Lectures and Oral Communications

OC 37 Pasteur Auditorium - Peptide/Protein Synthesis

COVALENT CAPTURE PURIFICATION: TOWARDS THE ROUTINE SYNTHESIS OF PROTEINS BY SOLID PHASE PEPTIDE SYNTHESIS

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Several groups have described methods for assisting the purification of full-length material after synthesis of a polypeptide on the solid phase. Generally, an affinity tag or chromatographic handle is attached in the last synthetic step and remains attached to the polypeptide after final deprotection and cleavage from the resin. These tags can have disadvantages of expense (hexa-His, peptidic immunoaffinity tag, biotin, those which require enzymes), or hydrophobicity (Tmob, biotin), or both, and must generally be removed after serving their purpose. Removal conditions described so far, which are applied to the deprotected polypeptide, have been harsh (cyanogen bromide, aqueous triethylamine or sodium hydroxide) or less reliable (enzymatic). We describe a new approach to the purification of full-length material, applicable directly after final deprotection and cleavage, which avoids chromatography and lyophilization of crude synthesis product. This new approach, called covalent capture purification, results in nearly quantitative purification yields. The covalent capture tag we employ allows facile isolation of full-length material, which is then purified in an HPLC polishing step to remove any full-length material alkylated during deprotection / cleavage. The tag can be removed rapidly under very mild conditions. Provided that appropriate synthetic procedures are used (high-yield coupling reactions, and capping to limit the creation of deletion sequences), our approach permits the preparation of very long sequences in high purity without resorting to lengthy ligation reactions. The tag removal conditions are compatible with native chemical ligation chemistry, so longer polypeptides may be used to reduce the number of ligation steps required, and cysteine residues in the target protein can be further apart.

OC 38

STRIKING PROGRESS IN THE DEVELOPMENT OF PEPTIDES AS DRUGS

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For almost half a century, since Du Vigneaud first presented his pioneering synthesis of oxytocin to the world in 1953, the pharmaceutical community has been excited about the potential of peptides as "Nature's Pharmaceuticals". Further discoveries, including Merrifield's solid-phase synthesis method, introduced a decade later, recombinant techniques for expressing peptides and proteins in microorganisms, and, most recently methods for producing peptides and proteins in transgenic animals and plants, have all combined to increase the availability and lower the cost of producing peptides. For many years, however, the major obstacle to the success of peptides as pharmaceuticals was their lack of oral activity and, therefore, relatively few peptides reached the marketplace as approved drugs. As a result, several major pharmaceutical companies abandoned their research efforts in the area, in favor of small molecule mimics of peptide or protein lead compounds. However, in recent years, advances in the areas of formulation and novel delivery systems have revitalized the field, leading to several highly successful "blockbuster" peptide drugs, such as Lupron® and Zoladex®. The resulting resurgence of interest in peptides as pharmaceutical products has, once more, provided a challenge to the peptide industry to develop economically viable methods for manufacturing relatively complex peptides in extremely large quantities. The challenge is being met, and a number of new peptide drugs are now in late-stage development, with target requirements, at commercial launch, in quantities of up to metric tons.

Recent developments in methods for the large-scale manufacture of peptides will be discussed, with particular emphasis on chemical synthesis techniques. Examples will be presented of late-stage development products, which, if successful, will be required in multi-kilogram to ton quantities.

OC 39 Pasteur Auditorium - Peptide/Protein Synthesis

LARGE SCALE CHEMICAL SYNTHESIS OF T-20, A 36 AMINO ACID HIV-1 MEMBRANE FUSION INHIBITOR

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The surface envelope of HIV-1 contains two virally encoded glycoproteins, gp120 and gp41. Major roles for the larger gp120 subunit are the recognition and attachment to specific receptors/co-receptors on the surface of host cells. A major role for the gp41 subunit is to accomplish fusion of the viral membrane with the cell membrane. This is an early step in the virus life cycle and leads to entry of the virus into the host cell. A critical step in the fusion process is a structural rearrangement of gp41 that involves coiled-coil packing of two non-contiguous heptad repeat domains. A peptide named T-20 is modeled after one of these domains and interferes with the gp41 rearrangement and thus membrane fusion and virus entry into the cell. The T-20 peptide has been found to be a potent and selective inhibitor of membrane fusion and infection both *in vitro* and *in vivo*.

Clinical trials evaluating dose, durability and safety are ongoing. Drug substance needs to support the T-20 clinical program are estimated to exceed 200Kg. An economic and robust synthesis of the 36 amino acid peptide has been developed and is currently being used to produce T-20 on a 100-200 Kg/year scale. A 10 fold scale-up is being planned for commercial manufacture.

This paper will detail the process used to synthesize T-20 on an unprecedented scale, the rationale behind route selection, and the difficulties encountered during scale-up.

