ORAL PRESENTATIONS

No 1

New Metal-Complexes and their Biological Activity

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The interaction of many inorganic compounds with cellular components in many cellular processes, have been such as DNA and proteins, as well as their involvement extensively studied. Information derived from *in vitro* studies concerning metal binding or interaction with DNA and DNA of living cells appears to form essentially the same adducts. Several anticancer drugs that are capable of DNA intercalation or causes DNA alteration are related to topoisomerase activities. The cytotoxic effects of some agents have been suggested to result from distortion of the DNA double-helix, which induces topoisomerases to break and relax DNA.

In the present study we use the high nuclearity complexes of Ni, from trinuclear to decanuclear fused metallacrowns to study different biological parameters such as interaction with DNA, anticancer and antibacterial activity (1,2). In addition, the biological effect of two classes of newly synthesized complexes of Pt(II) and Pd(II) with 2-acetyl pyridine 4N-ethyl thiosemicarbazone will be used as interference compounds to topoisomerases *in vivo*.

Antibacterial activity was performed against a range of Gram positive and Gram negative bacteria. Minimal Inhibitory Concentration (MIC) was determined using the method of progressive double dilution in liquid media. The antiproliferative activity was carried out using the XTT microculture tetrazolium colorimetric assay through which an assessment of drug-induced cellular growth inhibition is attained. Drug potency is expressed in terms of IC50 values (drug concentration resulting in 50% growth inhibition) calculated by dose-response curves obtained by XTT-assay.

References

1. Raptopoulou C.P., Paschalidou S., Pantazaki A.A., Terzis A., Perlepes S.P., Lialiaris Th., Bakalbassis E.E., Mrozinski J., Kyriakidis D.A.: *J. Inorg. Biochem. 71*: 15-27 (1998)

2. Theodorou A., Dertzis M.A., Kovala-Demertzi D., Lioliou E.E., Pantazaki A.A., Kyriakidis D.A.: *BioMetals 12*: 167-172 (1999)

No 2

The Brain Proteome

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About 30-50% of the genes in mammals are specifically expressed in the nervous system. A differential expression of these genes in distinct patterns is necessary for the generation of the large variety of neuronal phenotypes. Proteomic analysis of the brain compartments may be useful in understanding the complexity of the central nervous system. Proteomics belongs to the emerging New Technologies. It provides information in a high-throughput mode about the state of the gene products, i.e. expression levels and post-translational modifications, as well as changes in these parameters, resulting from various disorders or the effect of external factors. Proteomics has as goal the discovery of novel drug targets and diagnostic markers. It usually comprises two steps, analysis of a protein mixture by two-dimensional electrophoresis and identification of the proteins by mass spectrometry. We applied proteomics technologies to analyze human and rat brain. The human brain samples were derived from control individuals and patients with neurological diseases. The rat brain samples were from animals treated with toxic agents and from animals serving as models of human diseases. Up to now, in our laboratory,

approximately 500 human and 150 rat brain samples have been analyzed using the proteomic approach and several protein alterations were found related with neurological disorders and toxic events. The major results of these studies will be presented and the potential and limitations of the technology will be discussed.

No 3

Bulk Synthesis of Resins and Peptides: Application in Drug Discovery and Production

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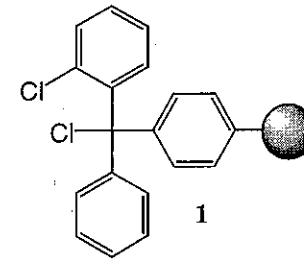
Past and present work on solid phase peptide and organic synthesis using the 2-chlorotrityl chloride resin *1* is presented in this report. In particular will be described, the development of peptide synthesis from the mg-scale for the evaluation of biological activity to the kg-scale synthesis of the pharmaceutical peptides octreotide, calcitonin and somatostatin and the ton-production of the first discovered entry inhibitor, anti- HIV 36 mer peptide pentafuside (T-20). This synthesis is performed by convergent methods using the 2-chlorotrityl resin and represents a milestone in the peptide production. The resin demand increased in parallel to several tons/year. Some important aspects of modern large-scale peptide and resin synthesis will by outlined.



Several applications of resins of the trityl-type in drug discovery will be reviewed.

