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Biokemija na Oddelku za biologijo – dobrodošli v svetu toksinov

Biochemistry at the Department of biology – Welcome to the world of toxins

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Nekaj o začetkih in zgodnjem obdobju

Ustanovitelj katedre za biokemijo je bil mednarodno priznani toksinolog in dolgoletni profesor biokemije na Oddelku za biologijo, prof. Dr. Drago Lebez. Na Biotehniški fakulteti Univerze v Ljubljani se je habilitiral kot profesor za biokemijo in postal tudi prvi predstojnik novo ustanovljene Katedre za biokemijo. S tem se je »tradicionalnim« biološkim disciplinam na oddelku za biologijo, pridružila nova razsežnost, preučevanje bioloških procesov na nivoju molekul. Začetki pa so bili zelo skromni, saj je imela katedra na razpolago le en prostor na hodniku 4. nadstropja Filozofske fakultete, kjer je dolga leta gostovala večina Oddelka za biologijo. Tudi opreme v tistem zasilnem lesenem boksu praktično ni bilo. Pa vendar je katedra rastla. Po nekaj letih je katedra dobila nove prostore v pritličju nekdanjega poslopja Biotehniške fakultete na Krekovem trgu 1, kjer je danes Teološka fakulteta. V teh prostorih je katedra doživela pravi razcvet, čeprav so bili prostori še vedno dokaj skromni. To so bili časi, ko smo delali v zasilnih prostorih s skromno opremo, veliko improvizacije in z malo denarja. A vendar je bilo to lepo obdobje, bili smo mladi in polni načrtov. Ob koncu bivanja naše katedre na Krekovem trgu smo se

Some words on the beginnings and the early period

The founder of the Chair of Biochemistry was the internationally renowned toxinologist and a long time professor of biochemistry at the Department of Biology, professor dr. Drago Lebez. He habilitated as a professor of biochemistry at the Biotechnical Faculty at the University of Ljubljana and became the first chairman of the newly established Chair of Biochemistry. The research of biological processes on the molecular level, conducted at the Chair, widened the scope of »traditional« biological disciplines at the Department of Biology. The beginnings were very modest, since the Chair only had one room available on the 4th floor of the Faculty of Arts, which was for many years the host to most of the Department of Biology. There was practically no equipment in that provisional wooden box we called an office, but still the Chair grew. After a couple of years it moved to a new location on the ground floor of the former building of the Biotechnical Faculty on Krekov Trg 1, which today hosts the Faculty of Theology. It was there, that the Chair flourished, even though the place was still pitiable. Those were the days of a provisional location, lacking equipment, a lot of improvisation and little funds.

morali zaradi selitve Biotehniške fakultete pod Rožnik, skupaj z genetiki preseliti nadstropje više. To je bila za nekatere že druga selitev v razmeroma kratkem času. Spet je bilo treba vse razmontirati in ponovno sestaviti, kar smo, kot vedno, opravili večinoma sami. V teh razmerah se seveda ni dalo normalno delati, saj smo bili praktično v zapuščeni hiši in smo le čakali kdaj bo narejen vsaj en trakt v novem biološkem središču kamor smo se namenili preseliti. Vaje smo imeli v nezakurjenih prostorih. Lahko bi rekli, da če si takrat potreboval poskusno žival, si jo lahko počakal kar na hodniku, saj so se po njih veselo podile podgane.

V novo, a skoraj popolnoma nedograjeno stavbo Biološkega središča, smo se vselili leta 1993. Tako smo se spet znašli na gradbišču, v nove prostore smo hodili kar po zidarskih lestvah. Dobro leto kasneje je bil zahodni trakt v celoti končan in življenje se je počasi normaliziralo. Najbrž je razumljivo, da so v vseh teh letih na naši katedri diplomirali številni študentje biologije, mikrobiologije in pedagoških programov. Na katedri so se usposabljali mnogi mladi raziskovalci, ki so kasneje magistrirali ali doktorirali. Pri nas so gostovali tudi tuji študentje in sodelavci. Veseli smo, da so bili vse te kolegice in kolegi z nami in so pripomogli k razvoju in napredku naše katedre ter hkrati tudi vemo, da so se pri nas dobro počutili in jim je bilo kar malo žal, ko so nas morali zapustiti. Ponosni smo, da sta profesorja Maček in Anderluh nosilca Zoisovih priznanj za pomembne raziskovalne dosežke, prof. Sepčičeva je nosilka mednarodne nagrade Daniel Jouvance za mlajše raziskovalce na področju morske biokemije, prof. Turk pa je bil pred kratkim za soavtorstvo gimnazijskega učbenika od »Molekule do celice« imenovan za Prometeja znanosti. Vsi člani katedre pa smo bili najbolj veseli nedavne pohvale s strani vas študentov, ki ste pohvalili naše pedagoško delo. Tega smo se res iskreno razveselili in lepšega plačila za naše delo si ne bi mogli želeti.