OC 40

SUGAR AMINO ACIDS: SYNTHESIS AND APPLICATIONS

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Sugar amino acids (SAAs) are sugar moieties containing at least one amino as well as at least one carboxyl group. They can be used as substitutes for conventional amino acids or peptide fragments[1]. Synthesis and applications for several of these building blocks [2] will be given. Some are shown in *Figure 1*.

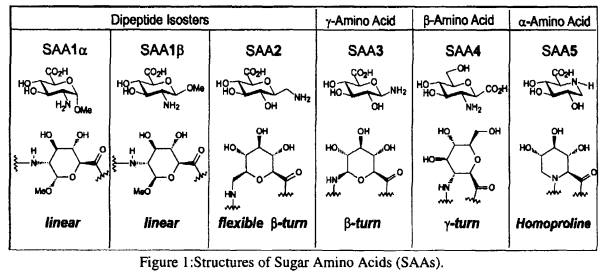

Figure 1:Structures of Sugar Amino Acids (SAAs).

They are mainly used

- as side chain modifications in peptides to improve bioavailability such as the tumor/tissue ratio of radiolabelled RGD peptides for metastasis diagnostics [3],
- as turn mimetics (*Figure 1*) to achieve higher activity, selectivity and bioavailability in cyclic bioactive peptides[4],
- as scaffolds for the design of non-peptidic biomimetics,
- as structural templates in form of linear and cyclic homooligomers.

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OC 41 Einstein Auditorium - Pharmacology of Peptides OC 42

DESIGN OF VASOPRESSIN AND OXYTOCIN AGONISTS, ANTAGONISTS, RADIOIODINATABLE AND FLUORESCENT LIGANDS WITH GOOD AFFINITIES FOR HUMAN RECEPTORS

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Potent and selective agonists and antagonists of the vasopressin (VP) V_2 (renal) and V_{1a} (vascular) receptors and of oxytocin (OT) (uterine) receptors which are effective in the rat, have found widespread use as pharmacological tools [1] and in the design of radioiodinatable [2] and fluorescent ligands [3] for use as receptor probes. However, species differences between the rat and other mammalian receptors, particularly the human VP V_{1a} and V_2 receptors [4, 5], has limited the usefulness of rat bioassay data and rat receptor binding data as predictors of activities in humans. This was particularly the case with VP V_2 antagonists. Thus, peptides which were highly effective V_2 antagonists *in vivo* in the rat [1, 5] were subsequently found to be agonists in humans [5]. The availability of cloned human VP V_2, V_{1a}, V_{1b} (pituitary) and OT receptors [6] and of a constitutively active human V_2 receptor [7] has greatly aided the design of ligands with good affinities for human VP and OT receptors. Using leads uncovered from classical rat bioassays [1], we now report the design and synthesis of a) V_2 agonists and antagonists with good affinities for human V_2 receptors, b) V_{1b} agonists and antagonists with good affinities for human V_{1b} receptors, c) radioiodinatable ligands with good affinities for human OT, V_{1a} and V_2 receptors, d) fluoresceinylated OT agonists and antagonists with good affinities for human OT receptors. A number of these new ligands could be useful pharmacological tools for cellular and molecular studies on human VP and OT receptors and may lead to the development of therapeutic agents for human use.

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2. Barberis et al., *Neuroendocrinology*, **62**:135-146 (1995).
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STRUCTURE-BASED DESIGN OF THE FIRST HIGH POTENT INHIBITORS OF BOTULINUM B-TOXIN

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Botulinum toxin type B is a dichain protein from *Clostridium botulinum* which consists of two disulphide-linked sub-units, a 100-kDa heavy sub-unit involved in binding and internalization and a 50-kDa light sub-unit involved in the zinc-endopeptidase activity of this toxin. It blocks the release of excitatory neurotransmitters in the peripheral nervous system resulting in the syndrome botulism which is characterized by flaccid muscular paralysis for which there is no therapy available to date. So we became interested in the inhibition of the proteolytic activity of botulinum B-toxin which is one of these toxins found in contaminated food. We both developed a highly sensitive and easily automatizable test to detect this toxin (in the picogram range) and to carry out a structure-based design of botulinum B-toxin synthetic inhibitors. In a preliminary approach we showed that the best zinc chelating group was a thiol on the β carbon of the β-amino acid at the S1 position. We also demonstrated that a free amino group was necessary at the N-terminal position. The best residue at the S1 position was demonstrated to be the para-carboxy benzyl group by testing a series of β-amino thiols. We tested pseudo-tripeptides interacting with the sub-sites S1, S'1 and S'2 of the enzyme. The use of combinatorial chemistry allowed the best groups interacting with the S'1 and S'2 sub-sites to be selected. By this way we have obtained the more potent inhibitors of botulinum B-toxin reported to date, Ki ≤ 10^{-7}M. These molecules were only tested *in vitro* as inhibitors of the endopeptidase activity of the light chain of the toxin. Their activity remains to be evaluated in more physiological conditions as for instance with neurons to determine *ex vivo* their ability to cross the membrane of the cells and to counteract the action of this toxin.

