VLPs starts at 70°C and fully completes by 90°C. Thus, in the case of PVX, VLPs thermal transition requires a lower treatment temperature (90°C) in comparison with TMV (94°C). The size of spherical PVX VLPs varies from 50 to 150 nm and unlike TMV SPs size does not depend on the initial virus concentration. The evidences that PVX VLPs are RNA-free and consist only of PVX coat protein were obtained. In this work we demonstrated that PVX VLPs have adsorption properties and could bind with different model antigens (recombinant Rubella virus antigen, recombinant Plum pox virus antigen, TMV coat protein). The adjuvant properties of spherical PVX VLPs were examined. Immunostimulating properties of native PVX virions were demonstrated and compared with spherical PVX VLPs. PVX spherical VLPs generated by thermal treatment of helical filamentous virions could become a promising platform for the development of functional active complexes. The work was funded by the Russian Science Foundation (grant no. 14-24-00007).

References

[1]. Atabekov *et al., J. Gen. Virol.* 2011, 92, 453–456.
[2]. Karpova *et al., J. Gen. Virol.* 2012, 93, 400–407.
[3]. Trifonova *et al., FEBS Journal*, 281 (Suppl 1), 421.

P14-042
Functioning change of serotonin metabolism in blood of patients with type 2 diabetes mellitus and ischemic stroke

A. Yurchenko, T. Tsarenko, O. Savchuk, L. Ostapchenko
ESC "Institute of Biology", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

It is known that cerebral atherosclerosis is one of the leading factors of ischemic stroke, and type 2 diabetes is an independent factor in their development. View of the relationship pathogenetic mechanisms of atherosclerosis and type 2 diabetes was expressed by several authors (Gallacher, 2013; Snell-Bergeon, 2014). Current studies indicate the involvement of serotonin in energy metabolism and the effect of serotonin on the concentration of glucose in the blood (Lam, 2007). These data give reason to assume that the serotonergic system may be involved in the pathogenesis of type 2 diabetes.

We have analyzed the content of serotonin, tryptophan, MAO activity and aggregation of platelets in the blood of patients with ischemic stroke, which suffer from type 2 diabetes. Studies have shown an increase of serotonin content of 46% and tryptophan to 5.6 times in patients with ischemic stroke and type 2 diabetes compared with the values of the control group. We found that activity of monoaminooxidase in patients with type 2 diabetes was reduced by 40% against to the activity in a group of healthy donors. Analysis of platelets aggregation showed an increase on 22% relative to this value of the donors.

These data may indicate a significant imbalance in serotonin metabolism in two groups of patients and the necessary for further research of metabolic enzymes of serotonin in the bloodstream and functioning platelet hemostatic links to additional information that could explain the differences in the content of these metabolites.

P14-043
Bioconversions of lipophilic dyes Nile Red and 25-NBD-cholesterol into mycobacteria

Y. Faletrov[1], A. Brzostek[2], R. Plocinska[2], M. Horetsky[1], V. Zavadskaya[1], E. Rudaya[1], J. Dziadek[2], V. Shkumatov[1]
[1]*Research Institute for Physical and Chemical Problems, Belarusian State University, Minsk, Belarus,* [2]*Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland*

Previously we have found that fluorescent *N*-alkylarylamine Nile Red (NR) is converted by mammalian cytochrome P450 steroid 17α-hydroxylase CYP17A1. Mycobacteria possess various P450s, so we tested of the dye with *M. smegmatis* and *M. tuberculosis*. It has been found that fluorescent oxidized derivatives of NR form during long incubations of the dye with both of the bacteria. Docking simulations have demonstrated that CYP130 & CYP125 can bound NR close to their heme cofactors affinely, pointing out on a possible involvement of the P450s in the oxidation of NR. Analogously, fluorescent steroid 25-NBD-cholesterol (25NC) has been confirmed to be converted *via* formation of its 4-en-3-one derivative, indicating that 25NC is a substrate for cholesterol oxidases and/or 3β-hydroxysteroid dehydrogenases from *M. smegmatis*. The bioconversions of the dyes by mycobacteria have reported for the first time. Due to uptake and metabolism of mammalian lipids by *M. tuberculosis* are essential for persistence of the pathogen, the established bioconversions should be taken into consideration if both NR and 25NC will be used for staining of lipids cells during a study of living *M. tuberculosis* or the host-pathogen interactions.