No 4

A Very Promising Cyclic MBP Peptide for Multiple Sclerosis

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This work describes biological evaluation of a series of potent cyclic MBP altered peptide ligands synthesized by novel methods. Synthesized amide-linked cyclic analogues are namely, cycloMBP(87-99), cyclo(87-99)[Arg^{91},Ala^{96}]MBP_{87-99} and cyclo(91-96)[Ala^{96}]MBP. Synthesis was carried out using the Fmoc/tBu methodology and the cyclization was achieved using O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronioum tetrafluoroborate (TBTU), 1-hydroxy-7-azabenzotriazol and 2,4,6-collidine as cyclization reagents. The cyclic peptides were designed using Nuclear Magnetic Resonance data obtained from linear peptides

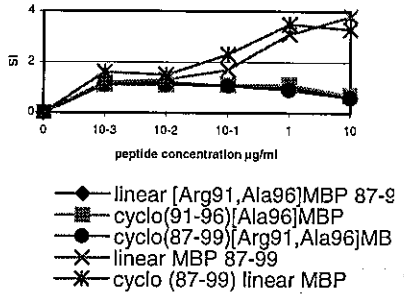
(MBP_{87-99} and [Arg^{91},Ala^{96}]MBP_{87-99})

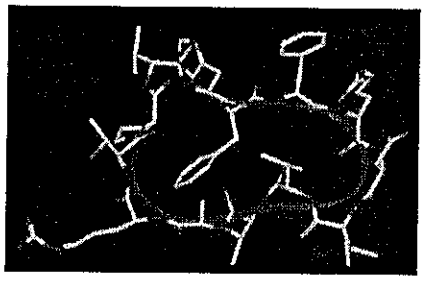
which revealed pseudocyclic conformation.

The *in vitro* effects of MBP altered peptide cyclization was then test by proliferation assay on human CD4+ T cell lines (Fig.2).

T cell lines were generated in vitro by a modified split-well technique and were specific to the linear peptide 87-99. Proliferative response of T cell lines to different MBP peptides was assayed by seeding in duplicate $1X10^4$ cells into 96 well

plates together with $5X10^4$ antigen presenting cells in complete medium, or at increasing concentration of linear and/or cyclic peptides. After 72 hours 0.5□Ci of ^{3}H-Thymidine was added to each well, and, after additional 6 hours ^{3}H-Thymidine incorporation was measured in a scintillation counter. Wells that showed a SI>2 were considered positive (SI = cpm of antigen stimulated wells/cpm of unstimulated wells).





Cyclo(87-99)[Arg^{91},Ala^{96}]MBP$_{87-99}$

Tested T cell lines (Fig. 2) proliferate only in the presence of linear MBP$_{87-99}$ peptide and cycloMBP$_{87-99}$. Cyclo(87-99)[Arg^{91},Ala^{96}]MBP$_{87-99}$ and cyclo(91-96)[Ala^{96}]MBP$_{87-99}$ seem to behave as antagonist of the linear MBP$_{87-99}$, in fact they show an activity similar to the linear antagonist [Arg^{91},Ala^{96}]MBP$_{87-99}$. Furthermore it is interesting to observe that cyclo MBP$_{87-99}$ acts as an agonist of the corresponding linear peptide analogue inducing a similar T cell proliferation on tested T cell lines.

References

1. Tselios T., Daliani I., Probert L., Deraos S., Matsoukas E., Roy S., Pires J.; Moore G., Matsoukas J.: *Bioorg. Med. Chem. 8*: 1903-1909 (2000)
2. Tselios T., Daliani I., Deraos S., Grdadolnik S., Mavromoustakos T., Melachrinou M., Thymianou S., Mouzaki A., Matsoukas J.: *J. Med. Chem. 45*: 275-283 (2002)

No5

Dermcidin, a New Antibiotic Peptide in Human Sweat

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Recently, a novel anti-microbial peptide DCD-1, derived from the Dermcidin (DCD) gene and secreted by sweat glands, has been described for the first time by Schittek et al. (*Nat. Immunol. 2:* 1133-1137, 2001).