OC 43 Einstein Auditorium - Pharmacology of Peptides OC 44

CONTRIBUTION OF CENTRAL AND C-TERMINAL REGIONS OF GASTRIN TO RECEPTOR RECOGNITION

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Gastrin (G17) with C-terminal GlyOH in place of an α amide was claimed to have little or no affinity for the CCK_2-receptor. Also, several des-Phe analogs of G17 were reported to be antagonists in functional assays based on rodent CCK_2-receptors. To further examine the contribution to receptor recognition being made by central and C-terminal regions of G17, this study was focused on the putative β-bend and the aromatic residue at position 17. γ-Lactam structure (γ-Lac) which mimics a β-turn, was introduced in place of Gly^{13} in truncated gastrin analogs with and without Phe^{17}. G17 analogs with addition of C-terminal GlyOH as well as with $GlyNH_2$ were also prepared.

Human CCK_2-receptor cDNA was delivered into CHO cells using activated dendrimer technology. The recombinant receptors were used in competition binding experiments with [^{3}H]CCK-8. The following rank order of potency was obtained:

CCK-8 > G17 ≈ [Leu^{15}]G17 > CCK-4 ≥ Ac[Leu^{15}]G17(11-17) ≈
≈ Ac[γ-Lac^{13},Leu^{15}]G17(11-17) > L-365,260 > G17GlyOH >> $G17GlyNH_2$

Ac[Leu^{15}]G17(11-16)NH_2 and Ac[γ-Lac^{13},Leu^{15}]G17(11-16)NH_2 did not bind to the receptor. G17GlyOH retained considerable affinity for the receptor but $G17GlyNH_2$ displayed dramatic reduction in the affinity compared to that of G17GlyOH.

The results indicate that stabilization of a β-turn in the central region of G17 is readily accommodated by the human CCK_2-receptor. The aromatic residue at position 17 is essential for binding to the receptor. Addition of Gly at the C-terminal end of G17 is well tolerated by the human receptor and a free carboxyl group on the Gly^{18} is preferred by the receptor to an amide.

Acknowledgments: this work was supported by the Cancer and Smoking Related Diseases Research Program of the State of Nebraska and the Carpenter Endowed Chair in Biochemistry, Creighton University.

BOVINE SERUM ALBUMIN DIFFERENTIALLY AFFECTS THE RATES OF DIPEPTIDYLPEPTIDASE-IV MEDIATED HYDROLYSIS OF GROWTH HORMONE-RELEASING FACTOR AND ITS ALA15-MODIFIED ANALOG.

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Plasma dipeptidylpeptidase-IV (DPP-IV) has been identified as a primary enzyme degrading growth hormone-releasing factor (GRF) both *in vitro* and *in vivo* by cleaving off its N-terminal Tyr-Ala dipeptide. Our earlier study [1] revealed that the half-life of Ala15,Leu27-bGRF(1-29)NH_2 **2** was about twice that of the parent compound Leu27-bGRF(1-29)NH_2 **1** when incubated in bovine plasma. It was puzzling why a Gly15→Ala15 substitution in the central part of **2** made this peptide less susceptible to DPP-IV-mediated cleavage at its N-terminus. We postulated that **2**, which is more helical than **1** [2], might be differentially bound and protected by plasma components, mainly BSA. To examine that, we set up an *in vitro* kinetic study to determine apparent hydrolysis rates of **1** and **2** incubated with purified DPP-IV in PBS either in the presence or absence of 4% BSA. The calculated half lives of **1** and **2** were respectively 18.2 and 19.3 minutes in PBS, as compared to 26.9 and 41.3 minutes, respectively in 4% BSA/PBS. Our data show that (i) in the absence of BSA **1** and **2** were hydrolyzed by DPP-IV at comparable rates; (ii) BSA slowed down the hydrolyses of **1** and **2** by 47.3% and 113.7%, respectively, as compared to the non-BSA condition; (iii) the hydrolysis rate of **2** was more affected by BSA which was reflected in its half-life of ca. 54% longer than that of **1**. We conclude that BSA binds both **1** and **2** to a different extent, possibly due to different peptide conformations, and this differentially affects the amount of each free peptide remaining available for DPP-IV cleavage. This effect is more pronounced for **2**, possibly due to its higher helical content and stronger binding to BSA. The fact that in bovine plasma the half-life of **2** was about 92%-112% longer than that of **1** indicates that in addition to BSA there might be other plasma components that also bind **2** stronger than **1** and thus provide additional temporary protection against DPP-IV cleavage.