Raziskovalno delo

Raziskovalno delo katedre je že od vsega začetka usmerjeno v preučevanje toksinov in ostalih drugih biološko aktivnih snovi, pri čemer

But still it was a beautiful period; we were young and full of plans. At the end of the Chair's stay at Krekov Trg we had to move a floor higher sharing it with the Chair of Genetics**.** For some this was the second move in a relatively short period of time. All had to be dismantled and put back together, which as always we did mostly by ourselves. The student labs were conducted in unheated classrooms. We used to joke that if one needed a test subject, they could find plenty in the hallways that were beset with rats. In such conditions, being situated in a half-abandoned building, normal work was virtually impossible. This left us only with plans to move, but we had to wait for at least one wing of the new biological center to be built.

We moved into the new, virtually unfinished building of the Biological Center in 1993. Again we found ourselves on a construction site, using construction ladders to reach our new premises. A year later the west wing was finally finished and life slowly normalized. Needless to say, in all these years many students from different programs (i.e. biology, microbiology, biology-chemistry, to name just a few) graduated and many young researchers, who later obtained M.Sc. and Ph.D. degrees, were trained at our Chair. Furthermore we hosted many foreign students and fellow researchers. We are happy to have done so, as these colleagues helped us develop and advance our Chair. Not only that, they felt good staying with us and maybe even regretted leaving us.

We are proud of Professor Maček and Professor Anderluh who both hold the Zois award for achievements in science, Professor Sepčič who has received the international Daniel Jouvance award for young researchers in marine biochemistry and Professor Turk who holds the Prometheus award of the Slovenian Science Foundation for the excellence in science communication for his co-authorship of the high school textbook »From the molecule to the cell«. All members of the Chair are honored about the recent praise from their students, which commended our pedagogical work. We are sincerely delighted and we could not wish for a better reward.

so nas večinoma najbolj zanimali toksini iz morskih organizmov, v zadnjih letih pa smo se začeli ozirati tudi po drugih virih. Laboratorij je specializiran in opremljen predvsem za izolacijo in karakterizacijo proteinov, ter za proučevanje njihove interakcije z naravnimi in umetnimi lipidnimi membranami. Biološko aktivne spojine večinoma izoliramo iz morskih nevretenčarjev. Sesilni morski organizmi kot so alge, spužve, ožigalkarji, mahovnjaki in plaščarji nimajo lokomotornega aparata, ki bi jim omogočil beg pred plenilci in lovljenje plena. Razvili so drugo strategijo – proizvodnjo širokega spektra strukturno zanimivih kemičnih snovi z najrazličnejšimi biološkimi učinki (citotoksično in citolitično delovanje, inhibicija rasti bakterij, alg, gliv ali virusov, inhibicija različnih encimov). S temi učinkovinami dodatno preprečujejo naseljevanje drugih organizmov na svojo površino ter bolj ali manj uspešno tekmujejo za prostor. Omenjene snovi so potencialno uporabne tudi za ljudi, npr. v medicini in farmaciji (zdravila) ter v kmetijstvu (npr. protivegetativna sredstva in insekticidi). V našem laboratoriju redno pregledujemo vodne in organske ekstrakte jadranskih morskih nevretenčarjev na vsebnost biološko aktivnih snovi. Morska organizma, ki sta naša »paradna konja«, sta morska vetrnica *Actinia equina* in morska spužva *Reniera sarai*. Iz področja raziskav, ki jih opravljamo na katedri, smo člani in domači ter tuji sodelavci katedre do zdaj objavili okrog 130 znanstvenih člankov, ki so bili objavljeni v mednarodnih revijah z razponom dejavnika vplivnosti od 0,5 do 15. V nadaljevanju bomo navedli le nekatere bolj pomembne izmed njih.

