OR01

AN EFFICIENT, DIRECT METHOD FOR SYNTHESIS OF DITHIASUCCINOYL (DTS) AMINES

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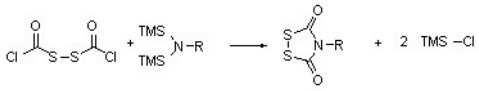
The 1,2,4-dithiazolidine-3,5-dione heterocycle, also referred to as a dithiasuccinoyl (Dts)-amine, serves as a readily removable amino protecting group of building blocks for the syntheses of peptides, glycopeptides, and PNA, and is also useful as a masked isocyanate, and (inversely) as a sulfurization reagent for trivalent phosphorus. Methods described to date for Dts-amine synthesis involve multiple steps, with concomitant reductions in yield and formation of byproducts. It seemed plausible that bis(chlorocarbonyl)disulfane (the two sulfur analogue of succinyl chloride) might serve as a reagent for the facile single-step elaboration of the heterocycle. Inspired by several precedents from the organosilicon chemistry literature that a trimethylsilyl group may serve as a "large proton," we report here on the successful, high-yield direct elaboration of the Dts heterocycle through reactions of bis(chlorocarbonyl)disulfane with bis(trimethylsilyl)amines [see Scheme]. The corresponding reaction directly with amines is unsuccessful, for reasons which are explained. Conditions for the new reaction were optimized, and the complete reaction pathway was elucidated through a series of model NMR studies, mass spectrometric analyses, and preparative experiments.



OR02

SOLID PHASE PEPTIDE SYNTHESIS ON MACROPOROUS METHACRYLATE MONOLITHS

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Ultra-short monolithic columns (disks) of optimized macroporous structure based on glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA) copolymer became quite popular as a chromatografic sorbent. The main advantage of these supports is their high hydraulic permeability and the dominance of convection over diffusion mechanism of mass-exchange at dynamic conditions that allows carrying out the separation at extremely high flow rates. After chemical conversions of original epoxy groups, GMA-EDMA monolithic disks can be used as efficient stationary phases for high-speed separations of biological molecules. The stability of the material in organic solvents allows their use for SPPS. Present report demonstrates the results of preparation of affinity supports via direct SPPS on macroporous GMA-EDMA monolithic disks. As the practical example, the synthesis of seven peptides - affinity ligands to plasminogen activators (PAs) – will be discussed. Fmoc-strategy was chosen as the experimental method. The affinity binding of synthesized ligands to PAs was evaluated using frontal analysis data. The results have been carefully compared with those established for affinity sorbents obtained by conventional way, e. g. by covalent immobilization of the same but preliminarily synthesized on Merrifield's resin, cleaved and purified peptidyl ligands. The practical applications of developed affinity sorbents will be also demonstrated. Moreover, specially designed non-flowing GMA-EDMA monolithic layers (biochips) were successfully used for parallel spot-SPPS. For this case, some practical examples will be shown and discussed.

OR03 P682

EFFICIENT SYNTHESIS OF DIFFICULT SEQUENCE-CONTAINING PEPTIDES THROUGH O-ACYL ISOPEPTIDES: APPLICATION TO THE SYNTHESIS OF ALZHEMEIR'S DISEASE-RELATED PEPTIDE, Aß1-42

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The synthesis of "difficult sequence"-containing peptides such as Alzheimer's disease-related peptide, Aß1-42 is one of the most troublesome matters in Peptide Chemistry, and the peptides are often afforded in low yield and purity in the SPPS. To solve this problem, we report a novel strategy for the synthesis of difficult sequence-containing peptides based on the synthesis of O-acyl isopeptides followed by an O-N intramolecular acyl migration reaction [1]. In the present study, model difficult sequence-containing peptides such as Ac-VVSVV-NH2 were synthesized in high yield and purity using this method, suggesting that the O-acyl isopeptides improved the nature of the difficult sequence during SPPS. This is due to their branched ester structures which modify the properties of parent difficult sequences such as unfavorable secondary structure. In addition, the obtained O-acyl isopeptides with an ionized amino group had higher water- and methanol-solubility (> 6,000-fold higher) than the N-acyl peptides and was purified effectively by HPLC. Finally, the parent peptides were obtained from the corresponding O-acyl isopeptides in PBS (pH 7.4) via an O-N acyl migration as highly pure precipitates. Furthermore, we successfully synthesized a natural difficult sequence-containing peptide, Aß1-42 via its O-acyl isoform at Ser26 ("26-O-acyl isoAß1-42") to overcome the intrinsic low solubility of Aß1-42. Consequently, it is understood that only one insertion of O-acyl group drastically improved the nature of difficult sequence in Aß1-42. Hence, this new method would contribute to the synthesis of larger peptides. [1] Sohma, Y., Sasaki, M., Hayashi, Y., Kimura, T., Kiso, Y. Chem. Commun., 124-125 (2004).

OR04

A ONE-POT TOTAL SYNTHESIS OF CRAMBIN

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Crambin is a model protein that has been extensively studied by x-ray crystallography, NMR, and computation of protein folding. We have reported an effective total chemical synthesis of crambin based on chemical ligation of three unprotected peptides [1]. However, the chemical tactics we used were laborious. Here, we report a one-pot synthesis of crambin that enables all the ligation steps and the folding of the full-length polypeptide, to be performed without purification of intermediate products. We made use of the 1,3-thiazolidine-4-carboxo- (Thz) group [2] to protect the N-terminal Cys of the middle peptide segment. The one-pot, three-segment ligation and folding was carried out on a tens-of-milligrams scale: the first ligation; conversion of Thz- to Cys-peptide; addition of the third segment to effect the second ligation; and, addition of folding cocktail to fold and form disulfides. The folded crambin molecule was purified directly from the total crude products. The synthesis took 48 hours total elapsed time. Overall yields from starting peptides to purified proteins were 35~45%. The covalent/tertiary structures of the synthetic protein were determined by LC-MS, 2D-NMR and x-ray crystallography. Efficient synthesis with facilitated handling methods will enable rapid studies of chemical variants of the crambin molecule, and will enable site-specific isotopic labeling for SS-NMR and FT-IR. [1] D. Bang, C. Neeraj, S. B. Kent, J. Am. Chem. Soc 126, 1377-1383 (2004) [2] M. Villain, J. Vizzavona, H. Gaertner, Proc. Second Int. Seventeenth Am. Peptide Symp., 107-108 (2001).

OR05

SEQUENCE-STRUCTURE-ACTIVITY RELATIONSHIPS FOR THE CYCLOTIDE KALATA B1

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Cyclotides are a diverse class of peptides characterised by their backbone cyclisation and cystine knot that contribute to their unusual stability. The properties of the framework are envisaged to provide a scaffold for the stabilisation of peptide epitopes with additional activities. To investigate the requirements for the cyclic cystine knot and its biological activities a complete set of alanine mutants of the prototypical cyclotide kalata B1 were prepared. Peptides were assembled using Boc-based solid phase peptide synthesis (SPPS) and the thiazip cyclisation. Utilising the cyclic backbone the synthesis could be started in six different positions (Figure 1 shows synthesis of loop 5 mutants). Using this approach, and combining the smaller loops, the total number of couplings was reduced from 667 (for the situation where every mutant is assembled individually) to 236.

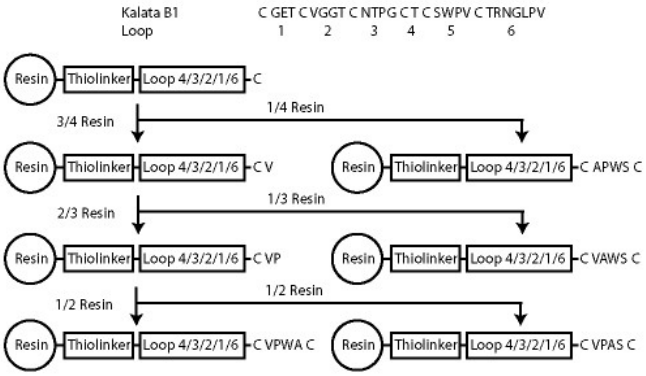

Figure 1. Synthetic Strategy for Loop 5 Alanine Mutants of Kalata B1.

OR06 P681

INTEGRIN ANTAGONISTS: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION

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Integrins, cellular adhesive receptors, play an important role in many physiological and pathological states. Currently there are 24 receptors known. We focused research on two receptors: fibrinogen receptor, aIIbb3, and vitronectin receptor, avb3. aIIbb3 has an essential role in platelet aggregation. Antagonists of aIIbb3 are already in use for treatment of thromboembolic disorders, e.g. tirofiban. avb3 is implicated in several pathologies, such as restenosis following percutaneous transluminal coronary angioplasty, cancer, osteoporosis, rhematoid arthritis and ocular diseases. Antagonism of avb3 is expected to help treat or even prevent these diseases. Endogen ligand binding to avb3 and aIIbb3 is mediated through a specific Arg-Gly-Asp (RGD) sequence. Based on the crystal structure of avb3 alone and in complex with the ligand cyclo(-RGDf[N-Me]V-) various binding models have been proposed for avb3 or aIIbb3 selective compounds consisting of a basic nitrogen group which interacts with the a subunit and the acid component which interacts with the b subunit. The specificity of antagonist interaction with the receptor lies in the distance between both pharmacophores and the nature of the basic nitrogen group. We used molecular modeling tools to design novel antagonists of avb3 and aIIbb3. We used various scaffolds, especially a rigid 2-methyl-3-oxo-3,4-dihydro-2H-pyrido[3,2-b][1.4]oxazine, on which the key pharmacophore elements were added. The synthesized molecules were evaluated by in vitro and in vivo tests and compared with the molecular modeling predictions.

OR07 P683

NOVEL WATER-SOLUBLE PRODRUGS OF CYCLOSPORIN ANALOGUES

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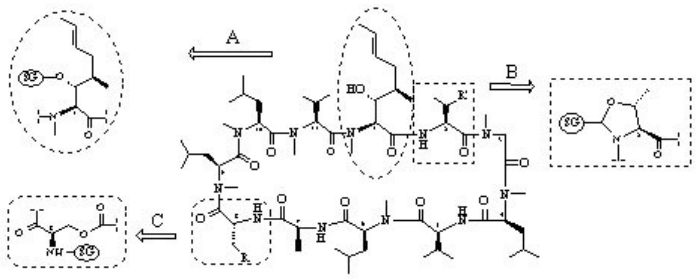
The cyclic undecapeptide Cyclosporin A (CsA) has a remarkable spectrum of diverse biological activities, including anti-inflammatory, anti-fungal, anti-parasitic as well as immunosuppressive activities. However, the low-water solubility of this drug is a serious problem causing undesirable pharmaceutical properties such as erratic oral absorption[1]. Here, we present the introduction of solubilizing systems into CsA (R, R' = H), CsC (R = H, R' = OH) and D-Ser8-Cs(R = OH, R' = H), applying direct derivatization (A), pseudo-proline (ψPro) insertion (B) and isoacylformation (C) (Figure). The chemical synthesis, physicochemical and pharmacological properties of a series of prototype systems containing various solubilizing groups (SG) are described and their potential use as prodrugs will be evaluated in terms of the chemical and enzymatic release of the parent Cs analogue. [1] Hamel, A. R.; Hubler, F.; Carrupt, A.; Wenger, R. M.; Mutter, M. Cyclosporin A prodrugs: design, synthesis and biophysical properties. Journal of Peptide Research 2004, 63, 147-154.



OR08 P679

EVOLUTIONARY COMBINATORIAL CHEMISTRY, A NOVEL TOOL FOR SAR STUDIES ON PEPTIDE TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER. 2. DESIGN, SYNTHESIS AND EVALUATION OF THE FIRST PEPTIDE GENERATION

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Successful drug development of peptides with therapeutic potential for treatment of CNS disorders require efficient delivery to the site of action. Drugs directed towards the CNS must overcome the blood-brain barrier (BBB). The BBB is formed at the level of the endothelial cells of the cerebral capillaries, these cells have tight junctions between them prohibiting any paracellular pathway. There is a great need for a systematic study of molecular structure and properties of peptide-based drugs that pass through the BBB, as no general tool to predict the transport has been established, and there are many exceptions to the existing rules. The novel approach, Evolutionary Combinatorial Chemistry, combines the selection and synthesis of biologically active compounds with artificial intelligence optimization methods as the Genetic Algorithms (GA). The solution is represented in a uniform way in all GA as a chromosome. Our chromosomes are sets of physico-chemical properties believed to be relevant for the transport across the BBB. To obtain the fitness of the peptides, the permeability through the BBB is determined using an in vitro BBB model. This model consists of Bovine Brain Endothelial Capillary Cells (BBEC) that are grown on a filter and form tight junctions due to the presence of astrocites. This assay has been optimized and the first generation of 24 peptides has already been synthesized and evaluated. We are confident that the use of the GA will allow us to increase our knowledge about the genome of peptides able to cross the blood-brain barrier.

OR09

METAL BACKBONE CYCLIZATION: NOVEL 99mTC LABELED GnRH ANALOG AS POTENTIAL SPECT MOLECULAR IMAGING AGENT IN CANCER

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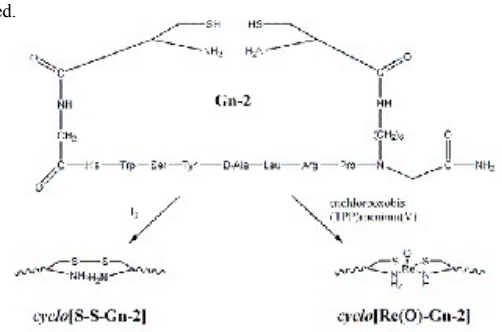
Gonadotropin-releasing hormone (GnRH) is a decapeptide that binds to specific receptors on the gonadotropes to regulate gonadotropin synthesis and secretion. Specific GnRH receptors are found in breast, prostatic and ovarian tumors. The aim of this study was to synthesize metal backbone cyclic (MBC) GnRH analogs with high affinity to GnRH receptors that can be radiolabeled with 99mTc. A library of 6 peptides based on the precyclic analog, Gn-2 was synthesized using SPPS protocols, purified by RP-HPLC, and characterized by ES-MS. The MBC GnRH analog, cyclo[Re(O)-Gn-2], was synthesized applying the recently reported MBC approach*. The binding capacity of cyclo[Re(O)-Gn-2] was compared to native GnRH, to Gn-2 and to the disulfide bridged backbone cyclic peptide cyclo[S-S-Gn-2]. Cyclo[Re(O)-Gn-2] was found to be the most active analog, with IC50 = 50nM (compared to IC50 = 10nM of the native GnRH). cyclo[99mTc(O)-Gn-2] was synthesized from Gn-2 and showed chromatographic behavior similar to its rhenium surrogate. We suggest that cyclo[Re(O)-Gn-2] and cyclo[99mTc(O)-Gn-2] might be useful for the imaging and treatment of GnRH positive prostate, breast and ovarian cancer. * Fridkin, G.; Bonasera, T. A.; Litman, P.; Gilon, C. J Comb Chem 2003, submitted.



OR10

NOVEL NEUROPEPTIDES WITH IRON-CHELATING MOIETY AS POTENTIAL AGENTS FOR CONTROL OF NEURODEGENERATIVE DISEASES

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Accumulating evidence suggests that iron dependent oxidative stress, increased levels of iron, and depletion of antioxidants in the brain may be major pathogenic factors in neurodegenerative disease. Neuropeptides such as vasoactive intestinal peptide (VIP), enkephalin, gonadotropin-releasing hormone (GnRH), and substance P (SP) have been shown to be associated with specific neuronal receptors. Thus, antioxidant-iron chelators conjugated to the peptides may directed toward their respective loci and have significant therapeutical potential against these diseases. In this study, a number of analogs of these neuropeptides bearing 8-hydroxyoxyquinoline moiety were synthesized, and their various properties related to iron chelation, and neuroprotective action were investigated. All the novel analogs were able to chelate iron and to form stable iron-complexes in solution. The analogs inhibited Fe/ ascorbate induced mitochondrial membrane lipid peroxidation with IC50 values (15 – 25 micromole) comparable to that of desferal, a prototype iron chelator which does not cross the blood brain barrier. In PC12 cell culture, the novel analogs at 1micromole were able to attenuate serum free stimulated cell death and improve the survival by 20 – 35%. They also provided protection against PC12 cell death induced by 6-hydroxy-dopamine (6-OHDA), with 1micromole of these analogs increasing the cell viability by 20-30%. EPR studies suggested that the novel peptide analogs, besides being good iron chelators, can also act as radical scavengers to directly scavenge hydroxyl radical. In conclusion, our data indicate that the new neuropeptide-derived chelators might serve as potential agents in the control of neurodegenerative disorders.