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NEW AMINO-ACIDIC ANTAGONISTS OF THE EXCITATORY AMINO ACID RECEPTOR

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We have obtained previously several amino-acidic antagonists of the Excitatory Amino Acid (EAA) receptor (1,2,3). The preliminary tests shown ionotropic non-NMDA (N-Methyl-D-Aspartate) [probably AMPA / kainic acid (α-amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid / 2-carboxy-4-isoprenyl-3-pyrrolidine acetic acid] receptor affinity, low neurotoxicity and a high antagonistic activity of some of them. In search for the next more effective amino-acidic antagonists of EAA receptor we now designed and synthesized several amino acid derivatives. We found several active compounds between aromatic amides of N-acetyl- or N-methyl- amino acids of a moderate hydrophobicity. N-methylation was carried out using CH_3I / NaH in the presence of tetrabutylammoniu bromide (TBAB), N-acetyl group was introduced in a typical way. Benzylamides were obtained by means of the mixed carboxylic-carbonic acids anhydrides method and purified by crystallization or column chromatgraphy. Pharmacological evaluation included maximal electroshock seizure test (Mes test), subcutaneous maximal Metronidasol seizure test (sc Met test) and neurotoxicity test (Tox test). The most promissing compounds were also tested quantitatively in mice after intraperitoneal (i.p.) administration. Three of obtained compounds shown a strong anticonvulsant activity. A number of high active anticonvulsants (antagonists of EAA receptor) was in percentage higher between the cyclic structures than between the previously obtained linear ones. They show also another kind of activity (anxiolytic-like and analgesic). Moreover the strongest anticonvulsant found in serie of amino-acidic antagonists of EAA receptor is present in group of derivatives of cyclic amino acids.

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NOVEL CATHEPSIN K INHIBITORS FOR CONTROL OF BONE RESORPTION

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Cysteine proteases are responsible for the regulation of a variety of biochemical processes. Cathepsin K, a cysteine protease of the papain superfamily, has been shown to be highly and selectively expressed within osteoclasts suggesting that this protease may be playing a critical role in the resorption of the bone matrix. We have disclosed novel classes of cysteine proteases that act by varied mechanisms (i.e. reversible, slow turn over and irreversible). Potent and orally bioavailable pyrrolidinone and piperidinone base inhibitors of cathepsin K have been among the structure types studied. Due to a facile epimerisation at the chiral center adjacent to the ketone neither of these series were deemed suitable for development. Peptidomimetics which are less prone to this epimerisation have led to a new series of potent human and rat inhibitors of cathepsin K. The design, synthesis and pharmacological evaluation of these inhibitors in rat models of bone loss will be presented.

Lectures and Oral Communications

L 11 Thursday Morning: Pasteur Auditorium L 12

THE RATIONAL DESIGN OF PEPTIDOMIMETIC INHIBITORS OF TNF-α PROCESSING AND AGGRECANASE

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Tumor necrosis factor alpha (TNF-α) is a key pro-inflammatory mediator in auto-immune diseases. The recent approval of injectible anti-TNF-α biologics (Enbrel® and Avakine®) and their remarkable success in patients with rheumatoid arthritis and Crohn's disease, has established this cytokine as a central therapeutic target in inflammatory diseases. TNF-α is produced in most cell types as a 26 kd membrane associated protein which is processed to a 17 kd soluble form by the recently identified metalloprotease TACE (TNF-α converting enzyme). TACE belongs to the ADAMs family (a disintegrin and metalloprotease domain) of Zn metalloenzymes (ADAM-17). Peptide based metalloprotease inhibitors that also target TACE have been shown to be effective in animal models of inflammation. Our approach to the rational design and development of peptidomimetic inhibitors of TNF-α processing has led to novel non-peptide small molecules that have good overall properties in vivo.

Related enzymatic targets we have studied are involved in the proteolysis and breakdown of proteogylcan, a major macromolecular constituent of cartilage. We recently reported (*Science* 284, 4 June 1999 1664) the discovery of a new metalloprotease termed "Aggrecanase" that is secreted by chondrocytes and shown to cleave proteoglycan to give peptide fragments consistent with those observed in the synovial fluid of patients with osteoarthritis. The discovery, rational design and development of peptidomimetic inhibitors of aggrecanase will be presented.

The therapeutic application of TNF-α and aggrecanase inhibitors will be discussed in the context of the development of new and selective disease modifying therapeutics for rheumatoid and osteoarthritis.

DESIGN AND SYNTHESIS OF NEW INHIBITORS OF THERAPEUTICALLY IMPORTANT PEPTIDASES.

