View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Repository of the Academy's Library



# 1. Chiroptical spectroscopic (ECD, VCD) studies

*Chiroptical Laboratory of Structure Analysis (CLSA)*

*(Investigator: Miklós Hollósi)*

A vibrational circular dichroism (VCD) spectrometer was purchased by the CLSA in 2005. The new instrument was installed in 2006. The VCD spectrometer determined the main research field of CLSA. The VCD instruments and softwares allowing ab initio calculations of VCD spectra (e.g. Gaussian 98) made possible broadscale application of VCD spectroscopy in chemistry and biochemistry. The simplest application of VCD is the determination of the optical purity of enantiomers of chiral molecules. However, the most important field of application of VCD spectroscopy is the determination of absolute configuration and absolute conformations in different conditions.

## 1.1 Determination of absolute configuration

VCD spectroscopy was applied first in 2006 for determination of the absolute configuration of one of the enantiomers of cyclic β-lactames obtained as side products of enantioselective lipolase-catalyzed ring opening reactions of racemic cyclic β-lactames [Vass, E., Hollósi, M., Forró, E., Fülöp, F.: Chirality 18 (2006) 773].

Other examples of the determination of absolute configuration are two papers reporting Rumanian-Hungarian joint studies on synthetic products [21,22].

## 1.2 Conformational studies

### *1.2.1 Determination of the conformation of cyclic peptides*

The most successful field of conformational analysis of peptides is the determination of the conformation of cyclic peptides. Relative to linear peptides with the same number of amino acids, the conformer population of cyclic peptides is limited, that simplifies the theoretical calculation of experimental VCD spectra. For an early summary of the VCD spectroscopy of cyclic peptides see the review article published in 2003 [Vass, E., Hollósi, M., Besson, F., Buchet, R.: Chem. Rev. 103 (2003) 1917].

A recent application of VCD spectroscopy is the conformational analysis of the antamanid-analogue cyclic peptide *cyclo*($Pro_2$-Gly-$Pro_2$-Gly). In DMSO it has a unique structure: two consecutive Pro residues are linked by *cis* peptide bonds (Gly-Pro and Pro-Pro). The other half of the molecule features an unusual Gly-Pro-Pro-Gly type I β-turn. This structure based on NMR spectroscopy was also confirmed by X-ray crystallography.

The VCD spectroscopy of the molecule was also studied in DMSO-d6 solution [unpublished results]. There is a very good agreement with the calculated spectra of the $A_2$ conformer at the B3LYP/6-31g* level of theory, using a polarizable continuum solvent model for DMSO. The positive couplet-like feature appearing at lower wavenumber, composed of a positive band at 1635 $cm^{-1}$ and a negative band at 1650 $cm^{-1}$, is mostly due to vibrations of the amide groups of the amino acid residues forming the β-turn structure.

Our knowledge of the VCD spectroscopy of γ-turns is limited. Representatives of γ-turn models are cyclic tetrapeptides which cannot form β-turns. We have synthesized cyclic tetrapeptides comprising two β-alanine residues. VCD spectroscopic studies were also performed [35]. VCD spectroscopy, in agreement with theoretical calculations revealed that low frequency amide I vibrations (at ~1630 $cm^{-1}$ or below) are indicative of a $C_7$ H-bonded inverse γ-turn with Pro in position 2, while γ-turns encompassing Ala absorb at higher frequency (above 1645 $cm^{-1}$).

The increasing interest in *peptidomimetics* prompted us to study the VCD properties of cyclic peptides comprising cyclic β-aminoacids. In collaboration with Professor Sewald at University Bielefeld (Germany) we studied the VCD spectroscopy and conformation of a series of cyclic peptides comprising *trans*-2-aminocyclohexane carboxylic acid (Achc) or *trans*-2-aminocyclopentane carboxylic acid (Acpc). These data were compared to the conformational information obtained by ECD and VCD spectroscopy. Experimental VCD spectra were compared to theoretical VCD spectra computed quantum chemically at B3LYP/6-31G(d) density functional theory (DFT) level. The good agreement between the structural features derived from the VCD spectra and the NMR-based structures underlines the applicability of VCD in studying the conformation of small cyclic peptides [36]. The cyclic models with *trans* β-aminoacids were prepared in Bielefeld together with cyclic peptides comprising *cis*-Achc or *cis*-Acpc. Contrary to the trans-models, there is a significant difference between the measured and calculated VCD spectra of *cis*-Achc and *cis*-Acpc derivatives. This can be the consequence of the structural diversity of the *cis*-models VCD calculation of *cis* models is in progress.

