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Myoinositol Phosphates as 'implicated' in Metabolic and Signal Transduction Pathways in Plants[Ψ]

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An account of the establishment of a novel metabolic cycle involving myoinositol phosphates and glucose-6-P in plants elucidating the probable pathway of synthesis and degradation of myoinositol hexakis phosphate has been documented. Glucose-6-P is used during seed formation by myoinositol(1)P synthetase for the production of myoinositol(1)P which is subsequently phosphorylated to myoinositol pentakis-phosphate, i.e. Ins(1,3,4,5,6)P_5 by phosphoinositol kinase and untimately to $InsP_6$ by another enzyme, i.e. $InsP_6$-ADP-phosphotransferase. $InsP_6$ is stored in the seeds as phosphate reservoir. During germination $InsP_6$ is hydrolysed by an enzyme phytase ultimately to myoinositol which is required for cell wall biosynthesis. As in the early phase of germination Ins(1,3,4,5,6)P_5 is also formed by the reversible reaction of $InsP_6$-ADP-phosphotransferase and hydrolysed by phytase to Ins(1)P prior to finally giving rise to myoinositol. This in turn can be converted to ribulose-5-P by myoinositol(1) P-dehydrogenase giving the feed-back to the production of glucose-6-P during early phase of germination through pentose shunt pathway. During the operation of this cycle ATP and NADH are generated, providing necessary energy at the early germination period.

Corollary to this metabolic cycle an intermediary phytase product, i.e. Ins(2,4,5)P_3 has been implicated to a pathway leading to Ca^{2+} mobilisation in plant cells. This is demonstrated when $InsP_6$-phytase complex was added after a definite time of hydrolysis which coincides with the time of optimal production of Ins(2,4,5)P_3 bound to phytase. The in vitro constituted Ins(1,4,5)P_3- or Ins(2,4,5)P_3-phytase complex has also been found effective in releasing Ca^{2+} from cellular stores, the release being 45% more as compared to that by free $InsP_3$ under identical conditions. Thus the alternative pathway of specific $InsP_3$ generation and its involvement in Ca^{2+} mobilisation has been proposed and elucidated.

In plants, a significant amount of inositol phosphates in the form of myoinositol hexakisphosphate ($InsP_6$ or phytin) accumulates as the reserve substance particularly in seeds and other tissues[1,2]. Phytin is a complex salt containing K^+, Mg^{2+}, Ca^{2+} and $InsP_6$ which is embedded in the protein matrix along with other minor cations[3]. Unequivocal identification of myoinositol pentakisphosphate and $InsP_6$ by nmr analysis in a variety of normal mammalian organs, tumors and cells lines as well as amoebae provides evidence that myoinositol polyphosphate is common cellular constituent not only of plants but also of animals and other unicellular species[4,5]. Invariably all the eukaryotic cell membrane contain inositol phospholipids or phosphoinositides from which myoinositol-trisphosphate is liberated by the action of an enzyme phospholipase C[6]. Therefore, it appears that myoinositol phosphates occur in different forms and obviously taking part in the diverse cellular functions[7,8] which are now being elucidated very rapidly after the discovery of myoinositol trisphosphate, i.e. Ins(1,4,5)P_3 as second mesenger in signal transduction pathway[9]. When we started work on myoinositol phosphates in early 1960s in Bose Institute, very little information was known. We feel that the present occasion will be befitting where an overall con-

[Ψ]Dedicated to Prof. D. P. Chakraborty with whom we have a pleasant association since 1950s working in the Bose Institute.

tribution from our group on myoinositol phosphates can be projected with the hope that it might create interests subsequently amongst the chemists in this country.

Establishment of a novel metabolic cycle in plants :

Around 1962-63, while studying the nucleic acid metabolism in the germinating mung bean seeds, a phosphate compound was found to be labelled with ^{32}P very rapidly. It was found to be identical in all respects with myoinositol hexakisphosphate (Fig. 1). That was very surprising

