SESSION 4:
Lipid signaling

ROLE OF PHOSPHOLIPASE C IN ABA SIGNAL TRANSDUCTION

[1]Iakovenko O. M., [1]Kretynin S.V., [2]Kabachevskaya E.M., [2]Lyakhnovich G.V., [2]Volotovski I.D., [1]Kukhar V.P., [1]Kravets V.S.

[1]The Institute of Bioorganic Chemistry and Petroleum Chemistry NAS of Ukraine, Murmanska str. 1, Kyiv 02094, Ukraine, e-mail: yon@bpci.kiev.ua

[2]Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus; Akademicheskaya str. 27, Minsk, BY-220072, Belarus, lmbc@biobel.bas-net.by

Phospholipase C (PI-PLC; EC 3.1.4.3) is a crucial lipid-associated enzyme that hydrolyzes membrane bound phosphatidylinositol- 4,5-bisphosphate (PIP2) to produce inositol-1,4,5-trisphosphate (IP3) and diacylglycerol. These two second messengers play key roles in amplifying extracellular signals and regulating of various intracellular processes triggered by abiotic and biotic stimuli. However the contribution of the phosphoinositide signaling pathway in plant acquisition of stress acclimation is not well understood at present.

One of the initial components of adaptation strategy is activation of plant protective mechanisms by ABA. Some reports point to the role of phosphoinositide-specific phospholipase C (PI-PLC) in ABA signal transduction. We focus our research is on the role of PI-PLC in ABA signaling in Zea mays L and *Pisum sativum L.* plant. The effect of ABA on the changes in level of polyphosphatidylinositols PI(4)P, PI(4,5)P2 and Ins(1,4,5)P3 of maize leaves were investigated. Ins(1,4,5)P3 levels have sharply increased within of ABA treatment in compared with control. Changes in Ins(1,4,5)P3 were associated with decrease in PI(4)P and PI(4,5)P2 levels. Our data indicate that the transient Ins(1,4,5)P3 production occurs on early stage of the ABA action as a result of PI-PLC activation.

One of the most sensible systems that activated after ABA perception is guard cells movement implementation. Application of ABA to stomata results in a decrease of the stomata pore that is achieved by inhibiting the processes associated with stomata opening and promoting the cellular events that occur during stomata closure. Neomycin sulfate, a well-known PIP2-PLC inhibitor, was used to examine the effects of PI-PLC and abscisic acid on stomata aperture in epidermal strips of pea leaves. Taken together, our data suggest that phospholipase C mediates the ABA effects on stomata aperture.

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PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLINOSITOL ARE ALLOSTERIC REGULATORS OF 5-LIPOXYGENASE FROM POTATO TUBER

Kharitonenko G. I., Kharchenko O. V.
Institute of Bioorganic Chemistry and Petroleum Chemistry NAS of Ukraine, 02094 Kyiv, Murmanska, 1, Ukraine, Email: anna@bpci.kiev.ua

Lipoxygenases are a class of non-heme iron dioxygenases that catalyze the incorporation of oxygen into 1,4-*cis,cis*-pentadiene containing fatty acids to form hydroperoxides. This reaction is a key step in the synthesis of signaling molecules such as jasmonic acid and related compounds, antimicrobial and antifungal compounds and a plant-specific blend of volatiles. Lipoxygenase products in plants are implicated in regulation of growth, senescence, and stress-related response. Under typical physiological pH, the fatty acids are located in membrane structures of the cell and therefore enzyme translocation to the membrane surface and interaction with its components, such as phospholipids (PL), will be an important for lipoxygenase activity regulation. Early was showed that phosphatidylcholine (PC) or phosphatidylinositol (PI) (0,005 – 0,3 mM) decrease or increase the steady-state velocity of linoleic acid ($C_{18:2}$) oxidation by 5-lipoxygenase (5-LO) from potato tubers, respectively.

The mechanism of interaction between enzyme and widespread phospholipids was studied in the present work. 5-LO was extracted from *Solanum tuberosum* tubers cv Lugovskay and purified by a 25-50% ammonium sulfate precipitation, dialysis, ion exchange chromatography (DEAE-cellulose pH 7,5), and hydrophobic chromatography (Butyl-sepharose pH 7,5). 5-LO shows positive cooperatively of linoleic acid ($C_{18:2}$) binding with a Hill coefficient of 4±0,22 in micelle system (0,02% Lubrol PX). PC and PI (0.1 mM) reduce a Hill coefficient under 1,196±0,14 and 1,67±0,13 respectively. It was demonstrated that PC decrease the maximum velocity of linoleic acid oxidation by 5-LO while PI increase this value.

