ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)
Biopolymers and Cell. 2017. Vol. 33. N 3. P 221–236

Materials of XI annual Conference of Young Scientists Institute of Molecular Biology and Genetics NAS of Ukraine

Biosensors based on modified screen-printed electrodes for differentiation of cerebrovascular disease

Y. Boichuk, O. Biloivan

Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine;
by1992@i.ua

The work is aimed at the development of a new generation portable multiparametric biosensor system for the diagnosis of brain ischemia by determination of the choline concentration in the blood samples of the upper vena cava and the activity and isoenzyme composition of lactate dehydrogenase (LDH) in the cerebrospinal fluid. The biosensors are based on screen-printed electrodes, which allows the measurements in 20–50 μl samples and low cost analysis.

The developed amperometric biosensor for choline determination is based on the carbon screen-printed electrodes and immobilized choline oxidase. To provide high sensitivity, selectivity and stability of the biosensor, the carbon electrode surface was modified with a cobalt-phthalocyanine mediator and Nafion (1 %)/nanodiamonds (ND) (1 %) composite films. The enzyme choline oxidase (EC 1.1.3.17) from Alkaligenes species with activity 13.1 U/mg was immobilized in BSA gel on the surface of a modified electrode. The proposed biosensor has a wide range of choline definition (0.002 ÷ 2.5 mM) at a low operating potential of 0.4 V, a low detection limit (0.2 μM) and a high stability over a long period of time (74 % of initial activity after 35-day storage).

The high performance nanocomposite sensor was developed for determination of LDH activity by NADH monitoring. The sensing surface required deposition of an aqueous solution of detonation nanodiamonds (agglomerates of 4–5 nm crystalline diamond) together with poly (allylamine hydrochloride), which provided an effective catalytic surface for NADH electrooxidation at Eapp = 0.45V vs. Ag/Ag+ (LOD 0.5 μM, linear range 0–900 μM), enabling the LDH activity detection over the range 0.3–10 U/ml. The assay employed cyclic voltammetry and chronocoulometry in 0.1 M phosphate buffer, pH 7.5 with NAD+ (10 mM)/L-lactate (80 mM) and sample pre-incubation (5 min) at 37 ˚C.

Alpha-E-catenin gene ablation leads to heart failure

V. Balatskyi[1], O. Palchevska[1], L. Macewicz[1], A. M. Gan[2], S. Goncharov[3], P. Pawelec[4], G. Portnichenko[3], T. Lapikova-Bryginska[3], V. Navrulin[2], V. Dosenko[3], P. Dobrzyn[2], O. Piven[1]

[1] Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine;

[2] Laboratory of Molecular Medical Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

[3] Bogomoletz Institute of Physiology of NAS of Ukraine, Kyiv, Ukraine;

[4] Laboratory of Cell Signaling and Metabolic Disorders, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

kostikov1212@gmail.com

Aim. Alpha-catenin links the cadherin-catenin complex to the actin cytoskeleton providing strong intercellular adhesion, but recent data suggest involvement of alpha-catenin to the regulation of signalling pathways. In this study we investigate a role of alpha-E-catenin in the heart during aging. **Methods.** In our work we use the alpha-E-catenin conditional knockout mice mated with the alpha-MHC-Cre transgenic mice. H&E, Oil Red O and van Giesons staining was performed to analyze the histological structure of heart. To determine whether the depletion of αE-catenin had an effect on the adult heart function, we have analyzed the basic cardio-hemodynamic parameters. The expression of genes were analysed with qPCR. Western blot was used to determine the level of proteins. **Results.** The mice with heterozygous and homozygous deletion of the alpha-E-catenin gene died before the 11th months of age. With HE, Oil Red and van Gieson staining we observed fibrosis lesions and lipid accumulation in the mutant hearts. The expression of the fetal genes (ANP, beta-MHC) elevated with the simultaneously decreased expression of alpha-MHC in the mice with heterozygous and homozygous deletion of alpha-E-catenin. Pumping, systolic and diastolic functions of the heart, in both αE-catenin full knockout and αE-catenin haploinsufficient groups were impaired and were clear signs of the heart failure development. The level of active beta-catenin and inhibited GSK3-beta were increased in both groups of the mutant mice, but more pronounced in homozygotes with the alpha-E-catenin deletion. We also observed upregulation of the canonical WNT-signaling target genes. Besides, the expression of a key regulator of lipids catabolism – PPARalpha was downregulated in the heart with alpha-E-catenin missing. The level of AMPK was increased whereas the level of active phospho-AMPK was decreased in both experimental groups of mice. The inhibition of phosphorylation at Ser-79 of acetyl-CoA-carboxylase decreased. In genera, we suggest the suppression of beta-oxidation of fatty acids in the hearts with heterozygous and homozygous deletion of the alpha-E-catenin gene. **Conclusions.** Overall, our data indicate that alpha-E-catenin heterozygous and homozygous deletion leads to the heart tissue abnormality, canonical Wnt signaling activation, violation of lipids metabolism and as a result the development of heart failure and premature mice lethality.

