, giving D-*myo*-inositol 1,2,4,5,6-pentakisphosphate (anticlockwise to give the lowest numbers) and the wheat bran enzyme is a 6-phytase, which yields L-*myo*-inositol 1,2,3,4,5-pentakisphosphate (clockwise) (Tomlinson & Ballou, 1962). These products are diastereomers. In this example symmetry is disturbed by removal of phosphate.

Another illustration of stereoselective attack is the oxidation of *myo*-inositol by atmospheric oxygen catalysed by *myo*-inositol oxygenase, an important enzyme in plants (Loewus & Loewus, 1983) and mammalian kidney (Charalampous, 1959). The product of the reaction is D-glucuronic acid, with *myo*-inosose-1 (more systematically designated 2,3,5/4,6-pentahydroxycyclohexanone) and H_2O_2 likely intermediates (Fig. 6). The ring is cleaved between C-6 and C-1, one atom of oxygen appearing in the carboxyl group, the other in water (Moskala *et al.*, 1981; Naber, 1983).

Biosynthesis of L-*myo*-inositol 1-phosphate

It is interesting that in enzymic reactions of *myo*-inositol the reactive site is usually the C-6–C-1 edge. We can speculate that Nature's predilection for the *myo*-inositol geometry follows from the stereospecificity of the enzymic process catalysing the biosynthesis of *myo*-inositol *de novo*. In this reaction D-glucose 6-phosphate, central to all carbohydrate biosynthesis, is isomerized to L-*myo*-inositol 1-phosphate (Eisenberg, 1967), a closure of the C-6–C-1 bond (Figs. 1 and 7). This reaction, together with the isomerizations leading to fructose 6-phosphate and glucose 1-phosphate, account for virtually all carbohydrates in Nature. Found in all plants and animals, L-*myo*-inositol 1-phosphate synthase is most abundant in the mammalian testis, but in which cell type the activity is located is still a matter of controversy (Eisenberg, 1967; Morris & Collins, 1971; Robinson & Fritz, 1979; Loewus *et al.*, 1983). The enzyme has been purified to homogeneity from mammalian testis (Maeda & Eisenberg, 1980; Mauck *et al.*, 1980) and yeast (Donahue & Henry, 1981).

The reaction shown in Fig. 7 has been studied intensively for the past two decades in an attempt to elucidate the mechanism of this isomerization. Additional stereochemical considerations, applicable to this reaction, will be introduced as the discussion progresses. The requirement for NAD^+, an oxidoreductive coenzyme, in a reaction involving no net change in composition, suggests the participation of oxidized intermediates in the process, but the direct demonstration of such intermediates has been uniformly unsuccessful owing to their tight binding to the enzyme (Sherman *et al.*, 1969). Indirect experiments in which the trapping of intermediates as labelled chemically reduced products was attempted have succeeded in showing with partially purified enzyme (Chen & Eisenberg, 1975) and with homogeneous enzyme (Eisenberg & Maeda, 1985) the intermediate role of *myo*-inosose-2 1-phosphate, presumed to originate from the cyclization of 5-ketoglucose 6-phosphate, another putative intermediate which, however, could not be detected. Additional supporting evidence from chemical model studies (Kiely *et al.*, 1978) led to the inference that the rate of cyclization of 5-ketoglucose 6-phosphate was far greater than the rate of reduction by NADH of *myo*-inosose-2 1-phosphate to L-*myo*-inositol 1-phosphate, precluding the possibility of demonstrating the