Ekvinatoksin, kakor ga je poimenoval že prof. Drago Lebez, ki je skupaj z dr. Igorjem Ferlanom (1974) objavil prvi članek o tem citolitičnem proteinu, je še vedno zvezda stalnica raziskav citolitičnih proteinov na naši katedri. V bistvu se pod tem imenom skriva več sorodnih in strukturno zelo podobnih proteinov, ki jih lahko izoliramo bodisi iz nematocist bodisi iz celih teles rdeče morske vetrnice (*Actinia equina*) (Maček & Lebez 1988). V strupu morskih vetrnic najdemo več farmakološko aktivnih snovi. Skupino proteinov, ki tvorijo pore, imenujemo aktinoporini. So zelo močni toksini z vrsto farmakoloških učinkov. Najbolj preučena je tvorba pore v naravnih in modelnih lipidnih membranah. Aktinoporine

Research work

The chair's research work has from the start been oriented into studying toxins and other biologically active substances with special interest put on toxins from marine organisms, even though we have in the past few years paid due regard to other sources as well. The lab is specialized and equipped especially for the isolation and characterization of proteins, and for the study of their interaction with natural and artificial lipid membranes. Biologically active compounds are mostly isolated from marine invertebrates. Sessile marine organisms like algae, sponges, cnidarians, bryozoans and tunicates do not have a locomotion apparatus, which would enable them to escape from their predators or catch their pray. They have developed a different strategy – the production of a wide spectrum of interesting chemical substances with very different biological effects (i.e. cytotoxic and cytolytic activity, the inhibition of bacterial growth, the inhibition of different enzymes etc.). These active substances additionally prevent the colonization of other organisms on their surface and allow them a more or less successful competition for their space.

The above mentioned substances are also potentially applicable as pharmaceuticals in medicine and pharmacy, as well as in agriculture (e.g. herbicides and insecticides). In our lab we regularly survey the content of biologically active substances in aqueous and organic extracts of the Adriatic marine invertebrates. We pride ourselves especially in the research of the *Actinia equina* sea anemone and the *Reniera sarai* marine sponge. Based on the research conducted at our Chair, together with our Slovenian and foreign colleagues, the Chair members have published around 130 scientific papers in international journals with the impact factor between 0.5 and 15. Some of the most important publications are presented bellow and listed in the reference section.

Several students of biology, microbiology and pedagogical programs have not only graduated at our Chair, but have also successfully obtained a masters and doctoral degree. The collaboration in numerous Slovenian and international research projects, the assignment of many young researchers and last but not least much praise for our entire pedagogical work, which we have received from

druži skupen mehanizem delovanja, ki poteka v večih stopnjah: vezava topnega monomera na membrano, oligomerizacija, vgradnja v membrano in povezovanje v končno poro, ki je prepustna za majhne molekule. Ekvinatoksinu II, smo v sodelavah najprej določili primarno in sekundarno strukturo (Belmonte & al. 1994), kasneje pa še prostorsko, terciarno zgradbo (Athanasiadis & al. 2001). Kaj kmalu smo ekvinatoksin II tudi klonirali in ga izrazili v heterolognem bakterijskem sistemu (Anderluh & al. 1996). V zadnjih letih poskušamo razumeti interakcijo ekvinatoksina z membranami na molekularnem nivoju. Predvsem nas zanima topologija membransko vezanega toksina, kateri deli toksina sodelujejo pri vezavi in oligomerizaciji in kateri tvorijo membransko poro. Rezultate smo objavili v prestižni reviji Journal of Biological Chemistry, ki je ena od najbolj pomembnih revij na področju biokemije in molekularne biologije (Hong & al. 2002; Anderluh & al. 2003; Malovrh & al. 2003; Kristan & al. 2004; Bakrač & al.2008). Ker se ekvinatoksin specifično veže na membranski lipid sfingomielin, je dobro molekularno orodje za označevanje regij celičnih membran, ki vsebujejo sfingomielin. Za to ga tudi komercialno tržimo v sodelovanju z ameriškim podjetjem Echelon Bioscience. Delo na ekvinatoksinu in drugih citolitičnih proteinih je skupino naredilo prepoznavno tudi v svetovnem merilu. Tako je v letošnjem letu prišlo do najpomembnejše objave skupine, ko je Gregor Anderluh, skupaj s sodelavcem iz Anglije, objavil pregledni članek o toksinih, ki tvorijo pore v lipidnih membranah, v eni od najbolj pomembnih biokemijskih revij Trends in Biochemical Sciences (Anderluh & Lakey 2008).

