Oral Presentation Abstracts

Lectures

PL

COMBINATORIAL APPROACHES AND SCREENING FOR T–CELL SUPERAGONISTS AND ANTAGONISTS

Günther Jung[1], Karl–Heinz Wiesmüller[2], and Burkhard Fleckenstein[3]

[1]Institute of Organic Chemistry, University of Tübingen, 72076 Tübingen, GERMANY
[2]EMC microcollections GmbH, 72070 Tübingen, GERMANY
[3]Institute of Immunology, University of Oslo, Rikshospitalet, 0027 Oslo, NORWAY

Synthetic peptide libraries represent excellent tools in elucidating the molecular interactions in T cell mediated immune response. Synthetic concepts and bioassays were developed to describe peptide–MHC–TCR interactions in a quantitative way. Complete experimental data sets of HLA–ligand motifs and T–cell recognition patterns from combinatorial peptide libraries serve as molecular basis for the development of synthetic vaccines, T–cell superagonists and peptidomimetic antagonists. Patient–specific peptides, peptidomimetics and vaccines of highest reactivity can be derived directly from the data sets via new prediction programs. The resulting lead structures can be developed to diagnostic tools and therapeutic for the treatment of viral infections, autoimmune diseases and cancer.

The influence of linearly oligomerized T–cell epitopes on T cell proliferation is studied. Lipopeptide collections are used to derive most efficient and nontoxic immunostimulants interacting with the Toll–like receptor TLR–2/6.

L–01

GENERATION OF PEPTIDES BY HUMAN ERYTHROCYTES. FACT AND ARTIFACTS

Vadim T. Ivanov, Andrei A. Karelin and Oleg N. Yatskin

Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry,
Russian Academy of Sciences Ul, 117997 Moscow, RUSSIAN FEDERATION

Within the frame of our studies in peptidomics a large number of bioactive hemoglobin fragments have been isolated from a variety of mammalian. In order to trace the biosynthetic pathways leading to these peptides we have studied in our preceding communications the peptide composition of human erythrocyte lysate (i.e. the intracellular peptide composition) as well as the peptides present in the supernatant of primary erythrocyte cell culture (i.e. peptides released from intact erythrocytes).

In this work we investigated in detail under various conditions (pH, protease inhibitors, denaturing agents) the proteolytic potential of erythrolysate and found that several peptides earlier identified as intraerythrocyte components in fact result from hemoglobin degradation by erythrocyte acidic protease(s) in the process of peptide isolation. A rational scheme excluding post–lysis proteolysis was developed for isolation of the peptide fraction. Further mass–spectrometric and microsequencing analysis resulted in determination of structure and concentration of about 50 endogenous intraerythrocyte hemoglobin fragments. With a few exceptions, none of them was described before.

The mechanism of their formation includes primary splitting of globin chains into two pairs of large peptides (50–100 amino acids long) followed by consecutive trimming by exopeptidases. The peptides released by erythrocytes follow a distinct biosynthetic route probably mediated by membrane bound protease(s). It was also found that the intraerythrocyte peptides cannot play a role of direct precursors of hemoglobin derived peptides present in tissue extracts. The biological activity and putative *in vivo* function of the discovered peptides will be discussed.

L–02

FROM α–AMINO ACIDS TO β–, γ–, δ–, ε–, ..., ω– AMINO ACIDS: SYNTHETIC METHODOLOGIES AND BIOACTIVE PRODUCTS

George Kokotos

Department of Chemistry, University of Athens, GR–157 71 Athens, GREECE

Non–natural amino acids play an important role in the design and synthesis of pharmacologically relevant molecules, analogues of bioactive peptides and peptide mimetics. The interest in homologated amino acids is increasing, as a result of the findings that peptides constructed from β– and γ– amino acids can adopt helix, sheet or reverse turn conformations. In addition, β– and γ–peptides may be suitable for pharmaceutical applications because they are stable against common proteases. In recent years we have developed novel methodologies for the synthesis of a variety of enantiopure non–natural amino acids. An approach to synthesize homologated γ–, ε–, and ω– amino acids is based on a Wittig–type or Horner–Wadsworth–Emmons olefination reaction of *N*–protected α–amino aldehydes with appropriate ylides, leading to chain homologation. A strategy to synthesize β– and δ– amino acids was based on the ruthenium–catalyzed oxidation of a phenyl group to carboxylic acid. Wittig olefination of Boc–protected amino aldehydes with semi–stabilized or non–stabilized ylides, followed by hydrogenation, produced saturated Boc–protected amines containing a phenyl group. The aromatic ring of these compounds was oxidized to carboxylic acid by $RuCl_3 \cdot xH_2O/NaIO_4$. The routes developed permit the insertion of any chain length between the amino and carboxy functionalities as a result of the original choice of the starting ylide chain length. The non–natural amino acids have been used in the synthesis of several bioactive compounds, such as digestive lipase inhibitors, phospholipase A_2 inhibitors and dendrimers. 2–Oxoamide derivatives based on γ–amino acids are potent inhibitors of human GIVA PLA_2 and represent a class of novel anti–inflammatory agents.

L–03

NEW HYDROPHILIC POLYSTYRENE POLYAMIDE RESINS. SYNTHESIS AND APPLICATIONS

Kleomenis Barlos, Dimitrios Gatos, Spyros Markos and Efi Scariba

Department of Chemistry, University of Patras, GR–26500 Patras, GREECE

Hydrophilic resins were produced by condensing polysterene (PS) with diamide–polyethyleneglycol (DA–PEG) derivatives of defined molecular weight. So, PS–DA–PEG of the required hydrophilicity and loading were obtained and their application in peptide olygonucleotide, organic synthesis and biochemistry was evaluated. Peptides, antibodies, oligosacharides etc. still bound on the resin, were recognized by biological systems allowing thus their application, especially in the development of diagnostics.

L–04

COMPUTER ASSISTED PEPTIDE SEARCH: IDENTIFICATION OF NEW ACTIVE PEPTIDES

Eric Carnazzi[1], Lionel Le Gallic[1], Stéphanie Valeau[1], Karine Clavier[1], Gilles Subra[1], Hervé Gaudry[1], Jean–Philippe Ripoll[1], Gilbert Bergé[1], Éric Ferrandis[2], José Camara[2], Christophe Thurieau[2] Jacques–Pierre Moreau[2], Karine Puget[1] and Jean Martinez[1]

[1]Laboratoire des Aminoacides, Peptides et Protéines (LAPP) UMR–5810,
Faculté de Pharmacie, 34093 Montpellier Cedex 5, FRANCE
[2]Laboratoires IPSEN–BEAUFOUR, 91966 Les Ulis, FRANCE

We have designed a new program for generating active peptides from protein and genomic sequence data. This program is able to search in the available data concerning protein structures and nucleotidic sequences to provide putative natural active peptide sequences ending by a C–terminal amide. Among the sequences that were available, we have synthesized quite an important number of peptides, either in 96–well plates on classical solid supports using an ACT instrument or by the Multipin technology, or using classical peptide synthesis. So far, more than 2500 peptides of variable lenght were obtained. As a general screening, these peptides were tested for their affinity to Guinea Pig brain membranes and for their activity on a second messenger system (cAMP, IP, Ca++) on transfected cells with total human brain cDNA and a reporter plasmid containing the luciferase gene. Among the tested peptides, two of them showed an interesting affinity for the brain membranes (in the nanomolar range) and a significant activity in stimulating cAMP accumulation in various cell lines. The details of the mining program will be presented, as well as the automated synthesis on solid support of libraries of amidated peptides and their general screening. The pharmacology of the two peptides that were identified as active will be presented in more details.