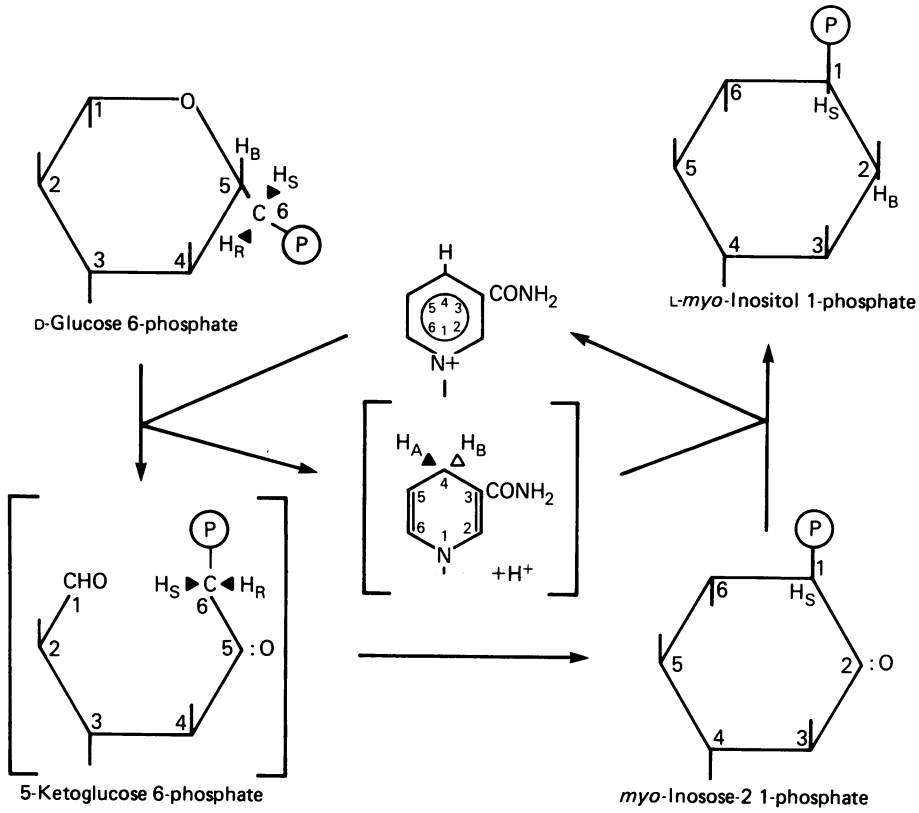

Fig. 7. Mechanism of isomerization of D-glucose 6-phosphate to L-*myo*-inositol 1-phosphate catalysed by L-*myo*-inositol 1-phosphate synthase

Undemonstrated intermediates are in brackets.

first intermediate. As illustrated in Fig. 7, the β anomer of glucose 6-phosphate is the reactive species in the isomerization (Wong & Sherman, 1985).

Besides the closure of the carbon–carbon bond, the transfer of hydrogen is the salient feature of the mechanism of Fig. 7. Oxidation at C-5 of glucose 6-phosphate by NAD^+ and reduction at C-2 of *myo*-inosose-2 1-phosphate by NADH imply the operation of a hydrogen shuttle between substrate and coenzyme; since C-4 of reduced nicotinamide carries a pair of hydrogen atoms, a stereoselective choice presents itself. H_B of NADH is apparently transferred while H_A remains uninvolved in the transfer (Byun & Jenness, 1981). The steric constraints of ring closure between C-6 and C-1 leading uniquely to the *myo* isomer of inositol 1-phosphate necessitate a similar choice between the paired hydrogen atoms at C-6 of 5-ketoglucose 6-phosphate; H_S is retained in the isomerization while H_R is lost to the medium (Loewus *et al.*, 1980). Since both reactants are chiral the paired hydrogen atoms in each are both geometrically and chemically distinct; they stand in diastereotopic relationship to each other and can be differentiated by any agent, chiral or achiral, or by any physical measurement. Chemical and physical evidence both attest to the differentiability of NADH hydrogens (Rob *et al.*, 1984; You, 1985). Not required for selection of the appropriate hydrogen atom, the enzyme confers absolute specificity based on chemical differences between paired atoms. In contrast, paired atoms at a prochiral centre (e.g. C-2 of ethanol, an achiral compound) are only differentiable on the basis of their geometry, requiring for their selective mobilization a chiral agent, usually an enzyme (Levy *et al.*, 1962). These hydrogen atoms stand in enantiotopic relationship to each other. In general, then, paired groups in achiral environments require chiral agents for differentiation; paired groups in chiral environments can be differentiated by any agent or physical measurement.