The gene codes for a 110 amino acid protein (DCD) that is proteolytically processed to form a 47 amino acid peptide found in secreted sweat, encompassing positions 63-109 of the pre-processed product. This first identified peptide was named DCD-1 and demonstrates various anti-microbial activities. It displayed dose- and time-dependent toxicity against organisms such as *E. coli*, *E. faecalis*, *S.aureus* as well as the fungi *C. albicans* at pH and salt conditions characteristic of human sweat. Thus, the dermcidin peptide probably plays a key role in the innate immune response of the skin. DCD shares no homology with any known anti-microbial peptides described to date and it as yet remains unclear as to its mode of processing or inter-individual variations in the amount of peptide processed.

Using the Fmoc-solid phase strategy, the 47-mer as well as a set of overlapping peptides have been synthesized and testet for its antibiotic properties.

To detect this peptide in sweat we used the SELDI (Surface Enhanced Laser Desorption/Ionisation) ProteinChip® System (Ciphergen Biosystems, Fremont, CA) which combines activated or chromatographic surfaces (ProteinChip® Arrays) with solid-state Time Of Flight (TOF) mass spectrometry. By this technique, we have been able to identify also several degraded dermcidin derived peptides possibly cleaved by exopeptidases in a very small sample volumes (1 ul). The DCD-1 concentration range in the sweat samples was approximately 0-9 ng/µl in the female and 0-23 ng/µl in the male.

A most interesting question is still open. What kind of structural elements are responsible for

this new type of antibiotic peptide? CD spectroscopy experiments and NMR studies are under investigation.

No 6

Animal Models in Understanding Autoimmune Diseases

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The generation and study of animal models for the study of autoimmune diseases, combined with modern approaches in gene targeting and expression in mice, has been fundamental to our current understanding of the mechanisms that underlie disease aetiology and pathogenesis in humans and to the identification and testing of new therapeutic targets. We have used transgenic and knockout technology to investigate the role of TNF and its receptors in the induction and progression of CNS inflammation and demyelination, such as that which develops in multiple sclerosis, and to assess its potential as a therapeutic target. Critical roles for TNF in the initiation of experimental autoimmune encephalomyelitis (EAE), a T cell-mediated disease targeting the CNS, and in the development of spontaneous CNS inflammation and demyelination in transgenic mice, will be demonstrated and discussed in the context of recent clinical trials using TNF blockade. Furthermore, studies in which EAE has been used for the design and testing of new myelin peptide mimetics as potential novel therapies for MS will be described.

No7

Inhibition of Signal Transduction as a Target of Cancer Therapy: The case of Glivec®

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Cell signaling describes the cell interactions that affects every cell function and is responsible for the communication of the cells with their neighbors (other cells and matrix molecules). The signal (information), which is due to responses to ligands that come to cell surface, is transmitted via the cell membrane to cytoplasm and often to the nucleus via the mechanism known as signal transduction (ST). The signal transmitted regulates several cellular events, such as cell proliferation and differentiation, apoptosis, altered gene expression, modified function of metabolic enzymes, modified cell morphology, activation of DNA synthesis, change in permeability of ions, altered synthesis of matrix macromolecules.

ST is a very complex procedure that involves a series of proteins; each acts on the next causing alteration on its conformation. The most important mechanism in ST is the addition of phosphates (Pi) via the action of protein kinases. ST starts with the direct activation of the enzymic action that is related with the receptor. One cell may expresses many growth factor receptors and, therefore, it may respond simultaneously or sequentially to more than one growth factor, indicating that there are many ST pathways that can work within a cell. Tyrosine phosphorylation is the most common ST mechanism and it is a strictly controlled event in normal cells. Kinases are enzymes responsible for the Pi transfer from ATP to tyrosine of the next protein. ATP and protein substrates are bound to specific domains of kinases and it is estimated that human genome involves about 2000 different tyrosine kinases (*ca* 50 have been examined). The action of protein tyrosine kinases is controlled from the well-matched binding domains of kinases with ATP and protein (otherwise the signal is not transmitted).