L–05

AN ATOMIC LEVEL APPROACH OF HYPERTENSION: THE ANGIOTENSIN CONVERTING ENZYME AND ITS INHIBITORS, ANGIOTENSIN II AND ITS RECEPTORS

Andreas G. Tzakos[1], Anastassios Troganis[2], Athanassios S. Galanis[3], Georgios A. Spyroulias[3], Paul Cordopatis[3], George Pairas[3], Evy Manessi–Zoupa[4] and Ioannis P. Gerothanassis[1]

Departments of [1]Chemistry and [2]Biological Applications and Technologies,
University of Ioannina, GR–45110, Ioannina, GREECE
Departments of [3]Pharmacy and [4]Chemistry, University of Patras, GR–26500, Patras, GREECE

In this lecture an atomic level approach of hypertension will be presented. Particular emphasis will be given to:

1. The modelisation of the 3D structure of the ACE_N domain of *s*ACE, based on the X–ray structure of testis ACE (*t*ACE).
2. Sequence and structural comparison between ACE_N and ACE_C and of other proteins of the gluzincin family which highlights key residues that could be responsible for the peptide hydrolysis mechanism.
3. Structural models of the interactions of nine ACE inhibitors (lisinopril, captoril, enalaprilat, ramiprilat, quinaprilat, perindoprilat, fosinoprilat, keto–ACE and RXP 407) both to ACE_C and ACE_N catalytic sites by automated computational docking.
4. Structural studies of the bioactive hormone Angiotensin II (AII) and its inactive precursor AI and implication for the receptor bound conformation through studies of the monoclonal antibody Fab131 and the homology modeled structure of the GPCRS AT_1.

L–06

STUDY OF INTERMOLECULAR INTERACTIONS OF PROTEIN–LIGAND AND PROTEIN–PROTEIN BY NMR SPECTROSCOPY

Manh Thong Cung[1], Nicolas Coudevylle[1], Aurélien Thureau[1], Maria Sakarellos–Daitsiotis[2], Constantinos Sakarellos [2], Vassilios Tsikaris[2] and Socrates J. Tzartos[3]

[1]Laboratoire de Chimie–Physique Macromoléculaire, UMR 7568 CNRS – INPL, 54001 Nancy Cedex, FRANCE
[2]Department of Chemistry, University of Ioannina, GR–45110 Ioannina, GREECE
[3]Department of Biochemistry, Hellenic Pasteur Institute, GR–11521 Athens, GREECE

In biological events, the most frequent targets are proteins, which interact in a specific manner with small ligands, drugs, proteins, nucleic acids. High resolution ^{1}H NMR is a useful tool for studying protein–ligand interactions under fast exchange conditions. To this aim, various experimental techniques such as transferred NOE (TR–NOE) and saturation transfer difference spectroscopy (STD), have been developed. TR–NOE provides information for intramolecular proton distance constraints of the ligand in the bound state, while STD has been used for mapping protein–ligand contacts in solution. The 1D STD NMR technique was also applied to get information about the binding specificity at the atomic level and to screen ligand libraries according to their affinity for the target protein. The STD pulse sequence can be easily introduced in 2D experiments such as 2D STD–TOCSY, 2D STD–HSQC. The preceding techniques have been successfully applied to structural analysis of the AChR epitopes in contact with an anti–AChR monoclonal antibody. The study of protein–protein and protein–nucleic acid interactions, normally requires isotope labeling (^{15}N or/and ^{13}C). Incorporation of heteronuclear half filters in 2D NOESY experiments is particularly attractive for studies of intermolecular complexes of biological macromolecules when only a selected labeled component is used. This technique allows the identification of the interacting residues and their localization at the complex surface.

L–07

MASS SPECTROMETRIC STUDIES OF NONCOVALENT PROTEIN–LIGAND INTERACTIONS

Antonis Tsarbopoulos[1,2]

[1]Department of Pharmacy, University of Patras, GR–265 00 Patras , GREECE
[2]GAIA Research Center, The Goulandris Natural History Museum, GR–14562 Kifissia, GREECE

Cellular functions are often triggered by weak noncovalent interactions involving enzyme and substrate, protein and ligand, protein and protein, and antigen and antibody. Diseases are often caused by the disruption of normal cellular processes that involve noncovalent complexes. Therefore, elucidating the structures and mechanism of these noncovalent complexes could be an important step towards understanding some of these disease processes, which in turn is a crucial step in modern drug design. An example of the implication of noncovalent interactions in diseases is the involvement of *ras* protein in transduction of cell surface signals in cancer research. In cancer cells, unlike in normal cells, *ras*–GTP (active state) is slowly hydrolyzed to the inactive state (i.e., *ras*–GDP), thus resulting in unregulated cell growth. The detection of these noncovalent interactions greatly assists the development of inhibitors for the relevant disease processes.

This has been achieved by applying the electrospray ionization (ESI) mass spectrometric (MS) technique, which has revolutionized the applicability of MS to the study of proteins of high molecular weight either by themselves or when they are bound to ligands. ESI MS allowed the detection of the *ras*–GDP and *ras*–GTP complexes and the monitoring of the stability of these complexes under different conditions of solvent and pH. The observation of these complexes by ESI MS has formed the basis for the screening of potential inhibitors that bind and inactivate the *ras* protein by preventing the exchange of GTP for GDP. In this presentation we will also present some preliminary data on the noncovalent complex formation between amyloid peptide (Aβ) peptide and some bioactive compounds, such as melatonin.

L–08

HIGH SENSITIVITY IDENTIFICATION AND QUANTITATION OF PROTEINS, PEPTIDES AND THEIR POST–TRANSLATIONAL MODIFICATIONS BY LC/MS/MS USING A NOVEL HYBRID TRIPLE QUADRUPOLE/LINEAR ION TRAP MASS SPECTROMETER

Christof Lenz and Volker Kruft

Applied Biosystems, Applera Deutschland GmbH, D–64293 Darmstadt, GERMANY

The identification, characterization and quantitation of proteins and peptides by mass spectrometry has traditionally been achieved by using a range of different techniques and instruments, e.g. a combination of MALDI–ToF, ESI–QqToF and ESI–Triple Quadrupole mass spectrometers. The presented hybrid mass spectrometer combines the analytical capabilities of Triple Quadrupoles (MRM quantitation, Precursor Ion Scanning for the detection of PTMs) with the MS/MS and MS/MS/MS capabilities of a high performance Linear Ion Trap for sequence determination and assignment.

Low and sub–femtomolar protein identification, detection and localization of PTMs as well as peptide and PTM quantitation can thus be achieved on a single instrument using automated LC/MS/MS experiments. Examples for the various applications will be presented.

L–09

LARGE SCALE PURIFICATION OF SYNTHETIC PEPTIDES BY REVERSED–PHASE CHROMATOGRAPHY FROM DEVELOPMENT TO INDUSTRIAL PROCESS

Alain Scarso

UCB–Bioproducts, UCB S.A., Belgium

The role of Peptides is more and more recognized as the basis of new therapeutic concepts in the pharmaceutical market and recent examples will be provided as an illustration. The global demand is therefore substantially growing and the need for capacity as well. UCB–Bioproducts mission is to provide its experience in scale–up of this demand by offering custom process development and manufacturing services related to synthetic peptides as active pharmaceutical ingredients (API).