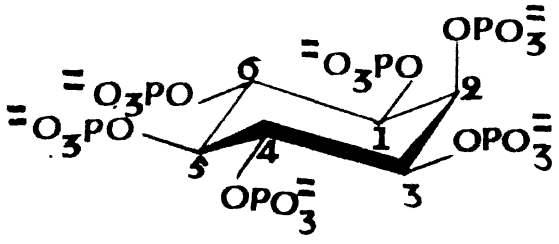

Fig. 1 Structure of myoinositol hexakisphosphate in the chair-form showing C2 position axial and others in equitorial positions

because information was available at the time that synthesis of $InsP_6$ occurs during the formation of seeds. Actually that provoked us to study this in a more systematic way. From the viewpoint of seed physiology, research in biosynthesis and breakdown of myoinositol hexakisphosphate has lagged far behind that of other major seed reserves such as starch and protein. Seed crops constitute a major renewable resource of our civilization. To fully understand the metabolic processes that control the major phosphate reserve in seeds is a major advance. In fact, a novel metabolic cycle emerged out of this work done by a number of our coworkers with their sincerity and devotion. The interesting part of the work is to synthesise labelled substrates either chemically or biochemically in our laboratory. This gives us an advantage to elucidate the metabolic pathway which was hitherto unknown.

An interesting phenomenon that occurs during seed germination is the dephosphorylation of $InsP_6$, which implies its importance in the regulation of phosphorus metabolism during seedling growth[10,11]. These observations led us to determine the different enzymatic activities associated with the metabolism of myoinositol phosphates during formation and germination of seeds. At the inception of this work in our laboratory, the endeavour was first directed toward the elucidation of enzymatic processes associated with the synthesis of $InsP_6$ during the formation of seeds Although the synthesis of $InsP_6$ during seed formation had been reported from several laboratories, the information regarding the enzymatic steps and the number of enzymes involved therein was very scanty. The involvement of an enzyme, phosphoinositol kinase, in the synthesis of $InsP_6$ during formation of seeds, has been reported from this laboratory[12-14]. This enzyme can phosphorylate 1P to higher homologues. The phosphoinositol kinase (P1-kinase), the enzyme responsible for the formation of higher inositol phosphate from lower one is found to be operative during the maturation as well as in the germination phase of mung been seeds. This enzyme, on purification, has been found to be associated with two different forms, namely, P1-kinase A and P1-kinase B. Both the forms of enzyme can phosphorytlate 1P to myoinositol pentakisphosphate $InsP_5$ (2-OH) instead of myoinositol hexakisphosphate which is the predominant phosphate ester found in seeds[15].

A second enzyme reported from this laboratory[16], namely, phytate ADP-phosphotransferase. has been found to be responsible for the phosphorylation of the hydroxyl group at position C-2 of $InsP_5$ (2-OH). Thus, the involvement of these two enzymes, phosphoinositol kinase and $InsP_6$-ADP-phosphotransferase, has been indicated for the complete phosphorylation of a specific 1P that is, Ins(1)P to the highest homologue, $InsP_6$. Since 1P was known to be the substrate of phosphoinositol kinase, which could not phosphorylate myoinositol, the route of the synthesis of 1P was then investigated Two enzymes were inplicated in this process.

namely, myoinositol kinase and Ins(1)P synthase; myoinositol kinase, which phosphorylates myoinositol to Ins(1)P, has been reported in germinating mung bean seeds. However, during maturation of seeds, this enzyme was found to be absent. So the existence of the second enzyme, Ins(1)P synthase, during maturation was looked for conversion of G-6-P to Ins(1)P by this enzyme during maturation of seed has been detected in this laboratory[12,14]. The complete biosynthetic pathway of $InsP_6$ as elucidated in this laboratory, involves the utilisation of G-6-P, the key compound of the glycolytic pathway for the synthesis of Ins(1)P, which is subsequently phosphorylated to synthesise $InsP_6$.