The obtained findings indicate to the mechanism of allosteric regulation of the 5-LO activity by PC and PI. The effect of PL on the maximum velocity of reaction depends on molecule charge. Probably the additional negative charge is essential for proton dependent stage of lipoxygenase catalyze. This is agreement with the activatory effect of acidic amphiphiles on the 5-LO from potato.

Evidently that the role of the membrane lipids composition in lipoxygenase catalyze consist in both binding protein to membrane surface and regulation of enzyme activity.

NITROGEN OXIDE AND WOUNDING INFLUENCE ON THE ACTIVITIES OF PHOSPHOLIPASES D AND A_2 IN TOMATO LEAVES

Khmelevskaya O.D., G.V. Lyakhnovich, E.M. Kabachevskaya, I.D. Volotovski
Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus; Akademicheskaya str. 27, Minsk, BY-220072, Belarus, Email: lmbc@biobel.bas-net.by

The great interest in research of plant NO-synthase signalling system is connected with a special role of nitrogen monoxide (NO) to have a wide spectrum of biological activities. Basing on literary data, it is possible to suppose, that phospholipid and NO-dependent pathways cooperate in defensive reactions of a plant organism in response to pathogen action and influence of pathotoxins and elicitors.

The aim of the present work was to study the influence of NO-donor sodium nitroprusside (SNP) and its antagonist methylene-blue (MB) on activity of phospolipases D (PLD) and A_2 (PLA_2) and also to reveal the possible interrelation between NO as a signal molecule and activities of PLD and PLA_2.

These studies were performed on leaves of tomato plants (variety "Vilina", resistant to *Phytophthora infestans*). The leaves were placed in water solutions of SNP (100 μmol), methylene-blue (10; 1; 0,1; 0,01; 0,001 mmol) and distilled water (control) for 30 min right after cutting and then were homogenized. Measurement of PLD and PLA_2 activity *in vitro* was carried out by spectrofluorometric method using fluorescent-labeled phosphatidylcholine analogue.

It was established, that wounding caused by cutting resulted in an increase of PLD and PLA_2 activities in comparison with the control already in 30 min after exposure (by 1,2 and 1,4 times, accordingly). Incubation of tomato leaves in the presence of 100 μmol SNP (without MB) results in significant increase of PLD activity commensurable with the effect caused by wounding.

At high content of MB (1-10 mmol) the effect of increase of PLD activity caused by wounding was completely inhibited and PLA_2 activity was considerably reduced.

But at more low content of MB (1-100 μmol) inhibition of phospholipase activities was inefficient.

PLD and PLA_2 activities in wounded tomato leaves remained to be high in the presence of 100 μmol SNP and various concentrations of MB as well as in absence of the mentioned compounds, and same, as in the presence of only one SNP (100 μmol). Inhibitory action of MB (all concentrations) was removed in the presence of 100 μmol SNP, and values of PLD and PLA_2 activity were commensurable with the effect caused by wounding.

The obtained data testify to close interrelation between NO-synthase and phospholipid metabolic pathways in plants.

The cross-talk between NO, phospholipid and phytohormone regulatory pathways of plant cell is discussed.

CHANGES OF UNSATURATION OF FATTY ACIDS OF SUGAR BEET CALLUS TISSUES LIPIDS AT BACTERIAL STRESS

Kolomiets J.V.[1], Vashchenko L.M.[2]
[1]*National Agricultural University, 15 Geroiv Oborony St., Kyiv, 03401, Email:julyja@zeos.net*
[2]*D.K. Zabolotnogo Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154 Akad. Zabolotnogo St., Kyiv, 03143, Email:Vaschenko@serv.imv.kiev.ua*

Adaptable properties of plants are caused by features of structure and a metabolism of lipid components of membranes. Stability of membranes connect with qualitative and quantitative changes in phospholipids and fatty acids composition. The increase of unsaturated fatty acids promotes increase of plants resistance to bacterial stress. It explain more friable packing of unsaturated fatty acids, than saturated, in bilayer and areas of contact phospholipids with proteins, that gives to a membrane the big plasticity, fluidity, flexibility.