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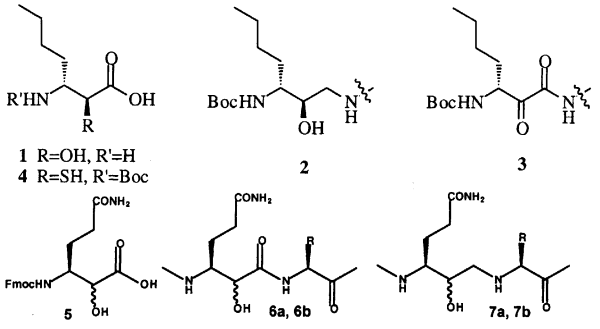
We describe the design and synthesis of novel, potential transition-state analogs and their use to prepare substrate-based inhibitors of Botulinum neurotoxin (BoNT) metalloprotease, methionine aminopeptidase-1, and bleomycin hydrolase. BoNT metalloprotease is the neurotoxic peptidase liberated in neurons and selective inhibitors are needed to prevent the effects of the holotoxin. Methionine aminopeptidase-1 removes the first amino acid from the growing peptide chain during protein biosynthesis, and selective inibitors of this enzyme may provide a potential new approach to antibiotic therapy. Bleomycin hydrolase is a novel cysteine peptidase that inactivates bleomycin *in vivo* and is a major effector for onset of tumor resistance to this drug. Syntheses and activities of the new compounds will be reported.



1 R=OH, R'=H
4 R=SH, R'=Boc

2

3

5 6a, 6b 7a, 7b

a: R = $(CH_2)_3NHC(NH)NH_3^+$ (BoNT/A)
b: R = CH_2Ph (BoNT/B)

OC 47 Pasteur Auditorium - Bioactive Peptides OC 48

IN VIVO ACTIVE ANTIMICROBIAL PEPTIDES AND THEIR BACTERIAL PROTEIN TARGETS

Laszlo Otvos, Jr, Philippe Bulet[a], David Craik[b], Ailsa McManus[b], Mark Rogers[c], Krisztina Bokonyi, Barry A. Condie, Insug O, Mare Cudic and Magdalena Blaszczyk-Thurin, The Wistar Institute, Philadelphia, PA, USA; [a]Institut de Biologie Moleculaire et Cellulaire, Strasbourg, France; [b]Centre for Drug Design and Development, Brisbane, Australia; [c]M-Scan, Inc., West Chester, PA, USA

At a time of the emergence of drug-resistant bacterial strains, the development of anti-microbial compounds with novel mechanisms of action is of considerable interest. Perhaps the most promising among these is a family of insect antibacterial peptides, which were shown to act on a target bacterial protein. One of the peptides, drosocin, is inactive *in vivo* due to the rapid decomposition in mammalian sera. However, another family member, pyrrhocoricin, is significantly more stable, has increased *in vitro* efficacy against gram-negative strains, and is devoid of *in vitro* or *in vivo* toxicity when administered alone. At low doses, pyrrhocoricin protected mice against *E. coli* infection, but, at a higher dose was toxic to compromised animals. Analogs of pyrrhocoricin were therefore synthesized to further improve protease resistance and reduce toxicity. A linear derivative containing unnatural amino acids at the termini showed high potency against *E. coli* infection and a complete lack of toxicity *in vivo* and an expanded cyclic analog displayed broad activity spectrum *in vitro*. Additional pyrrhocoricin analogs were made for general biochemical and structure-activity relationship studies. Through use of a biotin-labeled peptide, we isolated the elusive target protein from *E. coli*. According to mass spectrometry, Western-blot and fluorescence polarization, this protein is DnaK, the 70 kDa bacterial heat shock protein. The pharmaceutical applicability of this peptide family is underscored by the fact that pyrrhocoricin does not bind to the human equivalent Hsp70. However, the peptide may also interact with the lipopolysaccharide of the outer membrane of gram-negative strains, which may help the internalization process. The design of new drugs can be based on either the structure of the peptide-binding domain of the receptor or mimicking the structure of known ligands. To this end we determined the conformation of native pyrrhocoricin and drosocin by NMR and CD spectroscopy. Reverse-turns were identified as pharmacologically important elements at the termini, bridged by extended peptide domains.

DEVELOPMENT OF LEAD STRUCTURES FOR BRADYKININ AGONISTS AND ANTAGONISTS

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Bradykinin receptor agonists and antagonists are of great interest as potential drugs: agonists for the treatment of myocardial ischemia and antagonists for the treatment of inflammation such as allergic rhinitis, septic shock, trauma, bronchitis and asthma as well as in the therapy of some kinds of cancer. Because of the lack of a rationale for the design of agonists and antagonists or their interconversion we started a systematic search. We found two new lead structures for antagonists without any amino acid replacement at position 7. To discover the bioactive conformation of agonists and antagonists we stabilized analogues by a systematic lactamization between side chains and functionalized peptide bonds in the N- and C-terminal sequence, respectively. Biologically active cyclic analogues were analyzed by NMR. Starting from an active cyclic antagonist a 3-D-pharmacophore model was derived and used for the search of new nonpeptide lead structures. Biological activities were tested on smooth muscle organs, transfected COS-7 cells, on the release of cytokines from monocytes and also on the release of histamine from macrophages. Because histamine and cytokines can evoke important side effects of the potential bradykinin drugs we performed systematic SAR-studies for both reactions – the inhibition of the bradykinin induced cytokine release and the histamine release, to exclude some structures from further development. We are interested in developing potent B_2-receptor antagonists without histamine releasing activity to avoid inflammatory side actions. In contrast to enhanced histamine release, potent inhibition of cytokine release enhances the applicability of bradykinin antagonists as antiinflammatory drugs. For some antagonists inhibition of cytokine release was detected at concentrations as low as 10^{-15} M.