Mostly, germination of seeds is associated with enzymatic dephosphorylation of phytate by phytase. Although this enzymatic dephosphorylation had been reported from several laboratories, the mode of dephosphorylation during germination was an unsolved problem. The mode of dephosphorylation of phytate by phytase isolated from germinated mung bean cotyledons has also been reported from this laboratory[10,17,18]. The ultimate dephosphorylation product has been identified as myoinositol-2-phosphate. It has been established that phytase appears *de novo* during seed germination and that the detectable enzymatic activity appears after 4–6 h of imbibition. Other enzymes responsible for the utilisation of phytate phosphorus during germination reported from this laboratory[16,19,20], namely, $InsP_6$-GDP-phosphotransferase and $InsP_6$-ADP-phosphotransferase are very likely to be useful for the economical utilisation of phosphate from the phytate molecule. It has been established that these two enzymes transfer the phosphate molecule from position C-2 of the $InsP_6$ to a suitable acceptor such as GDP or ADP, synthesising GTP or ATP respectively. The dephosphorylation by these enzymes has been found to be a very early phenomenon during seed germination. These enzymes were detected even in ungerminated seeds. It is evident from the earlier experiments that dephosphorylation of $InsP_6$ by $InsP_6$-ADP-phosphotransferase or $InsP_6$-GDP-phosphotransferase starts immediately after imbibition of seeds, leading to the synthesis of $InsP_5$ (2-OH) and ATP or GTP. As the germination progresses, phytase activity appears in the seed, and the $InsP_5$ (2-OH) is further dephosphorylated, leading to the synthesis of Ins(1)P as the penultimate and myoinositol as the ultimate product. With these data available, a pathway involving G-6-P and myoinositol phosphates during formation and germination of seeds was proposed from this laboratory[16]. Though the conversion of Ins(1)P to G-6-P was speculated in the proposed cycle, no enzyme or enzyme system responsible for this conversion was known at that time. This was indeed achieved later when a specific Ins(1)P dehydrogenase was discovered in this laboratory[16,21-23]. The probable mechanism of combined dehydrogenation and decarboxylation of Ins(1)P leading to the formation of ribulose-5-phosphate is outlined in Fig. 2. The stoichiometry of this reaction suggests that 2 moles of NAD^+ are reduced for each mole of ribulose-5-phosphate produced[22,24]. However, the fact that NAD-dependent dehydrogenation is an initial requirement for decarboxylation has

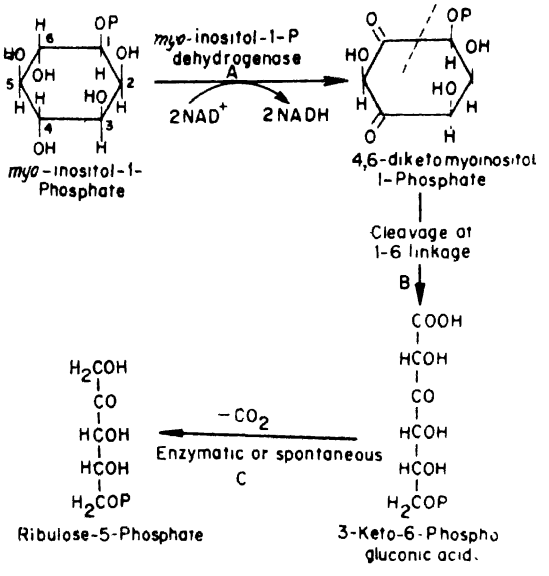

Fig. 2. Suggested pathways for conversion of myoinositol-1-phosphate to ribulose-5-phosphate. The sequences in the pathway are represented by A, B and C. The myoinositol molecule is cleaved between carbon atoms 1 and 6 by the enzyme, myoinositol-1-phosphate dehydrogenase.

been documented by the observation that omission of NAD from the reaction mixture results in a striking inhibition of decarboxylation. The presence of the enzyme system provides a link between the metabolic pathway of myoinositol phosphates and the pentose phosphate pathway during germination of seeds.

The significance of this pathway lies in the fact that it supplies the energy as ATP, reducing power as NADH, and pentose phosphate for nucleotide biosynthesis. It appears, then, that $InsP_6$ degradation during the early period of inhibition does play a significant role in seed germination and seedling vigour, which has been noted in the case of mung bean. An outline of the proposed metabolic cycle is given in Fig. 3. Information is now available as to how $InsP_6$ is synthesised from Ins(1)P during the formation of seeds as well as on the synthesis of IP_5

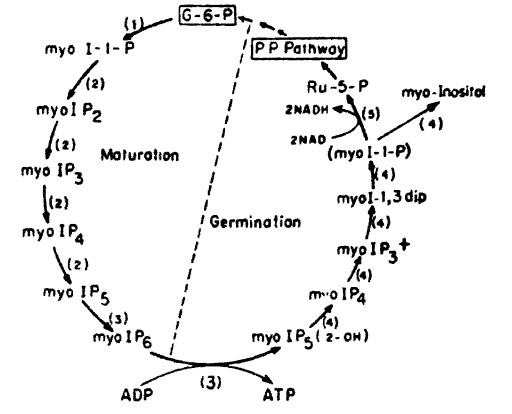

Fig. 3. Proposed metabolic cycle involving myoinositol phosphates during formation and germination of seeds; myo-IP_2, myo-IP_3, myo-IP_4, myo-IP_5 and myo-IP_6 or IP_6 correspond to bis-, tris-, tetrakis-, pentakis- and hexakis-phosphates of myoinositol respectively.

in the avian erythrocyte[25]. These reactions are obviously mediated through a number of enzymes, as stated earlier. However, many of the features of these reactions remain to be elucidated further.