Chronic myeloid leukemia (CML) is a proliferative disorder of haemapoietic stem cells. The cytogenetic abnormality of CML is due to the Philadelphia chromosome, which is a (9;22) chromosomal translocation. The expression of Bcr-Abl tyrosine kinase in CML has been recognized as a single molecular abnormality that causes transformation of a haemapoietic progenitor into a malignant clone. Bcr-Abl is responsible for the increased cell proliferation, the decreased apoptosis and altered adhesion of the bone marrow cells. Bcr-Abl is a therapeutic target for CML since it is detected in 95% of patients with CML, it is the causative abnormality of CML and it is constitutively activated intracellularly. Imatinib

(STI571, Glivec) has been developed to inhibit the signaling in CML acting through the replacement of ATP. Apart Bcr-Abl, the PDGF-receptor and the stem cell factor-receptor (c-Kit) are additional molecular targets for Glivec. Tumors associated with these targets involve gastrointestinal solid tumors (GIST), sarcomas, lung cancer, prostate cancer, gliomas and neuroblastoma, breast cancer, seminomas and germ cell tumors. Our studies are focused on the effect of STI571 inhibitor in invasion and metastasis of breast cancer cells.

No 8

On the Mechanism of Tumor Progression by Thrombin / Thrombosis

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Clinical, laboratory, histopathological and pharmacological evidence support the notion that a systemic activation of blood coagulation is often present in cancer patients (1). In addition thrombin was shown to promote tumor progression and metastasis in animals and epidemiological studies suggest an increased a risk of cancer diagnosis after primary thromboembolism (2).

We have proposed that the aforementioned results may be related to our finding that thrombin is a potent activator of angiogenesis (3). This is a thrombin receptor (RAR1)-mediated event and independent of fibrin formation (4). Many cellular effects of thrombin on endothelial cells can contribute to the angiogenic action of thrombin:

Exposure of endothelial cells to thrombin causes a time and dose-dependent decrease in the attachment of these cells to basement membrane components with a concomitant increase in MMP_2 activation (5).

Thrombin up-regulates the expression of integrin $a_v\beta_3$, the marker of angiogenic phenotype of endothelial cells (6).

Thrombin has chemeotactic and aptotactic effects on endothelial cells and up-regulates the expression of VEGF receptors (KDR & Flt1). Thus thrombin synergizes with the key angiogenic factor VEGF in endothelial cell proliferation (4). In addition, thrombin enhances the secretion of VEGF and MMP9 of prostate cancer cells (PC_3) (6). These results can explain the angiogenic and tumor promoting effect of thrombin and provide the basis for development of thrombin receptor mimetics or antagonists for therapeutic application.

References

1. Rickles F.R., Edwards R.L.: *Blood* 62: 14-31 (1983)
2. Sörensen H.T., Mellem K.L., Steffensen F.H., Olsen J.H., Nielsen G.L.: *N. Engl. J. Med.* 338, 1169-1173 (1998)
3. Tsopanoglou N.E., Pipili-Synetos E., Maragoudakis M.E.: *Am. J. Physiol. 264*: C1302-C1307 (1993)
4. Tsopanoglou N.E., Maragoudakis M.E.: *J. Biol. Chem. 274*: 23969-23976 (1999)
5. Maragoudakis M., Kraniti N., Giannopoulou E., Alexopoulos K., Matsoukas J.: Modulation of Angiogenesis and progelatinase a by thrombin receptor mimetics and antagonists. *Endothelium*, (in press), 2001
6. Maragoudakis M.E, Tsopanoglou N.E., Andriopoulou P.: Mechanism of thrombin-induced angiogenesis. *Biochemical Society Transactions* (in press), 2002

No 9

The Employment of Phage-displayed Random Peptide Libraries for the Identification of Antigens Associated with Inflammatory Demyelinating Diseases of the Central Nervous System

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Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS) in man. Clinical manifestation is due to destruction of the myelin sheath and sec-

ondary disordered repair or gliosis. The etiology of MS is unknown; however, autoimmunity, perhaps triggered by an exogenous infectious agent, may precipitate the disease. In immunologically and genetically predisposed individuals, an inflammatory immune response ensues. A specific humoral immune response in MS is the production into the CNS of immunoglobulins which appear as oligoclonal bands (OCBs) in agarose isoelectric focusing of cerebrospinal fluid. Although all five isotypes of immunoglobulin molecule can be detected in CSF, IgG is the most dominant isotype present. So far, the search for the identification of the target antigens for OCBs in MS has been inconclusive.

Subacute sclerosing panencephalitis (SSPE) is a rare delayed complication of measles virus infection which also causes progressive inflammation and demyelination of the brain and shows increased concentration of immunoglobulins demonstrated as OCBs in CSF electrophoresis. Approximately 95% of IgG of oligoclonal bands is synthesized locally, and 25-75% of the antibodies in the CNS is directed against measles virus.