The aim of our work is studying of bacterial stress impact, which induced *Pseudomonas wieringae,* on changes of unsaturation of fatty acid of sugar beet callus tissue 'Roberta'.

The object of research was callus tissue of sugar beet of hybrids 'Roberta'. We used Murasige-Scoog medium which contains the *P. wieringae* lifeless cells in concentration 0,4ml cell suspension/50ml medium and 0,5ml cell suspension/50ml medium for callus cells of sugar beet cultivation. *P. wieringae* lifeless cells was obtained by heating at temperature 100ºC in 2 hour. Callus cells lipids were extracted by Bligh and Dyer Method. Fatty acid methyl esters were prepared and identified by gas chromatograph Agilent 6890N with mass-spectrometer detector 5973 inert (Agilent, Waldbronn, Germany).

Lipids of callus of triploid hybrids Roberta have been characterized by presence of fatty acids with number of carbon atoms from C_{15} to C_{24}. In structure of callus tissues lipids the prevailing saturated acid was hexadecanoic acid – 18,89 %. Among nonsaturated acids prevailed 9,12-octadecadienoic acid – 23,20 %, cis-octadecenoic acid – 45,4 %. The contents of other fatty acids did not exceed 3 %.

Addition of *P. wieringae* lifeless cells in a nutrient medium caused change of the contents of fatty acids in callus tissues lipids of hybrids Roberta, is especial 9,12-octadecadienoic acids. At concentration of *P. wieringae* lifeless cells 0,4ml cell suspension/50ml medium the contents 9,12-octadecadienoic acids has been increased to 30,6 %, whereas cis-octadecenoic acids has been decreased to 30,7 % in comparison with the control. If concentration of *P. wieringae* lifeless cells were 0,5ml cell suspension/ 50ml medium, the contents of 9,12-octadecadienoic acids has been increased to 36,1 %, cis-octadecenoic acids has been decreased to 27,1 %. The increase in a ratio 9,12-octadecadienoic acids / cis-octadecenoic acids probably indicates to activation of work desaturatical systems or synthesis 9,12-octadecadienoic acids de novo.

Thus, adaptation of callus tissue of sugar beet triploid hybrids Roberta to *Pseudomonas wieringae* is connected to increase in nonsaturation lipids, mainly for the account 9,12-octadecadienoic acids.

CHANGES OF LIPOXYGENASE AND HYDROPEROXIDE LYASE ACTIVITIES DURING STORAGE AND GERMINATION OF POTATO TUBERS (*SOLANUM TUBEROSUM L.*)

Kopich V.N., Kharchenko O.V.
Institute of Bioorganic Chemistry and Petrochemistry National Academy of Science of Ukraine, 1 Murmanskaya str. 02660, Kyiv, Ukraine. E-mail: viktor@bpci.kiev.ua

Most phyto-oxylipins which constitutes a large class of diverse oxygenated polyunsaturated fatty acids and their derivatives are produced through the lipoxygenase pathway.

The oxygenation of polyunsaturated fatty acids by lipoxygenases (LOX) represents the first step in fatty acid metabolites synthesis. Hydroperoxide lyase (HPL) is a part of the lipoxygenase pathway that catalyses the conversion of fatty acid hydroperoxides into aldehydes and oxo-acids. Products of the lipoxygenase pathway play an important role as growth and senescence regulators, antimicrobial compounds and signal molecules.

Changes of both lipoxygenase and hydroperoxide lyase activity have been monitored during storage and germination of potato tubers (*Solanum tuberosum L.*).

LOX and HPL activity gradually increased beginning from apical dominance stage, reaching maximum at multiple sprout stage with a decline at later stages of development.

The maximum activity of both enzymes in bulbs occurs in multiple sprout stage. LOX activity increased more than two-fold in comparison with their activity in apical dominance stage, where HPL activity was not detected. In daughter tuber stage LOX and HPL activities decrease two- and three-fold, respectively. HPL activity was also synchronously enhanced with LOX activity in multiple sprout stage.

Moreover, LOX and HPL activities in seedlings and in bulbs were different. HPL activity in seedlings was more than two-fold higher in comparison with bulbs and vice versa for LOX activity.

These observations suggest that lipoxygenase pathway enzymes (lipoxygenase and hydroperoxide lyase) and their products play a substantial role during germination of potato tubers.