Acknowledgment: YF gratefully thanks FEBS for the financial support of the work during his short term fellowship to IMB PAN & Ukrainian Biochemical Society for membership.

References

[1] *FEBS J.* 280 (2013) 3109–3119.
[2] *BMC Microbiol.* 13 (2013) 43.
[3] *Annu Rev Cell Dev Biol.* 28 (2012) 411–437.
[4] *Trends Microbiol.* 19 (2011) 530–539.

P14-044
New antihistamine Kunitz-type polypeptides of the sea anemones, *Heteractis crispa* and *Stichodactyla mertensii*

O. V. Sintsova[1], V. E. Chausova[1], I. N. Gladkikh[1], M. P. Isaeva[1], V. M. Tabakmaher[1], M. M. Monastyrnaya[1], E. V. Leychenko[1], E. A. Pislyagin[1], E. S. Menchinskaya[1], S. Peigneur[2], J. Tytgat[2], E. P. Kozlovskaya[1]
[1]*G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Vladivostok, Russian Federation,* [2]*Laboratory of Toxicology and Pharmacology, University of Leuven, Leuven, Belgium*

The Kunitz-type protease inhibitors participate in regulation of vital processes, such as inflammation, blood coagulation, digestion and others. Poisonous animals have Kunitz-type polypeptides which, in addition to inhibiting serine proteases, can modulate voltage-gated channels (K_v, Ca_v, Na_v) and the TRPV1 receptor. Beside this, two Kunitz-type polypeptides from the sea anemone *Heteractis crispa* have shown antihistamine activity *in vivo*.

In this work we investigated cDNA transcripts of the sea anemones *H. crispa* and *Stichodactyla mertensii* and gene sequences encoding Kunitz-type polypeptides, which share a high sequence similarity with K_v channel blocker SHTXIII from *Stichodactyla haddoni*, were identified. The sequences of all mature polypeptides are characterized by point substitutions. Three chosen polypeptides of *H. crispa* and *S. mertensii* were produced in

the *Escherichia coli* expression system. They inhibited trypsin activity and did not have toxic effects in mice up to a dose of 5 mg/kg. Electrophysiological assays on eight potassium channel isoforms (K_v1.1–1.6, *Shaker* IR, h*ERG*) revealed the absence of modulatory activity. *In vitro* antihistamine activity study was conducted on bone marrow-derived macrophages from mice Balb/c and a statistically reliable inhibitory effect induced by the two polypeptides was observed. Noteworthy is the fact that the polypeptides are 10-fold more potent than fexofenadine (a selective antagonist of the H1 receptor). Electrophysiological analyses on the cloned H1 receptor in the *Xenopus laevis* expression system are planned to unravel the molecular interaction between the polypeptides and the H1 receptor.

This work was supported by the Russian Science Foundation (grant number 14-25-00037).

P14-045
A new multigene family of Kunitz-type IQ-polypeptides from sea anemones

A. N. Kvetkina, V. E. Chausova, O. V. Sintsova, E. V. Leychenko, M. P. Isaeva, E. P. Kozlovskaya
G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Vladivostok, Russian Federation

Protease dysfunction underlies various disorders including cancer, neurodegenerative and cardiovascular diseases. One of the biggest treatment challenges is to inhibit unwanted protease-related activity. A great number of protease inhibitors have been found in terrestrial and marine venomous animals including sea anemones that have been a promising source of new protease inhibitors. Previously we discovered the multigene superfamily of Kunitz-type GS-polypeptides from the tropical sea anemone *Heteractis crispa*. These polypeptides can inhibit different protease classes demonstrating analgetic, anti-inflammatory, antihistaminic and hypothermic activities.

