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INOSITOL

Synthesis, Functions and Clinical Implications

BIOCHEMISTRY RESEARCH TRENDS

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Preface

In this book, the authors present topical research in the study of the synthesis, functions and clinical implications of inositol. Topics discussed include hypoxia and angiogenesis related myo-inositol phosphates; inositol derivatives in mycobacterium tuberculosis; inositols and epilepsy; the contribution of inositol trisphosphate signaling to cardiac pathologies; the role of inositol 1,4,5-triphosphate receptor in hepatorenal syndrome; the pathophysiologic significance of inositol phosphates (IP) in Alzheimer's and bipolar disorder; design and synthesis of biotinylated inositol phosphates; the health benefits of inositol and inositol hexaphosphate; and the cellular functions of inositol.

Chapter 1 - One of the most fundamental physiological processes in the aerobic organisms is the delivery of oxygen to all tissues. Deprivation of oxygen leads to hypoxia which is a pathological condition playing a central role in numerous types of diseases, such as cancer (solid tumors), cardiovascular diseases and anemia. In this chapter the authors review the synthesis of a number of *myo*-inositol phosphates and their biological evaluation regarding hypoxia and angiogenesis in an independent or a combined relationship. Some of the compounds were found to be involved in the increased oxygen delivery by free hemoglobin or whole red blood cells, in the suppression of hypoxia-induced factor 1α (HIF-1α), in the inhibition of the phosphatidylinositol 3-kinase (PI3K) pathway, and in the reduction of angiogenesis. Consequently, the eradication or growth reduction of solid tumors, the prevention of sever anemia caused by sickle cell disease, and the enhanced exercise capacity of subjects with severe heart failure have been demonstrated. These results have evinced a leading role for *myo*-inositol phosphates in the treatment of diseases related to hypoxia and angiogenesis.

Chapter 2 - Inositol (Ins) is a polyol used for the preparation of Ins derivatives that carry out essential functions in eukaryotes. Although rarely found in bacterial species there are Ins derivatives that play critical roles in actinomycetes, a group of Gram-positive bacteria that includes *Mycobacterium* species such as *M. tuberculosis*, the causative agent of tuberculosis (TB). Consequently, there is much interest in understanding the functions and biosynthesis of Ins derivatives in mycobacteria to gain insights that can be used for the development of therapeutic agents. Mycobacteria can either obtain Ins from the environment through Ins transporters or synthesize it *de novo* via a two-step enzymatic pathway. Cellular Ins is converted enzymatically into phosphatidylinositol (PI), a precursor for more complex glycolipids, or incorporated as L-*myo*-inositol 1-phosphate (Ins-1P) into the small molecule mycothiol (MSH). These Ins derivatives carry out critical functions required for mycobacterial viability and virulence. The mycobacterial cell envelop contains an abundance of mannosyl-phosphatidylinositol containing glycolipids and lipoglycans, including phosphatidylinositol mannosides (PIMs) lipoarabinomannan, (LAM), and lipomannan (LM). Importantly, PIMs, LAM, and LM have been shown to function as immunomodulatory molecules, promote the entry of mycobacteria into phagocytic cells, and regulate phagosome maturation. Mycobacteria also produce the Ins derivative MSH, which is used by mycobacteria as the primary reducing agent and in the detoxification of xenobiotics. Due to these important functions, mycobacterial Ins derivatives are of therapeutic interest for vaccine development and as sources of potential targets for drug development. This chapter reviews mycobacterial uptake of Ins and biosynthesis of Ins derivatives, known functions of Ins derivatives in *M. tuberculosis*, and the progress to date on targeting Ins derivatives for vaccine and drug development.

Chapter 3 - Epilepsy is a heterogeneous group of disorders. It is the most common neurological disorder after stroke. Despite achieved progress in the treatment of epilepsy, about one third of the patients with epilepsy are resistant to existing pharmacotherapies. The current treatment of epilepsy focuses exclusively on preventing or suppressing seizures, which are the symptoms of the underlying disease.

The most important challenge is to prevent epileptogenesis, the process by which brain becomes epileptic. Drugs that prevent the development of epilepsy are not yet available.

The central nervous system is characterized with relatively high concentrations of myo-inositol (MI) as well as the means to synthesize it. MI serves not only as a precursor molecule for inositol lipid synthesis, but also as

a physiologically important osmolyte. Alterations in MI deposition may playrole in a number ofneuropathological conditions, either as a physiologically important osmolyte or as a precursor molecule for phosphoinositide synthesis.