CHILLING TOLERANCE DIFFERENCES OF VARIED POTATO CULTIVARS AND ITS GENOTYPES ACQUIRED THROUGH TRANSFORMATION

Sinkevich M.S., Deryabin A.N., Demin I.N., Trunova T.I.
Laboratory of Frost Resistance, Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya str. 35, Moscow 127276 Russia. Email: trunova@ippras.ru

As a part of chilling tolerance mechanisms research were determined two proving pathways – saturation index changes and accumulation of protective material (e.g. sugars). Effectiveness and impact of changes in these parameters in conjunction to relative chilling tolerance is discussed.

Our study was carried out with potato plants (*Solanum tuberosum* L.) cultivars Désirée and Desnitsa. Plants of Désirée cv. were transformed with vector carrying yeast invertase gene under the control of tuber-specific patatin promoter B33 class I, fused with proteinase II inhibitor leader peptide to provide enzyme location in apoplast. Plants were obtained in cooperative work of Max Plank Institute of Molecular Plant Physiolgy (Golm, Germany) and Laboratory of Growth and Development, Timiryazev Institute of Plant Physiology RAS, and gently provided by employees of the last. Plants of Desnitsa cv. were transformed with vector carrying dasA gene, encoding Δ12-acyl-lipid desaturase of fatty acids and thermostable lichenase reporter gene (licBM3). Both genes were placed under the control of constitutve 35S CaMV promoter. Agrobacterial transformation was performed according to method of micro-tuber transformation developed in Departnent of cell biology and biotechnology, Timiryazev Institute of Plant Physiology RAS. Both cultivars were grown in vitro at 22^{o}C under diffused fluorescent light (16 hours a day, LB-80 lamps, 4 klx) on MS nutrient medium, containing 2% of sucrose. Chilling was performed by exposition of plants at -7^{o}C during 30 min.

Potato plants of medium-speed maturating cv. Désirée is a product of Dutch selection, while early-to-medium maturating cv. Desnitsa plants appeared at Bryansk experimental station, so a priori we could suppose they would differ in chilling tolerance between cultivars and also between transgenic and wild genotypes of the same cultivar.

Comparison of the relative chilling tolerance of studied genotypes based on electrolyte leakage and lipid peroxidation intensity. As a result of experiments carried in our laboratory electrolyte leakage differences were shown for nontransformed plants of studied cultivars. Thus, control plants of Désirée cv. comparing with Desnitsa cv. demonstrated lower membrane permeability index that might mean higher tolerance.

Experiments with transgenic potato plant leaves either of Desnitsa or Désirée cv. revealed higher tolerance of their genotypes in comparison to their controls. Transformed plants possessed membranes that were more stable at low temperature than control ones. We suppose that described differences in membrane stability are corresponding to high concentration of soluble carbohydrates in case of Désirée cv. and low saturation index in case of Desnitsa cv., resulting in more effective signal and protective systems.

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INFLUENCE OF PLANT HORMONES AND GROWTH REGULATORS ON 9-LOX ACTIVITY OF POTATO MINITUBERS (SOLANUM TUBEROSUM L.)

Strashkevich O.V., Griz A.I.
Institute of experimental botany named V.F. Kuprevich the National Academy of Sciences Republic Belarus, 220072, Minsk, Academicheskaya Str., 3-28,Email: assolia@gmail.com

Plant lipoxygenases (LOXs) are a functionally diverse class of dioxygenases implicated in physiological processes such as growth, senescence, and stress-related responses. LOXs incorporate oxygen into their fatty acid substrates, and they produce hydroperoxide fatty acids that are precursors of jasmonic acid and related compounds.

Tuber formation in potatoes (Solanum tuberosum L.) is a complex developmental process that requires the interaction of environmental, biochemical, and genetic factors. Several important biological processes like carbon partitioning, signal transduction, and meristem determination are involved (Ewing and Struik, 1992). Tuber development at the stolon tip is comprised of biochemical and morphological processes. Both are controlled by differential gene expression (Hannapel, 1991; Bachem et al., 1996; Macleod et al., 1999) with most of the work focusing on the biochemical processes, including starch synthesis (Abel et al., 1996; Preiss, 1996; Geigenberger et al., 1998) and storage protein accumulation (Mignery et al., 1984; Hendriks et al., 1991; Suh et al., 1991). Much less is known about the morphological controls of tuberization, although it is clear that phytohormones play a prominent role (Koda et al., 1991; Xu et al., 1998, Sergeeva et al., 2000). Despite considerable work on the physiology of tuber development, the molecular mechanisms that control the changes in cell growth during tuberization have not been identified.