Lectures and Oral Communications

OC 49 Pasteur Auditorium - Bioactive Peptides OC 50

IDENTIFICATION OF AMINOACID RESIDUES INVOLVED IN THE INTERACTION BETWEEN VIP AND ITS HUMAN RECEPTOR VPAC1 USING PHOTOACTIVABLE VIP ANALOGS.

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Binding of peptides of the VIP family to class II G protein coupled receptor (GPCR) involves multiple domains in the N-terminal region and the extracellular connecting loops of receptors. Conversely, many aminoacids within peptides themselves take part in their high affinity binding to receptors. Therefore, it is essential to precisely determine which aminoacids of the peptide interact with which aminoacids in the receptor. The use of modified ligands carrying a photoactivable moeity (para-benzoylbenzoyl) allows, following the exposure to 345 nm UV rays, the creation of covalent bounds between the peptide and its receptor allowing the identification of anchor points. We previously described the VIP binding site in the human VPAC1 receptor by site-directed mutagenesis experiments and provided a molecular model. We tested here various photoactivable VIP derivatives obtained by substitution of the aminoacids 1, 6, 14, 22 or 24 by modified phenylalanines (1, 6 and 22) or lysines (14 and 24). Binding properties and adenylate cyclase activation parameters were determined for each modified peptide on CHO cells stably expressing a fusion protein between the human VPAC1 receptor and at its C-terminus the green fluorescent protein (GFP). The peptides substituted in position 22 and 24 had a slightly decreased affinity (5-10 times) compared to native VIP whereas peptides modified in position 1, 6 and 14 displayed respectively a 1000-, 50- and 1000-fold decreased affinity. We focused here on analogs modified in positions 6 or 22. After incubation of analogs with recombinant human receptor, irradiation with UV rays and analyses in SDS-PAGE both peptides labelled a 100 kD molecular entity corresponding to the expected molecular weight for the glycosylated receptor-GFP chimera. After treatment with PNGase F, the observed molecular weight was around 70 kD representing the deglycosylated chimera. Preliminary results of proteolysis with CnBr showed that the two different photoactivable VIP analogs bound to different CnBr fragments of the receptor. Further analysis is currently carried out to determine which aminoacid of the receptor was in contact with each photoactivable moeity. This work will provide precise physical input data to our current molecular model of the ligand binding site within the human VPAC1 receptor.

UNDERSTANDING RNA-BINDING PROPERTIES OF A PLANT VIRUS MOVEMENT PROTEIN USING SYNTHETIC PEPTIDES

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There are a wide variety of RNA-binding proteins that stabilize, protect, package or transport RNA molecules. The structural characterization of RNA-binding proteins and the understanding of how they interact with their target RNA to form functional complexes is a key problem in structural biology, with ramifications throughout molecular and cell biology. The viral-encoded movement proteins (MP) are a class of proteins that have a pivotal role in plant virus RNA cell-to-cell movement and the subsequent infection process. Detailed structural knowledge of plant virus MP and their interaction with viral RNA is scarce despite their important biological functions. We have chosen as experimental model the p7 MP from carnation mottle carmovirus (CarMV), one of the smallest MP known, for which we have previously shown RNA-binding properties. Three different peptides derived from the primary sequence of p7 where synthesized and assayed for RNA-binding. The N-terminal and the C-terminal segments of the p7 protein do not interact with RNA, while the Arg-rich central domain (peptide $p7_{17-35}$) binds to RNA with an affinity constant similar to that obtained for the whole p7 protein. The initial secondary structure characterization of p7 and its polypeptide segments, together with the RNA-binding experiments, allows us to define the peptide $p7_{17-35}$ as an α-helical inducible polypeptide that account for the RNA-binding properties of p7. To identify basic residues important for RNA binding, $p7_{17-35}$ derived peptides containing single alanine substitutions were synthesized and RNA binding potentials were determined by gel shift assays. In particular, it was demonstrated that one positively charged residue (R26) was more important than the others for RNA binding. Further information about the relevance of amino acids surrounding R26 was obtained from conformational studies on selected peptides. On the basis of these data and the results from the structural characterization of p7 free in solution and when bound to a short nucleic acid, we will discuss the influence of RNA-induced structural changes and the relative importance of several amino acid residues on the RNA binding properties of this plant virus cell-to-cell MP.