OR11

FROM OMEGA-CONOTOXINS TO MACROCYCLE MIMETICS

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The venom from the cone snails comprises more than a hundred different peptides that selectively target a number of different receptors and ion channels in mammals. This makes them interesting leads in the development of novel therapeutics. Several omega-conotoxins targeting the N-type voltage-gated calcium channel (VGCC) have been identified, including CVID and MVIIA from Conus catus and C. magus, respectively. The selectivity of omega-conotoxins for the N-type VGCC over the P/Q-type VGCC has led to their development as potential therapeutics for intractable pain. The omega-conotoxins are typically 24-29 residues with conserved six cysteine, four loop framework. Despite their selectivity and potency at the N-type VGCC, due to their peptidic nature, they are not ideal drug candidates. Extensive structure-activity relationship (SAR) studies of a selection of omega-conotoxins at the N-type VGCC have suggested that residues, including the conserved Lys2, that was initially believed to directly bind to the receptor and included in the pharmacophore instead play a structurally stabilising role and can thus be excluded from the omega-conotoxin pharmacophore. This suggests that the interaction surface of the peptide with the receptor is much smaller than previously anticipated. Based on this modified pharmacophore, we designed a series of small molecular equvialents built to mimic loop 2 of CVID. The SAR of the omega-conotoxins at the N-type VGCC and the design, synthesis and activity of the macrocycle mimetics, and their potential as future lead molecules as N-type VGCC inhibitors, will be discussed.

OR12

NOVEL MACROCYCLIC LIBRARIES WITH SPATIAL DIVERSITY (SIBS): APPLICATION FOR THE DISCOVERY OF IGF-1R INHIBITOR

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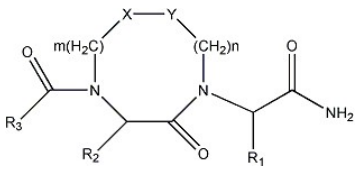
Combinatorial libraries of novel macro cyclic compounds with spatial diversity and improved ADME properties (called SIB technology) are described. These new compounds may be candidates for a variety of uses like human medicine, veterinary medicine and agriculture. Theoretically these libraries have six levels of diversity elements: (1) the ring chemistry, (2) the number of R groups, (3) the chemistry of the R groups, (4) the location of each R group on the ring. (5) the chirality of the R groups and (6) the size of the ring. Here we present the synthesis of libraries with the general structure shown in Figure 1 where X-Y = S-S. The R groups were chosen from the Tyr reach motif of the IGF-1R activation loop. Three libraries, comprising of six to sixteen compound each, were synthesized by SPS methodology. All the compounds were characterized by HPLC and MS and purified by preparative RP/HPLC. SIB 7 was active in biochemical and cell growth assays in the low micromolar range. It selectively inhibited the transphosphorylation of IGF-1 receptor and the activation of extracellular signal activated kinase (ERK) induced by IGF-1 in breast cancer cells. SIB 7 inhibited anchorage-dependent and –independent growth of breast cancer cell lines.



OR13

INTERACTIONS OF HANTAVIRUS GENE PRODUCTS WITH ANTI-APOPTOTIC PROTEINS

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Hantaviruses cause hemorrhagic fevers with renal syndrome and hantaviral pulmonary syndrome but the pathogenesis of the two diseases are not well understood. We have found that hantavirus infections induce apoptosis in cell culture, which involves direct interactions between viral and cellular proteins. Common to virus infections, the apoptotic outcome is strictly controlled by apoptotic and anti-apoptotic effects. The key players in apoptotic and anti-apoptotic effects belong among others to NF-κB mediated transcripts, which by cDNA array analysis are delayed in hantavirus infections. The goal of our research is to identify the virus-host protein domain and peptide ligand interactions, which are responsive in altered transcriptional regulation in apoptosis. The present study identifies TTRAP, TRAF2, BCl2 and actin interactions with hantavirus proteins. These interactions are characterized by yeast two-hybrid experiments, SPOT-peptide arrays and pull-down assays. In addition, kinetic data of different peptide and protein constructs derived from the proteins of interest are evaluated with surface plasmon resonance (Biacore).

OR14

IMMUNOGENICITY OF HIGHLY CONSERVED FRAGMENTS OF HEPATITIS C VIRUS ENVELOPE PROTEINS E1 AND E2

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Despite of the high variability of hepatitis C virus (HCV) E1 and E2 envelope proteins 8 highly conserved regions were revealed. Peptide scanning approach showed a limited number of B-epitopes, reactive to HCV-infected patient sera antibodies, inside these regions. Lack of antigenic activity of these sites can be explained by the absence of widely MHC II-specific T-helper epitopes nearby, since they are not revealed experimentally and not predicted by the known software. However, some E1 and E2 conserved sites possess CD81- and heparin-sulfate-binding activities, and this fact presents an important impulse to their use in artificial anti-HCV vaccines. Several peptides corresponding to E1 and E2 highly conserved and possibly functionally active sites have been obtained by solid-phase synthesis. Various peptide conjugates with carrier proteins and polymers have been used as immunogens for laboratory animal immunisations. Antibodies from hyperimmune antisera have been characterised with respect to their specificities. Peptide carriers have been shown to differ in their efficiency to stimulate anti-peptide antibody production. Success in obtaining antibodies against most carrier-attached highly conserved HCV E2 envelope protein fragments lacking the immunogenicity in the whole protein, shows a perspectivity of the artificial design approach for the construction of antigens able to produce anti-HCV antibodies with no isolate specificity. The work was supported by the Russian Ministry of Industry, Science and Technology Interdisciplinary Program “New Generation Vaccines and Medical Diagnosticums for the Future”.

OR15

THE MODE OF ACTION OF ENDONUCLEASE-VIII AS REVEALED FROM ITS STRUCTURE WITH AND WITHOUT DNA SUBSTRATE

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Reactive oxidation species induce structural damage in cellular DNA. These DNA damages are cytotoxic, miscoding or both and are believed to be at the origin of cell lethality, tissue degeneration, aging and cancer. In order to counteract immediately the deleterious effects of such lesions, cells have evolved a number of DNA repair mechanisms, one of which is Base Excision Repair (BER). The BER pathway is initiated by DNA glycosylases excising the modified base by hydrolysis of the N-glycosidic bond, generating a free base and an abasic site. Endonuclease VIII (EndoVIII, Nei) of E. coli is a base-excision DNA-repair enzyme that excises oxidized pyrimidines (T, C) from damaged DNA. Its substrate specificity overlaps with endonuclease-III (Nth), and it shares significant sequence homology with E. coli Fpg. We have recently determined the 3D structure of EndoVIII covalently crosslinked to specifically damaged DNA substrate at 1.25Å resolution, as well as the structure of the free enzyme (at 2.8Å resolution) and two of its catalytic mutants, E2A and R252A (at 2.3Å and 2.05Å resolution, respectively). EndoVIII consists of two domains that are connected by a 'hinge', a mostly β-sheet N-terminal domain and a α-helical C-terminal domain. The DNA-binding cleft lies between these two domains. The DNA in the complex is kinked, and the deoxyribitol moiety is everted from the duplex into an active site pocket and covalently bound to Pro1. The resulting space in the bound DNA is filled by Leu70, stabilizing the twisted conformation of the bound DNA. Two conserved glutamic acid residues, Glu2 and Glu5, are positioned near the deoxyribitol moiety, suggesting a role in protonation of the heterocyclic oxygen. Two DNA binding motifs are observed in EndoVIII, the Helix-2turn-Helix and the Zinc-finger motifs, both contribute to non-specific binding of DNA. A comparison between the structures of the DNA-bound and the free enzyme reveals a very significant conformational change, reflecting a potential inter-domain rotation of about 50?. This conformational transformation involves the exchange of a specific set of hydrogen bonds in the loop connecting the two domains, resulting in 'open' (DNA-free) and 'closed' (DNA-bound) forms of the enzyme. Such inter-domain flexibility has not been reported for any of the base-excision repair enzymes structurally characterized to date, and may present the first evidence for a DNA-induced conformational change of a DNA repair enzyme.

OR16

FROM FROGS TO FARNESYLATION: BIOPHYSICAL PERSPECTIVES ON HOW PEPTIDES INTERACT WITH MEMBRANES

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Why do frogs survive in dirty environments? How do proteins localize at the membrane interface? Antimicrobial and lipidated peptides provide excellent model systems to learn more about basic peptide-membrane interactions. Among other biophysical techniques, the analysis of the shape deformations of giant unilamellar vesicles using vesicle fluctuation analysis (VFA) allows us to characterize the membrane bending rigidity, kc, in the presence of (i) antimicrobial peptides: membrane destabilizers and (ii) lipidated peptides: membrane attachers/rigidifiers. Such biophysical studies reveal more about how these two different classes of peptides affect membrane physical properties.

OR17

BIOPHYSICAL STUDIES ON THE INHIBITION OF ß-AMYLOID AND THE PRION PROTEIN AGGREGATION

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Neurodegenerative diseases such as Alzheimer's disease (AD) and the Transmissible spongiform encephalopaties (TSEs) are related to protein misfolding. It is believed that the development of aggregates of the β-amyloid protein (βA) and the prion protein (PrP) is a key process in the pathology of Alzheimer's disease and spongiform encephalopaties, respectively. We have designed and synthesized β-sheet breakers peptides that aim to interact with βA aggregates and prevent the incorporation of monomer units in the intermediates or in the growing fibrils. These inhibitors contain N-Methyl groups to prevent hydrogen bond formation and some of them have been synthesized using D-amino acids to increase its resistance to proteases. Biological studies[1] using PC12 cells and biophysical studies using thioflavin T (ThT), turbidity and transmission electron microscopy (TEM) have allowed us to identify two good inhibitors of βA aggregation. We have also begun to study the aggregation process of a fragment of the prion protein by high resolution magic angle spinning NMR (HR-MAS). For this purpose we have designed a synthetic strategy to obtain free peptides reversibly bound to solid supports. Parallel aggregation studies are also carried out using more conventional techniques (ThT, turbidity and TEM). [1] Cruz, M.; Tusell, J.M.; Grillo-Bosch, D.; Albericio, F.; Serratosa, J.; Rabanal, F.; Giralt E.; Inhibition of β-amyloid toxicity by short peptides containing N-methyl amino acids. (2004, Journal of Peptide Science, in press)

OR18

COMPARISON OF DISTANCE GEOMETRY AND MOLECULAR DYNAMICS FOR CALCULATION OF PEPTIDE STRUCTURES FROM EXPERIMENTAL NMR DATA

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In a comparison of two structure calculation protocols, distance geometry (DG) based and pure molecular dynamics (MD), major differences were found for most members of a set of 21 conformationally restricted peptides for which NMR data had been collected in previous studies. The differences can be related neither to ring size nor to the presence of additional disulfide bridges even for cyclic peptides with similar sequences. A clear problem of the pure MD method is that for several of the investigated peptides energy barriers exist between low energy structures that cannot be crossed in reasonable time, even at simulation temperatures as high as 1000 K. In most cases molecular dynamics led to quite homogeneous structure ensembles while the DG based approach for these peptides often resulted in considerable structural diversity. Results of the MD method depend markedly on the force field used whereas the DG approach utilizes molecular dynamics only for refinement and is quite insensitive to differences in force field parameters. Because the peptide structures investigated here differ in number and size of cycles as well as in amino acid sequence, it seems justified to generalize that pure molecular dynamics calculations might result in incomplete structure ensembles and tend to underestimate conformational flexibility. Distance geometry based protocols on the other hand seem to explore the conformational space more thoroughly, but sometimes suffer from energetically less relaxed final structures.

L01

BRIDGES IN PEPTIDES: SEMISYNTHETIC PATHWAYS AND STRUCTURAL/BIOLOGICAL CONSEQUENCES

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The introduction of artificial bridges between the main-chains and also the end-groups of peptides and proteins is a versatile and useful technique for analytical investigations and preparative purposes. The present state of the art will be illustrated on the basis of selected examples. Analytical studies aim largely at the elucidation of spatial relationships and interactions within and between macromolecules in solution. Many bifunctional reagents with different nature, size and flexibility are available, either with two chemically identical or different reactive groups. Crosslinking reactive side-chains, as lysine and glutamic acid, is another possibility. Photoaffinity labelling has proved of special value to study ligand - receptor interactions. The inter- or intramolecular complexes are usually subjected to enzymatic cleavage, followed by separation and analysis of the fragments. These can be facilitated by introducing additional handles like biotin. The identification of fragments has reached a new dimension through the application of mass spectrometry. Semisynthesis of defined crosslinked polypeptides and proteins is still scarce. It has, so far, been explored rather extensively with insulin. While intramolecularly linked insulins are valid mini-proinsulins, some dimers of insulin analogues exhibit exceptionally high in-vitro biopotencies and receptor binding. Monofunctional reagents with affinities for other proteins provide pathways for non-covalent complexes. Thus, thyroxyl-insulins have a high affinity towards endogenous binding proteins, and the binding of insulin acylated with a fatty acid to serum albumin is finding therapeutic application.

L02

BRIDGES IN PEPTIDES AND SMALL PROTEINS: CONSTRUCTION BY CHEMICAL SYNTHESIS

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The realization that the bioactive three-dimensional structures of peptides and proteins are dictated principally by their linear sequences has provided the impetus for efforts to control such structures by the installation of covalent constraints. In this design, chemists take their cues from Nature, which relies predominantly on intramolecular cyclization of the main-chain amide or on cysteine-cysteine disulfide bridges (these latter can also be intermolecular). In addition to reproducing this repertoire of crosslinks, chemists explore linkages such as side-chain-to-side-chain amides, i.e., lactams, and oximes, among others. This lecture will summarize the general principles for the design and the chemical synthesis of peptide and protein bridges, illustrated with examples from the recent literature.

L03

MOLECULAR DESIGN OF PEPTIDES THAT CONTAIN 5'-AMINO-2,2'-BIPYRIDINE-5-CARBOXYLIC ACID AS AN UNNATURAL AMINO ACID: MODEL STUDIES ON PHOTO-FUNCTION AND STEREOCHEMISTRY OF THE METAL COMPLEXES

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We have proposed molecular design of peptides in which 5'-amino-2,2'-bipyridine-5-carboxylic acid (5Bpy) is introduced as an unnatural amino acid.[1] The peptides are expected to fold through coordination with metal ions to produce artificial proteins with definite highly ordered structures. In particular, the folded peptides with ruthenium tris(bipyridine) complex as the core are anticipated to exhibit photochemical functions such as strong and long-lived emission at longer wavelength, photo-sensing properties for some anions, and photo-induced electron/energy transfer. To investigate these photochemical properties, the ruthenium tris(bipyridine) complexes with amide groups at 5,5'-positions were synthesized as the model complexes. These ruthenium complexes show the similar photochemical properties to those of the parent ruthenium tris(bipyridine) complex, however the direction of the introduced amide groups (RC(O)NH- or RNHC(O)-) strongly affects these photochemical properties. The model complexes also exhibit photochemically sensing properties for some anions such as chloride and acetate anions. Such octahedral complexes with tris-chelated unsymmetrical bidentate ligands have two stereo-isomers, fac/mer, and two chiral enantiomers, Δ/Λ. Stereo-control of the octahedral metal complexes by the peptide sequences containing 5Bpy has been investigated through NMR, CD and HPLC studies of the stereochemistry of iron complexes with short peptides. These results of investigation will be utilized as a guiding principle to design novel artificial and photofunctional proteins. [1] H. Ishida, M. Kyakuno, S. Oishi, Biopolymers (Peptide Science), 76, in press (2004).

L04

CHEMICAL SYNTHESIS OF ELECTRON TRANSFER PROTEINS WITH FLAVIN, QUINONE AND HEME

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To continue our chemical synthesis of artificial electron transfer (ET) proteins [1,2], we report further progress of template-assembled synthetic proteins [3] with native-like cofactors: flavin, quinone and heme. Three different helices of 27 amino acids and a constrained cyclic decapeptide template containing two D-Pro-Gly [4] and four cysteines with three orthogonal protecting groups have been prepared as building blocks. Flavin was incorporated as Fmoc-protected flavin amino acid synthesized from either Lys [5] or diaminobutyric acid. Anti-parallel four-helix bundle proteins with 118 amino acid residues are achieved by ligation of the three different bromoacetylated helices to the cysteine residues of the template. Quinones including ubiquinone (0), menaquinone (0), plastoquinone (0), and 2,3,5-trimethyl-1,4-benzoquinone were covalently attached to a single free cysteine of the assembled proteins (after Acm cleavage) via thiol additon. Heme is finally incorporated by bis-His coordination. Flavin, quinones and heme are aligned in this order within the hydrophobic core of the proteins. Biophysical and electron transfer properties of these new constructs are under investigation. [1] Rau, H. K., et al. Proc. Natl. Acad. Sci. USA 95, 11526 (1998) [2] Li, W. et al. in Benedetti E. and Pedone C. (Eds.) Peptides 2002 (Proceeding of 27th European Peptide Symposium), Edition Ziino, Napoli, Italy, 192 (2002) [3] Mutter, M. and Vuilleumier, S. Angew. Chem. Int. Ed. Engl. 28, 535 (1989). [4] Peluso, S., et al. Chembiochem. 2, 432 (2001) [5] Carell, T., et al. J. Org. Chem. 63, 8741 (1998)

L05

A NEW APPROACH FOR PROLONGING THE ACTIONS OF PEPTIDES, PROTEINS AND LOW MOLECULAR WEIGHT DRUGS IN VIVO

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Most peptide and protein drugs are short-lived species in vivo, having a circulatory half-life of minutes. This is particularly valid for nonglycosylated proteins having a molecular mass of less than 50,000 daltons. A technology for elongating drug's lifetime in vivo has been developed. In this approach, several 9-fluorenylmethoxycarbonyl-SO_3H (FMS) moieties are covalently linked to peptides and proteins FMS-linked to amino groups of peptides and proteins, hydrolyses, at a slow rate, in aqueous media under physiological conditions with a t1/2 value in the range of 7-14 hrs, generating the nonmodified parent proteins. FMS containing peptides, proteins and low molecular weight drugs are long-lived species in vivo. Elongation takes place irrespective of whether the short-living character of the drug is attributed to receptor-mediated endocytosis, proteolysis at tissue surfaces, inactivation by serum proteases or clearance through glomerular filtration in the kidneys. Two or more FMS moieties should preferably be covalently linked for converting a short- to long-lived drug in the circulation. The loss of therapeutic potency that often accompanies FMS derivatization provides a profound advantage in this technology as conjugates are hydrolyzed and reactivated. a desirable pharmacological profile consisting of a flat and broad peak of circulating levels of the agent was ordinarily obtained following a single administration.