[C-6 of glucose 6-phosphate and C-4 of reduced nicotinamide of NADH have been classified as prochiral carbons because they are bound to paired hydrogen atoms and two unlike groups (Hanson, 1966). But where at least one of the unlike groups is chiral, as in the two examples cited, the term prochiral fails to meet the definition of prochirality set out earlier (see under 'Stereochemistry'); total molecular chirality precludes local prochirality (Mislow & Siegel, 1984). For this reason we have avoided the designation of these carbons as prochiral in the discussion of hydrogen transfer in the isomerization of glucose 6-phosphate to L-*myo*-inositol 1-phosphate. Individual hydrogen atoms are assigned configuration based on priority of groups bound to the particular carbon in question (Hanson, 1966). For C-6 of glucose 6-phosphate, O > C-5 > H,H; each hydrogen atom in turn is given the higher priority and assigned configuration depending on the direction of ordering of bound groups. The hydrogen which is part of the clockwise sequence O,C-5,H is called H_R; its partner in the anticlockwise sequence is H_S. For C-4 of NADH, C-3 > C-5 > H,H; the hydrogen of the clockwise sequence C-3, C-5, H is H_R and its anticlockwise partner H_S. But to avert the confusion in Fig. 7 certain to result

from using the same notation as for the C-6 hydrogens, the NADH hydrogens are instead designated H_A and H_B, respectively (Cornforth *et al.*, 1962).]

The carbonyl groups of 5-ketoglucose 6-phosphate and C-4 of the nicotinamide ring of NAD^+ are sites of further stereoselectivity affecting the nature of the cyclohexanehexol isomer produced. Atoms doubly bound to carbon such as carbonyl oxygen and aromatic carbon can be treated as a pair of like groups, only one of which participates in further reactions. In the ring closure at C-1 and in the reduction at C-5 of 5-ketoglucose 6-phosphate, the *re* faces are involved (Loewus *et al.*, 1980); in the transfer of hydride to C-4 of NAD^+ it is the *si* face that receives H_B (Byun & Jenness, 1981).

[Carbon atoms doubly bound either to oxygen or to other carbons are trigonal, in contrast with singly bound carbons which are tetrahedral. Trigonal carbon and its ligands occupy a single plane that can be attacked stereoselectively from either face; the stereochemical notions developed in this Review for tetrahedral carbon apply as well to trigonal carbon. Three situations will be considered. (1) For a carbonyl group linked to unlike symmetrical groups the two faces are related enantiotopically; achiral reduction, for example, gives an equal mixture of enantiomeric alcohols while chiral reduction selectively produces only one of the enantiomers, as in the enzymic reduction of dihydroxyacetone phosphate to L-α-glycerophosphate. (2) In those carbonyls linked to a chiral moiety, as both carbonyls of 5-ketoglucose 6-phosphate, the faces are related diastereotopically, differentiable by any reagent. (3) For a carbonyl group linked to compensating chiral moieties, as C-2 of *myo*-inosose-2, the faces are also related diastereotopically and therefore differentiable by any reagent. The reduction of *myo*-inosose-2, whether enzymic or chemical, leads to mixtures composed largely or even exclusively of *myo*-inositol compared with *scyllo*-inositol, depending on the method of reduction (Posternak, 1965*b*).

The faces are identified by the priority rule (Hanson, 1966); for 5-ketoglucose 6-phosphate the priority at C-5 is O > C-6 > C-4; at C-1, O > C-2 > H. In Fig. 7 the viewer sees that the priority at C-5 is anticlockwise; the face is therefore designated *si*. At C-1 the priority is clockwise, thus *re*; the faces remote from the viewer are *re* and *si*, respectively. At C-4 of NAD^+ the priority is C-3 > C-5 > H; the near face with clockwise order is *re* and the remote *si*.]