The chemical processes are based on homogeneous and/or solid–phase strategies. In the downstream part of the process (purification, isolation) development strategy must fulfil the following conditions: efficacy, scalability, productivity and reproducibility. The different development steps to reach this goal using reversed–phase HPLC purification will be described. Typical scheme of the purification cycles will be presented.

Large scale purification of Bivalirudin active pharmaceutical ingredient will be detailed as an example of industrial HPLC process performed on highly efficient reversed–phase medium. Bivalirudin is a 20 amino acids peptide obtained by homogeneous phase synthesis. The current size of a purification campaign is 25 kg (related to isolated API). Several hundred kilos have been produced until now. Yield versus capacity to remove critical impurities will be discussed, as well as the regulatory aspects associated to the downstream part of the process.

L–10

STRUCTURAL FEATURES OF THE INTERACTION BETWEEN SDF–1 ANALOGUES AND THE CXCR4 RECEPTOR

Pasquale Palladino[1], Antonia De Capua[1], Carlo Pedone[1], Raffaele Ragone[2], Filomena Rossi[1], Gabriella Saviano[3], Teodorico Tancredi[4] and Ettore Benedetti[1]

[1]Dipartimento di Chimica Biologica, C.I.R.Pe.B., Università Federico II di Napoli and I.B.B. CNR, 80134 Napoli, ITALY
[2]Dipartimento di Biochimica e Biofisica, Seconda Università di Napoli, 80138 Napoli, ITALY
[3]Dipartimento di Scienze e Tecnologie per l'Ambiente e il Territorio, Università degli Studi del Molise, 86170 Isernia, ITALY
[4]Istituto di Chimica Biomolecolare, CNR, 80078 Pozzuoli (NA), ITALY

Chemokines represent a family of structurally related glycoproteins with potent leukocyte activation and/or chemotactic activity. Among chemokine receptors, CXC receptor 4 (CXCR4) and its natural ligand stromal–derived cell growth factor 1 (SDF–1 or CXCL12) have been highlighted for a role in human breast cancer metastasis and for syncytia–inducing form HIV–1 in CD^+ T–cell. Recently we determined by fluorescence spectroscopy the apparent dissociation constant (K_d') of the bimolecular interaction between receptor– and ligand–derived synthetic peptides, which supports a simplified model for the interaction of chemokine SDF–1 with the CXCR4 receptor. We also identified a synthetic agonist of receptor CXCR4 able to activate ERK1/2 phosphorylation in a dose–dependent manner following stimulation of CHP100 cells. We have determined, as shown in the Figure, the solution structure of this short N–terminal SDF–1 analogue which adopts a turn structure similar to the X–ray structure of a SDF–1 fragment. This finding may be important for recognition in receptor binding.

Cluster structures satisfying the NMR experimental restraints for the SDF–1 analogue. The structure shown in heavy line indicates a mean structure for the SDF–1 peptides.

L–11

RXPA380, A POTENT AND SELECTIVE INHIBITOR OF ANGIOTENSIN–CONVERTING ENZYME C–DOMAIN: A NEW PERSPECTIVE IS OUTLINED

Dimitris Georgiadis[1], Vincent Dive[2] and Athanasios Yiotakis[1]

[1]Department of Chemistry, University of Athens, GR–15771, Athens, GREECE
[2]CEA, Département d'Ingénerie et d'Etudes des Protéines, 91191 Gif/Yvette Cedex, FRANCE

Somatic Angiotensin–Converting Enzyme (ACE) is a zinc metallopeptidase involved in the release of angiotensin II (AngII) and the inactivation of bradykinin (BK), two peptide hormones that play a key role in the regulation of blood pressure, renal and cardiovascular functions. Since the discovery that somatic ACE consists of two parts with distinct functions, the N–domain and the C–domain, our effort was focused on the development of domain selective inhibitors in order to investigate the functional roles of ACE domains as well as to examine their suitability as next–generation type drugs with improved clinical profile. In 1999, we reported the discovery of the first highly N–domain–specific inhibitor, phosphinic tetrapeptide RXP407, by screening several phosphinic peptide libraries. Unfortunately, such an approach failed to provide similar results for ACE's C–domain. This target was only achieved last year with the discovery of highly C–domain–selective inhibitor, phosphinic tripeptide RXPA380 (Cbz–PheΨ[PO(OH)CH_2]Pro–TrpOH). The synthesis of this molecule demanded the development of a novel, modified methodology since the classical approach was ineffective. Key steps of this stategy is the Michael addition of a silyl aminophosphonite to a suitable allylic acetate followed by nickel boride reduction of the rearranged double bond. With RXP407 and RXPA380 in hand, we were able to investigate the contribution of each active site to the cleavage of AngI and BK in vivo and in vitro. Moreover, the structural requirements for the remarkable selectivity of RXPA380 were defined, based on its comparison with several RXPA380 analogues. Undeniably, RXPA380 appears to render one of the most promising leads for the design of improved next–generation, domain–selective ACE inhibitors.

L–12

STRUCTURE ELUCIDATION OF THE BETA SUBUNIT OF FcεRI

William A. Gibbons[1] and Mire Zloh[2]

[1]New Technology Centre, University of St. Andrews, St. Andrews, Fife KY16 9SR, UK
[2]School of Pharmacy, London WC1N 1AX, UK

The high affinity IgE receptor (FcεRI) plays a crucial role in allergy and inflammation. A 3D structure of this four subunit receptor (αβγ2) is unknown and the structural studies of the beta subunit are presented here. The beta subunit has four hydrophobic membrane–spanning segments and two cytoplasmic tails. The four transmembrane domains have alpha helical structure and are connected by three loop sequences according to the proposed topological model. The structural studies (experimental and theoretical) of subunit domains such as its loop peptides, cytoplasmic peptides and transmembrane helices were performed. The 3D structure of the synthesized loop peptides and cytoplasmic peptides were elucidated based on CD and/or NMR data, which were used were then used as data basis for the higher level calculations. The transmembrane bundle of four helices of the beta subunit were characterised by mapping the relative lipophilicity of their surfaces using lipophilic probes and by docking of the individual helices. The data on the relative lipophilicity of the surfaces as well as the surfaces that favoured helix–helix interactions were used in combination with the spectroscopy–based structures of the loops and cytoplasmic domains to predict the helix arrangement and 3D structure of the beta subunit of the high affinity IgE receptor. Finally, the molecular simulations calculations yielded full 3D models of structures of the beta subunit, which should form a basis for the further modelling of the whole high affinity IgE receptor.