L06

PROTEIN OLIGOMERIZATION AND AMYLOID FIBRIL FORMATION THROUGH DOMAIN SWAPPING

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[1]Department of Organic Chemistry, University of Gdansk [2]Department of Crystallography, A. Mickiewicz University, Poznan, Poland [3]Department of Clinical Chemistry, University Hospital, Lund, Sweden

Neurodegenerative diseases as Alzheimer's disease, Parkinson's disease, Huntington's disease and others are caused by abberant peptide or protein conformation. Among amyloidogenic proteins which have the propensity to self-assemble into oligomers and amyloid fibriles is a human cystatin C (HCC). The crystall structure of HCC solved by us showed dimer formation via the mechanism of 3D domain swapping and this observation led to the suggestion that analogous domain swapping mechanism, but propagated in an open-ended fashion, could be the basis of the formation of cystatin fibriles. Considering the proposed model of fibrillogenesis, it seems possible that inhibition of the domain swapping process should suppress the entire process of dimerization and fibrillogenesis. This presentation will describe different approaches used by us in the study of domain swapping mechanism of HCC, as well as results of our investigations on the inhibition process of dimerization and oligomerization of HCC.

L07

SWITCH-PEPTIDES: ISN- INDUCTION OF CONFORMATIONAL TRANSITIONS RELEVANT IN DEGENERATIVE DISEASES

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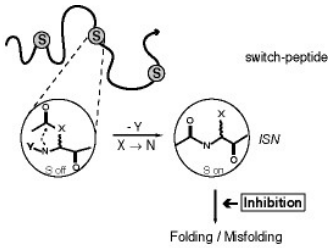
Due to their impact on neurodegenerative diseases, irreversible conformational transitions of peptides have moved into the center of interest. Here, we develop a novel class of switch-peptides, which allows to access folding events in the process of structure evolution, i.e. 'in statu nascendi' (ISN) of the molecule. According to the Figure, N(Y)- acylisopeptides (Y = H+ or enzymatically cleavable groups[1]) are assembled by SPPS resulting in flexible polypeptide chains of high water solubility. In triggering X α N acyl migration, the native peptide backbone is restored, setting off conformational transitions and folding processes at physiological conditions. In applying host-guest techniques, the concept serves as a diagnostic tool for elucidating the impact of external factors (inhibitors, ββ breakers) upon the onset of ββ sheet and fibril formation of amyloid β derived peptides. Results on conformational switches, structure inhibition and assembly relevant in degenerative diseases will be presented. [1] M. Mutter et al., Angew. Chem., submitted.



L08

UNUSUAL BEHAVIOUR OF A PEPTIDE SEGMENT FROM THE PRIMARY STRUCTURE OF HUMAN PRION PROTEIN

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The normal cellular prion protein (PrPC) is a host-encoded cell surface glycoprotein expressed mainly in the brain and to a lesser extent in many other tissues. The critical step in prion diseases that affect animals and humans seems to be the conversion of PrPC, into the abnormal prion isoform, PrPSc, which forms insoluble aggregates partially resistant to proteolysis. Recently, the high specific monoclonal antibody V5B2 has been prepared that discriminates between CJD and normal brain tissue samples without proteinase K digestion. 13-residue synthetic peptide P1 from the C-terminal a-helix of human PrP (CITQYERESQAYY), coupled to KLH has been used as an antigen. The solution behaviour of antigen P1 has been investigated by applying the combination of spectroscopic (CD-spectroscopy, UV-spectroscopy), electrophoretic (PAGE), immunochemical (ELISA), microscopic (scanning electron microscopy) and chromatographic (gel filtration) techniques. Our CD results reveal that peptide P1 in aqueous solution at neutral pH adopt some kind of ordered secondary structure, which can be affected by temperature, pH and ionic strength. The ordered structure of P1 has been for two folds better recognized by antibody V5B2 than unordered one. Furthermore, the gel filtration and native PAGE results confirm that P1 in aqueous solution is not in monomeric form. Additionally, we observed that P1 at pH 1.2 and in the presence of Cu2+ forms insoluble aggregates. The data obtained from scanning electron microscopy reveal that the low pH-induced aggregates are fibril-like, while Cu2+-induced aggregates are amorphous. The behaviour of peptide P1 in aqueous solution can be ascribed as a prion-like.

L09

A DESIGNED HEXAPEPTIDE INHIBITOR OF AMYLOID FORMATION AND CYTOTOXICITY OF ISLET AMYLOID POLYPEPTIDE (IAPP)

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The pathogenesis of type II diabetes is strongly associated with the process of aggregation of the 37-residue islet amyloid polypeptide (IAPP) into cytotoxic, beta-sheet-containing amyloid fibrils. Therefore, inhibition of IAPP amyloid formation and related cytotoxicity is an important task towards the development of therapeutic approaches in type II diabetes. We have shown that the rational N-methylation of two amide bonds of the IAPP beta-sheet and amyloid core sequence IAPP(22-27) or NFGAIL was able to convert short, amyloidogenic and cytotoxic sequences of IAPP into non-amyloidogenic and non-cytotoxic ones (Kapurniotu, et al., J. Mol. Biol. (2002)). The hexapeptide NF(N-Me)GA(N-Me)IL has been found to be able to interact with its precursor NFGAIL and inhibit its aggregation into cytotoxic amyloid. Here, we present our studies on the effect of NF(N-Me)GA(N-Me)IL on aggregation into beta-sheets and cytotoxicity of full sequence IAPP. Circular dichroism (CD) spectroscopy and electron microscopy (EM) show that NF(N-Me)GA(N-Me)IL is able to interact with IAPP and to inhibit its aggregation into beta-sheets and amyloid. Moreover, NF(N-Me)GA(N-Me)IL is found to inhibit the cytotoxic effect of IAPP aggregates on pancreatic beta-cells according to the MTT reduction assay and as assessed by apoptosis analysis. Studies with the non-N-methylated sequence NFGAIL and a homologeous sequence of rat IAPP suggest that the above effects are due to the introduced N-methyl rests. These results suggest that NF(N-Me)GA(N-Me)IL may find application as an inhibitor or a lead compound for the design of inhibitors of IAPP aggregation-mediated beta-cell damage and related pathogenesis in type II diabetes.

L10

LARGE-SCALE PRODUCTION OF PEPTIDES -- THE SUCCESS STORY

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During the 50 years since Du Vigneau published the synthesis of oxytocin – the first peptide pharmaceutical, which is still in widespread use today – peptides have matured into an important segment of the pharmaceutical industry. Despite some setbacks in the 1980s and early 1990s, as big pharma closed its peptide research groups worldwide, there has been a recent resurgence of interest in the field. Thus, of the more than 40 peptides that are in use as pharmaceuticals today, approximately 25% have received approval within the last decade, and numerous products are in late-stage development. Several peptides, such as Lupron® and Zoladex®, have even achieved the status of "blockbuster" drugs, while several more are poised to do so. The long-awaited realization of the promise of peptides as "Nature's Pharmaceuticals" can be largely attributed to advances in the areas of formulation and delivery. This has somewhat mitigated the fact that, as a class, peptides are generally not orally active – one of the factors that impeded their development as pharmaceuticals for many years. However, it is important to note that the introduction of new technologies, which have enabled drugs from this important class to be manufactured more economically, has been of equal importance to the field. The current status of peptides as pharmaceuticals will be discussed, together with examples of both approved products, and also a number that are in late-stage development. Manufacturing strategies will be presented, with particular emphasis on chemical synthesis techniques.

L11

'FUZEON' (TM), PEPTIDE PRODUCTION AT THE TON SCALE, SYNTHETIC STRATEGY, DEVELOPMENT AND MANUFACTURING CHALLENGES

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Fuzeon, or T-20, is a 36 amino acid peptide and is the first approved drug in a new class of antiretrovirals designed to block HIV fusion to host cells. Commercial material requirements for this drug are in the multi-ton range. The unprecedented manufacturing scale needed to produce these quantities of peptide has presented challenging scale-up issues, which resulted in some new manufacturing techniques. Synthetic strategy, scale-up issues and the impact T-20 has had on the field of peptides will be discussed.

L12

INVERSE STRATEGY OF AMINO-ACID RESIDUE ANCHORING ON SOLID SUPPORT AFFORDS NEW CHEMISTRY - FIRST EXAMPLES

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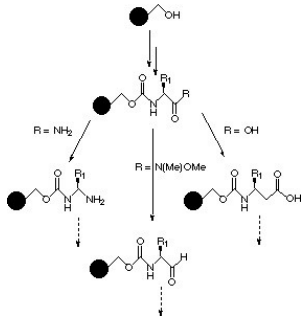
Conventional C to N terminus solid-phase peptide synthesis is based upon the anchoring of a carboxylic acid to a hydroxy or amino resin to give an ester or amide. However, the need to prepare a wide variety of low molecular weight chemical compounds by solid-phase synthesis encouraged us to develop a different strategy. For this approach, we investigated the anchoring of amino-acids via their amine function, opening by this way all the chemistry reactions on the free carboxylic acid group. In this preliminary report, we will discuss about our results on the Hofmann rearrangement of a single residue anchored to the resin via its amine function, the generation of aldehydes from Weinreb amides, the Arndt-Eistert homologation and use of these moieties in further chemistry. Other potential reactions are actually explored.



L13

ENGINEERING PROTEASES AS COUPLING REAGENTS IN PEPTIDE SYNTHESIS

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Chemical protein synthesis is a powerful method for assembling selectively modified proteins, providing a freedom for protein engineering inaccessible by standard site-directed mutagenesis. This benefit comes, however, together with the need for developing approaches that allow for the selective, universal and racemisation-free incorporation of chemically modified peptide fragments into the protein targets. Efforts in this direction pioneered the substrate mimetics-mediated protease catalysis as a novel ligation method that apparently breaks with the popular rule whereupon the specificity of an enzyme leads necessarily to a decrease in the method's flexibility. In fact, the approach tolerates a high structural diversity at the ligation site despite the pronounced substrate preferences of the biocatalysts.[1] Unwanted peptide bond cleavages based on the proteolytic activity of wild-type proteases, however, still remain as a serious side reaction limiting the universality of this biocatalytic approach. In this contribution we describe the synthesis behavior of genetically optimized proteases with decreased proteolytic activities. Furthermore, the effect of the enzyme's stereospecificity on the course of peptide ligations and modifications is presented. Surprisingly, the latter led to the finding that L-amino acid specific enzymes are better suited for reactions on all-D-peptides,[2] while those having an artificial specificity are more efficient towards all-L-peptides. Selected reactions prove the power of the novel biocatalysts for the coupling of both all-L- and all-D-peptides without the risk of proteolytic cleavages. [1]F. Bordusa, Chem. Rev. 102 (2002), 4817. [2]N. Wehofsky, S. Thust, J. Burmeister, S. Klussmann, F. Bordusa, Angew. Chem. Int. Ed. 42 (2003), 677.

L14

INDUSTRIAL SYNTHESIS OF DI- AND SHORT OLIGOPEPTIDES AT DSM

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DSM Research, Life Sciences - Advanced Synthesis, Catalysis and Development, Geleen, The Netherlands

DSM is one of the largest producers of di- and short oligopeptides and proteins in the world. In this presentation an overview of low-cost technologies for di- and short oligopeptides will be presented that are or may be applied within DSM on large scale, i.e., (solution-phase) chemical synthesis, chemo-enzymatic synthesis and fermentation. The choice for either one of these technologies is not only determined by inherent production costs but also by factors like scale, peptide complexity, required process R&D time and life-cycle of the product. These technologies will be exemplified by existing and future DSM products. For instance, of the high-intensity dipeptide sweetener Aspartame, currently produced by DSM on multi-1000 tonnes per year scale, the current and a novel chemo-enzymatic process will be shown as well as a radically novel fermentative option (Asp-Phe fermentation using microbial peptide synthetase technology). In the area of low-cost chemical peptide synthesis, the cheap and easily cleavable (acid labile) N-sulfamido protective group was developed as well as a cheap reagent (dimethyldichlorosilane) for the coupling of amino acids and amines via the cyclic activation/protection strategy (the 'holy grail' in chemical peptide synthesis), so without any N-protection. In the field of low-cost chemo-enzymatic synthesis the enzymatic deprotection of N-formyl-protected peptides by peptide deformylase has been identified as promising concept (also in a fully enzymatic "two-enzyme one-pot synthesis" variant). Additionally, the deprotection of N-phenacetyl protected peptides by penG-acylases or various origins was developed.

L15

APPROACHES TO PHARMACEUTICAL PEPTIDE DELIVERY

S. Zalipsky

ALZA Corporation, Mountain View CA, USA

Peptides and proteins hold considerable promise as biologically active substances with high potency and specificity. Their development as therapeutics, however, has been hampered by their limited solubility, tendency to aggregation, immunogenicity, limited stability, and susceptibility to proteolytic degradation, all of which complicate their delivery. This presentation will cover several innovative approaches to the controlled delivery of peptides over periods ranging from days to months. The primary focus will be on the following peptide delivery technologies developed by ALZA Corporation. 1. Transdermal Macroflux® technology that can be also viewed as a bolus needle-free administration. It is based on titanium microprojections that, when applied, to the skin penetrate into the epidermis to create drug transport pathways. 2. Parenteral delivery of polypeptides in a poly(ethylene glycol)–conjugated form. In a prodrug variation of this methodology, the polymer chains act as promoieties of the protein-based drug. 3. Degradable and nondegradable implant systems. Upon implantation, the ALZAMER® Depot gel gradually releases peptide over a period of a few weeks, during which time the implant itself is degraded. DUROS® technology allows gradual release of an active peptide for up to one year from a subcutaneously-implanted miniature titanium cylinder.

L16

THE THIRD WAVE OF NATURAL PEPTIDES: FROM HORMONES AND ANTIBIOTICS TO PEPTIDE POOLS

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Traditionally, since mid-50ies studies of naturally occurring peptides have been mainly revolving around two groups of substances: (i) hormones + neuropeptides (including toxins) found in higher organisms and (ii) antibiotics produced non-ribosomally by actinomycets and other lower organisms. More recently a rapidly growing family of peptide antibiotics derived (as hormones) from specific protein precursors and functioning in a broad variety of organisms was discovered and studied. Following the mainstream of postgenomic technologies we attempted in recent years total screening of various biological sources for peptide material and found that each tissue or cell culture produces a conservative set of oligopeptides resulting from endogenous proteolysis of common functional proteins (hemoglobin, actin, intracellular enzymes, etc.). In the present communication new data will be presented on peptides found in murine transformed cell cultures (WEHI-3) as well as in insect (Rhodnius prolixus hemolymph) and plant (Avena sativa acrospire oat) sources. Most of the peptide pool components affect proliferation rates in cell cultures. Data will be provided showing that part of these peptides acts by hormone-like receptoric mechanisms, while others penetrate the cell membrane and act intracellularly. The structural and mechanistic data obtained in this work will be discussed under the assumption of participation of pool components in tissue homeostasis.

L17

APOLIPOPROTEIN A-I AMPHIPATHIC A-HELICAL PEPTIDE MODELS: EFFECT ON LDL OXIDATION IN VITRO

M. Sakarellos-Daitsiotis, C.H. Alexopoulos, A. Tambaki, K. Harilogis, A. Tselepis, C. Sakarellos

Department of Chemistry, University of Ioannina, Greece

The inverse relationship between plasma levels of high density lipoprotein (HDL) and the risk of atherosclerosis and coronary artery disease has been attributed to several HDL functions, including antioxidant and antiinflammatory effects. An important role in these effects plays the apolipoprotein content of HDL, especially apolipoprotein A-I (apoA-I). With the aim to develop atheroprotective agents, we report on the design, synthesis, conformational analysis and biological effects of four amphipathic α-helix apoA-I peptide models:
Ac-ESK(Palm)KELSKSW_{10}SEM_{13}LKEK(Palm)SKS-NH_2 (1),
Ac-ESK(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 intrinsic fluorescence probe. The syntheses of the apoA-I peptide models were carried out 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 Cu2+-induced oxidation of the low-density lipoprotein (LDL) in vitro, and to prevent the oxidation induced inactivation of the LDL-associated platelet-activating factor acetylhydrolase (PAF-AH) at different concentrations were investigated and their atheroprotective role is discussed.