In this connection it is important to recognize the fallacy in assuming that in chemically synthesized doubly tritiated substrates, e.g. [6-$^3H_2$]glucose 6-phosphate, both atoms are equally labelled; on the contrary, owing to inherent chemical differences in the two faces of a precursor carbonyl group situated in a chiral environment, it is likely that reductive processes will lead to unequal labelling to the extent of 2:1 as shown by Lemieux & Howard (1963) in the reduction of a pentodialdose with $LiAl^2H_4$. In studying the mechanism of L-*myo*-inositol 1-phosphate synthase, Chen & Charalampous (1967) found in the medium one-half the ^3H incorporated into L-*myo*-inositol 1-phosphate from [6-$^3H_2$]glucose 6-phosphate and inferred from this apparent isotope effect the intermediate role of 5-ketoglucose 6-phosphate. Their inference was based on the assumption that the substrate C-6 hydrogens were at equal specific radioactivity, when in fact there could have been a 2-fold difference in specific activity.

Biosynthesis and breakdown of phosphatidylinositol

The notable exception to our earlier statement that the C-6–C-1 edge is the preferred site of enzymic attack on *myo*-inositol is the formation of phosphatidylinositol, in which the attack occurs on the C-3–C-4 edge (Fig. 8). The first demonstration of lipid-bound *myo*-inositol in mammals was made by Folch & Woolley (1942) who discovered *myo*-inositol in brain lipids. Phosphatidylinositol was later shown to be synthesized by a reversible stereospecific attack on C-3 of free *myo*-inositol by phosphatidyl-CMP (CDP-diglyceride) catalysed by a membrane-bound transferase (Figs. 1 and 8) (Agranoff *et al.*, 1958; Paulus & Kennedy, 1960). As shown by Ballou & Pizer (1960) in the proof of structure of phosphatidylinositol, the compound is a derivative of D-*myo*-inositol 1-phosphate; following the rule of lowest number priority the numbering in the derivative changes to anticlockwise. The systematic name is phosphatidyl-1-D-*myo*-inositol. Contrary to published rules of nomenclature (IUPAC–IUB Commission on Biochemical Nomenclature, 1977/1978) no phosphoinositides of the L series are known.

[These rules have been clarified by Agranoff (1978), who in an effort to assure that *myo*-inositol derivatives are numbered consistently on the axial OH side of the ring, has metamorphosed Fig. 2 into a mnemonic turtle, the head representing the axial OH at C-2 and the tail at C-5. These rules might have been more quickly adopted had they been illustrated with a symbol of speediness rather than slowness. Correct names and structures of the phosphoinositides have appeared in a recent newsletter (Nomenclature Committee of IUB and IUPAC–IUB Joint Commission on Biochemical Nomenclature, 1985).]

Phosphatidylinositol has long been of biological interest since it was found, with phosphatidic acid but not

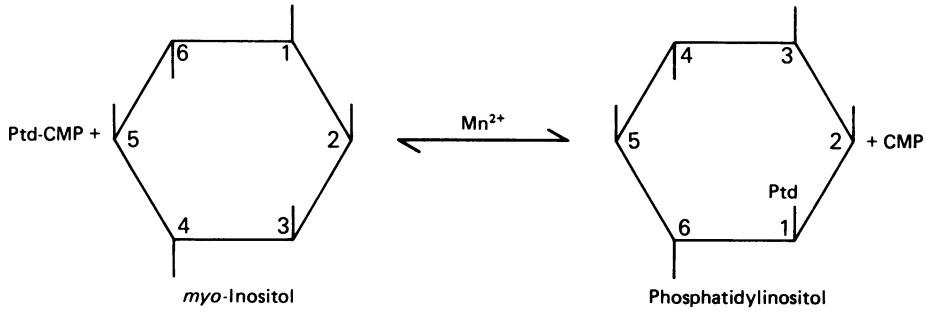

Fig. 8. Biosynthesis of phosphatidylinositol catalysed by phosphatidyl-CMP:*myo*-inositol phosphatidyltransferase