Vodotopne **polimerne alkilpiridinijeve soli** (poli-APS), smo leta 1994 dokaj slučajno izolirali iz vodnega ekstrakta morske spužve *Reniera sarai*, ki je kazal vrsto zelo zanimivih bioloških aktivnosti (Sepčić & al. 1997). V začetku smo bili prepričani, da gre za protein. Kljub temu, da temu ni bilo tako, smo izolirali strukturno zelo zanimivo polimerno organsko spojino, preučevanje njene strukture in funkcije pa traja in se nadgrajuje še danes. Poli-APS imajo dve izraziti potencialno uporabni aktivnosti: tvorijo pore v lipidnih membranah in ireverzibilno inhibirajo encim acetilholinesterazo, ki je udeležena

our students in the past years, all further prove the success of the Chair's work.

The Equinatoxin, as named by Professor Drago Lebez, which together with Dr. Igor Ferlan published the first article on this cytolytic protein in 1974, has since then been under investigation in our laboratory. The name in essence holds many related and structurally very similar proteins, which can be isolated either from nematocysts or from the whole bodies of the *Actinia equina* sea anemone (Maček & Lebez 1988). Pharmacologically active substances can be found in the venom of sea anemones. The group of pore forming proteins is called actinoporins. These are very strong toxins with a range of pharmacological effects. The formation of the pore in natural and model lipidic membranes is the most studied. What unites the actinoporins is a multi-step common activity mechanism, which includes the binding of the soluble monomer to the membrane, oligomerization, the insertion into the membrane and the formation of the final pore, which is permeable to small molecules. In collaboration with our collaborators we first defined the primary and secondary structure of the Equinatoxin II (Belmonte et. al. 1994), and finally the crystal structure was also determined (Athanasiadis et. al. 2001). Shortly after we cloned and the Equinatoxin II and expressed it in the heterologeus system. For the past few years we have been trying to understand the interaction of the equinatoxin with the membranes on the molecular level. What we are most interested in is the topology of the membrane bound toxin and which of its parts are involved in the binding and the oligomerization (Hong et.al. 2002; Anderluh & al. 2003; Malovrh & al. 2003; Kristan & al. 2004; Bakrač & al. 2008). Results of this studies have been recently published in the Journal of Biological Chemistry, one of the most important journals in biochemistry and molecular biology. Since the Equinatoxin II binds especially to the membrane lipid sphingomyelin, it is an appropriate molecular tool for marking the domains of cell membranes, which contain sphingomyelin. Fot this reason we comercially market it in cooperation with the American company Echelon Bioscience.

Our work on Equinatoxin II and other cytolytic proteins is acclaimed within the international

v prenosu živčnih impulzov v sinapsah (Turk & al., 2007). Inhibitorji acetilholinesteraze v višjih koncentracijah delujejo kot živčni strupi, v manjših odmerkih pa se lahko uporabijo za zdravljenje nekaterih nevromuskularnih bolezni kot so glavkom, miastenija gravis ali Alzheimerjeva bolezen. Afiniteta poli-APS do acetilholiensteraze v kombinaciji z njihovo membransko aktivnostjo se odraža v njihovem selektivnem protitumorskem delovanju na celice pljučnega raka, ki imajo na membranah izražen holinergični sistem (Paleari & al. 2006). V sodelovanju s skupino iz Aberdeena (Škotska) smo ugotovili, da poli-APS tvorijo prehodne pore v membranah sesalskih celic ter omogočajo njihovo stabilno transfekcijo s tujerodno DNA. Zato bi bili poli-APS lahko potencialno uporabni v genski terapiji (McLaggan & al. 2006). Na katedri trenutno podrobneje preučujemo interakcijo poli-APS z umetnimi in naravnimi lipidnimi membranami.