Several lines of evidence indicate the involvement of changes in inositols and other osmolytes in epilepsy and the effects of inositols in the regulation of induced seizures. The authors have revealed that the water extract of the medicinal plant *Aquilegia vulgaris* (a plant widely used in Oriental folk medicine as antiepileptic and soporific treatments) contains compounds altering binding of ligands to the benzodiazepine andgamma-aminobuturic acid (GABA) binding sites of the GABA-A receptors. The authors have identified two such compounds of this extract: (1) (MI) and (2) sleep-inducing lipid oleamide. Further the authors have shown that MI and scyllo-inositol (SCI) pretreatment significantly decreases the severity of seizures induced either by pentylentetrazolium or kainic acid (KA). As these effects were achieved by physiological concentrations of the administered inositols, the authors have hypothesized that MI and SCI could represent endogenous anti-convulsants.

Our original data showed that MI also could interfere with the process of epileptogenesis. The authors have first induced status epilepticus by KA and then applied MI daily treatment. The authors have shown that a 28-day MI treatmentsignificantly attenuates biochemical changes associated with the process of epileptogenesis in the hippocampus, and restores the amount of some drastically reduced proteins to the normal level. To these proteins belongs the GLUR1 subunit of glutamate receptors, calcium/calmodulin dependent protein kinase II (CaMKII), a $\gamma 2$ subunit of GABA-A receptors. The obtained results indicate that MI treatment could at least modify the epileptogenesis process induced bybrain insult. Further studies of MI and SCI action could lead to more successful translational research and development of inositols as future anti-epileptic compounds.

Chapter 4 – Inositol (1,4,5) trisphosphate is generated at the cell surface following stimulation and via activation of its receptors on intracellular stores, is the prime regulator of cellular Ca^{2+} responses. However, Ca^{2+} regulation in the heart depends most importantly on Ca^{2+}-induced Ca^{2+} release controlled by ryanodine receptors on the sarcoplamic reticulum, raising questions about the contribution of IP_3 and its receptors (IP_3-R). Furthermore, cardiomyocytes show only limited activity in generating IP_3 and the level of expression of IP_3-R is low. However, both IP_3 generation and IP_3-R expression are heightened in many cardiac pathologies, including hypertrophy, heart failure and in atrial

fibrillation. IP_3 has been suggested to contribute to disease progression by promoting arrhythmia, hypertrophy and chamber dilatation. Experimental studies have argued both for and against this proposal. Many factors are altered in diseased myocardium and the challenge is to identify those that critically influence outcomes. In the current review, the evidence that IP_3 and IP_3-R contribute to heart disease is critically re-evaluated.

Chapter 5 - Many hormone and neurotransmitter receptors utilize the phosphate-dylinositol signaling cascade. Phosphatidylinositol 4,5-bisphosphate is converted into two putative second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. IP_3 regulates various kinds of cellular responses. It induces the release of Ca^{2+} from the intracellular stores by binding to the IP_3 receptor (IP_3R) on the endoplasmic reticulum. Many cell types, often nonexcitable, including glomerular mesangial cells (GMC) and renal vascular smooth muscle cells (VSMC), depend on this pathway to couple external signals to intracellular Ca^{2+} release. IP_3Rs not only triggers the release of calcium from intracellular stores but also opens plasma membrane calcium channels. Hepatorenal syndrome (HRS) is a common complication of advanced cirrhosis, characterized by renal failure and major disturbances in circulatory function. Renal failure is caused by intense vasoconstriction of the renal circulation.

HRS develops in approximately 55% of all patients with FHF. In order to delineate the role of inositol 1,4,5-triphosphate receptor in HRS, the authors investigated renal expression of type 1 inositol 1,4,5-trisphosphate receptors (IP_3R1) in mice with FHF. FHF model was induced by intravenous injection of lipopolysaccharide (LPS) in D-galactosamine (GalN)-sensitized mice via the caudal vein. GalN/LPS treatment led to a marked renal dysfunction and hyperkalaemia, of which kidney perfusion pressure was also significantly enhanced. However, it did not see any significant change in renal histopathology of rats in FHF. Simultaneously, the expression of protein and mRNA levels of renal IP_3R1 increased markedly, serum TNF-α and endothelial-1(ET-1) levels reached a maximum level. The authors employed an inhibitor of IP_3Rs (2-APB) in this renal failure model, and found that reduction in the glomerular filtration rate (GFR) was reversed and hyperkalaemia recovered after administration of 2-APB; whereas, 2-APB did not affect the liver injury and the expression of IP_3R1. In contrast, intravenous injection of the TNF-α monoclonal antibody into the caudal vein significantly decreased the mortality rate of GalN/LPS-treated rats from 70% to zero, and significantly improved the compromised GFR. In TNF-α monoclonal antibody treated rats, serum TNF-α, ET-1 levels and expression of IP_3R1 were