*1.2.2 Conformational studies on linear models*

The simplest linear representatives of γ-turn models are N-acetyl-aminoacid-N'-methylamides (Ac-Aaa-$NHCH_3$). The corresponding pseudo-γ-turn models are N-acetyl-β-homoaminoacid-N'-methylamides (Ac-β-homoAaa-$NHCH_3$). In these models the γ-turn structures are replaced by pseudo-γ-turns which may contain $C_8$ H-bonding. Decker Majer and coworkers in CLSA started to synthesize γ-turn model compounds (Aaa = Pro, Ala, Phe, Val) and the corresponding pseudo-γ-turn models (β-homo-Aaa = Pro, Ala, Phe, Val). They also performed ECD and VCD spectroscopic studies. In collaboration with CLSA Tarczay and coworkers (Laboratory of Molecular Spectroscopy, Eötvös University) started IR and VCD spectroscopic studies of Ac-β-homoPro-$NHCH_3$ in cryogenic matrices and in solution [24]. In comparison of the measured and calculated VCD spectra three different *trans*-pseudo-γ-turn conformers and one *cis*-conformer can be identified at low temperature in Ar or Kr matrix. Contrary to Ac-Pro-$NHCH_3$, the content of the *cis*-conformer is >10%. In polar solution the *cis*-conformer becomes dominant. Based on the results the tendency of the formation of pseudo-γ-turn is smaller than the adoption of γ-turn in Ac-Pro-$NHCH_3$. The presence of C8 H-bonding can be identified in three conformers.

**1.3 Studies on chiral dirhodium complexes by ECD and VCD spectroscopy**

There is an increasing interest in dinuclear rhodium(II) complexes because of their chemical reactivity, high catalytic activity in many reactions and potential application as anticancer agents. We succeeded in preparing a series of chiral dirhodium complexes containing N-benzyloxycarbonyl-L-phenylalanine (Z-Phe-O) in equatorial position [$Rh_2$(O-Phe-Z)$_n$(OAc)$_{4-n}$, n=1-4]. Recently we reported the synthesis of a $Rh_2L_1$ complex comprising N-acetyl-L-phenylalanine (Ac-Phe-$O^-$) ligand and comparative ECD and VCD spectroscopic studies on the $Rh_2$(O-Phe-Z)$_1$(O-Ac)$_3$ and $Rh_2$(O-Phe-Ac)$_1$(O-Ac)$_3$ complexes (abbreviated as $RH_2Z_1$ and $Rh_2Ac_1$, respectively) [34]. Until recently, VCD spectroscopy has not yet been used for conformational studies of chiral dinuclear rhodium complexes. Calculations of the VCD spectra were performed at *ab initio* (DFT) level of theory using Gaussian 03 [B3LYP functional combined with the LANL2DZ basis set for the dirhodium core and the 6-31G(d) basis set for other atoms]. The

population-weighted sums of the computed VCD spectra of the conformers are in excellent agreement with the experimental VCD spectra. The combination of the VCD and ECD spectroscopic methods led us to the structural characterization of the complexes.

Publications in international journals: **7** (July 1, 2009 – December 31, 2010).

## 2. Protein NMR and protein modeling

*Laboratory of Structural Chemistry and Biology and Protein Modeling Group*
*(Investigator: András Perczel)*

### 2.1 Computational work on peptides and prioetin fragments

Collagen is a triple helical protein, highly hydrated in nature. Water molecules form bridges of different length around the POG repeats and self assemble into left-handed helical water threads. To explore the stability of these specifically hydrated places, we have designed suitable QM models: each comprises a triple helix formed by 18 residues surrounded by 8 to 12 explicit waters [10,28]. Two sets of amino acids were incorporated, i) standing for the core structural subunit of tropocollagen (POG-model) and one ii) for its natural enzyme recognition sites (AAG-model). We have determined the stability order of the water binding places. For example the strongest being -8.1 kcal mol(-1), while the weakest -6.1 kcal mol(-1) per hydrogen bond. In X-ray structures, each triplet of tropocollagen is shielded by six to nine water molecules. Furthermore, we have found that beside the mandatory six, the "surplus" three water molecules further strengthen the binding of all the others.