L18

SPECIFIC AND LONG LASTING IMMUNE RESPONSE TO SOLUBLE AMYLOID OLIGOMERS MIMICS: ARE WE CLOSER TO THE SECOND GENERATION OF VACCINES AGAINST DEGENERATIVE DISEASES?

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Coupling of nanoparticles and biomolecules has many applications. Nanoparticles may be used in protein-based recognition systems, DNA oligomerization and templating, gene-gun technology biomimetic systems, biotemplating, biolabeling and the construction of model systems . Here we demonstrate the coupling of a peptide (Aβ with a thioester C-terminus) to gold nanoparticles, to mimic the micellar structures formed when proteins aggregate. The polyclonal serum produced by vaccination of rabbits, mice, and Canine with the molecular mimics is called “anti-oligomer” because it is specific for the soluble oligomeric intermediates and has no detectable reactivity with soluble low MW or fibrillar Aβ species. Surprisingly, no anti-oligomer immunoreactivity against low MW Aß or Aß fibrils was observed for the unfractionated serum even after boosting the animals twelve times, indicating that the immune response to the molecular mimics is very specific, also the antibody titter was unchanged for more than 18 months after the last boost. Spherical soluble oligomers have been observed for many different types of amyloids. Surprisingly, anti-oligomer reacts well with all of the soluble oligomeric aggregates, regardless of sequence and does not react with either the soluble low MW species or the fibrils. This includes oligomeric and protofibrillar aggregates from synuclein, IAPP, poly glutamine, lysozyme, human insulin and prion peptide 106-126. At the same time it does not detect any proteins in soluble cells lysates. These results indicate that anti-oligomer recognizes a unique common structural feature of the polypeptide backbone in the amyloid soluble oligomers that is independent of the amino acid side chains.

L19

MOLECULAR CHARACTERIZATION OF COVALENT TRANSGLUTAMINASE (TG2)GLIADIN PEPTIDE COMPLEXES INVOLVED IN COELIAC DISEASE

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Covalent complexes of TG2 with gluten peptides may be involved in the formation of auto-antibodies against TG2 in coeliac disease patients ingesting gluten. We have characterized complexes between TG2 and two immunodominant gliadin peptides generated in vitro. Complex formation was quantified in spin column experiments. Complexes were separated by SDS gels and digested. Acyl acceptor sites in TG2 were identified by various experiments using mass spectrometry. Two types of covalent complexes were found: the peptides were either linked via a thiolester bond to the active site cysteine or via isopeptide bonds to particular lysine residues of TG2. After incubation of TG2 with an equimolar ratio of peptides, equal amounts of peptides were bound by thiolester and isopeptide linkage. At higher peptide to TG2 ratios, multiple isopeptide bond formation dominated. Altogether six lysine residues were identified as gluten-peptidyl acceptor sites. Deamidated peptides were released from the complexes by hydrolysis of the thiolester bond, whereas isopeptide bonds were found to be stable. Deamidated gluten peptides released from thiolester linked complexes may bind to HLA-DQ2/DQ8 molecules after endocytosis in TG2-specific B-cells. This type of TG2-gluten complexes is therefore the best candidate for participation in hapten-carrier mediated formation of TG2 antibodies.

L20

MAKING PEPTIDE VACCINES 'BETA': T CELL EPITOPES CONTAINING NOVEL AMINO ACIDS ELICIT ANTI-TUMOUR CYTOTOXICITY

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The ability to deliver minimal T-cell epitopes to the effector cells of the immune system negates the requirement for in situ antigen synthesis or further processing and also minimizes side effects and simplifies monitoring. One of the confounding issues with peptide-based vaccines is their poor bioavailability, which is associated with proteolysis and oxidative damage of the ‘naked’ peptides once taken out of the context of the intact antigen. Our approach to this problem has been to engineer protease resistance and non-natural residues into tumor antigen epitopes. Peptide bonds that incorporate beta-amino acids are resistant to proteolysis, yet this substitution only involves the addition of a methylene group adjacent to the C-alpha carbon of the natural residue. We have individually substituted each amino acid of tumor antigen peptides with the corresponding beta-amino acid or other non-natural amino acids. The immunogenicity of the analogues was established using T-cell lines and by priming naïve mice and testing their ability to destroy tumor cells that express the tumor antigen. To understand the molecular basis of these non-natural amino acid substitutions on peptide orientation and MHC conformation, crystal structures of selected peptide/MHC complexes have been solved to high resolution (1). Overall, the peptide analogues were well tolerated, demonstrated enhanced serum stability maintained strong MHC-binding and remained immunogenic. References (1)Webb, A. I.; Dunstone, M. A.; Chen, W.; Aguilar, M.-I.; Chen, Q.; Jackson, H.; Chang, L.; Kjer-Nielsen, L.; Beddoe, T.; McCluskey, J.; Rossjohn, J.; Purcell, A. W. J. Biol. Chem. 2004, M314066200.

L21

UTILIZING GENOMICS AND PROTEOMICS FOR PEPTIDE BASED DRUG DESIGN. DRUG DESIGN FOR DISEASE RECONSIDERED

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The classical approach to drug design: single target identification; lead ligand identification; ligand optimization via structural, conformational and dynamic consideration; has been shown to be inadequate or even counter-indicated for many diseases; e.g. neuropathic pain, cancer, AIDS, diabetes and mental illness. Using genomics and proteomics it has been shown that changes in several (many) genes/proteins are involved in disease states. A new approach to drug design is needed involving design of ligands which can interact as agonists and/or antagonists at two or more functionally different sites simultaneously. This approach will be illustrated by the design of ligands that can simultaneously act as agonists at opioid (μ and δ) receptors, and as antagonists at CCK receptors (CCK1 and CCK2) for treatment of neuropathic pain; and by design of multimeric ligands that can simultaneously act at two or more receptors (same or different). An examination of whether tissue specific ligands can be designed will be discussed. Supported by grants from the USPHS and NIDA.

L22

SAR OF IMPROVED COMPOUNDS FOR TARGETED RADIOTHERAPY OF HUMAN SOLID TUMORS EXPRESSING GASTRIN RELEASING PEPTIDE RECEPTORS

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The Gastrin Releasing Peptide Receptor (GRP-R) is over-expressed in many cancers, including prostate, breast and small cell lung cancer. Bombesin (BBN), a naturally occurring peptide, is a potent GRP-R agonist with homology to 12 of the 24 amino acids of native Gastrin Releasing Peptide (GRP). The last 8 amino acids (QWAVGHLM) have been shown to be critical for biological activity. Previously, it was shown that BBN[7-14] linked via 8-aminooctanoic acid to a DOTA-chelate containing a beta emitting isotope such as 90Y or 177Lu, binds to GRP-R(+) tumor cells in vitro and increases the survival of GRP-R(+) tumor bearing mice. We found that optimization of the linker between the DOTA-chelate and Bombesin [7-14] produced compounds with substantially improved efficacy in vivo. It is well known that a bile acid moiety binds to human serum albumin (HSA), increasing the half-life of compounds linked to HSA in blood. However, we have found that 3-amino-3-deoxy-cholic acid derivatives, used as linkers, also demonstrate this property, which is novel. SAR of different cholanoic linkers allowed the selection of DOTA-Gly-Chol-BBN[7-14] (where Chol is 3-amino-3-deoxycholic acid) as a potential candidate for clinical development. A study conducted with a HEPES solution of Gd-DOTA-Gly-Chol-BBN[7-14] revealed binding with HSA (Ka = 1.2 x 104 M-1; Rb=20 mM-1s-1). The chemistry of 177Lu-DOTA-Gly-Chol-BBN[7-14] and related analogs, and the pharmacological studies that demonstrate the utility of these compounds in targeted radiotherapy will be presented.

L23

EFFECT OF HYDROPHOBIC CHAINS ON PEPTIDE ANTIMICROBIAL AND ENDOTOXIN-NEUTRALIZING ACTIVITIES

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Hydrophobic partitioning between water and lipid bilayers combined with electrostatic interactions mainly govern the initial stages leading to binding and functioning of antimicrobial peptides. Furthermore, Gram negative bacteria contain lipopolysaccharides (or LPS) in their envelope, amphiphiles with a very hydrophobic section adjacent to a dense and negatively charged oligosaccharide moiety, which are the most potent elicitors of bacterial sepsis. As for antimicrobial activity, both hydrophobic and electrostatic interactions play a crucial role in a peptide LPS-neutralizing activity. Thus, using a series of analogs of a lactoferrin fragment (FQWQRNIRKVR), we have investigated how alteration or enhancement of such interactions affects a peptide antimicrobial and LPS-neutralizing activities. Thus, amino acid omissions, substitutions, and insertions were made to modify the peptide overall charge, hydrophobic core, and/or amphipathicity. Lipopeptide derivatives were also synthesized by inserting lipophilic chains varying in length and nature at the N-terminus. The peptides antimicrobial activities against a large panel of bacteria, LPS-neutralizing activities, and effect on lipid model membranes and LPS aggregates as determined by CD, FTIR, and NMR spectroscopy, microcalorimetry, and X-ray diffraction were comprehensively characterized. The peptides binding to LPS was also evaluated and correlated to biological data of bactericidal activity and inhibition of LPS-induced production of cytokines. Not only novel peptides and lipopeptides having both broad spectrum antimicrobial and endotoxin-neutralizing activities were generated, these SAR studies also provide valuable insights into the effects of hydrophobic chains on cationic peptide scaffolds on peptide-lipid interaction events and their associated biological functions. Supported by grant ANEPID (QLK2-CT-2002-01001) from the EC.

L24

DESIGNED SELF-ASSEMBLING PEPTIDES FOR STRUCTURE-FUNCTION STUDY OF BIOMINERAL REGULATORY PROTEINS: INVESTIGATION OF THE ROLE OF PROTEIN SELF-ASSEMBLY AND MULTIPLETS OF CHARGED AMINO ACID RESIDUES

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The bottom up approach to material synthesis is one of the frontier areas of research in the material science and technologies. The molecular level understanding and rationalization of the formation mechanism of biominerals will provide a key direction for controlled fabrication of new materials with unusual properties. Towards this direction we investigated the role of protein self-assembly and the sequence specificity of avian eggshell matrix proteins using model peptides. The peptides were designed, synthesized and conducted a detailed structure-property study to unravel the molecular mechanism of eggshell calcification. The primary structural features of a few eggshell matrix proteins were used to design the peptides. The solution structure of these peptides in solution was investigates using CD, intrinsic tryptophan fluorescence, quasi-elastic light scattering, 2D NMR and molecular modeling studies. The aggregation of peptides in solution is used to explain the observed results from the in vitro crystallization experiments. Based on this, we propose a particular structure of self-assembled peptide is crucial for the formation of calcite crystal aggregates. The understanding of the molecular mechanism of this biomineralization process led to the design of new self-assembling peptides with novel activities.

L25

APPLICATION OF AZACYCLOALKANE AMINO ACID-DERIVED PEPTIDE MIMICS IN THE STUDY OF G-PROTEIN COUPLED RECEPTORS

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Ligands for G protein-coupled receptors (GPCRs) represent a significant class of therapeutic targets because of their potential to regulate signal transduction processes implicated in human physiology. More than 60% of all currently available drugs work by interacting with known GPCRs. On the other hand, because GPCRs are large proteins that are typically membrane-bound, they have generally eluded characterization by crystallographic as well as spectroscopic methods. To develop effective ligands for GPCRs, we have studied peptide ligands obtained from screening and native sources using series of azacycloalkane amino acids. Employing these heterocyclic amino acids to constrain the backbone and side-chains of peptide leads, we have generated mimics with greater potency and selectivity. Our presentation will focus particularly on efforts to study the structural requirements for recognition at the oxytocin receptor (1), the opioid receptor-like ORL1 receptor (2) and the prostaglandin F2alpha receptor (3). The results of these initial studies will illustrate the potential of azacycloalkane amino acids as 'privileged' scaffolds for the discovery of GPCR ligands. (1) Bélec, L.; Maletinska, L.; Slaninova, J.; Lubell, W.D. J. Peptide Res. 2001, 58, 263-273. (2) Van Cauwenberghe, S.; Simonin, F.; Cluzeau, J.; Becker, J. A. J.; Lubell, W.D.; Tourwé, D. J. Med. Chem. 2004, 47, asap. (3) Peri, K.G.; Quiniou, C.; Hou, X.; Abran, D.; Varma, D.R.; Lubell, W. D.; Chemtob, S. Seminars in Perinatology 2002, 26, 389-397.

L26

USING DESIGNED PEPTIDE PANELS FOR DE-RISKING GPCR PROJECTS

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The GPCR family of proteins has traditionally provided the pharmaceutical industry with rich sources of targets for drug discovery. The successful discovery of therapeutic compounds with activity at these targets is evidenced by the considerable number of marketed drugs. These discoveries have been facilitated by the fact that the endogenous ligands are either biogenic amines or peptide ligands with less than 10 amino acids in length. In contrast, discovery of small molecule agonists or antagonists for GPCR targets that are characterized with endogenous peptide ligands of 10 or more amino acids has been considered difficult, if not risky. We developed a strategy of de-risking such projects by using a designed series of peptides which allow one to assess the probability of success or failure in rapid fashion. This strategy will be discussed, citing cases from obesity-related GPCR projects where the endogenous peptide ligands include Melanin-concentrating hormone (MCH), Prolactin releasing peptide (PrRP), and Neuropeptide W (NPW).

L27

DIFFERENT BINDING MODES OF ANGIOTENSIN II AND [SAR1]-ANGII TO THE AT1 RECEPTOR

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Homology modeling of the structure of the angiotensin II (Ang II) AT1 receptor, based on the high resolution rhodopsin crystal structure, indicated that it is unlikely that the binding of AngII to AT1 involves simultaneously all the receptor's residues reported in the literature to participate in this process. Site-directed mutagenesis using Ala substitution of charged residues Lys20, Arg23, Glu91 and Arg93 was performed to evaluate the participation of their side-chains in ligand binding and in triggering the cell's response. A comparative analysis by competition binding and functional assays using Ang II and the analogue [Sar1]-Ang II suggests an important role for Arg23 of the AT1 receptor in binding of the natural agonist. Our results indicate the occurrence of different binding modes for AngII and [Sar1]-AngII. In our docking model, interaction of the Ang II's Asp1 with the receptor's Arg23 brings the ligand's Arg2 guanidinium in contact with the receptor's Asp278. In the case of [Sar1-Leu8]-AngII the lack of the β-carboxyl group allows a closer interaction of the Arg2 residue with the receptor's Asp281. This particular behavior may also explain previous findings suggesting contrasting roles for the Asp278 and Asp281 residues on ligand binding.

L28

BIOPHYSICAL AND MUTAGENIC ANALYSIS OF A G PROTEIN-COUPLED RECEPTOR: PHOTOCROSSLINKING OF THE TRIDECAPEPTIDE ALPHA-FACTOR INTO STE2P OF SACCHAROMYCES CEREVISIAE REVEALS CONTACT POINTS BETWEEN THE PEPTIDE AND ITS RECEPTOR BINDING SITE

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Mating of Saccharomyces cerevisiae is initiated by binding of a peptide pheromone, alpha-factor, to its cognate G protein-coupled receptor (Ste2p). Analogs of alpha-factor (WHWLQLKPGQPMY) containing the photoactivatable group p-benzoyl-L-phenylalanine (Bpa) and biotin as a tag were synthesized using solid-phase methodologies. Bpa was inserted at positions one, three, five, eight, and thirteen of alpha-factor to obtain a set of cross-linkable analogs spanning the pheromone. The biological activity and binding affinities of all analogs for Ste2p were determined. Two of the analogs tested, [Bpa1]alpha-factor and [Bpa5]alpha-factor, showed three- to four-fold lower affinity compared to alpha-factor, whereas [Bpa3]alpha-factor and [Bpa13]alpha-factor had seven- to twelve-fold lower affinities, respectively. [Bpa8]alpha-factor competed poorly with [3H]alpha-factor for Ste2p. All of the analogs tested except [Bpa8]alpha-factor were alpha-factor agonists. Cross-linking studies demonstrated that [Bpa1]alpha-factor, [Bpa3]alpha-factor, [Bpa5]alpha-factor and [Bpa13]alpha-factor cross-linked to Ste2p; the biotin tag on the pheromone was detected by a NeutrAvidin-HRP conjugate on Western blots. Digestion of receptors crosslinked to [Bpax]alpha-factor (x = 1, 3, 13) with chemical and enzymatic reagents suggested that the N-terminus of the pheromone interacts with a binding domain consisting of residues from the extracellular ends of TM5 (transmembrane 5), TM6, and TM7 and portions of EL2 (extracellular loop 2) and EL3 close to these TMs and that there is a direct interaction between the position 13 side chain and a region of Ste2p (F55-R58) at the extracellular end of TM1. Site-directed mutagenesis results correlated with the crosslinking studies. The results have been incorporated into a model for alpha-factor bound to its receptor.