Genome-scale prediction of R-loops reveals their association with complex promoter architectures, G-quadruplexes and transcriptionally active enhancers

V. Bondarenko[1], T. Wongsurawat[2], P. Jenjaroenpun[2], Surya PavanYenamandra[3], V. Kuznetsov[3]

[1] Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine;

[2] Department of Genome and Gene Expression Data Analysis, Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR), Singapore;

[3] Department of Biomedical Informatics, College of Medicine, University of Arkansas for Medical sciences, Little Rock, AR, USA;
vladyslav.bon@gmail.com

R-loops are three-stranded RNA:DNA hybrid structures which can be essential for many normal and pathological processes. Previously, we developed a quantitative model of R-loop forming sequences (RLFS) (called QmRLFS), and predicted more than half a million strand-specific RLFS mainly located in ~75 % genes and their flanking regions, CpG islands and disease-associated genomic loci in the human genome.

Here, we carry out a comprehensive comparative analysis of QmRLFS-predicted RLFS with experimental data and demonstrate a high performance of QmRLFS predictions at the single gene and genome scales. The predicted RLFS are significantly co-localized with the promoter- and enhancer-like chromatin marks and with DNA tertiary structures such as G-quadruplexes, providing evidence for the mechanistic linkage and the existence of functional networks between DNA tertiary structures and R-loops in DNA regulatory regions. Predicted RLFS are also co-localized with transcriptionally active enhancers. The latter is indicative of the potential trans-regulatory functions of RNA:DNA hybrids in the formation of 3D chromatin loops in mammals.

Overall, our study provides a rationale for further discovery and characterization of many other unknown DNA regulatory elements directly or indirectly involved in the formation of the RNA:DNA interactome — the basis for an emerging quantitative R-loop biology and pathology.

Phthalocyanine complexes with axially coordinated ligands as inhibitors of amyloid fibrillization

S. Chernii

Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine;
chernii.sv@gmail.com

Self-assembling of different proteins into the insoluble linear aggregates amyloid fibrils is supposed to be the cause and major evidential symptom of such disorders as neurodegenerative diseases, amyloidoses, prion diseases and type II diabetes mellitus. One of the important approaches to affect such disorders is the discovery of molecules potentially able to prevent or suppress the amyloid formation.

Among the efficient scaffolds against the amyloid fibrils formation, the planar macrocyclic complexes phthalocyanines are suggested. The goal of this work is to study Hf- and Zr-phthalocyanine complexes with spatial geometry for their anti-amyloidogenic activity in the fibrillization reaction. The inhibitory activity of these compounds was assessed using the fluorescent dye-based *in vitro* assay, ultraviolet-visible (UV-VIS) spectroscopy and atomic force microscopy (AFM).

Phthalocyanines have shown the ability to essentially affect the intensity of insulin amyloid fibrillization and to change morphology of the formed aggregates; this effect is strongly determined by the nature of the out-of-plane ligands. We have determined the IC50 value for high-effective inhibitors Hfphthalocyanine bearing pyromelitic acid or Zrphthalocyanine bearing quinoliniumstyryl fragment (3.6 μM and 0.16 μM, respectively); these compounds demonstrate ~90 % suppression of fibrillization according to the fluorescent assay. UV-VIS spectroscopy study revealed that Hfphthalocyanines with a higher tendency to self-association demonstrated a higher activity of the insulin fibrillization inhibition compared to the poorly aggregating metal complexes. In the presence of phthalocyanines, the fibrillization reaction could be redirected to the formation of either small amount of filamentous fibrils with reduced diameter or oligomeric aggregates with diameter of about 3–12 nm. In the presence of Hfphthalocyanine coordinating chlorine atoms, the formation of large species with diameter up to 100 nm, attributed to amorphous aggregates that do not contain beta-sheets, was shown. We suggest that different effects of different phthalocyanine complexes on the protein fibrillization could originate from the nature of out-of-plane ligands, their geometry, charge and thus the ability to interact with aminoacid residues of proteins.

