Biomedical & Pharmacology Journal Vol. **7**(2), 433-437 (2014)

Inositol Phosphates and their Biological Effects

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http://dx.doi.org/10.13005/bpj/508

(Received: October 30, 2014; accepted: December 01, 2014)

ABSTRACT

The paper is dedicated to data analysis on inositol phosphates nature distribution, structure and biological functions as well as enzymes - phytases - capable to hydrolyze these hardly digestible compounds and their complexes. Pharmaceutical application of inositol phosphates in treatment and prevention of various inflammatory and cancer diseases is discussed.

Key words: Inositol phosphates, Phytases, Biological activity, Medical importance.

INTRODUCTION

Inositol phosphates are multifunctional molecules with different phosphate group content in inositol ring, discovered more than 150 years ago in plant and animal cells, including human cells. These compounds are involved in implementation of various cellular functions such as membrane transport, cell division, cytodifferentiation and cell death. Inositol phosphates play an important role in cellular signal transduction, regulation of cell proliferation, RNA export, DNA repair, ATP regeneration and protein folding[1,2]. Currently, attention of researchers is focused on study these compounds as medicines for chronic inflammation and cardiovascular disease treatment, for reduce blood cholesterol, prevent diabetes, deposits of kidney stones, Parkinson's disease and cancer[3-5]. Pharmacological properties of inositol phosphates depend on the number and location of phosphate groups in inositol ring. Some of low-phosphorylated inositol phosphates (InsP3, InsP4, InsP5) regulate important physiological processes in cell[6]. Of particular interest is the study of *myo*-inositol hexa*kis*phosphate (phytate) anticarcinogenic action. Phytate is the most common in nature and its chemical synthesis is unprofitable and results in presence of adverse products. Enzymatic synthesis of inositol phosphates with different phosphorylation degree by using phytate-hydrolyzed enzymes (phytases) is carried out under mild conditions and is cost-effective biotechnological process. Developments on the basis of microbial enzymes are promising in this direction[7,8]. Phosphatases that decompose phytate are widely represented in microorganisms and characterized by higher molecular activity at physiological pH values and stability in contrast to eukaryotic enzymes[9,10]. Moreover, a significant number of microbial genomes are sequenced, and their analysis allows to identify novel phytases with interesting properties and to conduct their successful cloning for intended use. In eukaryotic cells *myo*-inositol phosphates are destroyed by phytases up to compounds with different degrees of phosphorylation, which as well as phytate and free inositol have a positive effect on treatment and prevention of many diseases, including cancer[1].

Inositol phosphates and phytate-hydrolyzed enzymes

All organisms use different micro- and macronutrients of exogenous origin for normal

growth and development. Phosphorus, one of important cell elements, is necessary for normal functioning of basic biological processes and presents in composition of nucleic acids, phospholipids, coenzymes, hormones, ATP molecules. Plants receive this vital element from soil solution of salts, animals - from plant foods or supplements produced by modern biotechnological methods. In soil and plants the main form of phosphate is the organic compounds of inositol phosphates. These compounds are found in all plant organisms, in large quantities they are contained in cereals and fruits leguminous plants (from 0.4 to 6.4%)[11]. Two decades ago it was believed that inositol phosphates are not digested by single chamber stomach animals due to the lack or low level of phytases (enzymes for inositol phosphates utilization) in gastrointestinal tract. Recent years studies have shown that inositol phosphates get into mammalian organism by food with high content of fibrous cellulose and are present in animal cells at concentrations from 5 to 100 μM [2,12]. *In vitro* and *in vivo* studies of the effect of these compounds on diseases development and prevention showed their biological significance.

Inositol molecule is a cyclic alcohol, in which all six carbon atoms are linked to hydroxyl groups. Equatorial or axial (axis direction) location of these groups leads to formation of nine alternative conformations (stereoisomers)[13]. The most common inositol stereoisomer in plant and animal cells is *myo*-inositol with one axial and five equatorial hydroxyl groups. It is having the most stable conformation. *Scyllo*-, *chiro*- and *neo*-inositols are also detected in these objects, but in smaller quantities. Substitution of hydroxyl groups on phosphate groups in inositol ring leads to formation of inositol phosphates. Compounds from inositol monophosphates to inositol hex*akis*phosphates are formed depending on the number of phosphate groups in inositol ring. For *myo*-inositol hex*akis*phosphate in literature often use the term phytate or phytic acid and abbreviation InsP6 or IP6.