L29

RESCUE OF P53 MUTANTS BY PEPTIDIC CHEMICAL CHAPERONES: A NOVEL APPROACH FOR ANTI-CANCER DRUGS

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Protein misfolding is the cause of many human diseases, including cancer. A novel therapeutic strategy for such diseases is the use of chemical chaperones, which are small molecules that bind the misfolded protein and mediate its refolding, leading to its reactivation. The tumor suppressor protein p53 protects the cell against cancer by mediating cell-cycle arrest or apoptosis of genetically impaired cells. Mutations in the DNA-binding core domain of p53, which result in its unfolding and inactivation, have been implicated in approximately 50% of human cancers and their rescue is an important target in cancer therapy. Using rational design we have developed the peptidic chemical chaperone FL-CDB3, which stabilizes, refolds and reactivates mutant p53core [Friedler et al. (2002), PNAS 99, 937-942]. NMR studies showed that FL-CDB3 shifts the equilibrium of the oncogenic mutant R249S towards the native conformation [Friedler et al. (2004), J Mol Biol 336, 187-196]. In-vivo, FL-CDB3 was readily taken into cancer cells, induced the p53 target genes p21 and MDM2 in a p53-dependent manner, upregulated wild-type p53 and reactivated the oncogenic mutants R175H and R273H [Issaeva et al. (2003) PNAS 100, 13303-13307]. FL-CDB3 is a lead compound, which is a chemical chaperone for mutant p53. It shifts the equilibrium towards the native state and prevents the unfolding of destabilized p53 mutants. FL-CDB3 should refold p53 mutants immediately after their biosynthesis and maintain their native conformation until they reach the nucleus and transfer to their natural ligand (e.g. DNA), which binds tighter. We are currently developing improved FL-CDB3 derivatives.

L30

CRYPTO PROTEIN SUB-DOMAINS: SYNTHESIS AND STRUCTURAL STUDIES

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Cripto is the first member of a class of extracellular factors called EGF-CFC found in human and mouse embryonal carcinoma cells and male teratocarcinomas (1). It is strictly required in the early embryonic development and contributes to deregulated growth of cancer cells in adults, since it is highly over-expressed in breast, colon and other carcinomas in contrast to normal tissues where Cripto expression is invariably absent. All the EGF-CFC proteins contain two characteristic cysteine-rich regions: an Epidermal Growth Factor (EGF-like domain) and a CRIPTO/FRL1/Cryptic (CFC) domain. These domains (constituted by about 40 residues) are characterized by the presence of 3 disulphide bridges that confer rigidity to the polipeptide chain and impose the formation of loops presumably located in exposed region of the protein architecture. It was demonstrated that Cripto complexes its receptor Alk4 via its CFC domain and that the blockade of this interaction inhibits tumour growth. Therefore, the development of antagonists that block CFC-mediated signalling would have relevant therapeutic applications. Cripto domains have been prepared by solid phase chemical synthesis, purified and refolded. In order to determine the main structural properties featuring this new class of proteins conformational studies in solution have been undertaken by NMR and CD techniques, on both wild type and mutated domains carrying aminoacid substitutions on sites responsible of receptor recognition. [1] Adamson, E., D., Minchiotti, G., Salomon, DS., 2002, J. Cell. Phys. 190: 267-278.

L31

THE USE OF METAL-BINDING PEPTIDES AS ACTIVE CENTRE OF ARTIFICIAL METALLOENZYMES

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In the active centre of metalloenzymes the metal ions are bound by the coordinating amino acid side chains. In contrast low molecular wight peptides bind certain metal ions by the deprotonated amide nitrogens that increase the electron density and decreases catalytic ability of the metal ions. For this reason a number of non-peptide type imidazole or pyridine based compounds have been prapared to functionally mimic the enzymes. Recently, by solid phase peptide synthesis we prepared a number of short peptides designed for side-chain binding of copper(II) and zinc(II) ions. HHGH, HPHH or HHHHHH molecules offer enogh imidazole groups for the metal ions to avoid amide deprotonation at physiological pH. We investigated the metal binding solution equilibria and the structure of the species formed in the metal ion-peptide systems. The hydrolytic activity against model and natural phosphate esters was also checked. The active peptide will serve as a fusion tag to e.g. DNA binding proteins to achieve higher specificity.

L32

BIOORGANIC CHEMISTRY RESEARCH IN THE INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY - MEDICAL ASPECTS

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Institute of Organic Chemistry and Biochemistry was founded in 1950 by a group of researchers headed by Prof. F. Šorm. The Institute was given a task to perform an interdisciplinary fundamental research following the pattern organic chemistry – biochemistry – medicine. Design of peptide hormone analogues, study of genetic code and research on protein synthesis complemented by isolation and structure determination of natural compounds (e.g. azulenes) were directed to human or veterinary medicine. The late Josef Rudinger and his coworkers were the second in the world to synthesize oxytocin; neurohypophyseal hormone analogues DDAVP (Desmopressin), Methyloxytocin, Carbetocin and Glypressin (Terlipressin) were introduced to the veterinary and human medical practice. By studying the effect of nucleic acids antimetabolites on the biosynthesis of nucleic acids de novo the Institute stood at the dawn of rational cancer chemotherapy. 5-Azapyrimidine nucleosides (e.g. cytostatic drug decitabine) are written on the success list of the Institute. In addition to the application in leukemia and cancer chemotherapy, another potential outcome of the nucleoside antimetabolite action consists in their activity as antivirals. In the cell, nucleosides are activated by phosphorylation and transformed ultimately to their triphosphates which either directly inhibit the polymerisation to diverse nucleic acids or, as alternative substrates for the polymerases form chains with unsuitably altered properties. We studied undegradable nucleotide analogues which could act independently on the key-step of nucleoside kinase reaction. The successful principle of acyclic nucleoside phosphonates (ANP) was substantiated by three generally approved antivirals: cidofovir, tenofovir and adefovir.

L33

AN OUTLINE OF JOSEF RUDINGER'S LIFE

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Josef Rudinger was born in Jerusalem in 1924, but grew up and had his early education in Prague. In 1939 he was escorted to England by Tessa Rowntree and placed at Bootham School; he won a University Scholarship to read Chemistry at Newcastle in 1941. He flew in RAF Coastal Command operations 1942-1945, and then completed his degree at Newcastle. After two years' research there, in 1949 he returned to Prague, where František Šorm gave him a job to work on peptides. In 1957-8, he convened EPS-1 in Prague with Šorm formally in the chair; this was despite the fact that he was vigorously engaged against the State on behalf of his father Zdenek Rudinger, a political prisoner. Following the Russian invasion of 1968, Josef Rudinger found hospitality with Robert Schwyzer in Switzerland, and in 1970 he was made a Full Professor at the ETH in Zürich, where he died in 1975, just a few days after his 51st birthday. He was a charismatic polyglot with the gift of friendship, and had a genius for inducing cross-fertilisation in peptide science across disciplinary and international borders. A detailed Memoir will be published in the Journal of Peptide Science to coincide with the Symposium.

L34

JOSEF RUDINGER AND PEPTIDE ORGANIC CHEMISTRY

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In fifties and sixties, along with an affort to synthetize peptides, a lot of work was devoted to improve and find new methods for the synthesis. A brief reminder of some of these activities in the Prague peptide laboratory of the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences, encompassing J. Rudinger's years there will be given.

L35

NEUROHYPOPHYSEAL HORMONES - AGONISTS AND ANTAGONISTS: FROM DU VIGNEAUD TO THE PRESENT

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Oxytocin and vasopressin were first synthesized in the laboratory of Vincent du Vigneaud 50 years ago. This achievement not only ushered in the modern era of peptide chemistry, it also initiated a long lasting interest in structure/activity and design studies on these two peptides. Today, the availability as research tools, of a wide variety of selective agonists, antagonists, radioiodinated and fluorescent ligands for the OT, V1a, V1b, and V2 receptors for these two peptides, has helped to both stimulate and to maintain intense worldwide investigative interest in the multifaceted biological roles of oxytocin and vasopressin. This presentation will (a) pay tribute to the pioneering synthetic and structure/activity studies carried out by the du Vigneaud group in New York, the Rudinger group in Prague, and the Berde and Boissonnas group at Sandoz in Basle and (b) attempt to highlight some of the subsequent key findings in other laboratories, including my own, which have helped to advance the field. Finally, the indispensable roles of my collaborators; pharmacologists Wilbur H. Sawyer, Serg Jard, Claude Barberis, Gilles Guillon, Walter Chan and Hazel Szeto, the Merrifield solid phase method, the contributions of highly talented visiting and permanent peptide chemists from Hungary, England, Poland, Bulgaria and China in my laboratory, and continued funding from the NIH [GM-25280] over the past 30 years, will also be gratefully acknowledged.

L36

STRUCTURE-ACTIVITY RELATIONSHIPS IN PEPTIDES: PAST AND PRESENT CONCEPTS

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Questions concerning relationships between peptide structure and biological function were addressed shortly after the historic synthesis of neurohypophyseal peptides fifty years ago. To these aims, pharmacological assays were adapted, and response dynamics was systematically characterized in quantitative molecular pharmacological terms. New features, like stimulus-response coupling or transition agonism -> partial agonism -> antagonism were observed and later established as general pharmacological phenomena. Three phases can be recognized in the structure-function "philosophy". 1. Empirical changes of "natural" structures based on previous experience and intuition. 2. Semi-empiric methods resting mainly on linear free energy changes assumption (QSAR, substituent and fractional analysis). 3. Conformational studies of ligands and receptors, and computer-aided molecular modeling of their interactions. Whereas the first approach aided to clarify the role of individual functional groups and molecular segments, the second one enabled a quantitative treatment of structural data and to systematize effects of chemical interventions in a peptide molecule. The third approach reflects the contemporary activities in this research area.

L37

DDAVP – A SUCCESSFUL NEUROHYPOPHYSEAL HORMONE ANALOG: THE FATE OF RESEARCH

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[1-(3-mercaptopropionic acid), 8-D-arginine]vasopressin (DDAVP, Desmopressin), the first superactive vasopressin analog with very high and highly specific antidiuretic activity, was discovered, developed and introduced into social practice at the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Science in Prague between 1966-1972. DDAVP is successfully used for the substitution therapy of diabetes insipidus and for the treatment of enuresis nocturna. DDAVP preparation Desmopressin is the first officially recommended drug in mild Factor VIII deficiency. Hemostatic effects of DDAVP are exploited in the pre- and postoperational care of the patient. In clinical diagnostics DDAVP is used in the test of the concentrating capacity of the kidneys. DDAVP seems to have a positive effect in posttraumatic amnesia and retrograde amnesia after an electroshock therapy. In biochemical research DDAVP is frequently used in studies of vasopressin receptors, in pharmacological studies as a standard compound, etc. The reference literature on DDAVP and Desmopressin comprises over 3000 citations. DDAVP was produced in the Czechoslovakia and later Czech Republic from 1972 to 1999. The main producer of DDAVP now is Ferring AB. The annual output of DDAVP at Ferring AB amounts to 30-40 kg.

L38

FROM OXYTOCIN TO CARBETOCIN ; JOURNEY FROM DEVELOPMENT TO PRODUCTION

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The production process for Oxytocin was developed at Rudinger's peptide laboratory of the Institute of Organic Chemistry and Biochemistry in Prague and put to practice at Leciva (Czechoslovak pharmaceutical company) during the years 1958-1959. The synthesis was based on fragment condensation, using azide coupling, and contained many (at that time) advanced and original features. Structure activity studies during the forthcoming years were focused mainly on selectivity of action and better stability of oxytocin analogues towards enzymes. Rudinger and his group focused their attention on detailed study of position 2 and 3 and on the function of disulfide bridge. Elucidation of the importance of aromatic hydroxyl group of tyrosine for activity profile of Oxytocin led to the introduction of Methyloxytocin to human medicine as the safe uterotonicum in 1966. Replacement of disulfide bridge with thiomethylene group led to the birth of "carba analogs". First synthesized as the tool to elucidate function of disulfide bridge, carba modification was found to stabilize peptides containing this modification and in some cases significantly increase the peptide activity. That was the case of Carbetocin, which was later (1975-1977) introduced as enzymatically stable Oxytocin analog into both veterinary and human medicine. Time period since 1958 till the present days, marked by the milestones of discoveries in this challenging peptide field, will be reviewed in our presentation.

L39

PAST AND PRESENT TESTING OF NEUROHYPOPHYSEAL HORMONE ANALOGUES: FROM BLACKENED DRUM TO EXPRESSED RECEPTORS

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In the past 50 years several hundreds of analogues of neurohypophyseal hormones were synthesized and pharmacologically evaluated in order to determine the structure activity relationship and discover new drugs for human and veterinary use. In the beginning, exclusively pharmacological methods in vitro and in vivo for the vasopressic and oxytocic activities were used. Later receptor binding tests were developed using membrane preparations from the target tissues (rat, guinea pig) and radioactively labelled compound. This enabled to screen more compounds in shorter time interval and to compare their potencies. However this did not allow us to determine the character of the activity – agonistic, antagonistic, or partial agonistic. Only compounds having high affinity were then usually tested in pharmacological tests using rat model. The species difference was however a hindrance. Now-a-days, thanks to molecular genetic methods we have at our disposal cells having transiently or permanently expressed human vasopressin and oxytocin receptors. Cell cultures of these cells enable us to test at the same time the affinity to the relevant receptors as well as the activity, by determining competitive binding with selective radioligands and formation of the relevant second messenger (cAMP or IP3 or Ca2+), respectively. We have thus to our disposal standard pharmacological methods as well as the modern ones giving very reliable results. Anyway even this progress cannot rule out the pharmacological tests in vivo as only these tests will give us overall information about bioavailability, absorption, metabolism etc. Examples of activity determination will be given.

L40

FROM MODELING NEUROHYPOPHYSEAL HORMONES TO SIGNALLING MECHANISMS IN GPCRS

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The era of peptide chemistry effectively begun in the middle of the last Century with the pioneering syntheses of the nonapeptide hormones oxytocin and vasopressin (OT and VP, respectively) by du Vignaud et al. Since then, tens of thousands of biologically relevant endogenous peptides and their analogs have been synthesized and tools - including molecular modeling - have evolved, enabling systematic progress in their structure-activity correlations. We begin this communication with reminding our early attemts to define a pharmacophore of OT antagonists by modeling restrained bicyclic analogs, cleverly designed and synthesized by Hruby et al in the nineties. Due to progress in protein modeling, soon after early models of GPCRs did arrive. Thus, subsequently we illustrate, using selected examples, how selectivity of flexible OT and VP analogs could be explained by modeling and simulated relaxation thereof with respective VP and OT receptors. The crystal structure of the first class A GPCR, published by Palczewski et al in 2000 set again a new era, this time for studying signal transduction mediated by GPCR, pertinent to most endogenic neuropetides. To finish, we set forth a hypothesis on mechanism of activation of a Class A GPCR and its interaction with a relevant G protein. Acknowledgements. Supported by KBN grants DS 8372-4-0138-4 and BW/8000-5-0022-4. The computational time in CI TASK, Gdańsk, and in ICM, Warsaw, is acknowledged.

L41

MEDICAL ASPECTS OF NEUROHYPOPHYSEAL HORMONE RESEARCH

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Three compounds of neurohypophyseal hormone analogues have the most wide clinical use for almost half of the century: desmopressin (desamino-d-arginin-vasopressin, DDAVP), oxytocin ([3-isoleucine, 8-leucine]-vasopressin) and terlipressin (n-triglycyl-8-lysin-vasopressin). DESMOPRESSIN has been used for the treatment of central diabetes insipidus since 1967. Up to now it remained the most efficatious and adequate drug for its treatment. Besides,desmopressin is used for treatment of primary nocturnal enuresis in children and for nocturia in aged people. This indication represents the largest deal of prescriptions for DDAVP in our country. At relatively high doses, desmopressin increases 3 to 5 fold plasma Factor VIII concentrations in blood and is used in mild to moderate forms of haemophilia and von Willebrand´s disease before surgery. Injections of desmopressin given 4 hours after a lumbar punction significantly reduce the incidence and severity of post-lumbar headaches. In diagnostics, water deprivation test combined with subsequent administration of desmopressin is used for testing of renal concentration capacity what is epecially advantageous in children. OXYTOCIN is used in obstetrics for the induction or augmentation of labour, prevention or treatment of postpartum uterine atony and haemorrhage and to facilitate the milk-ejection reflex. TERLIPRESSIN is used for the management of haemorrhage, especially from gastrointestinal tract and for uterine bleeding, but may be used during abdominal and gynaecological surgeries as well.