Thus, the phthalocyanine complexes bearing out-of-plane ligands could be proposed as efficient inhibitors of amyloid fibrillization due to their strong potential to suppress fibril formation or the ability to redirect this process.

The study of the series of boron clusters – closo-borates: binding to globular proteins and influence on insulin fibrillization

M. Kuperman

Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine;
mvkuperman@gmail.com

Closo-borates are stable, low-toxic and water soluble high boron clusters. Such boron clusters are among the perspective boron-enriched target agents for boron neutron capture therapy (BNCT) of cancer, they are investigated as pharmacophores in biologically active molecules. Hence, the study of boron clusters, particularly their interaction with globular proteins is of high importance.

For this, we first explored the interactions of hydrogen closo-borates [B10H10]2-, [B12H12]2- and their derivatives (alkyl/aryl substituted and halogen clusters) with globular proteins – bovine/human serum albumins (BSA/HSA), immunoglobulin G, lysozyme, β-lactoglobulin. The fluorescent, circular dichroism (CD) spectroscopy, isothermal titration calorimetry (ITC) were used.

The method of protein fluorescence quenching shows the complex formation between closo-borates and studied proteins. The highest binding affinity was observed in the case of albumins; a quenching effect strongly depends on the nature of closo-borate substituents (up to 20 times for iodine derivatives and BSA). Characterization of albumin complexes by ITC shows a higher binding affinity to albumins (K ~ 104–106 M-1) and a larger number of clusters in complex (4–5 anions) for halogen clusters [B10Hal10]2- (Hal = Cl/Br/I) comparing with the parent closo-borate [B10H10]2- (K ~ 103 M-1, binding ratio 2:1). Thus, due to high binding affinity to serum albumins, the halogen closo-borates are proposed for further studies as the agents for BNCT with a higher "target delivery" potency comparing with the hydrogen closo-borates.

Then the impact of closo-borate [B12H12]2- on insulin fibrillization was studied by fluorescent, CD-spectroscopy and transmission electron microscopy (TEM). CD studies show that during fibrillization the conformation of free insulin changes from α-helix to β-sheet, whereas in the closo-borate presence the early conformational transition from α-helical structure to β-sheet and partially irregular one is observed. At the equilibrium phase, amyloid-sensitive cyanine dye shows a weaker fluorescent response to the insulin fibrillized with [B12H12]2- compared to the free insulin fibrils. TEM data point out fibrils formation in both the closo-borate absence and presence. In free state, insulin forms fibrils bundles consisting of branched fibrils, whereas in the closo-borate presence it forms large unbranched aggregates. Thus, closo-borate could affect the kinetic of insulin fibril formation and fibrils morphology via binding to insulin during fibrillization.

Creation of Gene Expression Database on Preeclampsia-Affected Human Placenta

O. Lykhenko, A. Frolova, M. Obolenska

Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine
lykhenko.olexandr@gmail.com

Publication of the gene expression raw data in public repositories made it possible to reuse these data for a cross-experiment integrative analysis and make new insights into biological phenomena. However, most popular of the present online resources serve as archives rather than ready for immediate access and interpretation databases. The data uploaded by independent contributors are not standardized and sometimes incomplete and need further processing before any further analysis. Hence, the need for a specialized database appears.

Given in this article is the description of the database created after processing a collection of 33 relevant datasets on preeclampsia-affected human placenta. The data processing includes the choice of relevant experiments from ArrayExpress database, the experiment sample attributes standardization according to MeSH term dictionary and Experimental Factor Ontology and the completion of missing data using information from the corresponding articles and authors.

The database of more than 1000 samples contains sufficient sample-wise metadata for them to be arranged into the relevant cross-experiment case-control groups ready for subsequent analysis such as a search for differentially expressed genes or inferring gene networks. Metadata include the information on biological specimen, donor's diagnosis, gestational age, mode of delivery and other sample characteristics.

MiR-15 is predicted to link neuroblastoma and breast cancer by binding to XIST and ANRIL lncRNAs

N. Malyshok, V. Kashuba

Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine;
n.malyshok@gmail.com

Noncoding RNA are known to be crucial molecules with diverse regulatory roles in oncology and neurodegenerative disease. The recent study suggest that lncRNAs ANRIL and XIST participate in the development of neuroblastoma, breast cancer and Alzheimer disease via binding disease-specific microRNAs.