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COMPUTER MODELING OF TRANSMEMBRANE PROTEINS FROM SCRATCH: TEST CASE OF BACTERIORHODOPSIN

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Results of comprehensive 3D modeling of transmembrane (TM) proteins starting from their sequences are presented in comparison with the only available high resolution X-ray structure of 7TM protein, namely of bacteriorhodopsin (BR). Each step of the approach represents a novel modeling procedure. First, TM helices have been located in the sequence by the non-statistical method. Then, each individual helix has been subjected to energy minimization. Rough packing of helical bundle(s) has followed, which employed the principle of dense packing of the ideal cylinders (in the membrane plane) combined with arranging the individual helices across the membrane. Fine packing of the helices has been achieved by energy calculations, each helix consisting of the "hard core" (backbone) and the "soft shell" (side chains). Finally, 3D structures of interhelical loops have been restored either as the "average" low-energy conformations available for the given loop, or by the direct build-up procedure, or by a more sophisticated algorithm starting from residue-residue contact matrices. The approach has predicted the low-energy BR structures differing from the X-ray structure by the rms values in the range of 3 - 4 Å (the "coordinate" rms's over C^{α}-atoms). Since most of the obtained structures readily reproduce specific conformational features of BR, the discussed test case confirms the possibility to utilize the discussed approach for predicting 3D structures of G-protein coupled receptors, which are also 7TM proteins.

THE PARATHYROID HORMONE RECEPTOR: MOLECULAR INSIGHT INTO LIGAND BINDING

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A molecular model of the G-protein coupled receptor for parathyroid hormone (PTH) has been developed with the aim of characterizing the mode of ligand binding. Numerous receptor fragments of the receptor (PTH1) have been structurally characterized using nuclear magnetic resonance, circular dichroism, and extensive computer simulations. To date most of the extracellular domain of PTH1 has been examined and the structural features characterized. Because of the design principles employed in the receptor fragments, these results can be seamlessly incorporated into the theoretical model of the receptor. This methodology of incorporating experimental data, consisting of the structural preferences of the extracellular loops and N-terminus of PTH1, into the model of the arrangement of the transmembrane helices (based on rhodopsin), has provided great insight into the possible modes of ligand binding and receptor activation. Using these results we hope to design PTH-based ligands with enhanced anabolic-bone activity and therefore provide leads towards a cure for osteoporosis.

MODELLING PEPTIDE CONFORMATIONS IN A PEPTIDE-LIKE SOLVENT.

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Thimet oligopeptidase (EC 3.4.24.15, EP 24.15) is a soluble, zinc-dependent, thiol-activated metalloendopeptidase belonging to the thermolysin-like family of oligopeptidases and incorporates the classic HEXXH motif at its catalytic site. It hydrolyses and inactivates a wide range of oligopeptides, including neurotensin, bradykinin, gonadotrophin releasing hormone (GnRH) and substance P, and is involved in the production of met- and leu-enkephalins from their precursor peptides.

Very little is known about the conformation of the catalytic site of EP 24.15. There are a few inhibitors of EP 24.15 known, of which *N*-[1-(*R, S*)-carboxy-3-phenyl propyl]-Ala -Ala-Tyr-*p*-aminobenzoate (cFP) is the most studied. The best working model suggests that the carboxyl group of carboxyphenylpropyl is essential for binding to the zinc ion of the catalytic site and the carboxyl group of *p*-aminobenzoate at the other terminus is essential for binding to the catalytic site.

We have previously carried out gas phase dynamics simulations with cFP which suggested that cFP could adopt a hairpin conformation in a hydrophobic environment, allowing both of the carboxylate groups to coordinate to the zinc ion simultaneously. We now present our results from molecular dynamics studies on cFP using high temperature simulations with explicit incorporation of the solvent *N*-methylacetamide (NMA) in the conformational search. NMA was chosen as an enzyme active site model as it combines hydrophobicity with hydrogen bonding ability.

Our results show that explicit representation of a hydrophobic solvent such as NMA significantly increases the number of hairpin minimum-energy conformations from 1% to 20% when compared with gas phase simulations. No hairpin conformations were found when the simulations were repeated in water. However, the presence of a coordinating metal ion promotes formation of the hairpin conformation almost to the exclusion of all other possible conformations, even in water, which lends further support to the suggestion that cFP coordinates to the zinc ion in the active site of EP24.15 through both carboxyl groups.