Še eden zanimiv vidik uporabe poli-APS je njihova antivegetativna aktivnost. V sodelovanju s skupino iz Genove (Italija) smo ugotovili, da poli-APS učinkovito zavirajo nastanek bakterijskega biofilma ter pritrjanje ličink raka vitičnjaka (*Balanus amphitrite*) na podvodne površine (Faimali & al.2003). Pritrjanje mikro- in makroorganizmov (predvsem školjk in rakov vitičnjakov) na podvodne površine predstavlja resen problem v industriji in pomorstvu, saj zmanjšuje hitrost plovil in povzroča škodo na potopljenih objektih. Zaščitni antivegetativni premazi, ki se uporabljajo za reševanje tega problema, vsebujejo večinoma aktivne snovi na osnovi kositra in bakra, ki so žal tudi toksične za različne vodne organizme. Evropska skupnost je že delno omejila uporabo premazov, ki vsebujejo kositer. Vse to je sprožilo raziskave na področju iskanja novih naravnih molekul z antivegetativnim učinkom. Raziskave so usmerjene predvsem v izolacijo takih naravnih snovi, ki imajo močne antivegetativne učinke, a le majhno toksičnost, in so dovolj enostavne, da se jih da pridobivati tudi s organsko sintezo. Rezultati inhibicije pritrjevanja in testi toksičnosti nakazujejo, da poli-APS spadajo med take spojine: njihov mehanizem inhibicije pritrjanja je reverzibilen in netoksičen. Vse to opogumlja nadaljnje raziskave tudi v smeri preiskušanja sintetičnih analogov v laboratorijskih poskusih. V sodelovanju s skupino iz Trenta (Italija) in Aberscience community. This leads to the recent success of our principal investigator Gregor Anderluh who, together with his colegue from England, published a review paper in the prestigious Trends in Biochemical Sciences (Anderluh & Lakey 2008). The paper describes the toxins that form pores in lipid membranes.

In 1994, alkylpyridinium polymers (poly-APS) were isolated from aqueous extract of marine sponge *Reniera sarai* which possesses several interesting biological activities (Sepčić & al., 1997). On the first glance it appeared that we were dealing with the protein compound, but later studies revealed a mixture of interesting polymeric organic compounds. The structural and functional studies of these compounds are still our ongoing project. Poly-APS show two particularly interesting activities, they form pores in lipid memebranes and ireversibly inhibit acetylcholine esterase (AChE), which plays an important part in transmition of neuronal signals in cholinergic synapses (Turk & al., 2007). In higher concentrations AChE inhibitors act as neurotoxins, but in smaller doses they can be useful as therapeutic agents for treatment of several neuromuscular disorders (i.g. glaucoma, myasthenia gravis, Alzheimer's disease etc.)

Poly-APS affinity to AChE in combination with their membranolytic activity are promising for selectivelly destroying certain lung cancer cells that express cholinergic system on their membranes (Paleari & al. 2006). In collaboration with a group of scientists from Aberdeen (Scotland) we found out that poly-APS form transient pores in mammalian cell membranes which enables a stable transfection with heterologeus DNA. Therefore, poly-APS might be a useful tool in gene therapy (McLaggan & al. 2006). Currently, we are studying the interaction of poly-APS with artificial lipid membranes and with several cell lines.

Antifouling activity of poly-APS opens another potential use of poly-APS In collaboration with our collegues from Genova (Italy) we found out that poly-APS prevents the formation of bacterial biofilms and attachment of barnacles larvae (*Balanus amphitrite*) to the submerged structures (Faimali & al. 2003). Fouling of micro- and macro organisms on underwater surfaces creates a serious problem in maritime industry an

deena (Škotska) smo nedavno uspeli sintetizirati dimerne in tetramerne analoge poli-APS in jih testirati na protivegetativno aktivnost, razvijamo pa tudi tehnične rešitve vklapljanja poli-APS oziroma sintetičnih analogov v različne premaze za preverjanje učinkovitosti zaščite potopljenih površin v morju (Sepčić & Turk 2006).