We used LncRNADisease, Lnc2Cancer, HMDD v2.0 and Mir2disease to predict lncRNA- and miR-associated disease. Additionally, we utilize TardetScan and lncRNABase (starBase) v 2.0 to search for lncRNA-miRNA interaction. The disruptions of lncRNA ANRIL expression (also named as CDKN2B-AS, locus CDKN2a/b (INK4/ARF), chromosome 9p21) have been associated with the development of neuroblastoma and Alzheimer disease. We predicted the interactions between ANRIL and microRNA in neuroblastoma and Alzheimer disease. ANRIL can act as a decoy while containing the sequences that mimic miRNA target sites to titer these miRs away from their primary targets thereby act as a molecular sponge. Using TargetScan 7.0, we predicted the target sites for hsa-mir-15-p/16-p/195-p/424-p/497-p/6838-p and hsa-mir-125-5p/4319 in ANRIL 3'UTR. Then, we used HMDD v2.0 and Mir2Disease databases to find a possible involvement of these miRs in Alzheimer disease and neuroblastoma. According to both databases, miR-125 is implicated to Alzheimer disease and miR-15 to neuroblastoma. Since ANRIL participates in the development of both above-mentioned disorders and can have microRNA sponge activity, it may positively regulate miR-125 and miR-15 targets by competing with them for microRNA binding sites thus restoring the expression of target genes.

LncRNA XIST is predicted to interact with miR-15 family microRNAs in breast cancer, whereas Lnc2Cancer showed the participation of ANRIL in the breast cancer molecular networks. This suggests that miR-15 could potentially be involved in the formation of various cancers and link these long non-coding RNAs. Thus, we predicted the target sites for miR-125 and miR-15 in 3'UTR of ANRIL lncRNA that could uncover its possible sponge activity in the development of neuroblastoma and Alzheimer disease. Additionally, we predicted the interaction of miR-15/ANRIL in neuroblastoma and miR-15/XIST in breast cancer where miR-15 can potentially serve as a link between two different cancers. We will further experimentally validate the predicted microRNA sites in ANRIL and XIST 3'UTR.

Hepatoprotective effect of oligoribonucleotides-D-mannitol complexes under thioacetamide-induced hepatotoxicity

T. Marchyshak[1], I. Shmarakov[2], Z. Tkachuk[1]

[1] Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine;

[2] Yuriy Fedkovych Chernivtsi National University, Ukraine;
march-tanya@mail.ru

Therapeutic application of oligonucleotides is a leading trend in the correction of metabolic disorders and related pathologies and is perceived as a unique foundation of innovative biomedicine. The oligoribonucleotides-D-mannitol complexes (ORNs-D-mannitol) display a vast spectrum of biological effects, including cellular metabolism stimulation with activation of endogenous synthesis of regulatory proteins, stimulation of reparation processes. The high biological activity of these complexes promotes studying the hepatoprotective activity under acute hepatotoxicity. The objective of this research was to investigate the hepatoprotective effects of the ORNs-D-mannitol under thioacetamide liver toxicity in mice. It was demonstrated that a dose of 200 mg/kg of these complexes decreases the lesions and inflammatory infiltration of liver parenchyma under thioacetamide-induced hepatotoxicity. The ORNs-D-mannitol attenuated a thioacetamide-induced free radical damage of hepatic biopolymers that is manifested in the reduction of TBA-reactive products, carbonyl derivatives and in the recovery of protein thiol groups, reduced glutathione. Investigating the thioacetamide toxicity in the liver cells we found that the ORNs-D-mannitol reduced the expression of proinflammatory (Il6, Tgf α) and profibrotic (Col1A1, αSma, Tgfβ1) genes at mRNA level by 65, 80, 75, 77 and 87 % respectively in comparison with control thioacetamide.

Thus, the results of this work demonstrate that the ORNs-D-mannitol have has the hepatoprotective effect during the acute liver injury. These complexes attenuate thioacetamide-induced free-radical damage of liver biopolymers and modulate the expression of some genes which are involved in the development of liver damage at the thioacetamide toxicity.