All inositol phosphates are strong natural chelators of metal ions[14]. These compounds create intramolecular links with polyvalent metal cations such as Ca(2+), Mg(2+), Zn(2+), Cu(2+), Fe(2+), Fe(3+), Mn(2+), Al(3+), so forming water-soluble chelate complexes in equimolar ratio of 1:1. In excess of metal cations slightly soluble and insoluble salts are formed. The presence of several metal cations in phytate molecule contributes to formation of insoluble complexes[15,16]. In addition, solubility of phytate complexes depends on environmental pH. Phytates of calcium, cadmium, zinc and copper are better soluble at pH values below 4.0-5.0, whereas, for example, magnesium phytate is soluble at pH 7.5. Phytate can also contact with positively charged proteins and amino acids, forming complexes with them[15,17]. These complexes are soluble at pH 5.0 and can be digested in gastrointestinal tract, but with increasing pH their solubility significantly reduced and inhibited effect of gastric proteolytic enzymes[17,18].

Phytases (*myo*-inositol hexa*kis* phosphatephos phohydrolazes) form a special group of phosphatases which are found both in pro- and eukaryotic cells. They possess phytate-hydrolyzed activity and are able to step-by-step phytate dephosphorylation. Mechanism of action of all phytases is based on enzyme hydrolysis of inositol chemical bonds with phosphoric acid residues. Phytases catalyze partial or complete hydrolysis of phytate and its complexes, forming as end products free inositol, metal cations, and less phosphorylated forms of inositol phosphate[15,19]. Hydrolysis by phytases is consecutive and stereospecific. So, formation of less phosphorylated forms of inositol phosphate and *myo*-inositol using phytases is a potential alternative to chemical synthesis. Phytases are divided into several groups of enzymes with different substrate specificity, need for metal ions, optimum pH action and mechanism of hydrolysis[19]. Unique properties of different groups of these enzymes allow them to efficiently split inositol phosphate at different pH values[20].

Phytases actively secreted into soil by microorganisms so releasing phosphorus from the soil organic compounds and thereby facilitating its absorption by plants. Phosphates in vegetable feed are digested by animals not only from inorganic salts, but also from a variety of organic compounds including inositol phosphates. For example, anaerobic bacteria of rumen with phytate-hydrolyzed activity can effectively hydrolyze

phytates in rumen at high phosphate content[20]. Although phytates are poorly digested by monogastric animals, it is shown that cells of animals, given phytate with food, contain inositol phosphates with different degree of phosphorylation[2,12]. In the absence of phytate (InsP6) in food its levels in cells of all organs and biological fluids is very low and inositol phosphates are found only in form of inositol pentakisphosphates (InsP5)[6]. If phytate enters to animals with food, its content in cells significantly increased. The authors concluded that endogenous synthesis of InsP6 is not determinative. The main thing is the presence of phytate in nutrient feed. In addition, it is shown that up to 50% of inositol penta*kis*phosphates in cells can be formed from phytate (InsP6) due to dephosphorylating by intracellular phytases, i.e. inositol hexa*kis*phosphate can be considered the predecessor of inositol penta*kis*phosphates. In turn, inositol penta*kis*phosphates are precursors to more low phosphorylated inositol phosphates (InsP4 and InsP3), which are regulators of vital physiological processes [6].

Inositol phosphates as the promising therapeutic drugs

Myo-inositol hexa*kis*phosphate (InsP6) and inositol phosphates are dephosphorylated in cells by phytases to intermediate products InsP1 - InsP5 and possess antioxidant properties[1]. It is shown that phosphate groups located at positions 1, 2 and 3 of inositol molecule are unique to InsP6 because phytate due to specifically binding to iron ions competitively inhibits formation of hydroxyl radicals. Therefore, according the authors, these compounds are strong physiological antioxidants[2].