On the structure and stability of sheet-like conformers of beta-peptides we have computed stable structures never seen before [30]. Single- and double-stranded structures were analyzed, and the seeds of large beta-layers and biocompatible nanomaterials were described. Both the mono- di and oligomeric forms of beta-alanine supramolecular complexes were evaluated by using the adequate M052X/6-31G(d) level of theory for peptides of this size. Sheet structures built up of strands with carbonyl groups monotonically facing the same spatial direction, polar strands, were previously assigned and synthesized by Seebach and coworkers. Now we have presented a novel beta-peptide sheet structure of alternating carbonyl group orientations, called as apolar strands. These

novel secondary structural elements of beta-peptides are structural analogs of beta-pleated sheets of proteins. Interestingly enough, the latter type of apolar strands are foreseen as very stable supramolecular complexes and are more firm by approximately 10 kcal.mol-1 than the aforementioned polar strands. Furthermore, apolar strands lack the inherent twisting of beta-layers, present in polar strands resulting in the tubular shape.

**2.2 NMR studies on proteins**

The modular C1r protein is the first protease activated in the classical complement pathway, a key component of innate immunity. Activation of the heteropentameric C1 complex, possibly accompanied by major intersubunit re-arrangements besides proteolytic cleavage, requires targeted regulation of flexibility within the context of the intramolecular and intermolecular interaction networks of the complex. We have expressed and purified the two complement control protein (CCP) modules, CCP1 and CCP2, of C1r in their free form, as well as their tandemlinked construct, CCP1CCP2, to characterize their solution structure, conformational dynamics and cooperativity [26,27]. The structures derived from NMR signal dispersion and secondary chemical shifts were in good agreement with those obtained by X-ray crystallography. Internal mobility of the modules, especially that of CCP1, exhibited considerable changes accompanied by interfacial chemical shift alterations upon the attachment of the C-terminal CCP2 domain. Our NMR data suggest that in terms of folding, stability and dynamics, CCP1 is heavily dependent on the presence of its neighboring modules in intact C1r. Therefore, CCP1 could be a focal interaction point, capable of transmitting information towards its neighboring modules.

Complement control protein modules (CCP) typically mediate protein-protein interaction during immune response in vertebrates. Using NMR chemical shift perturbation mapping, we present previously lacking experimental evidence for intermolecular interactions between the CCP1 and CCP2 modules of the human C1r serine protease (SP). The identified interface is clearly distinct from that observed in the covalently linked CCP1-CCP2 pair. Structural models of the CCP1-CCP2-SP segments of two C1r molecules built on the basis of shift perturbation data are fully consistent with an extended interaction interface and suggests the possibility of a structural rearrangement as a switch between functional states of human C1r.

Among biomolecules, proteins fulfill the most diverse roles. Recent advances in NMR spectroscopy demonstrate that these molecules can be characterized by motions on a wide range of time scales, and besides their three-dimensional (3D) structures, dynamics is also fundamental to truly understand their biological activity. This review focuses on NMR techniques suitable to assess the internal dynamics of proteins from picoseconds to minutes [23]. After a brief introduction of protein motions and different techniques used to capture them, we have described the dynamical aspects of a diverse set of NMR observables in detail. We also present some examples of investigations on various systems aimed to understand Dynamics– Structure–Activity Relationships (DSARs) of proteins.

Publications in international journals: 7 (July 1, 2009 – December 31, 2010).

## 3. Protein X-ray crystallography

*Laboratory of X-ray crystallography*

*(Investigator. Gábor Náray-Szabó)*

Prolyl oligopeptidase (POP), a cytosolic serine protease, degrades a variety of proline-containing oligopeptides by cleaving the peptide bond on the carboxy side of proline residues. It degrades neuropeptides involved in the processes of memory and learning. Inhibitors of POP are potential therapeutic agents in the treatment for dysfunction of the memory system.