Study on oligoribonucleotides-D-mannitol complexes effects on the influenza A virus H1N1 (A/FM/1/47) infectivity *in vitro* and expression of some host genes under the influenza infection *in vivo*

N. Melnichuk, L. Semernikova, Z. Tkachuk

Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine;
natalia.melnichuk8@gmail.com

The influenza virus (flu) hemagglutinin (HA) mediates both receptor (glycan) binding and membrane fusion for cell entry. The flu infection causes activation of NO synthase II (NOS2), arginase II (Arg2), xanthinoxidase (XO) enzymes involved in the production of free radicals, such as NO., O2.-, ONOO-. Free radicals involved in signal transduction pathways, activate the transcription factors such as NFκB and may lead to the lung tissue damage. Previously, it was found that the oligoribonucleotides-D-mannitol complexes (ORNs-D-mannitol) possess the] antiviral activity against the flu A (H1N1) virus *in vitro* and *in vivo*. However, the mechanisms of the ORNs-D-mannitol anti – flu activity are not clear yet. Current research is aimed at study of the ORNs-D-mannitol effects on theHA-glycan interaction, flu virus infectivity *in vitro* and expression of the nos2, arg2, xdh, nfkbia, nfnb1 genes in the mice lung epithelium cells under flu infection. To achieve this goal we applied the agglutination, TCID50, MTT, one- and two-step RT-PCR assays.

The ORNs-D-mannitol were found to hinder the interaction between HA and glycan. For example, the ORNs-D-mannitol decreased the HA titer of flu virus by 4 times. It was also detected that the ORNs-D-mannitol significantly decreased ($p < 0.05$) the infectious titer of flu virus vs. control flu virus. To validate the antiviral activity of the ORNs-D-mannitol in blocking viral binding and entry into the host cells, the ORNs-D-mannitol were studied and found to inhibit the viral replication by 98 % and to enhance by 94 % the cell viability during the flu infection *in vitro*. In the flu virus-infected mice the overexpression of all investigated genes was detected compared to the healthy ones. The ORNs-D-mannitol injection for prevention reduced the mRNA level of arg2, nos2, xdh, nfkbia, nfkb1 expression by 56, 48, 32, 70, 63 % respectively vs. the virus-infected mice. And the ORNs-D-mannitol injection for treatment reduced the mRNA level of arg2, nos2, xdh, nfkbia, nfkb1 expression by 26, 41, 38, 40, 52 % respectively vs. the virus-infected mice.

Our results show that the ORNs-D-mannitol decrease the flu virus infectivity affecting the HA-glycan interaction *in vitro*. The expression of all investigated host genes is modulated by the ORNs-D-mannitol under the flu A virus infection *in vivo*. It allows us to assume that by modulating the expression of nos2, arg2, xdh, nfkbia, nfkb1 genes, these complexes can protect lung from the free radical damage under the flu infection.

Detection and analysis of the fusion transcripts in prostate cancer

L. Mevs, G. Gerashchenko, E. Rosenberg, V. Kashuba
Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine;
LiliaMevs@meta.ua

Background. The gene fusion, involving an ETS family of transcription factors, is a frequent event in prostate cancer. It triggers the transcriptional programs, activating invasion, induction of DNA breaks and enhancing the ETS oncogenic functions. Therefore, it is important to study the ETS gene fusions for better understanding the prostate carcinogenesis and the patient response to hormone therapy.

Aim. To analyze a possible relationship between the ETS fusion transcripts and clinical characteristics of prostate cancer and to study how the expression of fusion transcripts is associated with the emergence and progression of prostate cancer.

Methods. Quantitative PCR (q-PCR) was used to analyze the expression of 6 fusion transcripts at the mRNA level. The amplified products were checked by gel-electrophoresis and direct sequencing. We analyzed 30 samples of the frozen prostate cancer tissues, obtained by prostatectomy. We studied highly, moderately and low differentiated tumors (Gleason grade was < 7, 7 and > 7, respectively).

Results. The expression of 6 fusion transcripts (TMPRSS2-ERG, TMPRSS2-ETV1a, TMPRSS2-ETV1b, TMPRSS2-ETV4a, TMPRSS2-ETV5b, TMPRSS2-ETV5c) was studied in the prostate cancer samples. Only one fusion transcript (TMPRSS2-ERG) was found and sequenced. This fusion was detected in 14 of 30 prostate cancer samples, i.e. with a frequency of 47 %. No correlation between TMPRSS2-ERG and clinical characteristics of the prostate tumors was found.

Conclusions. Only one out of six reported earlier fusion ETS transcripts was detected in a cohort of 30 Ukrainian patients. The frequency of TMPRSS2-ERG fusion transcript detection was 47 %. The further studies on the fusion transcripts are necessary.

Characteristic of the interaction between nuclear protein hnRNPK and ITSNs and its potential role in the nuclear accumulation of ITSN1

S. Pankivskyi, M. Dergai, O. Novokhatska, A. Rynditch

Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine;
s.pankivskyi@gmail.com

The family of ITSN proteins represents evolutionarily conserved scaffold proteins involved in the regulation of endocytosis, exocytosis, cytoskeleton remodeling and signaling transduction. Functioning of ITSNs in cellular processes is mediated by multiple protein-protein interactions.

