PROCEEDINGS
OF THE NATIONAL ACADEMY OF SCIENCES OF BELARUS

CHEMICAL SERIES 2016 N 3

FOUNDER IS THE NATIONAL ACADEMY OF SCIENCES OF BELARUS

The Journal has been published since January 1965

Issued four times a year

This issue is devoted to the 23rd Conference on Isoprenoids, which takes place in Minsk on September 4-7, 2016. The subject of the Conference is a widespread large group of natural compounds whose molecules consist of C5 isoprene units connected to each other in various ways. A number of vitamins, pheromones, allelopathins, receptor sensors as well as sterols, the key elements of cell structure, and many other physiologically active natural compounds, such as e.g. steroidal hormones of humans and animals, belong to isoprenoids. They are responsible for the reproduction, sexual differentiation, development, adaptation, regulation of mineral and protein metabolism, nervous activity, digestive system, i.e. virtually all the vitally important functions of a living organism. An attractive feature of the Conference is a variety of isoprenoid-related topics: from their search in natural sources, chemical synthesis and structural analysis to molecular biological, genetic engineering, ecological, and medicinal aspects.

All topics are considered by the specialists from different fields during common discussions offering a broad vision of a subject that is especially important for young scientists for imaging the current state and perspectives of natural products chemistry – a basement of efficient medications, ecologically friendly agrochemicals and biotechnologies for modern time and for future.

The current issue contains more than 80 abstracts of papers presented at the Conference (the author's style and spelling retained).

Contents

🕮 EFFECT OF BRASSINOSTEROIDS ON ION CHANNELS AND SIGNALLING IN ROOTS OF HIGHER PLANTS

Darya Straltsova[1], Palina Chykun[1], and Vadim Demidchik[1*]

[1]Belarusian State University, Minsk
e-mail: dzemidchyk@bsu.by

Brassinosteroids (BRs) are class of steroid hormones essential for the proper regulation of multiple physiological processes required for normal plant growth and development. Exogenous BRs can improve the quantity and quality of crops and ameliorates effects of stresses. Using native and synthetic analogues of BRs as a tool to improve plant yield seems to have a great potential for agriculture and biotechnology (Khripach, 2000). BRs have been intensively investigated for their biosynthesis, distribution and physiological functions using classical physiological tests, analyses of mutants and transgenic plants (*Arabidopsis thaliana* plants constitutively expressing aequorin). Recent data indicate that BRs are also sensed by the plasma membrane system catalyzing increase in the cytosolic free Ca^{2+} (in leaves of *Arabidopsis thaliana*). Zhao *et al*. (2013) have shown that the BR-induced elevation in the cytosolic free Ca^{2+} is abolished in knockout line lacking functional brassinosteroid receptor and after treatment with Gd^{3+} (blocker of Ca^{2+}-permeable nonselective cation channels) (Zhao, 2013). Zhang *et al*. (2005) using suspension culture cells of *Arabidopsis* have found that anion channel currents were inhibited by both 28-homobrassionolide and 28-castasterone and outwardly-directed K^+ conductance was stimulated by 28-homobrassionolide but inhibited by 28-castasterone (Zhang, 2005).

This study was to examine possible effects of brassinosteroids on the plasma membrane cation conductances in plant cells and related Ca^{2+} driven signalling events. Standard patch-clamp and aequorin chemiluminometry techniques were used (Shabala, 2006).

Here, we report the first electrophysiological characterisation of brassinosteroid-activated Ca^{2+}-permeable channels in higher plants. Wheat root protoplasts (tested by patch-clamping) and whole arabidopsis plants expressing Ca^{2+}-reporting protein, aequorin (analysed by chemiluminometry), were used in this study.

In the whole-cell patches (wheat root protoplasts), 1 μM 24-epibrassonolide, 28-homobrassionolide or 24-epicastasterone were applied exogenously. Only 24-epicastasterone modified transmembrane cation currents while 24-epibrassonolide and 28-homobrassionolide did not cause any reaction. Addition of 24-epicastasterone at cytosolic side through the patch-clamp pipette increased Ca^{2+} influx conductance, which demonstrated characteristics of depolarisation-activated Ca^{2+} channels. The pharmacological analyses have shown that brassinosteroid-activated Ca^{2+}-influx conductance was sensitive to inhibitors of Ca^{2+}-permeable cation channels. Blockers of K^+ channels did not inhibit this conductance.The plasma membrane conductance, which was activated by an endogenous 24-epicastasterone, showed bell-like shape with maximal activation at depolarisation voltages (bath: 20 mM Ca^{2+}). Labelling castasterone (and its derivates) with BODIPY (using castasterone-BODIPY conjugates which were synthesised chemically) showed that castasterone (and its derivates) can be transferred to the cytosol both in intact roots and protoplasts. This confirms that the effect of 24-epicastasterone at the cytosolic face can potentially be observed in real plants.