L–13

SEQUENTIAL OLIGOPEPTIDE CARRIERS (SOC_n) OF IMMUNOGENIC PEPTIDES WITH A BUILT–IN VACCINE ADJUVANT – APPLICATIONS TO RECONSTITUTED MODELS OF THE ACETYLCHOLINE RECEPTOR (AChR) AND THE La/SSB AND Sm AUTOANTIGENS

Constantinos Sakarellos[1], Dimitrios Krikorian[1], Konstantinos Poulas[2], Chryssa Voitharou[1], Iakovos Lazaridis[3], Socrates Tzartos[2,3] and Maria Sakarellos–Daitsiotis[1]

[1]Department of Chemistry, University of Ioannina, GR–45110 Ioannina, GREECE
[2]Department of Pharmacy, University of Patras, GR–26500 Patras, GREECE
[3]Department of Biochemistry, Hellenic Pasteur Institute, GR–11521 Athens, GREECE

We combined specific B and T cell epitopes for three different antigens, a "promiscuous" T cell epitope that could overcome genetic restrictions, and an "adjuvant" that could enhance and prolong humoral and cellular immune response, all anchored to a multivalent synthetic carrier, (trying to form an immunogen and consequently an effective vaccine). IL–1β(163–171), previously reported that is devoid of all pro–inflammatory effects of IL–1β but which maintain immunostimulatory activity of the intact cytokine, was added to the N–terminus of the SOC_n carrier, $(Lys–Aib–Gly)_4$ a carrier successfully applied in our laboratory for anchoring antigenic/immunogenic peptides. A promiscuous T cell epitope deriving from TT (Tetanus toxoid)(593–599) was also covalently attached to the C–terminus of SOC_n, as universal immunogen, resulting in the final modified carrier IL–1β(163–171)–SOC_4–TT(593–599). B and T cell epitopes derived from AChR, the main target of autoantibodies in myasthenia gravis and the La/SSB and Sm antigens, against which is directed the majority of autoantibodies in patients with Sjögren's and Systemic Lupus Erythematuous were coupled to the Lys–$N^{\varepsilon}H_2$ groups of the modified SOC_n carrier. The obtained constructs were administered in mice and rabbits following either the complete/incomplete Freund's adjuvant protocol or without any adjuvant. Induction of specific autoantibodies recognizing the priming constructs, as well as their cognate immunogens was detected in ELISA assays, even when immunization occurred without adjuvant, while production of antibodies against the carrier itself was negligible. It is concluded that IL–1β(163–171)–SOC_4–TT(593–599) is a promising multifunctional carrier for developing human vaccines.

L–14

RECOMBINANT POLYPEPTIDES IN THE STUDY OF THE NICOTINIC ACETYLCHOLINE RECEPTOR AND MYASTHENIA GRAVIS

Socrates J. Tzartos[1,2], Vassiliki Avramopoulou[1], Ira Gavra[1], Kaliopi Kostelidou[1], Olympia Kotsifa[2], Avgi Mamalaki[1], Konstantinos Poulas[2], Loukia Psaridi–Linardaki[1], Sotiris Sideris[2], Alexandros Sotiriadis[1] and Nikos Trakas[1]

[1]Department of Biochemistry, Hellenic Pasteur Institute, GR–11521 Athens, GREECE
[2]Department of Pharmacy, University of Patras, GR–26500 Patras, GREECE

The nicotinic acetylcholine receptors (AChR) are ligand–gated ion channels in muscles and neurons, formed by 5 homologous subunits. The muscle AChR has the subunit stoichiometry $\alpha_2\beta\gamma\delta$ or $\alpha_2\beta\varepsilon\delta$ whereas the neuronal AChRs are formed either by combinations of various kinds of α and β subunits (α2–6, β2–4) or by α–subunit homopentamers (α7–10). ACh binds at the α–subunits inducing the opening of the channel. Autoantibodies to muscle AChR cause myasthenia gravis (MG), whereas neuronal AChRs are involved in diseases like Alzheimer, Parkinson and Schizophrenia . We are expressing the extracellular domains (ECD, amino acids ~1–210) of human AChR subunits in the yeast *Pichia pastoris* and are using them for the development of novel therapies and for AChR structure–function studies: (a) The ECDs of all muscle AChR subunits (α,β,γ,δ,ε) were in near native–like conformation, bound conformation–dependent antibodies and the α–subunit also bound AChR ligands. The ECDs when immobilized on Sepharose beads could immunoadsorb the autoantibodies from MG patients' plasma. These immunoadsorbents may be used to replace the non–specific plasmapheresis therapy by antigen–specific autoantibody immunoadsorption. (b) The ECD of the neuronal subunit α7 did bind ligands but its water–solubility was limited. We subsequently constructed a mutant α7–ECD in which Cys116 was replaced by Ser and the hydrophobic Cys128–Cys142 loop was substituted by the corresponding hydrophilic homologue from the acetylcholine binding protein. The mutant exhibited improved ligand binding affinity and complete solubility forming pentamers which correspond to the ECD of the whole homopentameric α7–AChR. Crystallization efforts have been initiated.

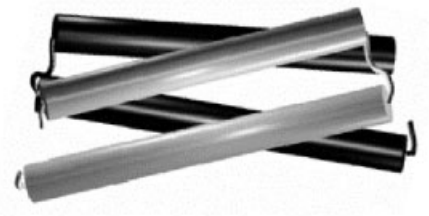
L–15

STRUCTURAL POLYMORPHISM OF A SMALL 4–α–HELICAL BUNDLE

Michael Kokkinidis[1,2], Nicholas M. Glykos[3], Metaxia Vlassi[4] and Dina Kotsifaki[2]

[1]Department of Biology, University of Crete, GR–71409 Heraklion, GREECE
[2]Institute of Molecular Biology and Biotechnology (IMBB), GR–71110 Heraklion, GREECE
[3]Department of Molecular Biology and Genetics, GR–68100 Alexandroupolis, GREECE
[4]NCSR "Demokritos", GR–15130 Athens, GREECE

Recurrent tertiary motifs in proteins presumably reflect convergent evolutionary solutions to basic requirements for protein structural stability. The 4–α–helical bundle motif, usually a sequentially connected array of four α–helices, represents the simplest tertiary motif. Naturally occurring 4–α–helical bundles serve a variety of functions and occur both in monomeric form and a variety of oligomeric assemblies. Basic structural simplicity and functional diversity make this motif a prime candidate for the at ab initio design of novel proteins. The ROP (repressor of primer) protein is a homodimeric RNA–binding protein involved in the regulation of the copy number of ColE1 plasmid. Rop is the paradigm of a canonical 4–α–helical bundle (Figure) and, as such has been the subject of numerous investigations ranging in their approach from structural and biochemical to thermodynamical and computational studies. Two regions of the molecule have attracted particular attention, not least because of the ongoing debate about their roles in folding and stability of bundles: a) The bend region of ROP b) The hydrophobic core. A combined crystallographic, mutagenesis and computational study of ROP aiming to discover the principles that stabilize the 4–α–helical bundle motif will be presented.



L–16

RADIOLABELLED PEPTIDES FOR TUMOUR THERAPY

Marion de Jong

Nuclear Medicine, Erasmus MC, Rotterdam, The NETHERLANDS

On their plasma membranes, cells express receptor proteins with high affinity for regulatory peptides, such as somatostatin. Changes in the density of these receptors during disease, e.g. overexpression in many tumours, provide the basis for new imaging methods. The first peptide analogues successfully applied for visualisation of receptor–positive tumours were radiolabelled somatostatin analogues. The next step was to label these analogues with therapeutic radionuclides for peptide receptor radionuclide therapy (PRRT). Results from preclinical and clinical multicentre studies have shown an effective therapeutic response using radiolabelled somatostatin analogues to treat receptor–positive tumours. Infusion of positively charged amino acids reduces kidney uptake, enlarging the therapeutic window. For PRRT of CCK–B receptor–positive tumours, such as medullary thyroid carcinoma, radiolabelled minigastrin analogues are currently being successfully applied. For the future, the combination of different therapy modalities holds interest as a means of improving the clinical therapeutic effects of radiolabelled peptides. With e.g. the combination of different radionuclides, such as ^{177}Lu– and ^{90}Y–labelled somatostatin analogues, a wider tumour region of high curability can be reached. A variety of other peptide–based radioligands, such as bombesin and NPY(Y_1) analogues, receptors for which are expressed on common cancers such as breast cancer, are currently under development and in different phases of (pre)clinical investigation. Multi–receptor tumour targeting using the combination of these analogues is promising for scintigraphy and PRRT of breast carcinomas and the lymph node metastases.