the other phospholipids, to undergo greatly increased turnover in membranes of many tissues stimulated by a variety of agonists: α-adrenergic and muscarinic cholinergic agents, among others (Hokin, 1985). Although the chemistry of the synthetic segment (Fig. 8) was clarified early, the process of breakdown has not been understood until recently (Fig. 1). Notwithstanding the presence in various systems of enzymes catalysing hydrolysis of phosphatidylinositol at either phosphoryl diester linkage, it was impossible to discern which of these was of primary importance in a particular system until the introduction of Li^+ as an inhibitor of *myo*-inositol 1-phosphatase. As an example of the clarification resulting from the use of Li^+ it is appropriate to cite the work of Hokin-Neaverson & Sadeghian (1984). In mouse exocrine pancreas stimulated with cholecystokinin free *myo*-inositol formation from phosphatidylinositol exceeded that of *myo*-inositol 1-phosphate while under the identical conditions, except for the addition of Li^+, the reverse was found. Experiments of this kind performed in many laboratories have led to the conclusion that phosphatidylinositol is broken down to diacylglycerol and *myo*-inositol in sequential reactions catalysed by phospholipase C and *myo*-inositol 1-phosphatase.

Inhibition of *myo*-inositol 1-phosphatase by Li^+

The application of Li^+ inhibition to this problem was the culmination of several stages of development beginning with the observation by Allison & Stewart (1971) that free *myo*-inositol levels decreased markedly in brains of Li^+-treated rats, followed by the demonstration by Naccarato *et al.* (1974), that mammary gland *myo*-inositol 1-phosphatase was specifically inhibited by Li^+ *in vitro*. Allison (1978) then showed that brain *myo*-inositol 1-phosphate increased, with the decrease in *myo*-inositol, in Li^+-treated rats. Hallcher & Sherman (1980) confirmed the inhibition of *myo*-inositol 1-phosphatase by Li^+ in bovine brain *in vitro*. Finally the observation of Sherman *et al.* (1981) that the D enantiomer of *myo*-inositol 1-phosphate accumulated in these brains, and not the L enantiomer, clearly implicated phospholipase C in the breakdown of phosphatidylinositol, a dramatic illustration of the importance of stereochemistry in biological mechanisms. A linkage between the *myo*-inositol pathway and the pharmacological action of Li^+ in treatment of brain disorders is now being sought and Li^+ as a tool of the cell biologist in studies of the phosphoinositides in cellular processes is now firmly entrenched (Berridge *et al.*, 1982). An explanation for the inhibitory action of Li^+ must await purification of the enzyme.

[The separation of the enantiomeric *myo*-inositol 1-phosphates on a chromatographic scale was achieved by g.l.c. (Sherman *et al.*, 1981) on a glass capillary column coated with a chiral silicone/valine copolymer, originally developed for the separation of enantiomeric amino acids. As discussed earlier, enantiomers react with chiral agents through the formation of diastereomeric intermediates or complexes, which in this instance resulted in separation by differential rates of migration through the column. As also mentioned earlier, the chiral agent need not be an enzyme. This differentiation of enantiomers by non-enzymic chiral agents was extended to paired enantiotopic groups in the same molecule in the classical studies by Schwartz & Carter (1954).]