Phage-displayed random peptide libraries have proved to be useful tools for mapping protein-protein interactions. The screening phage-displayed random peptide libraries can identify sequences that interact specifically with an antibody with no prior structural information of the target. The peptide library has been generated by introducing short synthetic degenerate oligonucleotides into the viral M13 genome (gIII gene). The short peptides with random aminoacid sequence are expressed as fusion parts of the pIII protein, which is displayed on viral surface and is accessible during screening experiments.

We used this approach to define the target antigens for: a) IgGs eluted from brain of one SSPE patient and one MS patient and b) protein eluted from IgG OCB from an MS case.

The peptides identified to react specifically with these antibodies were found to bear homology with a number of proteins. In this presentation we will see how these proteins are implicated with the immunopathology of the diseases.

No 10

Effect of Peptide Analogs of Myelin Basic Protein on Cytokine Secretion by Peripheral Blood Mononuclear Cells of Multiple Sclerosis Patients and Controls

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The objective of this study was the use of newly designed peptide analogs of disease-associated myelin basic protein (MBP) epitopes, as immunomodulatory agents in multiple sclerosis (MS).

We designed and synthesized 10 linear and cyclic analogs of the guinea pig MBP(72-85) and the human MBP(87-99) epitopes, as described [1]. All the peptides were added, at various concentrations, to human peripheral blood mononuclear cell (PBMC) cultures derived from 34 MS patients, 7 at presentation and 27 recall, and 7 healthy age-matched controls; their effect on cellular proliferation and cytokine synthesis was assessed using the BrdU proliferation kit (Roche) and human cytokine ELISAs (Endogen, R&D, Diaclone) respectively.

Overall, the peptide effect on cellular proliferation in both patients and controls was negligible when the peptides were added at various concentrations in cells cultured in plain culture medium. When the peptides were added to cell cultures in the presence of mitogens (PHA and/or PMA & ionomycine) their effect was variable. Briefly, 6/10 peptides increased proliferation rates in both patients and controls and 4/10 had no effect. Regarding cytokine synthesis, the addition of all 10 peptides in cell cultures (in the absence of mitogens) had no effect on cytokine production by PBMC of healthy controls while it resulted in heterogeneous alterations in the patterns of cytokines produced by the cells of the MS patients: Assuming that IFN-γ is the main inflammatory cytokine and IL-10 the main anti-inflammatory cytokine in MS, we focussed on the alterations caused in the secretion patterns of those 2 cytokines and made the following observations: 4/10 peptides caused a decrease in IFN-γ production by PBMC of MS patients and 4/10 caused an increase in IL-10 production. The peptides that caused a decrease in IFN-γ and an increase in

IL-10 production by PBMC of the same patients, were the peptides human cyclo (91-99)[Ala^{96}]MBP_{87-99} and the human cyclo (87-99)[Arg^{91},Ala^{96}]MBP_{87-99}. This effect was more pronounced in the patients at presentation compared to the recall patients that nevertheless showed the same response pattern.

In Conclusion, out of 10 similar peptides tested, only 2, the human cyclic peptide analogs (91-99)[Ala^{96}]MBP_{87-99} and (87-99)[Arg^{91},Ala^{96}]MBP_{87-99}, had a beneficial immunomodulatory effect in human MS PBMC and warrant further investigation.

References
1.Tselios et al.: *Bioorg. Med. Chem. Lett. 10*: 2713-2717 (2000)

No 11

Protein Tyrosine Kinase Inhibitors: A new Class of Anti-cancer Drugs and their Interactions with Cytotoxic Stimuli

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Protein tyrosine kinase (PTK) inhibitors are a new group of pharmacological agents that inhibit expression or catalytic activity of PTKs. PTK inhibitors can be classified into four major groups.

1) Small molecule drugs that inhibit ATP binding to the catalytic domain of the enzyme. New highly specific agents have substituted low-specificity agents of first generation PigTK-inhibitors as genistein, herbimycin-A, erbstatin etc. Quinazolines (ZD1839, *Iressa*), phenylaminopyrimidines (imatinib mesylate) pyridopyrimidines and pyrimidopyrimidines (PD158780), pyrrolopyrimidines and pyrazolo-pyrimidines (CGP59326), tyrphostins (leflumomide), indoles and oxindoles (SU5416) are some of the new class of small-molecular weight PTK inhibitors that are under clinical development.