It is known that phytate may reduce bioavailability of minerals converting them into an insoluble state. However, experiments on rats showed that given with food inositol phosphates prevent deposition of calcium salts in animal tissues[1]. Inositol trisphosphates (InsP3) are considered as agents that participate in calcium release and prevent formation of kidney stones[2]. In addition, inositol trisphosphates in cells can be phosphorylated to a higher degree of phosphorylation, up to inositol pyrophosphates containing high-energy pyrophosphate bonds (p-p). Recently have been identified such polyphosphates, as diphosphoinositol pentaphosphate (InsP7) and bi-diphosphoinositol tetraphosphate (InsP8), which are permanent components of inositol phosphate pool circulation[21]. It is shown that InsP7 which takes part in enhancing of insulin sensitivity is formed in the presence of inositol hexakisphosphate kinase 1 (InsP6K1)[22]. Studies have clarified that phytate is prophylactic against many diseases, normalizes cholesterol concentration in blood, lowers pathological activity of platelets and stimulates immune system, regulates cell growth and angiogenesis in bladder cancer, inhibits cancer cells growth and increases cell death of human adenocarcinoma colon[2,12,23,24].

The efficiency of phytate (InsP6) and free inositol in prevention and treatment of malignancies and other chronic diseases is shown. Phytate and free inositol in human and animal food are reduces the risk of these diseases. It is shown that phytate inhibits growth of bladder cancer cells *in vitro* and *in vivo*[12]. In animals receiving phytate and free inositol with food a low incidence of cancer was found. The pathways of cell malignancy were determined. Phytate reduces the rate of cell proliferation, controls cell cycle and regulates uncontrolled cell division[1].

One of important characteristics of malignancy is the ability of cancer cells to form metastases which penetrate into normal cells. By the use of mouse cancer metastasized cell lines it was shown a significant decrease in number of metastasized colonies, if the food contains phytate. *In vitro* experiments showed inhibition of metastasis in cancerous breast cells by phytate[25]. Moreover, *in vitro* in colon cancer cells phytate inhibits activity of matrix metalloproteinase MMP9. This metalloproteinase facilitates metastases penetration in vascular system, so phytate represents a potential prophylactic by prevention of migration and invasion of cancer cells[26].

The most commonly used chemotherapeutic drugs, such as tamoxifen, are aggressive and act the tumor cells as well as normal cells, including blood cells. A major complication of chemotherapy in this case is thromboembolism. Experiments with rats showed that phytate (InsP6)

inhibits platelet aggregation to 45%, so reducing the risk of not only coronary heart diseases, but also the occurrence of ischemic stroke[2]. The use of phytate with anticancer drugs was found to enhance therapeutic effect for breast cancer treatment[27].

Free inositol has a moderate anti-cancer effect. But free inositol increases antitumor activity when combined with phytate as has been shown *in vitro* to different models of cancer (colon cancer, breast cancer and lung metastases)[11,27]. For instance, patients with colorectal cancer were given combination of two compounds (InsP6 + Inositol) as an adjuvant to chemotherapy. Ultrasound examination and computed tomography scan performed after treatment showed a significantly reduced cancer cells growth rate and marked regression of tumor[1]. Another group of patients after surgery for breast cancer were also taken with food a mixture of two compounds, phytate and inositol (InsP6 + Inositol). The analysis confirmed that these patients decreased adverse reactions after chemotherapy and improved quality of life[27].

Thus, the use of chemotherapeutic drugs in conjunction with complex InsP6 + Inositol showed a significant improvement for patients: hemoglobin level have increased, liver enzymes, bilirubin, urea and creatinine normalized.

CONCLUSION

So, *in vitro* and *in vivo* is found that inositol phosphates can be applied in treatment of inflammatory diseases and cancer and in combination with inositol are promising for use in clinical therapy. Combination of these compounds also has anticarcinogenic effect against chemotherapy, controls formation of metastases and improves quality of life. Although due to lack of knowledge intended use of these compounds is still limited, however, we can confidently conclude that inositol phosphates have great potential in treatment and prevention of cancer

This work was supported by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (project No 14-83 0211/02.11.10083.001) and partly by the Russian Foundation for Basic Research (project no. 12-08-00942a). This work was performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University.

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