L–17

RECEPTOR–TARGETED TUMOR IMAGING EMPLOYING TETRAAMINE FUNCTIONALIZED PEPTIDES LABELED WITH TECHNETIUM

Theodosia Maina, Anastasia Nikolopoulou and Berthold Nock

Institute of Radioisotopes–Radiodiagnostic Products, NCSR "Demokritos", GR–15310 Athens, GREECE

The high density expression of peptide hormone receptors on the surface of neoplastic cells provides the molecular basis for the in vivo tumor targeting employing radiolabeled peptides. The impact of this approach in nuclear oncology has been recently established by the advent of radiolabeled somatostatin analogs in the diagnosis and therapy of neuroendocrine tumors. Furthermore, several recent studies report on alternative radiopeptide – peptide receptor systems for targeting frequently occurring malignant disease with radionuclides. Our work so far has been focused on the development of a spectrum of peptide analogs labeled with the gold standard of diagnostic nuclear medicine ^{99m}Tc. For stable incorporation of this radiometal, peptides were modified at the N–terminal with open chain tetraamines. This donor system has the ability to bind ^{99m}Tc almost quantitatively at low concentrations forming a monocationic octahedral complex. This metal chelate effectively survives in the biological milieu both in animals and in man and favors rapid excretion of radiopeptides into the bladder via the kidneys and the urinary system. Several such radiopeptides based on somatostatin, bombesin, neurotensin and minigastrin have been developed and their properties exhaustively studied in cell and animal tissue preparations providing ample information about their receptor affinity, internalization capability and metabolic stability. Furthermore, their biodistribution studied in healthy and xenograft–bearing rodents pinpointed radiopeptides that combined high and target–specific uptake with rapid clearance from background tissues. Several such compounds are currently being validated as candidates in the receptor–targeted tumor imaging in man and results so far have been very promising.

L–18

IDENTIFICATION AND STRUCTURAL MODIFICATION OF ANTIBODY EPITOPES OF MUC2 GLYCOPROTEIN

Katalin Uray[1], Ádám Bartos[1], Andrea Kiss[1], Regina Tugyi[1], Dóra Iván[1], Emke Windberg[1], Zsuzsa Majer[2], Alan Perkins[3] and Ferenc Hudecz[1,2]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Budapest 112, P.O.Box 32, H–1518, HUNGARY
[2]Department of Organic Chemistry, Eötvös L. University, Budapest, HUNGARY
[3]Medical School, University Hospital, Nottingham, NG7 2UH, UK

The MUC2 glycoprotein expressed mainly by the epithelium of the colon is built up of variable number tandem repeats (^{1}PTTTPITTTTTVTPTPTPTGTQT^{23}) with non–repetitive regions towards the N– and C–termini. Several MUC2 specific monoclonal antibodies raised against peptide K^{12}VTPTPTPTGTQTPT^{25}–KLH conjugate have recognised samples from human tumor tissues. These findings prompted us to localize the sequence of antigenic sites responsible for antibody binding and provide molecular basis for understanding the different recognition between tumours and healthy tissues. We have identified a pentamer sequence of ^{18}PTGTQ^{22} as epitope related to mAb 996, while mAb 994 recognised pentapeptides with the TX^{1}TX^{2}T motif. In order to optimise antibody binding we have produced epitope peptides with flanking regions of L–Ala, L–X or D–Ala residues or oligoethylene glycol. We found that that these modifications could improve significantly antibody binding and lead to "superantigens" but also result in protection against proteolysis under *in vitro* conditions.

Supported by the Hungarian Research Fund (OTKA No. F 034886, T037749, T–043576) and the Ministry of Culture (FKFP 0153/2001).

L–19

CRH PEPTIDES IN INFLAMMATION

Achille Gravanis[1], Christos Tsatsanis[2], Antonis Makrigiannakis[3], Ekaterini Chatzaki[1], Ioannis Charalampopoulos[1], Erene Dermitzaki[2] and Andrew N. Margioris[2]

Depts. of [1]Pharmacology, [2]Clinical Chemistry and [3]Gynecology, School of Medicine, University of Crete, GR–71110 Heraklion, GREECE

Hypothalamic corticotropin releasing hormone (CRH) belongs to the CRH family of neuropeptides, which also includes Urocortin, and Urocortins I and II. Two distinct sub–types of CRH receptors encoded by separate genes have been described to date, CRF1 and CRF2, and an additional high–affinity binding site is provided for by the soluble CRH–binding protein (CRF–BP). The CRF system is also expressed in peripheral tissues, including the female reproductive tract (uterus–placenta), the adrenals, the GI tract (stomach, colon) and the immune cells (lymphocytes–macrophages). CRH peptides are differentially expressed in these tissues and differentially regulate various local inflammatory phenomena. Indeed, CRH and Urocortin participate in the inflammatory response of the uterus during blastocyst implantation, in epithelial gastric cell protection in H. Pylori gastritis, or in colitis. Recent experimental findings from our group associate CRH peptides with immune and endocrine cell apoptosis and survival in these tissues. Indeed, CRH and Urocortin are effective regulators of Fas/FasL and Bcl–2 pro– and anti–apoptotic proteins, and their up–stream effectors, such as transcription factor NF–kB and PKC kinases. The recent development of micromolecular CRH agonists and antagonists offer new therapeutics tools for pharmacological interventions in the pathophysiology of inflammation in these tissues.

L–20

STRUCTURE AND BINDING OF THE COMPLEX BETWEEN A SYNTHETIC C–TERMINALLY TRUNCATED HEVEIN AND CHITOOLIGOSACCHARIDES: DEFINING THE MINIMUM HEVEIN DOMAIN WITH MEASURABLE AFFINITY

David Andreu[1], Miquel Vila–Perelló[1], Nuria Aboitiz[2], Patrick D. Groves[2], Juan Luis Asensio[2], Francisco Javier Cañada[2] and Jesús Jiménez–Barbero[2]

[1]Department of Experimental and Health Sciences, Universitat Pompeu Fabra, 08003 Barcelona, SPAIN
[2]Centro de Investigaciones Biológicas, CSIC, 28040 Madrid, SPAIN

Plants respond to pathogenic attack by producing defense proteins able to bind reversibly to chitin, a β(1–4) linked N–acetyl glucosamine (GlcNAc) polysaccharide which is a structural component of fungal cell walls or invertebrate (insect, nematode) exoskeletons. A number of those defense proteins share a highly conserved Gly, Cys–rich common structural pattern organized around a four disulfide core known as hevein domain or chitin binding motif. The motif is found in lectins such as *Urtica dioica* or wheat germ agglutinins, in addition to hevein itself or its natural variant pseudo–hevein. Its small size and related synthetic availability make it an excellent model system for the study of protein–carbohydrate recognition, particularly the role of factors such as protein size, structure and dynamics, or stacking and hydrogen bonding interactions. Further motivation for the study of hevein domains comes from the role of hevein as major latex allergen and from the antifungal activity of class I chitinases or Ac–AMP antimicrobial peptides, all of them bearing hevein motifs.