Using bioinformatic approaches, it was shown that N-terminal region of ITSNs containing EH domains and a coiled-coil region (CCR) is found in all eukaryotes suggesting an important role of the region in endocytosis. The emergence of other protein binding domains in ITSNs led to the expansion of their partners and cellular functions.

It was previously shown that ITSN1 localized in a cell nucleus and potentially might contribute to the regulation of early response genes expression. However, ITSN proteins do not contain DNA or RNA binding domains, as well as functional nuclear import and export signals, which suggests that the nuclear function and nuclear-cytoplasmic shuttling of ITSN1 are mediated by other proteins.

Analysis of the protein interaction databases demonstrated that 96 potential partners of ITSN protein might localize in the cell nucleus whereas 40 partners possessed known nuclear function. One of such proteins is heterogeneous nucleoriboprotein K (hnRNPK) involved in the regulation of thetranscription, mRNA maturation, and translation, as well as the actin cytoskeleton remodeling and the modulation of signaling cascades.

Using GST-fused SH3 domains of ITSN1 and ITSN2, we have shown that hnRNPK binds ITSN2 SH3D domain but does not bind to ITSN1 SH3 domains. However, it has been found that GST fused hnRNPK binds recombinant protein ITSN1ΔEH but does not interact with full-length ITSN1 and recombinant ITSN1ΔSH3, implying that the interaction between hnNRPK and ITNS1 requires the presence of all SH3 domains whereas EH domains may impair the interaction.

Immunofluorescent analysis of 293 and MCF7 cells demonstrated that overexpression of hnRNPK stimulates nuclear accumulation of ITSN1 whereas NLS and proline-rich region are important for the process. Moreover, the treatment with antibiotic LMB or overexpression of hnNRPK stimulates the nuclear localization of recombinant protein ITNS1ΔSH3 compared to ITSN1ΔEH, which suggests an importance of EH domains in the nuclear localization of ITSN1.

Therefore, the data characterize the hnNRPK-ITSNs interaction and its role in the nuclear accumulation of ITSN1. However, a functional role of the interaction requires further studies.

Discovery of benzylidenebenzofuran-3(2H)-one (Aurones) as inhibitors of human protein kinase CK2

M. Protopopov, V. Vdovin, G. Volynets, V. Bdzhola, S. Yarmoluk

Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine;
mykola.protopopov@gmail.com

Protein kinase CK2 is a pleiotropic, constitutively active serine/threonine protein kinase involved in different cell signaling pathways which regulate proliferation, survival, apoptosis, tRNA and rRNA synthesis and cellular transformation. CK2 is involved in the development of different types of cancer, inflammatory responses, pain and virus infections. Therefore, protein kinase CK2 is an attractive molecular target for the development of small-molecular inhibitors which can be important compounds for pharmaceutical application.

Earlier, we synthesized 51 derivatives of benzylidenebenzofuran-3(2H)-one (aurones). In this study, we have tested these compounds for inhibitory activity toward protein kinase CK2 using *in vitro* kinase assay and optimized their physicochemical parameter LogP. Among tested 51 aurone derivatives we have found 6 compounds inhibiting CK2 with IC50 values of less than 10 nM.

To find CK2 inhibitors with improved logP parameters, a series of novel 88 substituted benzylidenebenzofuran-3(2H)-ones has been synthesized and tested *in vitro* toward human protein kinase CK2. It was revealed that 3 compounds with improved logP parameter inhibit CK2 activity with IC50 values of less than 10 nM. The most active compounds inhibit CK2 with IC50 of 3.5 nM. The structure-activity relationships of 139 aurone derivatives have been studied and binding mode of this chemical class has been predicted.

Enzymes of purine and taurine biosynthesis in human placenta of the first and third trimesters of gestation

S. Ralchenko[1], S. Zubenko[2], R. Sadretdinova[2], B. Olifirov[3], M. Obolenskaya[1]

[1] Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine;

[2] NTUU "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine;

[3] OlesHonchar Dnipropetrovsk National University, Dnipro, Ukraine;
ralchenkos@gmail.com

Background. Biosynthesis is a part of metabolic function of placenta that is yet poorly characterized though it may be a crucial point for the placental and fetal development. Previously, we investigated interrelated metabolic pathways which connect one-carbon unit metabolism, nucleotide synthesis and turnover of sulfur-containing substances. Revealing a significant abundance of mRNAs of GART and ADO we hypothesize the functioning of corresponding enzymatic conversions in human placenta.