V zadnjih letih potekajo obsežne biokemijske in genetske raziskave usmerjene v preučevanje rasti in razvoja komercialno pomembnih užitnih gob. Iz bukovih ostrigarjev (*Pleurotus ostreatus*) in njivnic (*Agrocybe aegerita*) smo izolirali nova proteina **ostreolizin** in **egerolizin**. Oba imata molekulsko maso 15 kDa in sta predstavnika egerolizinske družine proteinov. Specifično se izražata v času tvorbe primordijev in plodnih teles, ter imata verjetno pomembno vlogo v razvojnem krogu gobe (Berne & al., 2002).

Proteina sta v nanomolarnih koncentracijah litična za eritrocite in nekatere celične linije ter toksična za glodalce, če jih vbrizgamo intravenozno (brez skrbi, še naprej uživatje v obari ali rižoti iz ostrigarjev: ostreolizin se hitro inaktivira s kuhanjem in z nizko pH-vrednostjo v želodcu). Ugotovili smo, da se ostreolizin specifično veže na vezikle sestavljene ali iz holesterola in sfingomielina ali holesterola in nasičenih glicerofosfolipidov in jih lizira. V umetnih in naravnih lipidnih membranah takšna kombinacija lipidov obstaja sočasno v tekoči neurejeni in v tekoči urejeni fazi, ki je značilna za lipidne rafte. Rafti so membranske domene z vrsto pomembnih bioloških funkcij (signalizacija, transmembranski transport, vezava in vstop toksinov, patogenov in ostalih ligandov). Rezultati naših raziskav na celičnih linijah z okvarjeno sintezo holesterola ter na umetnih lipidnih mono- in dvoslojih kažejo na to, da ostreolizin specifično prepoznava lipide v tekoči urejeni fazi in se veže na lipidne rafte. Zadnji poskusi s fluorescenčno označenim ostreolizinom nakazujejo na to, da bi protein v sublitični koncentraciji, ali njegova rekombinantna netoksična mutirana oblika, bili lahko uporabni kot molekularno orodje za študij lipidnih raftov, trenutno ene najbolj preučevanih tem na področju biologije membran (Sepčić & al. 2003; Sepčić & al. 2004; Berne & al. 2005).

Poleg teh treh »paradnih konjev«, ki so skoraj stalnica naših raziskav, na katedri preučujemo tudi nekatere druge proteinske molekule, kot sta bak-

transportation. Fouling of ship hulls creates drag, increases fuel consuption and decreases the average speed of the ship. In addition it also creates damage on submerged structures. Nowadays, the main active ingredients in antifouling coatings are based on Zn or Cu compounds that are highly toxic for the marine environment. In addition very effective, but also highly toxic antifouling coatings based on tributyltin (Sn) compounds were already banned from EU markets. It is therefore not surprising that this bustered an intensive research in order to find natural compounds with antifouling activities. The ideal compound should have strong and broad antifouling activity but minimal toxicity. In addition, its structure should be simple enough to obtain large quantities by means of organic synthesis. The laboratory antifouling and toxicity tests of natural poly-APS revealed that they have a great potential and satisfy the majority of requests for an ideal antifouling agent. Result encouraged us to continue testing using synthetic analogues. Several such compounds were recently synthesized in collaboration with organic chemists from Aberdeen (Scotland) and Trento (Italy). We are also trying to find proper technical solutions to incorporate poly-APS in a coating suitable for field experiments (Sepčić & Turk 2006).

Part of our recent recearch is dedicated to the biochemical and genetic studies of growth and development of commercially important fungi. From two species of such edible fungi *Pleurotus ostreatus* and *Agrocybe aegerita* we have isolated ostreolysin and aegerolysin, two new proteins with mol. w. of 15 kDa that belongs to aegerolysin protein family. They are expressed during the development of primordia and are probably important for the fungal fruiting (Berne & al. 2002).

In nanomolar concentrations both proteins are lytic for red blood cells and several other cell lines. They are toxic for rodents if they are applied intravenously (don't worry you can still enjoy this mushrooms in a stew, since cooking and low pH in the stomach destroy their toxic properties). We found out that ostreolysin binds specifically to the lipid vesicles which are composed of cholesterol and sphingomyelin or to the vesicles composed of cholesterol and saturated glycerophospholipids. These type of vesicles were also prone to lysis

terijski protein **listeriolizin** (Bavdek & al. 2007) in človeški obrambni protein **perforin** (Beseničar & al. 2008), ki delujeta, seveda vsak na svoj način in z drugačno vlogo, citolitično.