Finally, there has recently been a rapid progress in understanding of receptors that generate intracellularly signals from inostitol lipids. One of these lipids phosphatidyl-inositol 4,5-bisphosphate, is hydrolysed to diacylglycerol and inositol trisphosphate as part of a signal transduction mechanism for controlling a variety of cellular processes including secretion, metabolism, phototransduction and cell proliferation. Diacyl-glycerol operates within the plane of the membrane to activate protein kinase C, whereas inositol trisphosphate is released into the cytosol to function as second messenger for mobilising intracellular calcium which can modulate a number of enzymic function including protein kinase activity. The question arises as to whether $InsP_3$ is generated by the phytase action from $InsP_6$ bypassing the phosphoinositide pathway in plant. The preliminary indication is that *in vitro* product of phytase from $InsP_6$ can influence Ca^{2+} mobilisation. If this is so, what is needed to demostrate, while in association with phytase, $InsP_3$ can elicit the Ca^{2+} release from the cell or intracellular stores.

Elucidation of calcium release from microsomal fraction by myoinositol trisphosphate-phytase complex in plant :

Research on $InsP_6$ in mung bean has resulted in elucidation of a novel metabolic cycle and indicated that it can form a basis to manipulate phytase in order to generate alternatively $InsP_3$ from $InsP_6$. It has been established that one of the intermediary products of phytase with $InsP_6$ is $Ins(2,4,5)P_3$[26] instead of $Ins(1,4,5)P_3$ which is implicated as second messenger in the network of Ca^{2+} mobilisation in signal transduction pathway[9]. $Ins(2,4,5)P_3$ can also act as an elicitor in intracellular Ca^{2+} mobilisation in mung bean[27]. The problem was how $Ins(2,4,5)P_3$ still associated with phytase can act as seocnd messenger. In fact, mobilisation of Ca^{2+} from microsomal/vacuolar fractions was detected when $InsP_6$-phytase was added after a definite time of hydrolysis which coincides with the time (20–30 min) of optimal production of $Ins(2,4,5)P_3$ bound to phytase. The in vitro constituted $Ins(1,4,5)P_3$- or $Ins(2,4,5)P_3$-phytase complex was

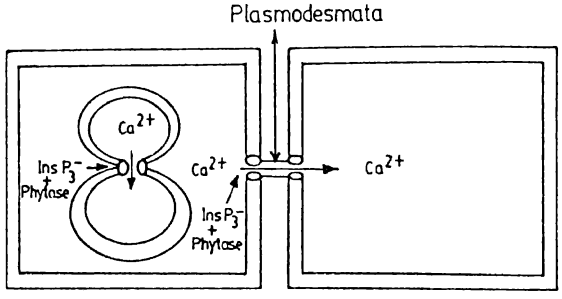

Fig. 4. A model showing intercellular or intermicrosomal calcium release after interaction of $InsP_3$ phytase complex with the receptor. Due to hydrolytic action of phytase on $InsP_6$ putative complex is formed. This complex, i.e. InsP3-phytase elicits calcium release from microsomes/vacuoles or from one cell to other through plasmodesmata to maintain the calcium pool within the cell.

also effective in releasing Ca^{2+} [27]. $InsP_3$-phytase complex releases 45% more microsomal Ca^{2+} than that released by free $InsP_3$ under identical conditions; other inositol-phosphate-phytase complexes were ineffective. Furthermore, $InsP_3$-phytase complex is recognised by the putative receptor associated with microsomal fraction[28] suggesting that the $InsP_3$-phytase complex can act as an elicitor in intracellular or intercellular Ca^{2+} mobilisation in plant systems where phytase and $InsP_6$ occur (depicted in Fig. 4) and can take part in the overall network of signal transduction process in plants[29].

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