The aim of the study was to determine expression of the *GART* gene, involved in purine biosynthesis, expression of the *ADO* gene, involved in taurine synthesis, and enzymatic activity of ADO in placental tissue.

Methods. Protein levels of GART and ADO were determined by western blot analysis. Enzymatic activity of ADO in tissue was assayed by detecting the product of the reaction – hypotaurine. Amounts of hypotaurine and taurine were measured by HPLC-MS.

Results. Proteins corresponding to two different transcripts of the *GART* gene were observed in human placenta. The abundance of both long (GARS-AIRS-GART) and short (GARS) variants was higher in samples from the first trimester than from the third one. The amount of GARS-AIRS-GART decreased more than GARS. Enzymatic activities of GARS, AIRS, GART domains are part of the metabolic pathway of *de novo* purine biosynthesis. GARS and GARS-AIRS-GART proteins are produced from the same gene due to alternative cleavage and polyadenylation of its transcripts. Also, the presence of ADO protein was shown in placenta from different terms of gestation. Its abundance remains at the same level in both cases. Moreover, the activity of ADO in placenta was demonstrated for the first and the third trimesters. It is slightly greater at the beginning of pregnancy. Hypotaurine, the product of reaction catalyzed by ADO, is a precursor of taurine. So, it is likely that taurine can be synthesized in placenta through the pathway of coenzyme A degradation which involves ADO enzyme. Concentration of taurine in placenta was more than two times higher at the end of pregnancy in comparison with early stages of gestation. Taurine has a whole number of physiological functions, including osmoregulation and cytoprotection.

Conclusions. The presence of proteins GARS, GARS-AIRS-GART, ADO and enzymatic activity of ADO in human placenta at early and late gestation indicates respective synthetic pathways in placenta important for the proliferation and growth processes during pregnancy."

Study of interaction between manitol and nucleosides by fluorescent probe

V. Shchodryi[1], O. Kachkovskyi[2], Y. Shaydyk[3], Y Slominskyi[4], Z. Tkachuk[1]

[1] Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine;

[2] Institute of bioorganic chemistry and petrochemistry of NAS of Ukraine, Kyiv, Ukraine;

[3] Institute of physics of NAS of Ukraine, Kyiv, Ukraine;

[4] Institute of organic chemistry of NAS of Ukraine, Kyiv, Ukraine;
shodryj1992@gmail.com

Background. Therapeutic drugs which are based on ribonucleic acid are highly effective, non-toxic and have a wide range of biological effects. In particular, RNA-containing drugs can increase immune reactivity, have antiviral and anti-inflammatory activity and can regulate main metabolic ways in various pathological conditions.

Methods. Linear absorption spectra were obtained with a Specord 210 Plus UV−visible. The steady-state fluorescence and excitation anisotropy spectra were measured with Jasco FP-8200.

Results. We have observed the decrease in the fluorescence intensity of the dye water solution with ribonucleosides; this points to stack interaction between the dye molecule and ribonucleosides base. The increasing concentrations of nucleosides are accompanied by a regular decrease in the fluorescence intensity up to saturation. The decrease in the fluorescence intensity at saturation depends on a nucleoside type and hence it differs for solutions of A, C, U and G. The maximum effect was obtained for the fluorescent sensor solution with riboadenosine.

Additional decrease in intensity was observed in complexes with D – mannitol. The largest difference in the fluorescence intensity was observed in riboadenosine and its complex with D-mannitol. The spectral effect may indicate the interaction between oligonucleotsides and mannitol. Based on quantum-chemical calculations, it is established that the hydrogen bonds between manitol molecule and two centers can be generated. Also, the calculations show appreciable changes in charge distribution in base molecules, especially in the atoms which can generate hydrogen bonds. This can be accompanied by the change in stack interaction between sensor and ribonucleoside molecules.

Conclusion. The spectral study of water solutions of mixture of dye probe with ribonuleosides has shown the stack interaction between the studied molecules. Introducing the manitol in investigated solutions causes the additional spectral effect which points to the interaction between ribonucleosides and mannitol by generating two hydrogen bonds.