Here we report on the discovery of a novel sea anemone Kunitz-type inhibitors family named IQ-polypeptides. In *H. crispa* cDNA library, we revealed transcripts encoding for IQ-polypeptides characterized by a large positive charge (8.5–9.3). The mature IQ-polypeptides consist of 58 residues with conserved spacing of six cysteine residues. Unlike GS-polypeptides, IQ-polypeptides have a propart peptide following the signal sequence. The IQ-polypeptide genes belong to a multigene superfamily of Kunitz-type GS-polypeptides based on a very high similarity of its signal peptide-coding sequence. Using cDNA libraries from different sea anemones, such as *H. magnifica* and *Stichodactyla mertensii,* we found that IQ-polypeptide genes are widely presented in different species of Stichodactylidae. Comparing the mature IQ-polypeptides of sea anemones, we revealed that they were high conserved and differed from each other by point amino acid substitutions probably indicating similar biological activities. Further obtaining and characterizing of recombinant IQ-polypeptides as potential therapeutic agents are of great interest.

This work was supported by the Russian Foundation for Basic Research (grant number 14-25-00037).

P14-046
Inhibition of DNA-topoisomerase I/II activity with selected bistacrine-thiourea/urea derivatives and their biological effect

J. Janočková[1], J. Plšíková[1], D. Kučerová[2], J. Koval'[2], R. Jendželovský[2], J. Korábečný[3], K. Kuča[3], S. Hamul'aková[4], P. Fedoročko[2], M. Kožurková[1,3]
[1]*Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik, Košice, Slovakia,* [2]*Department of Cell Biology, Institute of Biology and Ecology, Faculty of Science, University of P. J. Safarik, Košice, Slovakia,* [3]*Biomedical Research Center, University Hospital in Hradec Králové, Hradec Králové, Czech Republic,* [4]*Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik, Košice, Slovakia*

Topoisomerases influence the topological state of DNA by relaxing torsion tension in supercoiled DNA and their activity is essential for cell viability. Both forms of the enzyme, Topo I/II, may also have the capability to damage the genome leading to cell death in both healthy and cancerous cells. Well-known anticancer agents such as acridine or camptothecine derivatives act by interfering with DNA synthesis and by inhibiting Topo I/II activity(1). Previous studies have shown that some tacrine derivatives can act as dual-effect Topo I/II inhibitors, thereby suggesting that these agents may show potential for development as novel anticancer agents(2).

Bistacrine-thiourea/urea derivatives (**1**–**4**) are a novel class of cytotoxic agents which combine both 9-amino-1,2,3,4-tetrahydroacridines linked with thiourea/urea and various lengths of alkyl chains. In this study, Topo I/II inhibition mode assays were performed and verified that the novel compounds are topoisomerase suppressors rather than poisons. The cytotoxic action of **1**–**4** on human leukemic cancer cell line HL-60 were assessed using different techniques, such as MTT assay, the detection of mitochondrial membrane potential, cell viability measurements and cell cycle distribution analysis after 24, 48 and 72 h incubation. The studied derivatives were found to be more cytotoxic than the positive control, tacrine. Binding studies of **1**–**4** with ctDNA were also performed in order to characterize the effect mechanism using a variety of techniques (UV-Vis and fluorescence spectroscopy, thermal denaturation, linear dichroism and viscometry).

References

[1] Esselen, M., Barth, W.S., *Adv. Mol. Toxicol*, 8, 2014, 123–171.
[2] Janočková, J., *et al*., *Bioorg. Chem*., accepted.

This study was supported by VVGS-PF-2014-435, VVGS-2014-173, VEGA 1/001/13, APVV-0280-11, UHHK 00179906.

P14-047
Two novel antioxidants with diverse biological effects on curcumin-induced apoptosis in C2 skeletal myoblasts; signaling mechanisms involved

M. Peleli[1], I.-K. Aggeli[1], A. N. Matralis[2], A. P. Kourounakis[2], I. Beis[1], C. Gaitanaki[1]
[1]*Animal & Human Physiology, School of Biology, National and kapodistrian University of Athens, Athens, Greece,* [2]*Medicinal Chemistry, School of Pharmacy, National and kapodistrian University of Athens, Athens, Greece*

Excessive levels of reactive oxygen species (ROS) contribute to a number of pathological conditions including muscle disorders. Since redox equilibrium imbalances may seriously affect skeletal muscle performance of daily activities, there has been a substan-