L42

RELEASE OF VASOPRESSIN WITHIN THE BRAIN: STIMULI, MECHANISMS, AND FUNCTIONAL CONSEQUENCES

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Vasopressin (AVP) is released within the brain from dendrites, perikarya and axons upon stimulation, including emotional and physical stressors. Microdialysis in freely behaving rats and mice, antagonist treatment, antisense targeting and viral vector approaches have been used to study behavioral consequences of central AVP release including its manipulations. Particularly after its release within hypothalamic and limbic brain areas, AVP has been shown to trigger a wide variety of emotional responses and stress coping strategies. If centrally released AVP is physiologically involved in emotionality, it should play a critical role in animals selectively and bidirectionally bred for either high (HAB) or low (LAB) anxiety-related behavior. Indeed, in HAB rats (i) AVP expression and release are higher in the hypothalamic paraventricular nucleus (PVN) compared to LAB rats, (ii) intra-PVN treatment with an V1 receptor antagonist resulted in a decrease in anxiety and (iii) neuroendocrine aberrations are clearly driven by AVP overexpression and overrelease. Moreover, treatment of HAB rats with the antidepressant paroxetine normalized AVP mRNA expression, ACTH/Cort responses and anxiety-related behavior. Thus, we decided to investigate the AVP locus as a candidate gene for anxiety. Indeed, a number of single nucleotide polymorphisms in the AVP gene promoter of HABs were detected. One specific polymorphism conferred reduced binding of the transcriptional repressor CBF-A, thus resulting in AVP overexpression in vitro and in vivo. Remarkably, also in a recently established mouse model of anxiety, AVP was found to be overexpressed and overreleased in the PVN of HAB mice, indicating a more general phenomenon.

L43

HUMANIN AND ITS DERIVATIVES, A NEW LINEUP OF NEUROPROTECTIVE PEPTIDES

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Humanin (HN) is a neuroprotective factor consisting of 24 amino acids. We isolated its cDNA by a functional screening searching for antagonistic factors against neuronal cell death caused by a familial Alzheimer's disease (AD)-linked mutant. HN suppresses neuronal death induced by all AD-related insults including familial AD-linked mutant genes and amyloid-?, which is a major component of senile plaque and is implicated in pathogenesis of AD. On the other hand, HN does not affect neuronal death caused by non-AD related insults such as polyglutamine repeat and familial amyotrophic lateral sclerosis-linked SOD1 mutants. Thus, the neuroprotective action of HN is not omnipotent but preferential against AD-related insults. When HN cDNA under the mammalian-expression promoter is transfected to cells, HN peptide is intracellularly expressed and secreted from cells, suggesting that HN is a self-secretive peptide. HN exerts neuroprotective activity extracellularly. Accordingly, even synthetic HN peptide suppresses cell death when added to the culture media. Multiple findings imply that HN binds to a putative receptor and activates intracellular signaling molecules for neuroprotection. Structure-function analyses indicate that self-dimerization is required for HN to exert its action, and the substitution of Ser14 by Gly or D-Ser increases its neuroprotective potential by 1000-fold. Immunostaining with anti-HN antibody showed the presence of immunoreactivity in neurons in the occipital lobe of AD brain, from which HN cDNA was originally isolated, but not in age-matched control brain. Detailed characteristics of HN and its derivatives and their potential for AD therapy will be discussed.

L44

NOVEL NEUROPROTECTIVE PEPTIDES: FROM GENES TO BEHAVIOR AND POTENTIAL THERAPEUTICS

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Activity-dependent neuroprotective protein (ADNP, JBC 276, 708, 2001) was discovered as a glial mediator of neuropeptide - induced neuroprotection. Vasoactive intestinal peptide – (VIP) stimulates glial cells to produce ADNP which contains neuroprotective epitopes (J. Neurochem 72, 1283, 1999). ADNP is critical for brain formation: without it, the developing embryo dies (Dev Brain Res. 144, 83, 2003). Peptide scanning identified an eight amino acid sequence, NAP (NAPVSIPQ), as the smallest neuroprotective element of ADNP. NAP protected against neuronal death induced by the beta amyloid peptide (the Alzheimer's disease-related toxin). The structural characteristics required to inhibit toxic beta amyloid aggregates are included in NAP: 1] at the ends of the molecule, there are polarized amino acids, Q and N; 2] NAP has a 6-amino acid long hydrophobic core, with two P residues that are important in preventing beta sheet formation. NAP inhibited beta amyloid aggregation using: (1) fluorimetry; (2) electron microscopy; (3) high-throughput screening of beta amyloid deposition onto a synthetic template; and (4) Congo-Red staining of neurons. Thus, as part of its neuroprotective properties, NAP modulates toxic protein folding in the extracellular milieu (Peptides 24, 1413, 2003). NAP was found in the brain following intranasal administration; increased cognitive functions (JPET, 293, 1091, 2000) and provided protection against anxiety (Neurosci. Lett. 2004). Neurodegenerative diseases are unified by neuronal death. The neuroprotective properties of NAP and its biophysical attributes make it an excellent drug candidate for development towards the treatment of neurodegenerative conditions. Support: Gildor Chair, ISOA, ISF, Allon Therapeutics, Inc.

L45

HYPERHYDROXYLATED PEPTIDES: A NEW STRATEGY FOR NEURONAL TARGETING BY VENOMOUS MARINE MOLLUSKS

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Venomous marine mollusks belonging to the genus Conus (Cone Snails) utilize a unique neurochemical strategy to capture their prey. Their venom is composed of a complex mixture of highly modified peptides (conopeptides) that interact with a wide range of neuronal targets and have application as neuropharmacological agents. Posttranslational modifications present in conopeptides are essential for neuronal targeting selectivity and affinity. Here we described an unprecedented set of modifications based upon hydroxylation of polypeptidic chains that define a new neurochemical strategy for prey capture. We have isolated four novel conopeptides: gla-1/gla-1' from the venom of Conus gladiator and mus-1/mus-1' from venom of Conus mus. These conopeptides contain the modified amino acid γ-hydroxyvaline (Hyv=V*). The complete sequences of these conopeptides were determined by a combination of nano-NMR and MS/MS methods. Additionally, we have isolated three related conopeptides from Conus villepini (vil-1, vil-2 and vil-0) that are similar in properties to the gla/mus conopeptides; however, vil-1 incorporates γ-hydroxyleucine (Hyl=V*) instead of Hyv. These peptides contain a double modification of the polypeptide chain in contiguous residues, γ-OH-Xaa-D-Trp, that defines a new class of conopeptides that we have termed γ-Hydroxyconophans. These are the first examples of a polypeptide chain containing Hyv. These conopeptides are unusual because (i) they are not constrained as most conopeptides (ii) They are hyperhydroxylated as they have a high content of hydroxylated residues, and (iii) their sequences has no close match in any database. γ-Hydroxyconophans adds further diversity to the known neurochemical strategies used by cone snails to capture their prey.

L46

NEUROTOXIC OLIGOMERIC PEPTIDES FROM THE BRI GENE CAUSE NEURONAL LOSS IN FAMILIAL BRITISH AND DANISH DEMENTIAS

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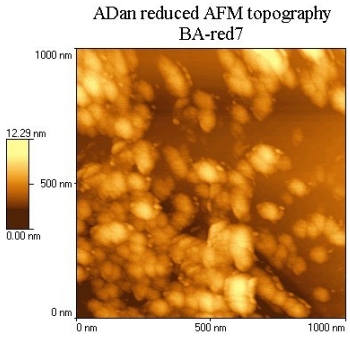
Mutations in the BRI gene yield a transmembrane protein 11 residues longer than the wild type. Furin cleavage yields 34-residue peptides, Abri and Adan. These peptides have been synthesised and shown to yield neurotoxic soluble aggregates.Oligomers are detected by Tricine-PAGE, gel filtration, and electrospray mass spec. AFM (Figure)show bunches of elongated speroids, that when added to human neuroblastoma cells in culture, give rise to apoptotic cell death. Molecular models show that the 11-residue extension distinguishing mutant Abri and Adan from wild-type BRI, are involved in hydrophobic and electrostatic self-assembling interactions. Aggregation of these peptide in the brian produces human dementing disease with spastic paralysis and hearing defects



L47

CHOLESTEROL IS IMPORTANT FOR THE TOXICITY OF A BETA AND ITS BINDING TO CELL MEMBRANES

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Accumulation of the amyloid protein (Abeta) in the brain is central to the pathogenesis of Alzheimer's disease. The presence of aggregated Abeta in association with cerebral blood vessels causes weakening of the vessel wall and also increases the risk of stroke due to intracerebral haemorrhage. Our studies have shown that Abeta is toxic to vascular smooth muscle cells in culture and one possible mechanism underlying this toxicity may be that Abeta can bind to membranes which in turn may have detrimental effects on cell function. In this study, surface plasmon resonance was used to study Abeta binding to membranes using either synthetic lipid mixtures or plasma membrane preparations isolated from vascular smooth muscle cells. Lipid vesicles were coated onto an L1 biosensor chip and the amount of binding of Abeta peptides to the lipid membrane was measured. The results showed a very good correlation between membrane binding and toxicity. Furthermore, binding was strongly influenced by the concentration of cholesterol in the membrane. Moreover, cholesterol biosynthesis inhibitors (statins) decreased membrane binding and also decreased toxicity. In all cases, binding was closely dependent on the concentration of cholesterol in the membranes. Ageing the peptides by incubation for 5 days increased the proportion of oligomeric species and also increased toxicity and membrane binding. The results strongly support the view that Abeta toxicity is a direct consequence of binding to lipids in the membrane and that reduction of membrane cholesterol with cholesterol-lowering drugs may be of therapeutic value.

L48

PEPTIDE LIBRARIES: PAST, PRESENT, AND FUTURE

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Peptide chemist who happened to be at the right time at the right place to witness the birth of combinatorial chemistry and who participated in the early years of transformation of chemist's mindset to accept the concept of millions-at-a-time instead of one-at-a-time, is giving his view of the dynamic field of combinatorial chemistry and specifically peptide libraries. The history of the conceptual discoveries as well the state of the art combinatoorial technologies will also be discussed. The author is apologizing in advance for any controversies this lecture may create, and at the same time he is welcoming them since (in his own humble opinion) controversies stimulate creative thinking, and only creative thinking brings scientific progress.

L49

VOLATILIZABLE SOLID AND SOLUBLE POLYMERIC SUPPORTS FOR ORGANIC SYNTHESIS

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An alternative approach to support phase synthesis will be presented. These methods include "volatilizable" solid and soluble polymeric supports, as well as both "traceless" and "non-traceless" linkers. The concept of volatilizable solid supports entails the use of any solid support/linker combination that will yield not only the desired synthetic product on the solid or soluble polymeric support, but will permit the complete transformation in the final cleavage step of both the support and/or the linker into components that are readily removable due to their inherent volatility. Examples will be presented in which functionalized silica gels and polydimethylsiloxane oils are used as synthesis supports. Silica gel is completely transformed with aqueous or anhydrous hydrogen fluoride (HF) to tetrafluorosilane (SiF_4, boiling point = -86 degrees C). While not typically considered of significance, elimination of the final extraction step during the combinatorial synthesis of hundreds to thousands of compounds will greatly decrease for the time required for the tedious extraction of such samples following their cleavage and will increase the yield of the desired products (especially when preparing small amounts of very large numbers of compounds). These approaches enable the facilitated synthesis of a broad range of therapeutically and diagnostically useful compounds. Examples will also be presented using this approach for cost effective solid phase synthesis of preparative amounts of material.

L50

ENGINEERING CYCLIC TETRAPEPTIDES CONTAINING CHIMERIC AMINO ACIDS AS PREFERRED SCAFFOLDS

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Cyclic tetrapeptides are the smallest and most rigid cyclic peptides that still contain trans-amide bonds. Density functional theory (DFT, B3LYP/6-31G*, GAUSSIAN 98) was used to explore the conformational surface of cyclic tetrapeptides containing heterochiral dipeptide units, such as c(Pro-pro-Pro-pro), and the barriers to interconversion of the various conformers. Solvation effects were examined using the polarizable continuum model. The goal was to stabilize the backbone in different conformers for the four amide bonds, all-trans, all-cis, cis-trans-cis-trans (ctct), trans-cis-trans-cis (tctc), cis-trans-trans-trans, etc. by chemical modification such that rigid backbone templates could be used as 'priviledged scaffolds' by incorporation of chimeric amino acids to orient side chains for receptor recognition. c(Pro-pro-Pro-pro) had previously been shown to exist as two mirror-image conformers ctct and tctc in water. DFT suggested that the all-trans conformer would be most stable in vacuo, but confirmed the experimental observation in water. Substitution of an N-methyl amino acid, or pipecolic acid (Pip) for proline increased the stability of the all-trans conformer implying that the five-membered exocyclic rings of the four proline residues were the source of the cis-amide bond preferences. Replacement of the carbon alphas of proline with nitrogen (AzPro) enhanced stability of the all-cis conformer dramatically and this effect analyzed in detail (Ye and Marshall, submitted). By appropriate choice of amino acid (Pro, AzPro, Pip, Aib, NMe-AA), a series of cyclic tetrapeptide scaffolds could be designed that stabilized a unique conformer of the four amide bonds. Side-chain substitution patterns compatible with the unique conformer have been explored.

L51

MAPPING AND RECONSTRUCTION OF A DISCONTINUOUS AND HIGHLY CONFORMATIONAL BINDING SITE ON FOLLICLE STIMULATING HORMONE SUBUNIT-BETA (FSH-BETA) USING SYNTHETIC SCAFFOLDS

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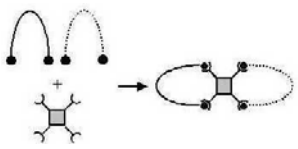
The identification of protein-protein interaction sites has been of great scientific interest for vaccine and drug research. In 1984 Geysen, Meloen and Barteling introduced the use of solid-phase libraries of overlapping peptides covering an entire protein, which allowed the systematic mapping of antibody binding sites on proteins.1,2 However, the mapping of discontinuous and highly conformational binding sites is still a major challenge. Recently, we have developed new screening technologies ("loopscan", "double-loopscan", etc.) using synthetic scaffolds that allow the mapping of discontinuous binding sites. The potential of our new screening technologies is illustrated with recent findings from mapping studies on Follicle Stimulating Hormone (FSH) and human Chorionic Gonadotrophin (hCG). Both glycoprotein hormones play a crucial role in reproduction, which makes them attractive targets for contraceptive vaccination. They share a common heterodimeric structure consisting of an identical alfa-subunit noncovalently bound to a hormone-specific beta-subunit. We have now succeeded in the synthesis of discontinuous binding site mimics for both hormones with binding affinities in the nanomolar range using our unique set of scaffolds. References: [1] H. M. Geysen, R.H. Meloen, S. J. Barteling, Proc. Nat. Acad. Sci. USA 1984, 81, 3998-4002. [2] R.H. Meloen, R. J. M. Liskamp, J. Goudsmit, J. Gen. Virol. 1989, 70, 1505-1512.



L52

A NOVEL PROCESS FOR MANUFACTURING OF HIGH DENSITY MULTI-PURPOSE CHEMICAL MICRO-ARRAYS

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We have pioneered and continuously advanced the utilization of planar synthesis supports, cellulose membranes in particular, for the multi-step solid-phase assembly of chemical compounds by several types of special combinatorial and parallel synthesis techniques such as the Filter Disc Method (1983), the SPOT-synthesis (1990), the Cut&Combine Method (1998). The planar, addressable format of the synthesis supports provides opportunities not easily available by e.g. beads, most prominently the synthesis without any reactor walls by so called inclusion volume synthesis.[For a review on this topic see: Lebl, M. (2001) Biopoplymers]. Utilization of this type of material in the further miniaturization, automation and integration of high throughput synthesis & biological screening processes for immunological, functional genomics, proteomics and drug discovery studies will be exemplified. This is particularly concerned with the implementation of a novel process for manufacturing and application of peptide/compound libraries in the form of chemical micro-arrays. Our integrated process involves special new surfaces for synthesis; chemistries for peptides and small organic molecules; special linkers for the presentation of immobilized and soluble compounds; printing of thousands of array replicates from only nmol scaled synthesis in densities up to 1000 spots per cm2 comparable to standard micro-arrays on glass slides. For example, we have synthesized and printed about 3000 peptide fragments covering the major virulence proteins of Yersinia enterocolitica for the immune-profiling of pathogen challenged mice. The new chemical micro-arrays perform as reliably as previous synthetic macro-arrays made by SPOT-synthesis on cellulose membranes.