We have synthetically modified key interacting residues of hevein domains, with the goal of outlining the minimum scaffold capable of effective chitooligosaccharide binding. A first step in this direction is a C–terminally truncated, 32–residue version of hevein (HEV32), for which an efficient solid phase synthesis has been developed. The 3D structure of this modified lectin in both its free and chitin trimer $(GlcNAc)_3$–bound state has been studied in water solution by NMR and molecular dynamics simulations, and the thermodynamics of the binding process has been characterized both by ^{1}H–NMR and fluorescence methods. A comparison with the corresponding properties of natural hevein from latex has also been performed.

L–21

NEUROPROTECTIVE PEPTIDES RELATED TO ALZHEIMER'S DISEASE

Jiina Slaninová[1], Lenka Boroviková[1], Gabriela Krejová[2] and Jií Patoka[2]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, CZECH REPUBLIC
[2] Department of Toxicology, Purkyn Military Medical Academy, 500 01 Hradec Králové, CZECH REPUBLIC

Central change at Alzheimer's disease (AD) represents, except of formation of amyloid plaques and neurofibrillary tangles, huge and progressive loss of neurons. With regard to several theories formulated recently concerning the eminent impulse to the development of AD, several types of substances have been tested for neuroprotective activity, such as inhibitors of acetylcholin esterase, cholesterol synthesis reducing substances, inhibitors of proteolytic enzymes and antiinflamatory agents. This lecture is devoted to substances of peptidic nature, especially those based on the structure of humanin. Several *in vitro* tests using cell cultures and *in vivo* tests using rats used for the evaluation of their activity will be described. E.g. we have examined the effect of [Gly^{14}]humanin and its analogues on spatial memory and orientation impairment induced by 3–quinuclidinyl benzilate (QNB) in the multiple T–maze. This drug is used for experimental induction of central anticholinergic syndrome with negative impact to memory and learning. Cholinergic neuronal systems play an important role in the cognitive deficits associated with AD and other neurodegenerative diseases. Our study of spatial recognition and memory is a useful method to evaluate potentially antiamnesic drugs. In our study, all drugs were injected i.p. and were found to be active. The fact, that the peptides were active after i.p. application is very significant for drug development.

INTERACTION OF CALRETICULIN WITH B CELL LINEAR EPITOPES OF Ro60 kD AUTOANTIGEN, ENHANCES THE ANTIGENICITY AND THE RECOGNITION BY ANTI–Ro60 kD AUTOANTIBODIES

Efstathia V. Staikou[1], John G. Routsias[1], Athina A. Makri[1], Apostolos Terzoglou[1], Maria Sakarellos–Daitsiotis[2], Constantinos Sakarellos[2], George Panayotou[3], Haralampos M. Moutsopoulos[1] and Athanasios G. Tzioufas[1]

[1]Department of Pathophysiology, University of Athens, GR–11527, Athens, GREECE
[2]Department of Chemistry, University of Ioannina GR–45110, Ioannina, GREECE
[3]Biomedical Sciences Research Centre, Alexander Fleming, Vari, GREECE

Calreticulin is a molecular chaperone to newly synthesized polypeptides. Previous studies have shown that calreticulin is a putative protein member of the Ro/La RNP complex. In this study we investigated (a) whether calreticulin interacts specifically with epitope analogues of the Ro60 kd autoantigen and (b) whether this interaction enhances the conformation dependent recognition of these epitopes. Highly purified native calreticulin was found to bind preferentially with peptides derived from the sequence of the Ro60 kD175–184aa(10p) and 216–232aa(17p). This interaction was favoured by the combination of heat treatment, divalent cations and ATP. La/SSB epitopes as well as the common epitope of Sm did not interact with calreticulin. Thirty–eight anti–Ro60 kD positive and 23 anti–Ro60 kD negative sera of patients with autoimmune rheumatic diseases were tested. All anti–Ro60 kD positive sera recognized the complex calreticulin–17p, while 95% of the same sera also bound the complex calreticulin–10p. Tested individually, calreticulin, pep10p and pep17p presented very low reactivity (8%, 11% and 29%, respectively) against anti–Ro60 kD positive sera. Anti–Ro60 kD negative sera did not react significantly either with calreticulin, 10p and 17p, or with the complexes calreticulin–10p and calreticulin–17p (<5%). These results suggest that native calreticulin can be complexed with B–cell antigenic determinants of Ro60kd autoantigen, inducing their conformation–dependent recognition by anti–Ro60 kd autoantobodies. This work also indicates that calreticulin may play an active role in the initiation and perpetuation of the autoimmune response.

Oral Presentation Abstracts

Short Oral Presentations

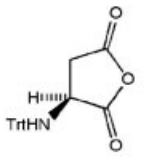
SL–01

ASYMMETRIC SYNTHESIS OF AMINO ACID AND PEPTIDE ANALOGS USING COMMERCIALLY AVAILABLE ACIDIC AMINO ACIDS AS CHIRAL TEMPLATES

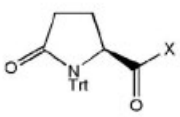
Constantinos Athanassopoulos and Dionyssis Papaioannou

Department of Chemistry, University of Patras, GR–26500 Patras, GREECE

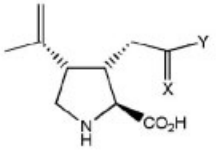
DCC mediated activation of *N*–trityl–aspartic and –glutamic acid produced the corresponding isolable anhydride **1** and pyroglutamic acid derivative **2**, respectively. The former, was reacted with a variety of nucleophiles to produce several interesting derivatives of non–proteinogenic amino acids suitable for use in peptide synthesis. The latter, was converted through a one–pot procedure to the corresponding isolable benzotriazolyl ester **3**, which was shown to be a convenient synthon for the introduction of pyroglutamyl residues in medicinally interesting hormones, as exemplified with the efficient synthesis of TRH (**4**) and analogs, incorporating L– or D–His and *cis*– or *trans*–4–hydroxy–L–proline. Finally, a synthetic protocol is described which allows the incorporation of the γ–amide (Kan, **6**) and the γ–benzyl ester [Kai(Bzl), **7**] and ether [Kol(Bzl), **8**] of kainic acid (Kai, **5**) into peptide chains, as exemplified with the synthesis of the Substance P (SP, **9**) analogs [Kan^5]–SP$_{5–11}$, [Glp^5, Kan^6]–SP$_{5–11}$, [Kan^6]–SP$_{6–11}$, [Glp^5, Kai(Bzl)^{11}]–SP$_{5–11}$ and [Glp^5, Kol(Bzl)^{11}]–SP$_{5–11}$.