Development of amperometric biosensors for the determination of lactate and pyruvate

Y. Topolnikova, D. Knyzhnykova, I. Kucherenko, O. Soldatkin
Institute of Molecular Biology and Genetics of NAS of Ukraine,
Kyiv, Ukraine
topolnyk.ya@gmail.com

Pyruvate and lactate are two of the key molecules in many metabolic pathways, therefore measurement of their concentration is very important for clinical diagnosis. An increase in the lactate level is related to prevalence of anaerobic processes in tissues caused by hypoxia (type A of lactic acidosis) or failure of lactate clearance system – renal and hepatic diseases, poisoning with some toxins, diabetes, *etc.* (type B of lactic acidosis). So, measurement of lactate is useful in clinics for the assessment of the patient's state and prognosis of a probability of shock and death [1]. Lactate-to-pyruvate ratio (LPR) is valuable for the discrimination of two types of lactic acidosis. Nowadays a value of LPR in venous blood is used for diagnosis of inborn dysfunctions of pyruvate dehydrogenase complex and other form of neonatal lactic acidosis; also LPR is helpful for diagnosis of the septic cases [2]. An increased level of pyruvate is observed in case of B1 shortage, arsenic and mercury poisoning, respiratory alkalosis and liver dysfunctions.

Thus, it is a current task to develop the methods of the simultaneous determination of lactate and pyruvate in blood. Biosensors are perspective methods of metabolite measurements, because they combine the high selectivity, rapid response time, and easy analysis, as well as no necessity of a sample preparation.

The aim of the present work is development of the biosensors for determination of lactate and pyruvate. The biosensor consists of enzymes (lactate oxidase and pyruvate oxidase) immobilized onto the surface of amperometric disc electrodes. Different methods of enzyme immobilization have been compared. It shows that lactate oxidase can be immobilized by cross-linking via glutaraldehyde, whereas pyruvate oxidase requires milder conditions like encapsulation in PVA-SbQ. The main analytical characteristics of the biosensor are studied, i.e. reproducibility of responses, linear range, minimal limit of detection.

It is planned to apply the developed biosensors for the lactate and pyruvate determination in blood serum samples.

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2. *Debray F-G, Mitchell GA, Allard P et al.* Diagnostic Accuracy of Blood Lactate-to-Pyruvate Molar Ratio in the Differential Diagnosis of Congenital Lactic Acidosis.*Clin Chem.* 2007;**53**(5):916–921.

Production and characterization of single-chain variable fragment antibodies against interferon beta-1b and interleukin-10

M. Usenko[1], N. Filipenkova[2], O. Gorbatiuk[1,3], O. Okunev[3], V. Kordium[1]

[1] Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine;

[2] Educational and Scientific Center «Institute of Biology and Medicine», Taras Shevchenko National University of Kyiv, Kyiv, Ukraine;

[3] State Institute of Genetic and Regenerative Medicine, NAMS of Ukraine, Kyiv, Ukraine;

usenko.m.alex@gmail.com

Interferon β-1b (IFN-β) is used as the first line treatment of multiple sclerosis (MS), which is the most widespread disabling neurological condition of the working age population around the world. For example, spreading MS in Ukraine increased more than twice during last decades. One of the proved effects of IFN-β longitudinal MS treatment is an increase in the number of the interleukin-10 (IL-10) secreting cells. IL-10 is well-known anti-inflammatory cytokine, playing an essential role in a number of autoimmune and inflammatory diseases. The development of new effective test systems for detection of IL-10 and IFN-β is of current importance.

Single-chain variable fragments (scFv) of antibodies to IFN-β and to IL-10, both fused with alkaline phosphatase (AP), were obtained. ScFv were chosen due to their obvious advantages, such as small size, possibility of easy obtaining in high concentrations in bacterial systems of expression. Besides, they can be genetically fused to the marker protein, that allows using them for one-step immunodetection of biological agents. DNA sequences for fusion proteins synthesis were constructed via gene cloning and PCR amplification. E. coli BL21(DE3) cells were transformed by obtained plasmids pCANTAB-ScFv(IL-10)-AP and pCANTAB-ScFv(IFN-β)-AP for the expression of scFv-AP. Fully functional molecules were obtained in periplasmic fraction of the cells and analyzed by immunochemical methods, being authentically detected in dot blots without additional purification steps. They also withstand longtime freezing in periplasmic extract. It was shown that such fusions of scFv-AP were perspective bifunctional molecules, suitable for detection of corresponding cytokines via ELISA. The scFv-AP can be obtained in preparative amounts by high productive and simple bacterial expression that simplifies the laboratory and industrial production of these immunoreagents, perspective for the development of new test-systems for the detection of IL-10 and IFN-β in different samples.