L53

DISTCODE: DISTANCE ENCODING OF SINGLE BEADS FOR IDENTIFICATION OF SPLIT-COMBINE LIBRARY MEMBERS

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Analysis of combinatorial split and combine libraries is cumbersome and often the desired structural information cannot be obtained form the minute amount of material bound to a single bead, neither by mass spectrometry nor by solid phase MAS-NMR, in particular for more complex molecules. In fact this is probably the single most important reason for the failure of split and combine libraries as a discovery tool in industrial laboratories. Therefore encoding techniques including quantum dot labelling, chemical tags, radio frequency labelling, and diffusion spheres have been attempted. However, these labelling techniques are difficult to use practically for a large assembly of beads. We therefore have introduced encoding termed distance encoding where a identifiable distance matrix is located in the form of fluorescence in the interior of large beads, thereby providing each bead with an identity. This form of encoding requires reading of the code for each chemical transformation carried out. Active substances identified by high trough-put screening techniques from a split mix library can be identified by reading the distance matrix in the active bead and identifying the same bead using the matrices generated during synthesis. Reading of the code is independent of the orientation of the bead and identification can be performed automatically at very high rates. A large set of library beads can be reproducibly recognised using this method. Encoding a variety of peptidic and peptide mimetic libraries and automated identification of library members and active hits will be presented.

L54

ANTIMICROBIAL PEPTIDES: A LESSON FROM NATURE FOR FUTURE ANTIBIOTICS

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Lytic peptides are a large group of toxins used in the defense and offense systems of all organisms, including plants and humans. A major group within these peptides includes antimicrobial peptides which kill a large repertoire of cells. Some of them kill selectively bacteria, others only fungi and/or mammalian cells, and some are non selective to any type of cells. Despite extensive studies, the parameters involved in their cell specificity are not yet clear. Furthermore, all L-amino acids antimicrobial peptides could not be used intravenously to combat bacterial infection due to the loss of their activity in serum. Mode of action studies in our laboratory suggested a unique 'carpet' mechanism that can explain their cell-selective toxic effect. Based on this mechanism, we designed a novel group of cationic lytic peptides and lipopeptides that are composed of D- and L-amino acids (diastereomers). These diastereomers can be designed to kill specifically bacteria or fungi without harming normal cells, in vitro and in vivo. Furthermore, some of them kill specifically also cancer cells in vitro and cure solid tumor in mice xenogragts. Their reduced toxicity, ability to preserve activity in blood, to significantly resist enzymatic degradation, as well as simplified sequence make them potential candidates for future therapeutics against microbial infection and cancer.

L55

A GENERAL STRATEGY FOR DEVELOPMENT OF ANTI-VIRUS PEPTIDES BASED ON X-EE-XX-KK CONCEPT FOR THE ALPHA-HELICAL ENV SEQUENCE: PRACTICE IN AIDS AND SARS

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Recently we have been confronted with increasing epidemic threat of serious infectious disease caused by emerging mortal viruses including HIV-1 and SARS-associated coronavirus (SARS-CoV). A general strategy for development of anti-viral drugs based on a common infection mechanism has been desired, which provides new methodologies for the prevention and treatment of other newly emerging viruses. A wide variety of viruses are presumed to establish their infection with target cells through membranes fusion between viruses and target cells mediated by formation of supramolecular structures of Env proteins of the viruses. For example, membrane fusion of HIV-1 and target cells has been well known to be mediated by formation of a six-helix bundle resulting from the coiled-coil interaction between N-region and C-region in the extracellular domain of gp41 (HIV-1 Env protein). We found that incorporation of replacement by artificial heptad sequence, X-EE-XX-KK, into α-helical C-region of gp41 allowed the remodeled peptides with enhanced α-helicity to exhibit high anti-HIV-1 activity, [1] and expected that this remodeling strategy, (referred to as X-EE-XX-KK concept), would be widely applicable to other virus using fusion machinery similar to gp41 of HIV-1. Spike (S) protein (Env protein) of SARS-CoV is supposed to be involved in the fusion process in a way to similar to gp41. In this study, we applied the same strategy using the X-EE-XX-KK concept to potential α-helical sequence of the S protein, and found the designed peptides exhibit strong anti-SARS-CoV activity. [1] A. Otaka and N. Fujii, et al. Angew. Chem. Int. Ed. 2002, 41, 2937-2940.

L56

POSTTRANSLATIONALLY MODIFIED MICROCIN E492, A SIDEROPHORE-PEPTIDE WITH POTENT ANTIBACTERIAL ACTIVITY

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Microcin E492 (MccE492), a hydrophobic and anionic 84-residue antimicrobial peptide from Klebsiella pneumoniae, was first isolated as an unmodified peptide (7886 u). We have isolated a posttranslationally modified form of MccE492 (MccE492m, 8717 u) with more potent antimicrobial properties than MccE492. MccE492m is the first example of a novel type of antibacterial peptides that we call siderophore-peptides. MccE492 and MccE492m were submitted to chymotrypsin digestion. A 1771.6 u fragment purified by RP-HPLC from the MccE492m digest contained the entire 831.2 Da posttranslational modification. This fragment was submitted to MS analyses, using hybrid ESI-QqTOF and nanoESI-ion trap instruments, and to an NMR study at 800 MHz. CID experiments on the [M+2H]2+ ion gave two independent series of b- and y-ions. The y-ions were shifted to upper m/z ratios consistent with an 831.2 Da mass increase, while the b-ions were unshifted, indicating the modification is localized on the MccE492 C-terminal serine. The NMR data together with further MSn experiments identified the complete structure of the modification. It carries N-(2,3-dihydroxybenzoyl)-L-serine, which belongs to a group of catechol-type siderophores involved in iron uptake by enterobacteria. Moreover, MSn experiments in presence of FeCl3 showed that the modification was able to capture Fe3+. We show that recognition of both MccE492 and MccE492m is mediated by iron/siderophore receptors at the surface of sensitive bacteria. Therefore, we propose that improvement of MccE492 antimicrobial activity upon modification results from an increase of the microcin/receptor affinity.

L57

A TARGETED LIBRARY OF Y-STYLOSTATINS AS POTENTIAL ANTIBIOTICS

M. Cruz[1], P. López[1], M. Rubiralta[1,2], E. Giralt[1,3], A. Dieza[1,2]

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Certain small cyclopeptides have been reported to be antibiotics of a "new generation", in that their activity on the prokaryote cell membrane should not allow antibiotic resistance [1]. We have designed two small libraries of cycloheptapeptides with potential antibiotic activity, based on the sequence of stylostatin 1 [2]. The libraries are built using both the natural amino acids and our 3-aminolactam pseudodipeptides [3]. Their synthesis and the evaluation of their activity on biomembrane models using BLM and liposomes will be discussed. [1]Ganz, T. Nature, 2001, 412, 392-393. [2]P. Forns, J. Piró, C. Cuevas, M.García, M. Rubiralta, E. Giralt, A. Diez, J. Med. Chem., 2003, 46, 5825-5833. [3]a. Piró, J.; Rubiralta, M.; Giralt, E.; Diez, A. Tetrahedron Lett., 1999, 40, 4865-4868. b. Piró, J.; Rubiralta, M.; Giralt, E.; Diez, A. Tetrahedron Lett., 2001, 42, 871-873.

Stylostatin 1	*cyclo*-(Pro^1-Asn^2-Ser^3-Leu^4-Ala^5-Ile^6-Phe^7)
ψ-stylostatins "2Polar +5Apolar" (16 compounds)	*cyclo*-(Pro^1-**X**^{2}-Ser^3-Leu^4-Ala^5-Ile^6-Phe^7)
	cyclo-(Pro^1-**X**^{2}-**{Ser^3-Leu^4}**-Ala^5-Ile^6-Phe^7)
ψ-stylostatins "3Polar +4Apolar" (16 compounds)	*cyclo*-(Pro^1-**X**^{2}-Ser^3-**Thr^4**-Ala^5-Ile^6-Phe^7)
	cyclo-(Pro^1-**X**^{2}-{**Ser^3-Thr^4**}-Ala^5-Ile^6-Phe^7)

X= Asn, Asp, Lys, Arg

{Ser-Y} = 3 differently functionalised 3-aminolactams

L58

COMPARISON OF ENTRY EFFICIENCIES OF CELL PENETRATING PEPTIDES

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We have previously shown by molecular dissection of the Antennapedia homeodomain that the peptide corresponding to the domain 43-58 (RQIKIWFQNRRMKWKK) named Penetratin translocates across the plasma membrane of eukaryotic cells by an energy independent pathway. No chiral recognition was found to be required for internalization. [1] From these results, several other short basic cell-penetrating peptides (CPPs) have been described. Conflicting results have been reported in literature concerning their relative translocation ability. [2] We have recently developed a new method based on MALDI-TOF mass spectrometry for the quantification of peptide internalization. This method allows a fast comparison of the intrinsic properties of CPPs: (1) affinity for plasma membrane (2) rate of cellular up-take (3) rate of cellular efflux and (4) intra-cellular stability. CPPs can be subdivided in two classes: peptides quenched into the cell ("Trojan" peptides) and peptides able to efflux against the transbilayer potential ("Shuttle" peptides). Using our new method, we have determined the intracellular concentration of a cargo peptide that has been delivered by different "Shuttle" peptides. Differences in the internalization mechanisms will be discussed from these data. [1] Derossi, D.; Chassaing, G.; Prochiantz, A. Trends Cell Biol 1998, 8, 84. [2] Cell-Penetrating peptides Processes and Applications Ülo Langel CRC PRESS pharmacology & toxicology series 2002.

L59

A CYCLIC TETRA-CYSTEINE CONTAINING TASP SCAFFOLD AS AN FE3+ TRANSPORTER

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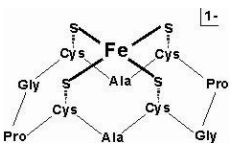
Iron deficiency is one of the greatest worldwide nutritional problems affecting more than 3.5 billion people. The main source of dietary iron is nonheme iron, the absorption of which is strongly influenced by dietary constituents. Muscle protein has long been known to enhance absorption of iron and this enhancing effect has been ascribed to chelating properties of cysteine which facilitates intestinal absorption. It is our aim to determine if a synthetic cysteine-containing TASP scaffold has the same effect. We prepared a TASP scaffold based on a cyclic decapeptide containing four appropriately placed cysteine residues. The rigid backbone of the scaffold creates a favorable tetrahedral coordination environment for the iron (see figure). The UV-vis spectroscopy of the Fe2+ and Fe3+ complexes give spectra similar to those obtained for the reduced and oxidized form of the iron-sulfur protein, rubredoxin. CD spectroscopy revealed that the stoichiometry of the Fe3+ complex is 1:1. EPR spectroscopy showed that the Fe3+ in the complex is coordinated in a skewed tetrahedral conformation. The Fe3+ complex is stable even under aerobic conditions. The efficiency of the complex to enhance iron absorption is under investigation in an in vitro Caco-2 cell model system and in an in vivo perfusion model in mice. Preliminary studies showed an enhanced effect by the complex. The chelating property of the scaffold opens up new routes for the release and transport of Fe2+ and Fe3+ into cells.



L60

CELL-PENETRATING PEPTIDES AND THEIR APPLICATION IN GENE REGULATION

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Intracellular transport of conjugated cargoes to cell penetrating peptides, CPPs, has opened new possibilities in biomedical research. Many cargo types, e.g. antisense and siRNA oligonucleotides, small and large peptides/proteins, have been shown to be efficiently transported intracellularly upon attachment to CPPs. This versatility of possible cargoes makes development of CPPs possible and desirable for gene therapy and other applications. Several cell penetrating peptides [1,2], CPPs, are known today. We have contributed with introduction of transportans and pVEC, which have been used for improvement of cellular distribution of peptide nucleic acids (PNA), peptides and proteins [3], The applications in gene regulation of cell-penetrating peptides are discussed . [1] Lindgren,M., Hällbrink,M., Prochiantz,A. and Langel,Ü. (2000) Cell penetrating peptides. Trends Pharmacol. Sci. 21, 99-103. [2] Cell-Penetrating Peptides. Processes and Applications, Ü.Langel, Editor, CRC Press, Boca Raton, London, New York, Washington, D.C., 2002 [3] Järver,P., and Langel,Ü. (2004) The use of cell-penetrating peptides in gene regulation. Drug Discovery Today, 9(9), 395-402.

L61

AMPHIPATHIC PROLINE-RICH CELL PENETRATING PEPTIDES AS POTENTIAL DRUG CARRIERS

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Cell penetrating peptides (CPPs) emerged one decade ago as important delivery systems to aid non-permeant hydrophilic drugs to cross the cell membrane. Among them, non-viral CPPs present as major advantages their low toxicity, accessible synthesis, high flexibility for modification, in order to covalently attach hydrophilic drugs, and easy scale-up. In 2002 our group was the first to report a polyproline (Pro14) cell penetrating peptide (1). Moreover by transmission electron microscopy an amphipathic Proline-rich sequence (VHLPPP)8 i.e. the N-terminal domain of the maize g-zein proved to interact with liposomes (2). This promising results prompted us to investigate the potential as CPPs of a series of amphipathic Pro-rich peptides of sequence (VXLPPP)n where n = 1-3 and X = Lys, His or Arg labelled at their N-terminus with 5(6)-carboxyfluorescein. The cell penetrating properties of these amphipathic Pro-rich peptides have been studied in HeLa cells using microplate fluorimetry, flow cytometry and confocal fluorescence microscopy (3). Studies concerning the mechanism of internalization, toxicology and metabolic stability will be presented. Their abilities as carriers in gene delivery are under current investigation. (1) L. Crespo, et al., J. Am. Chem. Soc. 2002, 124, 8876-8883. (2) M. J. Kogan et al., Biopolymers 2004, 73, 258-268. (3) J. Fernández-Carneado et al., Angewandte Chemie Int. Ed., 2004, in press.

L62

DIRECTION SELECTIVITY IN DNA BINDING BY CHIRAL LYSINE-BASED PNAS: INFLUENCE OF THE INTRINSIC PREFERENTIAL HANDEDNESS OF THE PNA STRAND CONTROLLED BY THE STEREOGENIC CENTERS

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Peptide Nucleic Acids (PNAs) are oligonucleotide analogues with a pseudopeptide backbone which have attracted much interest due to their ability to bind complementary oligonucleotides forming very stable PNA-DNA and PNA-RNA complexes. Many modifications have been proposed in order to improve their binding affinity and/or specificity. One of the most interesting modification is the introduction of stereogenic centers at the 'alpha' carbon . Recently we demonstrated that a chiral PNA bearing three adjacent D-Lys-based residues in the middle of the strand ("chiral box" PNA) exherts a very high mismatch recognition and direction control, binding only to a fully complementary antiparallel DNA sequence . In this presentation, we report the synthesis of a "chiral box" D-Lysine-based PNA and the homologous "chiral box" L-Lysine-based PNA with a high optical purity obtained by submonomeric solid phase strategy. Optical purity was checked after synthesis by means of chiral GC-MS methods and the e.e. of lysine was found to be 94% for both PNAs. Melting experiments, CD measurements and ESI-MS experiments of PNA-PNA and PNA-DNA duplexes containing both D-Lys and L-Lys-based "chiral box" PNAs revealed a relationship betweeen the absolute configuration of the stereogenic centers, the preferred helical handedness of the chiral PNAs, the direction control (antiparallel vs.parallel) and the mismatch recognition ability in PNA-DNA duplexes. The results will also be discussed outlining the similarities with the restro-inverso concept applied to peptide analogues.

L63

TUMOR SELECTIVE ACCMULATION OF PNA-OLIGOMERS CONJUGATED TO A SOMATOSTATIN RECEPTOR AFFINE PEPTIDE

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Enormous progress has been made in the development of antisense oligodeoxynucleotides (ODNs) as therapeutic agents inhibiting gene expression. Unfortunately, the therapeutical application of ODNs is still held back because of the low cellular uptake and the lack of specific transport into particular cells. Here we report a drug targeting system using somatostatin receptors (SSTRs) which are overexpressed in various tumors. Peptide Nucleic Acid (PNA) oligomers were covalently linked to octreotate, a highly potent analogue of somatostatin. Using BOC-chemistry, the peptide was assembled by solid phase synthesis and subsequently extended with PNA-monomers as a continuation of the peptide. Using the chloramine-T method, the 125I-labelled conjugate was obtained by direct iodination of a N-terminal Tyr residue. The receptor affinity was determined with a rat cortex membrane binding assay. The octapeptide was synthesized on Wang resin and oxidized with Tl(TFA)3 to form the cyclic disulfide. A 14-mer peptide nucleic acid (PNA) directed against the AUG start codon of the protooncogene bcl-2 was conjugated to the N-terminus of the peptide. Cleavage with trifluoromethanesulfonic acid, followed by the reversed-phase HPLC purification, produced PNA-octreotate conjugates in high overall yields. The unmodified as well as the tyrosine- and the 7-amino-4-methyl-coumarin-3-acetic acid-conjugated PNA-peptide-chimera similarly inhibited the binding of ^{125}I-3-Tyr-octreotide to rat cortex membranes. These binding studies revealed that the conjugation of the PNA-moiety does not influence the specific binding with high affinity to SSTRs. Biodistribution studies revealed the tumor-selective uptake of the targeted oligonucleotides in rats bearing the SSTR-positive pancreatic tumor cell line CA20948.