Infrastrukturni center za površinsko plazmonsko resonanco

Leta 2004 smo v okviru katedre za biokemijo na Oddelku za biologijo Biotehniške fakultete Univerze v Ljubljani ustanovili tudi infrastrukturni center za površinsko plazmonsko resonanco (ang. Surface Plasmon Resonance, SPR), ki je namenjen osnovnim raziskavam interakcije bioloških makromolekul in razvoju analitskih metod za potrebe farmacije, živilske industrije, biotehnologije in varstva okolja. S pomočjo SPR merimo interakcije bioloških makromolekul, katerih relativna molekulska masa je večja od 180 Da. Še zlasti je ta metoda uporabna za ugotavljanje interakcij med biološkimi makromolekulami ali celo med večjimi delci. Na katedri za biokemijo Center uporabljamo pri študiju delovanja citolitičnih proteinskih toksinov, odprt pa je za vse zunanje uporabnike. Tudi s tega področja smo objavili že kar nekaj člankov, prostor pa ne dopušča, da bi navedli vse (Beseničar & al. 2006; Beseničar & al. 2008). Do zdaj so v centru poskuse izvajali raziskovalci iz 15 slovenskih raziskovalnih skupin in tri skupine iz tujine.

Mednarodno sodelovanje

Dandanašnji brez uspešnega domačega in med-narodnega sodelovanja v modernih raziskavah seveda ne gre. Na katedri za biokemijo tako sodelujemo s številnimi domačimi in tujimi ustanovami. S kolegi z Inštituta Jožef Stefan sestavljamo programsko raziskovalno skupino »Toksini in biomembrane«, s številnimi kolegi po svetu pa sodelujemo v posameznih raziskavah, kjer se dopolnjujemo z opremo in ekspertizami na različnih področjih znanosti.

that follows the binding. In artificial and natural membranes such a combination of lipids coexists in liquid disordered and orderd phases. This is the main characteristics of lipid rafts, which are membrane domains responsible for many important biological functions (cell signalization, membrane transport, binding site and entry point for many toxins, pathogens and other ligands) Our experiments using artificial lipid mono- and bilayers or cell lines that lack cholesterol synthesis pathway revealed that ostreolysin specifically recognizes lipids in liquid ordered phase and binds to lipid rafts. Latest experiments using fluorescent labelled ostreolysin indicate that both ostreolysin in sublytic concentration or its nontoxic recombinant mutant could be used as a tool for studying lipid rafts. These are currently one of the »hot spots« in membrane biology research (Sepčić & al. 2003; Sepčić & al. 2004; Berne & al. 2005).

In addition to the above mentioned »celebrities« of our research we have also started to study some other protein molecules that possess membranolytic activities, i.e. bacterial cytolytic protein lysteriolysin (Bavdek & al. 2007) and perforin, a lytic protein which is involved in human immune response (Beseničar & al. 2008).

The Infrastructural Center for Surface Plasmone Resonance

Within the Chair of Biochemistry at the Department of Biology of the Biotechnical Faculty the Infrastructural Center for Surface Plasmon Resonance (SPR) was established in 2004. The Center's aim is basic research of the interaction of biological macromolecules in the development of analytical methods for the use in the pharmaceutical and food industry, biotechnology and environmental protection. With the help of SPR we measure the interaction of biological macromolecules, whose relative molecule mass is larger than 180. This method is especially useful for finding the interactions between biological macromolecules or even larger particles. The Chair of Biochemistry uses the Center to study the activity of cytolytic protein toxins, but the Center is also open to outside users. We have published a sizeable number of papers dealing with this

research area, but unfortunately limited space does not allow us to mention all (Beseničar & al. 2006; Beseničar & al. 2008). So far scientists from 15 Slovenian institutions and three groups from abroad have performed their experiments in the Center.

International cooperation

In the present day modern research is unimaginable without domestic and foreign cooperation. At the Chair of Biochemistry we consequently combine forces with various Slovenian and foreign institutions. We cooperate in the research program team »Toxins and biomembranes« with the colleagues from the Jožef Stefan Institute and with several colleagues around the world in individual research complementing each other with equipment and expertise in different scientific fields.

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