2) Monoclonal antibodies that target the extracellular domain of receptor PTKs (trastazumab-HER2, C225-EGFR).

3) Inhibitors of the SH2 domain of PTKs (AP22408).

4) Peptides that antagonize the binding of the substrate to the kinase.

The study of the interactions of PTK inhibitors with cytotoxic stimuli (as chemotherapeutic drugs, irradiation, Fas-ligation) is helping us to define the pathways of apoptotic cell death after DNA damage and the optimal way of co-administration with both chemotherapy and radiotherapy. We have shown that PTK-inhibitors promote cell death after γ-irradiation, UV-irradiation and chemotherapeutic drugs that damage DNA structure. Cellular response to DNA damage includes activation of several pathways for apoptosis, cell cycle arrest, DNA-repair mechanisms, survival pathways, etc. We have shown that PTK-inhibitor genistein inhibits the translocation of NFκB to the nucleus (which transcribe several survival signals) and deregulates G_2-cell cycle phase checkpoint, in cells that were treated with camptothecins (chemotherapeutic drugs that are potent topoisomerase I inhibitors). Both of these actions promote apoptotic cell death and decrease cell survival.

No 12

Contribution of the Growth Factor HARP in Angiogenesis

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HARP (Heparin Affin Regulatory Peptide) is a growth/differentiation secreted protein with distinct lysine-rich clusters within both the NH_2- and C00H-terminal domains and apparent molecular weight 18 kDa. It was initially purified from bovine uterus and neonatal rat brain and found to be

highly conserved among species. It has a high affinity for heparin and is localized in the extracellular matrix through interactions with glycosaminoglycans. It is expressed in developing tissues and displays important function in the growth and differentiation processes of several cell types.

The implication of HARP in the regulation of the angiogenic process has been clearly demonstrated in both *in vitro* and *in vivo* studies. Moreover, both protein and mRNA of HARP have been detected in blood vessels of different tissues, suggesting a role in the formation or/and maintenance of blood vessels. We have previously shown that although HARP stimulates angiogenesis by microvascular endothelial cells, it has a slight inhibitory effect on tube formation by human umbilical vein endothelial cells (HUVEC), suggesting that the microenvironment may significantly affect the actions of HARP. In this study, we show that HARP downregulates the expression of the VEGF receptor KDR and inhibits HUVEC proliferation, migration and tube formation on matrigel, induced by the 165-amino acid form of vascular endothelial growth factor ($VEGF_{165}$). In addition, HARP inhibits the binding of ^{125}I-$VEGF_{165}$ to the VEGF receptors of HUVEC, as well as of heparan sulfate deficient CHO cells that over-expressed VEGF receptors. The mechanism of this inhibition seems to be a direct interaction of HARP with $VEGF_{165}$ through the two â sheet domains of HARP containing the thrombospondin repeat. Further structure-function studies using synthetic peptides or mutagenesis analyses are required to identify the regions of HARP involved in its biological activities.

No 13

Investigation of Novel DNA Gyrase Inhibitors

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DNA gyrase is an essential enzyme with no direct mammalian counterpart and is therefore an attractive target for the design of antibacterial drugs. It catalyses the ATP-dependent introduction of negative supercoils into bacterial DNA as well as the decatenation and unknotting of DNA. Several classes of DNA gyrase inhibitors have been found i.e. coumarins, quinolines and cyclothialidines but all of them have their own limitations connected with toxicity, permeability and solubility, which prevent their widespread clinical use.

To overcome the limitations of known inhibitors we are searching for novel structures. We are using the methods of high resolution NMR spectroscopy to identify binding of novel compounds to the enzyme. Applied approach consists of several steps: determination of the chemical shifts of the protein ^{1}H, ^{15}N, ^{13}C nuclei, identification of the enzyme binding site, determination of chemical shift changes of the protein upon binding of novel compounds and determination of the location and orientation of the molecules in the enzyme active site.

We have already identified binding of new compounds, which are potentially safe drug candidates, because they are traditional dietary constituents of human life. We are investigating their biological activity in relation to their spatial orientation in the enzyme binding site to evaluate their potential as gyrase inhibitors and as building blocks of new leads for rational drug design.