Earlier we solved the crystal structures of POP in complex with three N-acyl-pro-pyrrolidine-type, inhibitors of nanomolar activities [5]. These molecules bind to the S1-S3 substrate binding subsites of the enzyme. The crystal structures helped us to understand the alteration in the binding affinity at the S3 site as a function of the size, shape and polarity of the N-terminal group of the inhibitors. Inhibitors with flat ring systems are stacked on the bottom of the S3 site. For these inhibitors, the optimal linker chain length is three C-C bonds. Insertion of an imido moiety into the junction of the ring system allows hydrogen bond formation with water molecules of the first solvent shell of the enzyme, indicated by a shift of the ring position in the complex as compared with the hydrophobic benzyloxycarbonyl group. The optimal length of the linker chain increases to four C-C bonds if there is a bulky group in the terminal position. Molecular dynamics

calculations indicate that this is due to the better fit into the binding pocket. There is notably good agreement between the calculated and experimental free energies of binding; the average error in the $IC_{50}$ values is around 1 order of magnitude. A 4-fold enhancement of the inhibitor activity upon replacement of the 4-$CH_2$ group of the proline ring by $CF_2$ is a consequence of a weak hydrogen bond formed between the fluorine atom and the host enzyme.

Calmodulin (CaM), a ubiquitous protein, is present in all eukaryotic cells. It modulates the action of over 100 proteins in a $Ca^{2+}$ dependent manner. Analyzing the structural and functional information of its complexes with various binding targets shed light to the basis of its ability to interact with such different peptides: (i) its central region is not only a flexible spacer between the main binding sites, but it can participate in specific binding itself; (ii) methionines of the main binding pockets facilitate adaptation to ligands of various size, shape and polarity. Upon $Ca^{2+}$ binding CaM undergoes a major conformational change effecting the target binding sites. Novel models propose that characteristically distinct behaviour of CaM towards its variety of targets is based on its target-specific $Ca^{2+}$ affinity beside the diversity of CaM-target interfaces. By critical assessment of elements of the binding sites of various target peptides as well as small molecule ligands we outlined the possibilities of drug design strategies [19].

We characterized structural aspects of molecular recognition in the terms of steric, electrostatic and hydrophobic aspects of the host/guest interaction [15]. Various protein/ligand systems were shown as examples where host/guest complementarity and similarity within a group of guest molecules where determined in terms of one or more of these aspects. Steric fit can be illustrated by the complex of acylaminoacyl peptidase with product-like peptide inhibitors occupying the S1-S3 substrate binding subsites of this serine protease. Heparin potentiation of C1 inhibitor, a serine protease inhibitor in serum can be explained by a novel mechanism based on neutralization of positively charged contact regions of the protease/inhibitor complex by binding polyanions like heparin. It is interesting to compare the steric and hydrophobic aspects of protein/ligand interactions in the highly adaptable binding region of calmodulin and the conformationally more conservative S1-S3 sites of prolyl oligopeptidase [5,19].

Further investigating serine proteases we defined a simple method of structural characterization of any Ser-His-Asp (or Ser-His-Glu) triads in protein structures by determining the configuration of four spatial points of the triad [16]. The method was used for screening the Protein Data Bank. We found that the geometric properties of just these four points of the enzymes are characteristic to their family.

Aromatic interactions are well-known players in molecular recognition but their catalytic role in biological systems is less documented. We studied the effect of the conserved aromatic stacking interaction between dUTPase and its nucleotide substrate on the mechanism of hydrolysis [29]. Our crystal structures with three variants of residue 145 (His: native, Trp and Ala) of *Mycobacterium tuberculosis* enzyme revealed, that (i) the geometry of the face-to-face offset stacking interaction between the substrate uracyl moiety and dUTPase is conserved; (ii) the aromatic residue involved in this interaction is interchangeable with another aromatic residue without structural and/or enzymatic alterations; (iii) loss of the π-π interaction does not affect the ground-state active-site conformation in the alanine mutant. Our kinetics studies revealed that loss of the aromatic side chain results in a significant 20–30-fold decrease in the rate constant of the chemical step. Using combined approaches of crystallography, kinetics, optical spectroscopy and thermodynamics calculations we concluded that the stabilization effect of the reactants complex by the aromatic residue is weak and it is increased in the transition state. As dUTPase does not go through large conformational changes during its enzymatic cycle, its catalytic effect is likely due to long-range electrostatic stabilization and/or by geometry optimization of the transition state. This report shows that one of the important contributions to this electrostatic effect comes from the investigated aromatic interaction. The abundance of similarly positioned aromatic interactions in various nucleotide hydrolyzing enzymes (e.g. most families of ATPases) raises the possibility of the reported phenomenon being a general component of the enzymatic catalysis of phosphate ester hydrolysis.