In response to a report (Alexander *et al.*, 1980) that

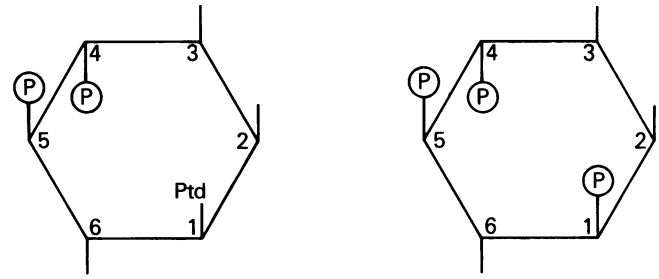

Fig. 9. Structures of *myo*-inositol derivatives important in receptor-mediated Ca^{2+} mobilization

$^6Li^+$ is more toxic than $^7Li^+$ in mice, we examined the lithium isotopes for differential inhibition of *myo*-inositol 1-phosphatase *in vitro* and found no difference (Parthasarathy & Eisenberg, 1984), a result confirmed by Sherman *et al.* (1984). Contrary to Alexander *et al.* (1980) we also found no differential lethality of the lithium isotopes in mice; LD_{50} for ^6LiCl, ^7LiCl, and natural LiCl (93% $^7Li^+$, 7% $^6Li^+$) was 17.7 mequiv./kg. Their recommendation to replace inexpensive natural lithium salts with costly ^7Li for therapeutic use is without merit.

Non-stereospecificity of *myo*-inositol 1-phosphatase

The central theme of this Review has been the stereoselectivity of enzymic reactions with enantiotopic groups within the same molecule. The same considerations apply to enantiotopic groups in different molecules, i.e. enantiomers; chiral agents attack them at different rates and with enzymes the selection is usually absolute. An exception to this rule is the non-stereospecificity of the hydrolysis of enantiomeric *myo*-inositol 1-phosphates by specific *myo*-inositol 1-phosphatase (Eisenberg, 1967; Hallcher & Sherman, 1980; Eisenberg & Parthasarathy, 1984); both the D and L isomers are nearly equally active substrates for the enzyme. Because of the dissimilar geometry of these substrates it is clear that the active centre of the enzyme must be uniquely designed to recognize a subtle, but unknown, structural feature common to both isomers or to produce a common transition form during the course of the reaction. Further work is needed to explain this unusual lack of optical selectivity.

Second messengers

The stimulated turnover of phosphatidylinositol is the gross manifestation of a subtler process involving two derivatives of phosphatidylinositol: phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate (Fig. 1). Although Ca^{2+} mobilization had been observed for a long time as a concomitant of stimulated phosphatidylinositol turnover in many systems (Michell *et al.*, 1981), it is only recently that the relationship between the two phenomena has been established. With the wealth of experimental evidence provided in the past 3 years there seems to be no doubt that receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate mobilizes Ca^{2+} from the endoplasmic reticulum to the cytosol and stimulates protein kinase C, the result of liberation of *myo*-inositol trisphosphate (Streb *et al.*, 1983; Burgess *et al.*, 1984; Joseph *et al.*, 1984) and diacylglycerol (Nishizuka, 1984), respectively. Both of these compounds are generated rapidly in low concentra-

tion and are quickly removed, properties required of molecules that signal cells to carry out designated functions. The active species of *myo*-inositol trisphosphate is known systematically as D-*myo*-inositol 1,4,5-trisphosphate, reflecting the configuration of the parent phosphoinositide, phosphatidyl 1-D-*myo*-inositol 4,5-bisphosphate (Fig. 9). A recent n.m.r. spectroscopic study by Lindon *et al.* (1986) confirms the *myo* structure of the trisphosphate and the presence only of equatorial phosphates, none of which occupy positions 2 and 3 (Fig. 9). The chemical events linking the appearance of *myo*-inositol trisphosphate to the release of Ca^{2+} remain obscure.