Potato LOXs are encoded by a large multigene family. Several LOX cDNAs have been isolated from potato tubers, roots, and leaves (Geerts et al., 1994; Casey, 1995; Kolomiets et al., 1996a; 1996b; Royo et al., 1996; Fidantsef and Bostock, 1998). LOX expression has been detected in developing tubers, and several groups have proposed that LOXs are involved in potato tuber growth (Bachem et al., 1996; Kolomiets et al., 1996a; Royo et al., 1996), but until now, there were no reports that demonstrated this involvement. Although LOXs are known to function in diverse physiological processes, this study is the first definitive proof of LOX involvement in the regulation of tuber development.

Several hormones, i.e., gibberellin, homo-brassinosteroid and growth regulator – salicylic acid, reportedly play a role in tuberization. The potato tuber discs were incubated with GA (10 mg/l), homo-brassinosteroid (10^{-7} M) and salicylic acid (10^{-7} M) during three hours. Proteins were extracted from minitubers as describes by Grimes et al. (1993). LOX activity was determined by Shimizy et al. (1990). Spectrophotometric measurements of the increase in A_{234}, caused by the formation of conjugated diene structures.

Our data witness that plant hormones (gibberellin, homo-brassinosteroid) and growth regulator (salicylic acid) brings about the change of the activities LOX. It was found that GA brought about an increase LOX activity (2.40 μmol sec^{-1} g^{-1} protein) with linoleic acid as substrates. Herewith exists the protein expression under action GA. Homo –brassinosteroid influenced on LOX activity (2.04 μmol sec^{-1} g^{-1} protein) relatively control variant (0.47 μmol sec^{-1} g^{-1} protein), but did not manifest themselves on the protein expression. SA weakly actuates LOX (0.70 μmol sec^{-1} g^{-1} protein), however powerfully expressed of the proteins, occurs the appearance an integer spectrum new proteins.

Overall, these results suggest that the expression of the tuber LOX genes is important in controlling tuber development.

NEW IN HORMONAL REGULATION OF α-AMYLASE IN GERMINATING WHEAT GRAIN

Yrgynbaeva S.M.
M.A. Aitkhozhin's Institute of molecular biology and biochemistry, Almaty, Ministry of Education and Science Republic of Kazakhstan. 86 Dosmukhamedov st., 050012 Almaty, Republic of Kazakhstan. Email: shynary@rambler.ru

One of the hot points of modern biology is the investigation of hormonal regulation of processing and activation of the enzymes. In this reason it is very interesting to investigate hormonal regulation of α-amylase in germinating wheat seeds. This enzyme plays key role in catabolism of starch – the basic energetic source for development of plant during its heterotrophic nutrition.

It is well known that the main hormone in regulation of α-amylase is the gibberellic acid (GA). It was supposed that this enzyme switches on the biosynthesis of α-amylase. However as it was shown by us almost all molecules of α-amylase are in latent state in the cells of aleurone layer of wheat seeds. And *de novo* synthesis of α-amylase is very low. Thus, a question arise on the other mechanism of GA action.

We suggested, that GA induces the phospholipase A in germinating wheat seeds. The phospholipase A converts phospholipids into their lisoforms. Lysophospholipids have the properties of strong detergents. They very easily solubilise the cell membranes.

We tested the effect of the lysophospholipids which was formed by GA and strong artificial detergent triton X-100. Both detergents have the same effects. They convert latent amylase to active state. And on the electrophoregramme appear 4-5 new bands of α-amylase.

It is well known, that during germination the molecules of protein undergoing glycosilation process in Golgi apparatus. Tunicamycine strongly inhibits this process. We tested the effect of tunicamycine on germinating wheat seeds and showed that the glycosilated α-amylase bands did not appeared in this case.

Now the interest of scientists sharply increasing to a new powerful bioregulator - nitrogen oxide (NO). We tested effect of NO on germinating wheat seeds and showed that NO causes the essential changing in electrophoretic spectra of α-amylase but the total activity of amylase not changed. It speaks that NO causes the modification of charging of molecules of α-amylase.

We also investigated very interesting effects of action of abscisic acid (AbA) on α-amylase in germinating wheat seeds. So, it was established, that under action of AbA there was a sharp decrease of α-amylase activity during germination. This speaks that AbA cancels the effect of GA during wheat seeds germination.