decreased. The authors had demonstrated in a recent study that TNF-α could enhance IP_3R1 expression at mRNA and protein levels in human mesangial cells (HMCs), mediated through TNFR1/PC-PLC/PKCα-dependent and TNFR2-dependent IP_3R1 pathways. Isolated perfusion kidney experiments demonstrated that in normal rat kidney preperfused with TNF-α, stimulation with ET-1 could raise the perfusion pressure evidently and overexpress IP_3R1 protein and mRNA levels. Similar results were seen in GMCs and VSMCs incubated by TNF-α, the calcium release markedly increased after treated with ET-1. The findings in the present study demonstrated that TNF-α-dependent IP_3R1 up-regulation might be a specific target in future studies of renal dysfunction during the end-stage of liver disease.

Chapter 6 - Alzheimer's disease (AD) is the leading cause of adult onset dementia and the incidence is expected to rise in US, Japan and other developed countries. AD is characterized by the accumulation of amyloid–beta peptides forming plaques. Though the neuropathology of AD was discovered over a century ago, the molecular aspects of the disease development are unclear. It is speculated that amyloid-beta peptides target cholinergic neurons leading to neural degeneration. Defective calcium signaling in neurons has been shown to play a critical role in the pathogenesis of AD. There is now increased interest and research on inositol phosphates (IPs) and calcium signaling since IPs are known to regulate Ca^{2+} homeostasis in endoplasmic reticulum, cell signaling, and cell death mechanisms. Thus, dysregulation of the IP mediated calcium homeostasis and associated cell physiology seems to play a pathological role in the development of many chronic illnesses particularly neurodegenerative diseases. In addition to IPs, aluminum toxicity also has been shown to contribute significantly to the perturbance of calcium metabolism, mitochondrial dysfunction, and inflammasome activation. There appears to be a convergence of dysregulated calcium metabolism, aluminum toxicity, and inflammasome activation leading to beta amyloid deposits and associated pathology in Alzheimer's. Similar to Alzheimer's, IPs also have been shown to influence the pathogenesis of other neurological diseases including bipolar disorder. At the cellular level, there might be common shared pathways and underlying mechanisms related to IP. In this review, the pathophysiologic significance of IPs in the development of AD and bipolar disorders is discussed.

Chapter 7 - Inositol phosphates play important roles as second messengers in intracellular signal transduction. In order to study the relative affinity and specificity in binding inositol phosphates and diverse inositol phosphate-binding proteins by Surface Plasmon Resonance (SPR) analysis and pull-down

analysis, biotinylated inositol phosphates, to be immobilized on avidin-based sensor chip or resin, were designed. The synthesis of the biotinylated inositol phosphates was accomplished by assembling the inositol phosphate building block, synthesized starting with optically resolved *myo*-inositol derivatives, and the biotin building block through a phosphate linkage. The inositol phosphate moiety of each biotinylated inositol phosphate showed specific binding to Pleckstrin Homology (PH) domain of PLCδ (phospholipase Cδ), PH domain of Grp1 (general receptor of phosphoinositides 1), and precursor of Gag protein ($Pr55^{Gag}$) of human immunodeficiency virus type 1 (HIV-1), as revealed by SPR analysis and pull-down analysis.

Chapter 8 - Inositol hexaphosphate (InsP6, IP6, phytic acid) is found in abundance in beans and cereals. InsP6 occurs at much lower levels in most mammalian cells, in which they regulate cellular functions. Altered inositol production has been reported from patients with diabetes mellitus, chronic renal failure, galactosemia, and multiple sclerosis.IP6 has drawn much attention due to its activity in cancer prevention and control of experimental tumor growth, progression, and metastasis. InsP6 increases differentiation of malignant cells which may then revert to the normal phenotype. IP6 treatment of all the cell lines tested so far demonstrates that it is cytostatic and not cytotoxic. InsP6 inhibits growth and induces terminal differentiation of HT-29 human colon cancer cells. Studies of the expression of tumor suppressor gene demonstrate up-regulation of wild type p53 and down-regulation of the mutant form. Its efficacy in treating central nervous system disorders including Alzheimer's disease, depression, panic disorder, and obsessive-compulsive disorder has been reported. Its usefulness as an analgesic, in pediatric respiratory depression syndrome,to mitigate adverse effects associated with lithium treatment, and in preventing neural tube defects in embryonic mice has been documented. IP(6) exhibits immunoenhancing and hypocholesterolemic activities, minimizes pathological calcification and kidney stone formation, and lowers pathological platelet activity.However,the risk of inducing uterine contractions restricts its usefulness in pregnancy.