1



2 X=OH
3 X=OBt



5 X=O, Y=OH
6 X=O, Y=NH_2
7 X=O, Y=OBn
8 X=H_2, Y=OBn

Glp-His-Pro-NH_2

4

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

9

SL–02

SYNTHESIS OF PROTECTED δ–GLYCAMINO ACID BUILDING BLOCKS FOR INCORPORATION INTO A PEPTIDIC CHAIN

George Paloumbis[1,2], Andrew D. Culshaw[2] and Paul Cordopatis[1]

[1]Department of Pharmacy, University of Patras, GR–26500 Patras, GREECE
[2]Novartis Institute for Medical Sciences, London, UNITED KINGDOM

Among the major classes of biomolecules, carbohydrates allow almost unlimited structural variations due to their chiral diversity and high density of functional groups. Sugar amino acids (also called *glycamino acids*) can be found in nature largely as structural elements. They are molecules that contain at least one amino group as well as at least one carboxyl group thus, they can be considered as monomeric building blocks of chimeras between carbohydrates and proteins. In some cases the sugar amino acids are linked to each other, and in other cases to amino acids. Besides their immediate intrinsic different pharmacological properties, glycamino acids can be used as building blocks for the synthesis of modified analogues of biologically active peptides and/or oligosaccharides. This thesis concerns the synthesis of a δ–glycamino acid in order to incorporate it into the *N*–terminus of a peptidic chain and investigate its potential use in medicinal chemistry. The *N*–terminus of the final sugar molecule will be protected with the base labile Fmoc group while the *C*–terminus will be in its free acid form for direct coupling with the *N*–terminus of the desired peptide. Additionally, the remaining three hydroxyl groups will be orthogonally protected with acid labile groups such as *p*–methoxy benzyl (PMB) or *t*–Butyl. To date we have synthesised the free glycamino acid with Fmoc protection on the amino group and benzyl protection on the remaining hydroxyl groups. Our current approach is two steps away from the target molecule. In parallel, we are investigating a slightly different approach, in which the acid labile protection of the hydroxyl groups is performed in the first few steps of the synthesis.

SL–03

α– AND β– ASPARTYL PEPTIDE ESTERS FORMATION VIA ASPARTIMIDE RING OPENING

Panagiotis Stathopoulos, Serafim Pappas, Sarantos Kostidis and Vassilios Tsikaris

Department of Chemistry, University of Ioannina, GR–45110 Ioannina, GREECE

A well–documented problem in the synthesis of aspartic acid– containing peptides is the aspartimide formation. This undesirable reaction has been proved to occur under both acidic and basic conditions and is dependent on the β–carboxyl protecting group, the acid or base used during the synthesis as well as the peptide sequence. Hydrolysis of α– aminosuccinimide yields a mixture of α and β– aspartyl peptides. On the other hand, cyclization of an aspartyl residue to succinimide in the polypeptide chain is particularly useful in the diketopiperazine formation. The mechanism for this reaction is an intramolecular attack of the peptide amino terminus on the aminosuccinyl carbonyl group. In addition, modified peptides containing the Asu moiety exhibit a type II β– turn conformation in a polypeptide chain. In a previous study, we demonstrated, for the first time, that treatment of the Asu–containing peptides with methanol in the presence of 2 % DIEA results in α– and β– aspartyl methyl ester peptides. Taking advantage of these results, we decided to test the aspartimide ring opening conditions using different types and concentration of alcohols (primary and secondary) and bases (DIEA, collidine, 4– Pyrolidino pyridine, 1– Methyl– 2– Pyrolidone, Piperidine) at different temperatures and times of reaction. The best results were obtained with DIEA while the aspartimide ring opening with secondary alcohols was realized only at high temperature values.

SL–04

STUDY OF THE NONCOVALENT INTERACTION BETWEEN AMYLOID–β–PEPTIDE AND MELATONIN BY MASS SPECTROMETRY

Fotini N. Bazoti[1,2], Jonas Bergquist[3], Karin Markidis[3] and Antonis Tsarbopoulos[1,2]

[1]Department of Pharmacy, University of Patras, GR–26500 Patras, GREECE
[2]GAIA Research Center, The Goulandris Natural History Museum, GR–14562 Kifissia, GREECE,
[3]Department of Analytical Chemistry, Institite of Chemistry, Uppsala University, SE–751 21 Uppsala, SWEDEN

Alzheimer's disease (AD), a leading cause of senile dementia, is associated with neurodegeneration, loss of cognitive ability and premature death. The cause of AD is presently unknown, but the existing hypotheses have centered on the amyloid beta protein–containing senile plaques and the protein tau–consisting neurofibrillary tangles, facilitaded by a multitude of risk factors. Although, several proteins are involved in the pathology of AD, amyloid–*β*–peptide (A*β*), is currently believed to play a central role. Among the mechanisms proposed to justify A*β*'s neurotoxicity, the hypothesis that A*β* aggregation is related to oxidative stress gains momentum. In light of the suggested link between oxidative stress and AD, it is proposed that antioxidants, and even more endogenous antioxidants, such as Melatonin (M), which has been reported to possess neuroprotective and anti–amyloidogenic properties, may offer a therapeutic regime for protection against the risk of this disease.

Noncovalent interactions between peptides/proteins and ligands, can reveal pathological conditions or can be implicated in therapeutic approaches. Mass spectrometry is a powerful tool for monitoring these interactions in real time, enabling the definition of the stoichiometry as well as the topology of the interacting species. In the present study, the possibilitty of the non–covalent complex formation between A*β* and M is assessed by mass spectrometric techniques (LC–TSQ–MS and LC–FTICR–MS). That will provide a screening method for the activity of anti–amyloidogenic indole analogs, and will aid towards the development of a preventive or therapeutic treatment for AD.

SL–05

SYNTHESIS AND SPECTROSCOPIC STUDIES OF PEPTIDES THAT REPRESENT THE TWO ACTIVE SITES OF SOMATIC ANGIOTENSIN CONVERTING ENZYME

Athanassios S. Galanis[1], Georgios A. Spyroulias[1], Evy Manessi–Zoupa[2], George Pairas[1], Ioannis Gerothanassis[3] and Paul Cordopatis[1]

Depts of [1]Pharmacy, and [2]Chemistry, University of Patras, GR–26500 Patras, GRECE
[3]Department of Chemistry, University of Ioannina, GR–45110 Ioannina, GREECE

Angiotensin–I Converting Enzyme (ACE) is a Zinc Metallopeptidase playing an important role in the regulation of blood pressure due to its action in the frame of the Renin–Angiotensin System. ACE inhibitors are considered among the most potent antihypertensive drugs and apart their major action, exhibit beneficial lateral effects in the prevention of cardiovascular disease in various classes of hypertensive patients. ACE is encountered in two distinct forms in humans, the somatic and the testis form. These differ from the structural point of view, mainly in size and number of catalytic sites. ACE belongs to *gluzincins* metalloenzymes with the characteristic **HE***XX***H** motif *(zincins)*, where the two histidines are the potential protein ligands. Hooper N. M. introduced the term gluzincins for the *zincins* whose third zinc ligand is a glutamic acid found in the consensus binding motif sequence **E***XXXX*.

We designed and synthesized peptides of 36, 37 and 46 amino acids that represent the sequence of the two zinc catalytic centers of ACE somatic type in order to investigate the structural features of the somatic ACE N– and C– terminal active sites. NMR and CD spectroscopy used in order to obtain experimental data concerning the secondary structure, the Zn(II)–coordinating residues and characterize the overall fold of the synthesized peptides. For further structural information, peptides with substitution performed on key–residues native ACE_N active site were also synthesized and studied by NMR. Finally, a comparison with the structural characteristics of the recent published testis ACE crystal structure is also presented.