L64

DEVELOPMENT OF AGENTS TARGETING EGF RECEPTOR AND PDGF RECEPTOR

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Over the past 18 years we have developed strategies to target protein tyrosine kinases with tyrphostins (tyrosine phosphorylation inhibitors). EGFR directed tyrphostins were initially developed by us and are currently being developed by a number of pharmaceutical companies[1]. I will describe the activities of these compounds as therapeutic agents and as possible imaging agents [2] . Similarly, I will also review the development of PDGFR directed tyrphostins[3] and their utility as potential anti-cancer agents and their current development as anti-restenosis agents in drug eluting stents[4]. References [1] Levitzki, A., and Klein, S. (2004). From Tyrphostins to Gleevec: Signal Transduction Therapy - From Concept to the Clinic. In Life Sciences for the 21st century, E. Keinan, I. Schechter, and M. Sela, eds., pp. 175-189. [2] Ortu, G., Ben-David, I., Rozen, Y., Freedman, N. M., Chisin, R., Levitzki, A., and Mishani, E. (2002). Labeled EGFr-TK irreversible inhibitor (ML03): in vitro and in vivo properties, potential as PET biomarker for cancer and feasibility as anticancer drug. Int J Cancer 101, 360-70. [3] Levitzki, A. (2004). PDGF receptor kinase inhibitors for the treatment of PDGF driven diseases. Cytokine and Growth Factor Reviews, In Press. [4] Banai, S., Chorny, M., Gertz, S. D., Fishbein, I., Gao, J., Perez, L., Lazarovichi, G., Gazit, A., Levitzki, A., and Golomb, G. (2004). Locally Delivered Nanoencapsulated Tyrphostin (AGL-2043) Reduces Neointima Formation in Balloon-injured Rat Carotid and Stented Porcine Coronary Arteries. Cardiovascular Research, In Press .

L65

FROM COMBINATORIAL SYNTHESIS TO CELLULAR PROBES: DESIGNING CYCLIC AND MULTIVALENT PEPTIDE LIGANDS AS INHIBITORS OF SIGNAL TRANSDUCTION PROTEINS

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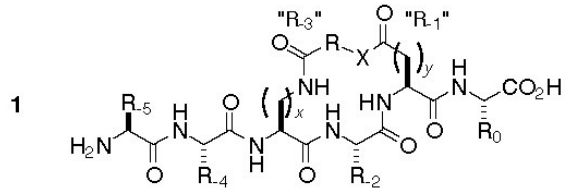
Many of the proteins that contribute to signal transduction processes in mammalian cells do so by participating in transient protein-protein interactions. One of the key mediators for this is the PDZ domain, a smaller structure within longer polypeptides that serves to selectively bind a desired target partner. The PDZ domain family is believed to comprise approximately 400 members, and sequence and structural differences between them allows for a large range of affinity and specificity. Our program has focused on designing and developing novel small peptide ligands to serve as inhibitors of these domains for use in cellular research. Here we report on two categories of peptide molecular probes for targeting PDZ domains, bridged cyclic peptides (1) and multivalent peptides. Using isothermal titration calorimetry and ELISA, we have determined that several of these compounds exhibit good affinity, and have been selected for in vivo studies. In addition to our amino acid bridged cyclic peptides (1), we have recently devised a second series that allows for expansion of molecular structure from the bridge unit. We also present our results on the combinatorial chemical preparation and the application to cellular assays of these peptides.



L66

MODULATION OF THE APOPTOSOME ACTIVITY USING PEPTIDES AND PEPTOIDS

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[1]*Fundación Valenciana Investigaciones Biomédicas-CSIC, Valencia*
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The elucidation of protein interactions is fundamental to understanding biological processes. Within a cell, a single protein may interact with many other molecules, and these interactions control de cellular pathways. The discovery of medium size and small molecules that modulate protein-protein interactions is of great interest for both basic research and biotech applications. Accordingly, the prevailing approaches have been structure-based design and combinatorial methods. However in those biological pathways where the key proteins are of unknown structure, a combination of peptide design based in genetic and biochemical data and combinatorial methods are the preferred options. We are interested in the identification of modulators of protein-protein interactions that have been described as point of control in apoptotic pathways. Apoptosis is a fundamental process for both human health and disease and is initiated and regulated by protein-protein interactions. We have identified by a combination of rational design and combinatorial methods, families of peptides and peptoids that inhibit in vitro the activity of the apoptosome, a macromolecular complex that activates mitochondria-dependent apoptosis pathways. Progress in the in vivo activity and in the structure-activity relationship will be discussed.

L67

BINDING, INHIBITION AND CATALYSIS OF S. GRISEUS AMINOPEPTIDASE AS REVEALED FROM ITS COMPLEXES WITH MECHANISM-BASED ANALOGS

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Streptomyces griseus aminopeptidase (SGAP) is a double-zinc exopeptidase that cleaves the N-terminal residue from protein and polypeptide substrates, with a high preference towards large hydrophobic amino-terminus residues. It is a monomer of low molecular weight (30 KDa), it is heat stable, it displays a high and efficient catalytic turnover, and its catalytic activity is modulated by calcium ions. These properties make SGAP an excellent representative enzyme for mechanistic studies of co-catalytic metaloproteinases, and a very attractive enzyme for various biotechnological applications. We have recently analyzed the high-resolution 3D-structure of SGAP and its complexes with a series of substrates, inhibitors and analogs. These studies enabled us to map the active site of SGAP and to identify some of its functional groups which are involved in enzyme-substrate interactions. Such studies indicate, that Glu131 and Tyr246 are critically involved in catalysis, and that Glu132, Asp160 and Arg202 are important for substrate binding and orientation, as well as the stabilization of the tetrahedral transition-state. These results allowed us to propose, for the first time, a general catalytic mechanism for the proteolytic reaction of SGAP. The extension of the series of amino acids and phosphonates as substrates in SGAP complexes, and especially the comparison of the different binding modes of L- and D-substrates, provided important additional information about relevant binding subsites and potential binding modes. These structural results led to detailed mapping of the active site, clarified aspects of selectivity and specificity, and enabled rational structure-based inhibitor design for SGAP and its pharmaceutically important homologs.

L68 P680

LOW MOLECULAR WEIGHT CXCR4 ANTAGONISTS BASED ON T140 ANALOGS THAT WERE IDENTIFIED AS TRIPLE-FUNCTIONAL AGENTS HAVING INHIBITORY ACTIVITIES AGAINST HIV INFECTION, CANCER METASTASIS AND RHEUMATOID ARTHRITIS

H. Tamamura[1], K. Hiramatsu[1], T. Araki[1], S. Mizokami[1], S. Oishi[1], S. Ueda[1], Z. Wang[2], S.C. Peiper[2], J.O. Trent[3], A. Hori[4], N. Kanzaki[4], M. Fujiwara[4], H. Nakashima[5], N. Yamamoto[6], A. Otaka[1], N. Fujii[1]

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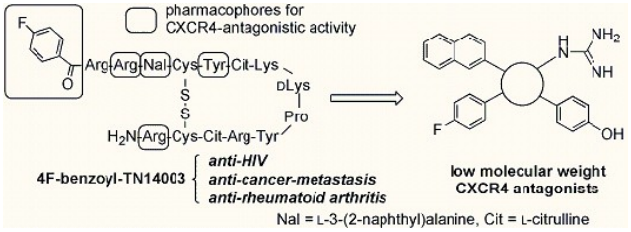
A chemokine receptor CXCR4 has multiple critical functions in normal and pathologic physiology including HIV infection, cancer metastasis and rheumatoid arthritis. A 14-mer peptide, T140, and its bio-stable analog, 4F-benzoyl-TN14003, were initially found to be strong CXCR4 antagonists that block HIV-entry.1 These peptides also showed remarkable inhibitory activity against cancer metastasis and progression in breast cancer, pancreatic cancer, melanoma, acute lymphoblastic leukemia, small cell lung cancer, etc. For instance, slow release administration of 4F-benzoyl-TN14003 by subcutaneous injection using an Alzet osmotic pump was found to significantly reduce pulmonary metastasis of breast cancer cells in SCID mice.2 Furthermore, this peptide showed inhibitory effects against rheumatoid arthritis: 4F-benzoyl-TN14003 administration suppressed the delayed-type hypersensitivity response induced by sheep red blood cells in mice, and reduced collagen-induced arthritis in mice. Taken together, 4F-benzoyl-TN14003 analogs have the potential of becoming promising agents for chemotherapy of AIDS, cancer and rheumatoid arthritis. In addition, we also report development of low molecular weight CXCR4 antagonists based on pharmacophore identification of 4F-benzoyl-TN14003. (1) Fujii, N., Nakashima, H. & Tamamura, H. (2003) Expert Opin. Investig. Drugs (Review), 12, 185. (2) Tamamura, H., Fujii, N., et al. (2003) FEBS Lett., 550, 79.



L69

NEW METHODS IN PROTEOMICS AFFINITY CAPTURE COUPLED TO MASS SPECTROMETRY (ACESIMS, ICAT AND VICAT) FOR PEPTIDE AND PROTEIN ANALYSIS

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The presentation will address new methods for protein quantitation in complex mixtures from biological samples, such as cell cultures, homogenates, and blood serum. The isotope-coded affinity tags (ICAT) method is based on in vitro tagging of denatured proteins at cysteine residues, followed by enzymatic digestion, affinity purification, and peptide analysis by tandem mass spectrometry. ICAT has been instrumental in protein quantitation relevant to the emerging field of functional proteomics. Examples will be given of the development of the ICAT strategy and applications to the specific identification and quantitation of cysteine-containing peptides from several proteins that were differentially expressed in yeast. The main focus will be on new developments of methods for absolute, mass-spectrometry based, protein quantitation based on Visible Isotope-Coded Affinity Tags (VICAT) for cysteine and lysine containing peptides from human and insect proteins. The performamce of VICAT will be compared to the other currently used methods of protein quantitation, such as AQUA and Western blots.

L70

STUDIES OF PEPTIDE YY IN SOLUTION AND WHEN BOUND TO PHOSPHOLIPID MICELLES

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Peptide YY (PYY) presents a member of the neuropeptide Y family of peptides. In this work we describe the structure of porcine PYY in solution and when bound to phospholipid micelles. Moreover, we compare the structure in the two environments with those determined by us for neuropeptide Y (NPY) previously. NPY and PYY are highly similar in their pharmacology displaying (sub)nanomolar binding affinities at all Y receptor subtypes. As a result of the comparison we find that the structures of both peptides in solution are distinctly different whereas they are almost identical in the micelle-bound state. Differences in their conformation are mainly due to the presence (PPY) or absence (NPY) of the so-called PP-fold, a structural feature in which the otherwise unstructured N terminus folds back onto the C-terminal α-helix. During our studies we discovered that all peptides, which were displaying the PP fold when unligated in solution, underwent conformational changes such that the N terminus was flexible in the micelle-bound state. Our work aimed at identifying residues important for the PP fold will be presented and experiments for characterizing the transition between the form free in solution and the membrane-bound state are presented. Our data accumulated so far present sound evidence that hormones from the NPY family are indeed recognized from the membrane-bound state and that it therefore is reasonable to use those conformations for structure-activity studies.

L71

A NOVEL THREADED RING STRUCTURE FOR THE ANTIMICROBIAL PEPTIDE MICROCIN J25

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Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia

Microcin J25 is a 21 amino acid peptide that has potent antibacterial activity against Gram-negative bacteria, resulting from its interaction with RNA polymerase. It exhibits remarkable stability for a peptide of its size lacking disulfide bonds and has been shown to retain biological activity after prolonged heating and treatment with chemical chaotropes. We show here that the remarkable stability of the peptide is due to a unique structure in which there is a covalent bridge between the side chain carboxyl of Glu8 and the N-terminus. This creates an eight amino acid embedded ring that is threaded by the peptide chain at the C-terminal tail of the molecule, forming a noose-like feature. The three-dimensional structure determined from NMR data shows that slippage of the noose is prevented by two aromatic residues flanking the embedded ring. Unthreading does not occur even when the molecule is enzymatically digested with thermolysin, creating a two-chain, non-covalently linked peptide that may be likened to an axle (one chain) threading a wheel (2nd chain). The unique threaded structure of native microcin j25 fully accounts for all observed NMR and biophysical data and is consistent with the remarkable stability of this potent antimicrobial peptide. The NMR and MS studies that led to the structural characterization of microcin j25 are reported and the structure is compared with the cyclotide family of circular proteins in which there is threading of a disulfide bond through an embedded ring.

L72

OPTIMIZATION OF PEPTIDE ARRAYS ON A NOVEL PROTEIN CHIP FOR A PRACTICAL PROTEIN CHARACTERIZATION SYSTEM

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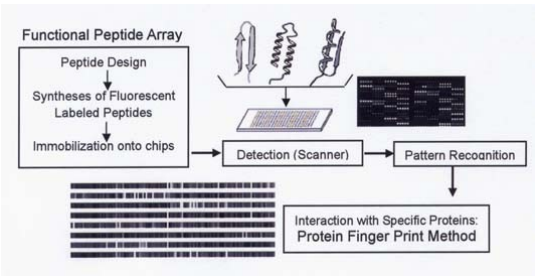
Novel protein-chips using peptide arrays have been produced (Figure 1), in which the peptides are used as sensing elements. We have successfully developed the protein finger print method, which can replace conventional methodologies such as mass spectrometric or surface plasmon resonance analyses [1-2]. Among several key technologies, which give difficulties in realizing the practical protein chip system, we have focused on the practical manufacturing process in the present paper. Thus, optimization of the fluorescent labeled peptides as capture molecules (choice of dyes and incorporation chemistry of dyes) and surface chemistry (immobilization of peptides) was assessed with respect to reproducibility, stability and cost performance. Additionally, several novel chip materials have been developed. References [1] Takahashi, M., et al., Chemistry and Biology, 10, 53-60, 2003. [2] Nokihara, K., et al., Solid-Phase Synthesis and Combinatorial Chemical Libraries 2004, ed: Epton, R., Mayflower Scientific, UK, in press



L73

HEPATITIS C VIRUS FUNCTIONAL MAPPING DATABASE: AN INSTRUMENT IN VACCINE DESIGN

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Hepatitis C virus (HCV) shows an extreme genetic polymorphism among isolates from different patients and even from one and the same patient during an infection course. HCV envelope proteins that evidently play the principal role in the virus attachment and entry to target cells have the greatest amino acid sequence variability. This complicates the study of virus-host cell interactions and vaccine design. A functionally-oriented HCV polyprotein amino acid sequence database (HCVMAP) has been designed. HCVMAP contains both structural and antigenic mapping data of HCV proteins and is accessible via WorldWideWeb. Primary data set consists of about 9,000 non-redundant HCV polyprotein full-length and fragmented sequences, as well as B- and T-epitopes taken from original publications. Processed data set contains aligned sequences and epitope mapping results with occurrence frequencies of mapped regions, with an easy navigation along the whole HCV polyprotein. Due to HCVMAP alignments several highly conserved envelope protein regions have been revealed. However, our own and other research data together with epitope predictions using HCVMAP sequences allow us to conclude that the whole envelope protein-based vaccine may be inefficient in producing anti-viral isolate-nonspecific antibody immune response because of unfavourable relative locations of possible B- and T-helper epitopes. Nevertheless, the revealed conserved probably functionally important sites can be used for synthetic anti-HCV vaccine design. HCVMAP can serve a template for designing functionally-oriented databases of other infectious agents, which are suitable for vaccinology research. Supported by the Russian Federal Interdisciplinary Program "New Generation Vaccines and Medical Diagnosticums for the Future" Grant.

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DESIGN AND NMR STRUCTURES OF SOMATOSTATIN (SRIF) RECEPTOR-SELECTIVE LIGANDS

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We discovered somatostatin (SRIF, H-Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH) in 1972 and mini-somatostatins (significantly shortened analogs of SRIF) in 1977. We described the synthesis of the first analogs with dissociated activities suggesting the presence of several SRIF receptors also in 1977. These contributions opened a new field and the basis for the development of drugs such as octreotide, lanreotide and others. SRIF analogs are used in the treatment of a variety of pathological conditions by modulating one or more of the five known membrane-associated receptor subtypes (sst1-5) and for receptor-targeted scintigraphy and radionuclide therapy. The actual function, distribution and specificity of the different SRIF receptors, however, are still far from being fully understood due to the lack of potent, labelable and selective agonists and antagonists to each receptor. Using structure activity relationships, we have successfully designed sst1- and sst4-selective agonists and sst3-selective antagonists that are amenable to specific labeling and with binding affinities equal or higher than that of SRIF-28. The use of betidamino acids (based on the aminoglycine scaffold) in the identification of leads for these unique molecules was critical. NMR spectrometry of sst1 and sst4 structurally-constrained ligands with high binding affinity led to the identification of two distinct consensus bioactive conformations different from that identified for sst2-selective analogs.