Irvine *et al.* (1984*a*) have examined the specificity of *myo*-inositol trisphosphate-stimulated Ca^{2+} release and found that alterations in the location of the phosphate groups from the 1,4,5 positions (Fig. 9) had a markedly deleterious effect. *myo*-Inositol 2,4,5-trisphosphate was only one-fifth as active as the 1,4,5 isomer. The 2,4,5 isomer was isolated from brain along with the 1,4,5 isomer and is probably an artifact of the isolation procedure, since a phosphoryl group in diester linkage is subject to transphosphorylation from the 1 position of *myo*-inositol to the 2 position during alkaline hydrolysis of the phosphoinositide, as discussed by Posternak (1965*c*). Additionally there is no evidence in animal systems for *myo*-inositol 2-phosphate, the penultimate hydrolytic product of *myo*-inositol 2,4,5-trisphosphate. They also tested the bisphosphates and found 1/60 the activity with the 4,5 isomer and none with the 1,4 isomer. From these findings they concluded that 4,5 *trans*-vicinal substitution and transannular substitution preferably at C-1 are essential to Ca^{2+}-mobilizing activity. All bis- and trisphosphates mentioned are of D configuration.

Adding to the complexity of the second messenger role of the *myo*-inositol polyphosphates already described is the discovery of additional water-soluble phosphates in rat organs subjected to receptor mediated muscarinic stimulation, with possible implication in Ca^{2+} mobilization. In parotid gland fragments Irvine *et al.* (1984*b*) have tentatively identified *myo*-inositol 1,3,4,-trisphosphate, of unknown configuration. In cerebral cortical slices Batty *et al.* (1985) have preliminarily identified *myo*-inositol 1,3,4,5-tetrakisphosphate, probably of D configuration. The early appearance of this compound after stimulation suggests a role both as second messenger and precursor of the trisphosphates. Both groups of investigators recommend caution in interpretation of their structural studies, which are based solely on chromatographic comparisons. The nature of the lipids from which these esters might be derived is unknown. An easy way to confirm the characterizations is reduction of the aldehydic intermediates produced in their degradation procedures with NaB^3H_4. This manoeuvre will provide sufficient label for purification of carrier-diluted putative altritol and xylitol to constant specific radioactivity and at the same time enable the determination of the configuration of altritol representing *myo*-inositol 1,3,4-trisphosphate but not xylitol (meso) representing *myo*-inositol 3,4,5-trisphosphate.

myo-Inositol polyphosphatases

An essential requirement of second messengers is that they be rapidly destroyed after performing their signal function in order to restore the system to the basal state in preparation for a new stimulus. In the liver, in which the most recent work has been done (Storey *et al.*, 1984; Seyfred *et al.*, 1984; Joseph & Williams, 1985) this function is served by specific monoesterases that sequentially convert *myo*-inositol 1,4,5-trisphosphate to the 1,4-bisphosphate and ultimately to *myo*-inositol via the 4-phosphate (Storey *et al.*, 1984; Sherman *et al.*, 1985) or the 1-phosphate (Fig. 1). There is no information on configurational specificity of these enzymes except with respect to *myo*-inositol 1-phosphate, as discussed earlier. In the liver the trisphosphatase appears to be confined to the plasma membrane and the other phosphatases to the cytosol, a result that makes it difficult to visualize the movement of *myo*-inositol trisphosphate from the plasma membrane where it is generated to the endoplasmic reticulum where it stimulates Ca^{2+} release, if it is destroyed in the plasma membrane. Seyfred *et al.* (1984) suggest that the activity may also be in the cytosol but is kept inhibited by polyamines during Ca^{2+} release. Connolly *et al.* (1985) report a soluble trisphosphatase in platelets which is inhibited by Ca^{2+}, but do not suggest how Ca^{2+} inhibition of the enzyme is compatible with restoration of the system to the basal state. Further studies will undoubtedly resolve these seeming contradictions.

Conclusion

We have attempted to present in this Review what we hope is a simplified, straightforward, and adequately illustrated explanation of configuration and conformation and their part in the stereochemistry of *myo*-inositol, its phosphoric esters, and the inositol phospholipids. We have applied these principles to biosynthetic and catabolic processes. The correct nomenclature should follow from our development of these subjects. Our further hope is that workers in this field, both veteran and newly attracted, pause to digest this information in an effort to rid this important area of biochemistry of the confusion and error that, with time, have gathered around it.

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