SL–06

APOA–I AMPHIPATHIC α–HELICAL PEPTIDE MODELS AS ATHEROPROTECTIVE PROBES: CONCEPT, SYNTHESIS, CONFORMATIONAL AND BIOLOGICAL STUDIES

Charalampos Alexopoulos, Afroditi Tampaki, Konstantinos Harilogis, Alexandros Tselepis, Maria Sakarellos–Daitsiotis and Constantinos Sakarellos

Departmet of Chemistry, University of Ioannina, GR–45 110 Ioannina, GREECE

Apoliporotein A–I (ApoA–I), the major protein component of high density lipoprotein (HDL), consisted of 243 aminoacids in a series of amphipathic helices, is thought to play an important role in lowering the risk of atherosclerosis and coronary artery disease. In order to develop atheroprotective agents, we report on the design, synthesis, conformational analysis and biological studies of four α–helix apoA–I peptide models: Ac–ESK(Palm)KELSKSW[10]SEM[13]LKEK(Palm)SKS–NH_2**(1)**, AcESK(Palm)KELSKSM[10]SEW[13]LKEK(Palm)SKS–NH_2 **(2)**, Ac–FKEFSKSMSEWFKEF–NH_2 **(3)** and Ac–FKEFSKSASEWFKEF–NH_2 **(4)** where Glu and Lys residues constitute the hydrophilic face, while Met, Phe, Leu, Trp as well as Palmitoyl–groups the spatially segregated hydrophobic phase of the amphipathic α–helix. Met could serve as additional oxidant–scavenger for protecting LDL from irreversible oxidative damage and Trp as an intrinsic fluorescence probe. The syntheses of the apoA–I peptide models were carried out on Rink amide Resin following the Fmoc–strategy and an orthogonal protection system. The helical characteristics of the apoA–I peptide models in their reconstituted form in POPC, DMPC, DMPG were studied by CD spectroscopy. The ability of the ApoA–I peptide models to inhibit the Cu^{2+} induced oxidation of the low–densirty lipoprotein (LDL) in vitro, and to prevent the oxidation induced inactivation of the LDL–associated platelet–activating factor acetylhydrolase (PAF–AH) were investigated and their atheroprotective role is discussed.

SL–07

SYNTHESIS OF TETRA– AND TRIPEPTIDE ANALOGS OF SUBSTANCE P FRAGMENTS AND THEIR PEPTOID–PEPTIDE HYBRIDS

Paraskevi Vakalopoulou and George Stavropoulos

Department of Chemistry, University of Patras, GR–265 00 Patras, GREECE

Synthetic peptides are under investigation as possible anti–tumor agents. The Substance P (SP) analog [D–Arg^{1}, D–Phe^{5}, D–Trp^{7,9}, Leu^{11}]SP (antagonist D) and the C–terminal analog [Arg^{6}, D–Trp^{7,9}, MePhe^{8}]SP$_{6–11}$ (antagonist G) inhibit the tumor growth and cell proliferation of Small Cell Lung Cancer (SCLC) *in vitro* and *in vivo*.

In the present study a series of tetra– and tripeptide analogs have been synthesized, based on the sequence of antagonist G, using the stepwise synthesis or the fragment condensation method either in solution or in SPPS. A series of tetra– and tripeptoid–peptide hybrids have been synthesized corresponding to the above peptide analogs. All the synthesized analogs were purified (HPLC) and identified (ESI–MS).

The peptoid–peptide hybrids are oligomeric peptidomimetics containing the N–substituted glycine residue. The incorporation of N–substituted glycine in peptide chains has been proved to improve their stability against proteases. Thus the analogs have incorporated the peptoid monomer [N(CH$_2$–Ph)–CH$_2$–CO–] (NPhe) instead of the amino acid residue of [HN–CH(CH$_2$–Ph)–CO–] (Phe).

SL–08

ANTIBODIES AGAINST SEQUENCES DERIVED FROM CARDIAC TROPONINS AS TOOLS FOR THEIR DETECTION IN SERA OF PATIENTS WITH CARDIOVASCULAR DISEASES

Constantinos Strongylis[1], Chryssa Voidarou[2], Demokritos Tsoukatos[1], Katerina Naka[2], Lambros Michalis[2], Ketty Soteriadou[3], Maria Sakarellos–Daitsiotis[1], Constantinos Sakarellos[1] and Vassilios Tsikaris[1]

[1]Department of Chemistry, University of Ioannina, GR–45110 Ioannina, GREECE
[2]School of Medicine, University of Ioannina, GR–45110 Ioannina, GREECE
[3]Hellenic Pasteur Institute, GR–11521 Athens, GREECE

Troponin complex plays an important role in regulating skeletal and cardiac muscle contraction. It consists of three different subunits (I, C, T) each of which is responsible for different functions. Cardiac troponins are released in the bloodstream during irreversible cardiac muscle damage and they are being detected in patients' sera 4–6 hours after the cardiovascular incident. The development of biological assays for the detection of cardiac troponins is based on the application of specific antibodies against the whole complex or individual subunits. The high homology between cardiac and skeletal muscle troponins, establish the problem of cross–reactions. We utilized homology computer software to determine regions of cardiac troponin I and T, which present the minimum homology compared to the skeletal isoforms. From the entire regions that have been determined, we have chosen the 19–31 (RRRSSNVRAYATE) and 118–131 (TKNITEIADLTQKI) regions of cardiac troponin I isoform for production of antibodies. To this aim the selected peptide sequences were conjugated to Sequential Oligopeptide Carrier (SOC), Ac(–Lys–Aib–Gly–)$_4$–OH. The constructs have been synthesized by SPPS method, purified by HPLC and identified by ESI–MS. The immunizations with the above analogues gave high titer immune responses. Evaluation of the specificity and affinity of the produced antibodies is now in progress.

DESIGN OF NEW LIPOSOME FORMULATION AND ENCAPSULATION OF THE SYNTHETIC ANTICANCER DRUG LEUPROLIDE ACETATE AND STUDY OF ITS THERMAL EFFECTS ON MEMBRANE BILAYERS

Vasiliki Saroglou[1], Sophia Hatziantoniou[1], Mihalis Smyrniotakis[2], Thomas Mavromoustakos[2], Aikaterini Zompra[3], Vassiliki Magafa[3], Paul Cordopatis[3] and Costas Demetzos[1]

[1]Department of Pharmacy, University of Athens, GR–157 71, GREECE
[2]Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, GR–11635 Athens, GREECE
[3]Department of Pharmacy, University of Patras, GR–26500 Patras, GREECE

The aim of this study was to design and to develop a new liposomal formulation for the encapsulation of the oligopeptide Leuprolide Acetate (Glp–His–Trp–Ser–Tyr–D–Leu–Leu–Arg–Pro–NHEt) which is used in the treatment of advanced prostate cancer and endometriosis, in order to achieve an adequate delivery system for controlling the release of the drug, and lead to more beneficial pharmacokinetic properties.

Different types of liposomes were prepared in order to select the most appropriate for drug encapsulation. Liposomes encapsulating leuprolide acetate have been prepared by the Reverse Phase Evaporation Method. The size measurements of Small Unilamellar Vesicles (SUVs) by using the dynamic light scattering method, showed that liposomes composed of Egg-PC/DPPG 9/0.1 (molar ratio) are sufficiently small (z–average mean: 82.3 nm (PI: 0.32). The encapsulation efficiency (%) of leuprolide acetate was estimated while the drug to lipid ratio has been determined. Experiments of solid Nuclear Magnetic Resonance (NMR) and Differential Scanning Calorimetry showed that there is a significant interaction between the oligopeptide and the model DPPC membrane bilayers. A new drug delivery system based on liposomes was developed. Small Unilamellar Vesicles (SUVs), prepared following the Reverse Phase Evaporation Method, were encapsulated in Large Unilamellar Vesicles (LUVs), (z average mean > 1500 nm; ζ–potential -25.8 ± 10.8 mV) using the Thin Film Hydration Method. Images of the new liposome formulations were taken using a fluorescence microscope.