Chapter 9 - Calcium-mobilizing receptors stimulate the hydrolysis of phosphatidylinositol 4,5-bisphosphate to 1,2-diacylglycerol and inositol 1,4,5-trisphosphate (Ins 1,4,5P3). Ins 1,4,5P3 released to the cytosol serves as a second messenger to release calcium from the endoplasmic reticulum. Growth factors such as platelet-derived growth factor, bombesin and vasopressin in fibroblasts or antigen in lymphocytes use the aforementioned transduction mechanism to produce intracellular mitogenic signals. The signaling mechanism via histamine H1-receptors is also used to mediate endothelial cell

functions. Insulin regulates the phosphatidylinositol(PI) 3-kinase involving a physical association between the insulin receptor and the PI 3-kinase and tyrosine phosphorylation. Inositol is a nutrient required for keratinocyte proliferation. The Ins1,4,5P3/Ca2+ signal pathway functions to regulate such diverse processes as egg maturation and fertilization, growth, secretion, metabolism, neural activity, and excitation-contraction coupling in skeletal muscle. Myo-inositol serves as a clinically relevant osmolyte in the CNS, and its hexakisphosphate and pyrophosphorylated derivatives may play roles in such diverse cellular functions as DNA repair, nuclear RNA export and synaptic membrane trafficking. Glycerophosphoinositols induce cell proliferation in thyroid cells,modulate actin cytoskeleton organization in fibroblasts,and reduce the invasive potential of tumour cell lines. Glycerophosphoinositols promote cytokine-dependent chemotaxis in T-lymphocytes induced by SDF-1alpha-receptor activation.

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Chapter 1

Hypoxia- and Angiogenesis-Related *myo*-Inositol Phosphates: Synthesis and Biological Evaluation

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Abstract

One of the most fundamental physiological processes in the aerobic organisms is the delivery of oxygen to all tissues. Deprivation of oxygen leads to hypoxia which is a pathological condition playing a central role in numerous types of diseases, such as cancer (solid tumors), cardiovascular diseases and anemia. In this chapter we review the synthesis of a number of *myo*-inositol phosphates and their biological evaluation regarding hypoxia and angiogenesis in an independent or a

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combined relationship. Some of the compounds were found to be involved in the increased oxygen delivery by free hemoglobin or whole red blood cells, in the suppression of hypoxia-induced factor 1α (HIF-1α), in the inhibition of the phosphatidylinositol 3-kinase (PI3K) pathway, and in the reduction of angiogenesis. Consequently, the eradication or growth reduction of solid tumors, the prevention of sever anemia caused by sickle cell disease, and the enhanced exercise capacity of subjects with severe heart failure have been demonstrated. These results have evinced a leading role for *myo*-inositol phosphates in the treatment of diseases related to hypoxia and angiogenesis.

1. Introduction

1.1. *myo*-Inositol Phosphates

myo-Inositol phosphates is a family of mono- to hexaphosphorylated *myo*-inositols. They play a key role in biological systems as they function as secondary messengers in important cell-signaling pathways and are implicated in diverse cellular functions, such as cell growth, apoptosis, migration, and differentiation [1–3]. A number of different *myo*-inositol phosphates, which are distinguished by the number and position of the phosphate groups, were found in different organisms.

This work focuses on a variety of polyphosphorylated *myo*-inositols (with the number of phosphates ≥ 3) which have been found to be related with the phenomena of hypoxia and angiogenesis.

1.2. Angiogenesis and Hypoxia

Angiogenesis is the process of blood vessel formation. It plays a central role in a variety of physiologic events, such as organ development, differentiation during embryogenesis, growth, tissue remodeling and wound healing [4, 5]. However, angiogenesis is also involved in several pathological conditions, such as proliferative retinopathies, age-related macular degeneration, rheumatoid arthritis, cancer, and arteriosclerosis [5–8]. It consists a multi-step process, during which capillary endothelial cells (ECs) migrate and proliferate to form three-dimensional structures capable of carrying blood, i.e. the generation of a mature vasculature from a primitive vascular network occurs [4].