We also tested the effect of different brassinosteroids on cytosolic free Ca^{2+}, using *Arabidopsis thaliana* plants constitutively expressing aequorin. Brassinolide and castasterone, and its derivates (24-epibrassonolide, 28-homobrassionolide, 24-epicastasterone, 28-homocastasterone) were tested. All six brassionosteroids induced elevation of the cytosolic free Ca^{2+} in arabidopsis root cells. In the present study we demonstrated that 24-epicastasterone being more potent than 24-epibrassonolide and 28-homobrassionolide. 10 μM of exogenous BRs was the minimal concentration at which statistically significant changes of the cytosolic Ca^{2+} were observed.

The obtained results suggest that the plasma membrane of root cells contains the brassinosteroid-activated cation-permeable channels, which can be involved in cell ion homeostasis and signalling.

REFERENCES

(1) Shabala, S., Demidchik, V, Shabala, L, Cuin, T.A., Smith, S.J., Miller, A.J., Davies, J.M., Newman, I.A. *Plant Physiol.* **2006**, *141*, 1653-1665.
(2) Khripach, V, de Groot, A.E., Zhabinskii, V.N. *Ann Bot.* **2000**, *86,* 441-447.
(3) Zhao, Y., Li, J. *Plant Physiol.* **2013**, *163*, 555-565.
(4) Zhang, Z., Qi, Z., Berkowitz, G.A. *Plant Cell Physiol.* **2005**, *46*, 1494-1504.

🕮 PHYTOSTEROL BIOCONVERSION AS A PLATFORM FOR PRODUCTION OF VALUABLE STEROIDS: NOVEL FINDINGS AND PROSPECTS

Marina Donova*, Dmitry Dovbnya, Galina Sukhodolskaya, Sergei Khomutov, Andrei Shutov, Eugeny Bragin, Tanya Ivashina, and Nicolai Strizhov

G. K. Skryabin Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, 142290, Russia
*e-mail: *donova@ibpm.pushchino.ru*

Phytosterols are recognized now as most attractive, available and low-cost raw materials for steroid pharmaceutical industry. Phytosterols are produced in huge amounts from plants, such as soya, pine, or wastes of cellulose production plants. Their microbial transformation is an effective tool for the production of high-valued steroidal drugs and their precursors. Although a range of biocatalytic methods has been developed, selection of suitable microorganisms, as well as creation of new engineered strains is of great importance for generation of improved bioprocesses and production processes for obtaining known and new metabolites with potent biological activity. The achievements in genetic and metabolic engineering of steroid-transforming strains in combination with novel approaches in the enzymatic and whole-cell biocatalysis provide a platform for highly effective and selective biotransformations.

Actinobacteria are known to catabolize phytosterol via the 9(10)-secosteroid pathway.[1] Along with degradation of the aliphatic side chain, different modifications of steroid core occur during sterol bioconversion, such as 3β-hydroxy-5-ene to 3-keto-4-ene moiety transformation, Δ^1-dehydrogenation, 9α-hydroxylation. Metabolic blocks allow production of valued steroids by exploiting of the cascade reactions which are the part of the degradative pathway.[2]

Biotransformation of phytosterols by actinobacteria, and especially, by the selected strains of *Mycobacterium neoaurum* VKM Ac-1815D, 1816D and 1817D provides effective production of androstenedione (AD), androstadienedione (ADD) and 9α-hydroxyandrostenedione (9-OH-AD), respectively. These androstane steroids are the key intermediates in the synthesis of various steroid drugs.

Based on the whole genome sequencing, as well as on transcriptomic profiling, the specific genes and gene clusters had been revealed which are essential for specific steroid modifications.[3,4] The data were applied for the generation of engineered strains with improved biocatalytic possibilities for production of AD, 20-hydroxymethyl pregn-4-ene-3-one (20-HMP, BA) and their analogs. The knock-out of *fadD3* gene in *Mycobacterium smegmatis* mc2 155 allowed effective production of 4a-hydroxy-6a-methyldecahydro-cyclopenta[f]chromen-7(8H)-one (HMDC) in a single step from sterols. The recombinant strains capable of single-step converting of phytosterol, or cholesterol to testosterone, 1-dehydrotestosterone, progesterone were generated using heterologous expression of eukaryotic steroidogenic systems in mycobacterial hosts. Consequent bioconversions of phytosterol with two microbial strains, - *Mycobacterium neoaurum* VKM Ac-1815D and *Aspergillus ochraceus* VKM F-830, in one bioreactor vessel enable effective production of 11α-hydroxy-AD which is a key precursor in the syntheses of halogenated corticoids. Effective production of dehydroepiandrosterone (DHEA) from phytosterol has been provided by the combination of the protection-deprotection of the oxygen functionality at C3 with selective side chain degradation of the 3-substituted sterols using *Mycobacterium neoaurum* VKM Ac-1815D. Re-