Acylaminoacyl peptidase (AAP) is a further member of the prolyl oligopeptidase family of serine peptidases. The size selectivity of the enzymes of the family is achieved by the fact that the active site is located in a cavity between the hydrolase and β-propeller domains of the enzyme. Some of these peptidases have closed structure so that the two

domains must be opened up to allow the substrate to access the active site. This open state however is inactive due to distortions of the catalytic triad. The only example with stable open conformation so far is dipeptidyl peptidase IV. We solved the crystal structure of *Pyrococcus horikoshii* AAP a hexameric enzyme [V. Harmat, B. Hornung, É. Tichy-Rács, A. Kiss-Szemán, Z. Szeltner, A.L. Kiss, K. Domokos, G. Náray-Szabó, L. Polgár: Acylaminoacyl Peptidase from Pyrococcus horikoshii Displays Unique Hexameric Structure Associated with a Distinctive Mechanism for Substrate Selectivity. In preparation].

The structure shows stable open global conformation, owing to two unusually long extensions of the propeller domain, which contacts the hydrolase domain of the neighbouring molecule in the hexamer and stabilizes the global open state and active conformation of the enzyme. The substrate can access the active sites through channels within the hexamer ensuring size selectivity.

Publications in international journals: 4 (July 1, 2009 – December 31, 2010).

## 4. Biochemical studies

Department of Biochemistry

*(Investigator: László Nyitray)*

### 4.1 Structural stability of myosin-2 coiled coil domains and regulation of the motor

We have previously determined the first high-resolution structure of any myosin coiled coil domain, namely the proximal S2 from scallop muscle myosin-2, a model system to study $Ca^{2+}$-regulation of the myosin motor protein.

During this project we extended this work and determined the structure of the same scallop peptide in three additional crystal environments and were able to visualize the entire N-terminus in one chain of the dimeric peptide. We have also compared the melting temperatures of this scallop S2 peptide with those of analogous peptides from three other isoforms. Taken together, these experiments, along with examination of sequences, point to a diminished stability of the N-terminal region of S2 in regulated myosins, compared with those myosins whose regulation is thin filament linked. It seems plain that this isoform-specific instability promotes the off-state conformation of the heads in regulated myosins [1].

### 4.2 Salt-bridge stabilized single stranded α-helix: a novel protein structural motif

The tail domain of myosin-6 contains a highly-charged 60 residue long sequence which was predicted to form a coiled coil structure. We have found that region forms a highly stable single stranded α-helix instead of coiled coil. A few highly charged natural peptide sequences were also α-helical structures in water. During this project we have shown show that these sequences represent a novel structural motif called ‘‘charged single a-helix’’ (CSAH). We developed two conceptually different computational methods capable of scanning large databases: SCAN4CSAH and FT_CHARGE. Using the consensus of the two approaches, a remarkable number of proteins were found to contain putative CSAH domains. The main functional features of CSAH motifs include the formation of relatively rigid spacer/connector segments between functional domains, extension of the lever arm in myosin motors and mediation of transient interactions by promoting dimerization [20].

A thorough analysis of cross-predictions between CSAH, coiled-coil and disordered protein segments using various prediction algorithms. We proposed that the simultaneous use of the programs COILS, IUPRED and CSAH predictors to achieve acceptable prediction accuracy and minimize the extent of cross-predictions. The relevance of observed cross-predictions might be that disordered sequences can adopt coiled-coil conformation relatively easily during protein evolution [33].

We have performed microsecond classical molecular dynamics simulations of five naturally occurring and two synthetic charged single α-helices (CSAHs) in aqueous solution. We found that naturally occurring and synthetic CSAHs possess similar structural and dynamic characteristics. the structural and dynamic properties of CSAHs arise from a combination of short- and long-range electrostatic interactions, in contrast with early propositions which suggested that salt bridges dominate the stabilization mechanism of CSAHs.