No 14

Folding and Redox-State Dependent Structural Changes of Electron Transfer Haem Proteins

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Characterization of the structural and dynamical differences between the two cytochrome involved in the electron transfer process in different oxidation states is a fundamental step for the elucida-

tion of the electron transfer pathway and the molecular recognition process between the partner molecules. Investigation of structural differences between oxidized and reduced cytochromes has been pursued in solution through high resolution NMR Spectroscopy and determination of the corresponding structural models. In the case of *Horse Heart Cytochrome c* parallel investigation by X-ray diffraction suffers through the lower stability of one of the two redox partners and solid-state structure determination of this protein, although yielded a structural model, it was hard to unambiguously attribute to the oxidized or to the reduced species. On the other hand, the solid state conformation of the oxidized *cyt c* has been determined in its complex form with cytochrome *c* peroxidase and structure should exhibit differences either with the same structure in its free state or with that in solution.

Horse Heart Cytochrome c folds in a compact, rather spherical, structure characterized by the presence of a hydrophobic core consisted by the N- and C-terminal helices, the 60's helix and one heme edge. The heme is covalently attached to the protein matrix by two thioether bonds involving sulphydryl groups of Cysteine residues (Cys14 and Cys17). The fifth heme iron ligand is His18 and the sixth Met80. Comparison of the solution structure of *Horse Heart Cytochrome c* in both redox states with the X-ray or solution structures of other cytochromes (*Saccharomyces cerevisiae* iso-1-cytochrome *c, Monoraphidium braunii* c_6, etc.) has revealed meaningful redox dependent changes of heme propionate 7. Propionate 7 has been proposed to constitute a pathway for electron transfer in several proteins such as cytochrome *c* and cytochrome *c* oxidases. Among these proteins studied in solution the conformational differences identified for equine *cyt c* are relatively large. The stability of the hydrophobic heme cavity is strongly dependent by the conformation and the inter-helical contacts between the two terminal helices. The hydrophobic environment facilitates electron transfer processes and protects electrons from being exposed to reducing agents.

No 15

On the Molecular Basis of Hypertension: NMR and Molecular Modeling Studies of Angiotensin II (AII) and Several Biologically Important Analogues in Solution-Comparison with AII Bound to Antibodies and the AT_1 Receptor

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In this lecture we will summarise:
(1). NMR and molecular dynamic studies of the conformational properties of the octapeptide hormone angiotensin II (AII) (Asp[1]-Arg[2]-Val[3]-Tyr[4]-His[5]-Ile[6]-Pro[7]-Phe[8]), and its receptor agonistic ([Cys(S-tbu)[5]]AII, [Sar[1],Cys(S-tbu)[5]]AII) and type I antagonistic analogues ([Cys(S-tbu)[5],Leu[8]]AII and [Sar[1],(S-tbu)[5],Leu[8]]AII) in aqueous solution[1,2]. Structure calculations suggest that AII and its analogues exhibit very similar structural elements of fragments 3-8. These residues have been proposed as the most immunogenic epitope for AII.
(2). A comparative study of the conformational properties of angiotensin II in the solution state with the structure of angiotensin II: (a) bound to the antibody Fab receptor, which have been determined by X-ray crystallography[3] and (b) docked to its AT_1 receptor (which was obtained through homology modeling). These studies could provide information on the mechanism of the selection of AII at the receptor site.

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References

1. Samanen J., Regoli D.: Structure-activity relationships of peptide angiotensin II receptor agonists and antagonists. In: (Ruffdo, R.R., ed.). Angiotensin II Receptors. Vol. 2, pp.11-97, CRC Press, Bocca Raton, FL, (994
2. Tzakos A., Troganis A., Bonvin A., van Nuland N., Gerothanassis I.P., Assimomytis N., Magafa V., Manessi-Zoupa E., Pairas G., Amzel L.M., Cordopatis P.: Manuscripts in preparation.
3. Garcia K.C., Ronto P.M., Vervoust P.J., Brünger A.T., Amzel L.M.: Three dimensional structure of an angiotensin II-Fab complex at 3 Å: Hormone recognition by an anti-idiotypic antibody. *Science 237*: 502-507 (1993)