### 4.3 Structural, kinetic and thermodynamic studies of the LC8 dynein light chain hub protein

LC8 dynein light chain (DYNLL) is a highly conserved eukaryotic hub protein with dozens of binding partners and various functions beyond being a subunit of dynein and myosin 5a motor proteins. We have compared the kinetic and thermodynamic

parameters of binding of both mammalian isoforms. Peptides containing either of the above motifs bind to DYNLL2 with micromolar affinity, whereas a myosin 5a peptide and the noncanonical Pak1 peptide bind with Kd values of 9 and 40 μM, respectively. Binding of the KXTQTX motif is enthalpy-driven, although that of all other peptides is both enthalpy- and entropy-driven. Compared with monovalent ligands, a significant avidity effect was found (e.g. Kd values of 37 nM for a dimeric myosin 5a fragment). Ligand binding kinetics of DYNLL can best be described by a conformational selection model consisting of a slow isomerization and a rapid binding step. We conclude that the thermodynamic and kinetic fine-tuning of binding of various ligands to DYNLL could have physiological relevance [31].

To better characterize the DYNLL binding motif we have studied its binding preference by an in vitro evolution method, phage display. The peptides were presented in bivalent manner fused to a leucine zipper. The phage-selected consensus resembles the natural one, but is extended by an additional N-terminal valine, which increases the affinity in a bivalent format into a subnanomolar $K_d$ range. We elucidated the affinity enhancing role of valine by comparing the crystal structures of two complexes. Based on the in vitro evolved sequence pattern we predict a large number of novel DYNLL binding partners in the human proteome. Based on NMR spectroscopic and X-ray diffraction studies we have shown that the DYNLL binding domain of myosin 5a is intrinsically disordered and its binding to DYNLL includes additional contacts compared to previously determined DYNLL-partner complexes [32].

Publications in international journals: 4 (July 1, 2009 – December 31, 2010).

## 5. Immunological studies

*Department of Immunology*

*(Investigator: Gabriella Sármay)*

### 5.1 Design, synthesis and biological function of phosphopeptide mimotopes disrupting intracellular signaling

SHP-2 protein tyrosine phosphatase is a „double edged sword", it may inhibit signal transduction by dephosphorylating certain signaling proteins, and may activate signaling by dephosphorylating thus inactivating negative regulatory proteins. Increased

activity of SHP-2 is described in many tumors. Phosphopeptides binding to SHP-2 SH2 domains may activate or inhibit phosphatase activity, therefore in order to design specific modulators, it is important first to reveal the exact structure of SH2 domain-phosphopeptide complexes. The aim of our work was to isolate recombinant single or double SH2 domains of SHP-2 phosphatase for structural studies.

*Production and purification of recombinant SH2 domains of SHP-2.*

The domains were multiplied by phusion enzyme upon 35 cycles, and the products were digested by Not I-el and BamH I. The purity was checked by agar gel-electrophoresis . The success of PCR and the digest were controlled. The PCR products were ligated into pBH4 vector, and then transformed in XL1-Blue cells. The constructs were transformed into Rosetta BL-41 cells. After the expression of the protein the cells were frozen then sonicated to release proteins, which were then purified by Ni-affinity chromatography, followed by FPLC on HiTrap Q columns. The proteins obtained were checked for purity by gel electrophoresis.

*Determination of binding affinity constant $K_D$ of the phosphopeptide corresponding to a sequence in the Grb2 associated binder (Gab1), an important adaptor protein, to SH2 domains.*

The quality and quantity of the purified SH2 domains was sufficient for affinity measurements. The affinity constant was determined by fluorescence polarization technique. The Gab-1 phosphopeptide (GDKQVEY(p)LDLDLD(NH2)) bound to the C+N terminal double SH2 domains with a Kd= 0.2823± 0.044 μM.

Further plans: isolation of the single SH2 domains in sufficient quantity, identification of the peptide-binding site by NMR. Unfortunately we had unexpected difficulties, it took a long time to isolate the proteins, thus NMR studies are not completed yet.

**5.2 Identification of cytoplasmic proteins that bind to the phosphopeptides**

The following phosphopeptides were tested:

**Oct-R8C-GDLDpe (phosphoester)**:Octanoyl-RRRRRRRR-C-GDKQVEY(p)LDLDLD(NH2)

**Oct-R8C-GDLDsp (tiophosphate)**: Octanoyl-RRRRRRRR-C- GDKQVEY(sp)LDLDLD (NH2)

**Oct-R8C-ELPN(pe) (phosphoester)**: Octanoyl-RRRRRRRR-C-ELDENY(p)VPMNPN (NH2)

We have found earlier by SPR (Surface Plasmon Resonance) methods that GDLDpe (Y627) bound to SHP-2 with high affinity, and it was also shown that the sequence of ELPNpe (Y447) bound to SH2 domain of PI3-kinase.

Next we examined whether the two phosphopeptides and shortened or modified (tioophosphate) versions of GDLD may bind to cytoplasmic proteins. The interacting proteins were affinity purified by a "pull down" assay, and the associated protein was identified. In accord with our previous experiments all short peptides bound to PLCγ, while only the 10 amino acid long peptide bound to SHP-2 phosphatase. On the other hand, this DKQVEY(p)LDLDLD peptide was not able to activate SHP-2 phosphatase, thus it might be able to compete with the natural substrate of SHP-2 without activating the phosphatase.

Mass spectroscopy data confirmed the results of Western blot experiments, and have shown that both GDLDpe and its modified, tiophosphate version, GDLDsp bound equally well to SHP-2. In addition, GDLDpe also binds to PLCγ, while GDLDsp is not.

Functional studies with phosphopeptides binding to SH2 domains

Previous experiment have shown that the cell membrane permeable carrier peptides composed of octanoyl acids and eight Arg (OR8) is able to transport peptides bound to them into the cells. Thus OR8--GDLDpe, - GDLDsp and –ELPNpe when transported into B-cells blocked BCR-induced Erk phosphorylation as detected by Western blot. OR8-ELPNpe also blocked Akt phosphorylation.

DT40 chicken B-cell line is a general model to study gene function. We compared the effect of cell permeable phosphopeptides on Erk and Akt activation using SHP-2 -/- DT40 cells. As in the previous experiments the cells were pretreated with cell membrane permeable phosphopeptides for 60 min, and then activated by anti-chicken IgG. This experiment proved that the inhibitory effect of the GDLD phosphopeptides was depending on the presence of SHP-2, while that of ELPNpe was not.

Based on these experiments we suggest that the cell membrane permeable Gab1 phosphopeptides may modulate the early steps of survival and growth signals at the level of Akt and Erk. Phosphopeptides may regulate cell's function on different ways, activating/inhibiting the SHP-2, or competing with its substrate. To understand the

mechanism of the phosphopeptide mediated modulation we have to carry out more experiments with short analogs, or modified version of the peptide.

**5.3. Structural studies on biotinylated collagen epitope peptide (ARGLTGRPGDA), extravidin-peptide complex and extravidin; comparison by CD spectroscopy**

The collagen induced arthritis (CIA) is a well accepted model of the human autoimmune disease, rheumatoid arthritis. Our aim was to investigate the effect of small artificial immune complexes composed of arthritogenic peptide epitope of collagen and a single chain antibody fragment (scFv) of the FcgRII/III specific monoclonal, 2.4G2 on the onset and severity of CIA. To form complexes we used biotinylated epitope peptide and scFv linked by extravidin.

First we characterized the collagen peptide, the extravidin and the complex of the two. The collagen peptide showed the curve typical for collagen.

We compared the CD spectrum of extravidin and extravidin-peptide complex.

The amount of peptide present in the complexes cannot explain the differences we have seen between the spetrum of extravidin and extravidin-peptide complexes.

The differential spectrum shows that there was a slight change in the structure of the protein as a result of peptide binding, the amount of ordered structures at 225 nm (mostly probably beta turns) increased. These data indicate that the binding of the collagen peptide to extravidine has a stabilizing effect on the structure.

The results of functional studies are submitted for publication, and have shown that the complexes induced peptide specific antibody synthesis, aggravate the disease, and stimulated an increased cytokine/chemokine secretion. The results contribute to the understanding of the pathomechanism of CIA.

(E. Szarka[1]*, Zs. Neer[1]*, M. Adori[1], A. Angyal[1], J. Prechl[2], E. Kiss[1,2], Zs. Barad[1], D. Kövesdi[1], P. Balogh[3], and G. Sármay[1,2]

Small complexes composed of the arthritogenic peptide epitope of collagen and a single chain Fv fragment of an Fcγ receptor II/III-specific monoclonal antibody modulate collagen induced arthritis via inducing cytokine/chemokine release *Arthritis research and therapy*. Submitted for publication)

Publication in international journals: 1 (January 1, 2009 – December 31, 2010).

**All publications of the NI OTKA project between July 1, 2007 and December 31, 2010: 36**