OLIGOMERIZATION STATES OF TRANSMEMBRANE α-HELICES STUDIED USING PEPTIDE MODELS

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Transmembrane (TM) domains of integral membrane proteins generally consist of one or more *ca.* 20-residue hydrophobic segments which span the lipid bilayer as α-helices. In dimeric and multi-spanning membrane proteins, helix-helix packing occurs via tertiary contacts of TM α-helices driven primarily by van der Waals "knobs-into-holes" interactions. Our working hypothesis is that individual TM segments act as autonomous folding domains which contain specific arrays of side chains that serve as recognition elements for such packing. Since only a handful of membrane proteins have been crystallized, we have approached this question through synthesis using Fmoc chemistry of peptides of prototypic structure KKK-(*TM*)-KKK. This design overcomes the inherent insolubility of TM segments, thereby allowing facile peptide purification and characterization. Sequences selected for synthesis are identified using *TM Finder*, a program developed in our laboratory which uses experimentally-derived dual requirements for both helicity and hydrophobicity[1] to identify putative TM segments (http://www.bioinformatics-canada.org/TM/). To demonstrate the effectiveness of this approach, TM segments of several proteins previously shown to oligomerize were synthesized within this construct. Circular dichroism spectroscopy confirmed that the peptides inserted into the membrane-mimetic environment of micelles in an α-helical conformation. Furthermore, we found that peptides retained their expected oligomerization states as shown by SDS-PAGE analysis, *e.g.*, the glycophorin A-derived TM peptide KKK-ITLIIFGVMAGVIGTILLISYGI-KKK migrated as a dimer[2] on SDS-PAGE. The overall results indicate that Lys residues do not mitigate against the membrane-interactive properties of the segments, while inherent interhelical packing properties of the segments are retained. This method may find general utility for studies on TM segment oligomerization states.

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MOLECULAR ANATOMY OF BLOOD

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We have developed an in vivo selection system in which phage capable of selective homing to different tissues are recovered from a phage display peptide library following intravenous administration. Using this strategy, we have isolated several organ and tumor-homing peptides. We have shown that each of those peptides bind to different receptors that are selectively expressed on the vasculature of the target tissue. The tumor-homing peptides bind to receptors that are upregulated in tumor angiogenic vasculature. Targeted delivery of doxorubicin or proapoptotic peptides to angiogenic vasculature using these peptides in animal models decreased toxicity and increased the therapeutic efficacy. Vascular targeting may facilitate the development of other treatment strategies that rely on inhibition of angiogenesis and lead to advances in cancer treatment. Our technology is also likely to extend the potential for targeting of drugs, genes, and radionuclides in the context of many diseases.

CASPASES AND THEIR ROLES IN APOPTOSIS

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The discovery that caspase-1, formerly known as interleukin-1β converting enzyme (ICE), is related to the product of the *Caenorhabditis elegans* death gene CED-3 has led to intense interest in the role of proteases in mammalian apoptosis. It is now clear that members of the caspase family of cysteine proteases, which at present includes eleven homologues of human origin, play essential roles in both apoptosis and inflammation. In apoptosis, these enzymes participate in a cascade that is triggered in response to proapoptotic signals and culminates in cleavage of a discreet set of proteins, resulting in disassembly of the cell. The rapid advances that have been made in unraveling the biological roles of caspases, together with an intimate knowledge of their structure, mechanism and specificities, have facilitated efforts to rationally manipulate apoptosis for therapeutic gain.

The antimicrobial peptides derived from chromogranins and proenkephalin-A

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INSERM 338 "Biologie de la Communication Cellulaire" Strasbourg France

Antibacterial and antifungal activities have recently been associated with the soluble matrix of bovine granules and detected in the soluble material secreted from stimulated chromaffin cells. In secretory granules these peptides are generated from cleavage of chromogranins (CGA, CGB), proenkephalin-A (PEA) and are released together with catecholamines into the circulation (1). Several natural antimicrobial peptides have been identified and we focused on predominantly released peptides located at the very conserved N- and C-terminal domains of the precursors : vasostatin-1 (CGA1-76) (2, 3), pro-chromacin (CGA79-431) (2), secretolytin (CGB614-626) (4, 5) and enkelytin (PEA209-237) (6, 7). Structural analysis have clearly shown that for some peptides posttranslational modifications (O-glycosylation and phosphorylation) were essential for the antimicrobial activity (2, 6, 7). Recently, we reported that vasostatin-1, a multifunctional peptide that exhibits vasoinhibitory activity of isolated blood vessels, inhibition of parathyroid hormone secretion, modulation of cell adhesion and neurotoxicity in neuronal/microglial cell co-cultures is able to kill filamentous fungi and yeast (3). The predominant presence of vasostatin-1 in chromaffin granules, in numerous neuroendocrine tissues and its release from sympathetic nerves suggests an important biological function for this peptide. Furthermore, the presence of chromogranins and proenkephalin- derived peptides in various biological fluids implicated in defence mechanisms (cerebrospinal fluid, synovial fluid, milk) and in immune cells secretions (3, 7) is a striking finding. Thus, the characterization of new non-toxic antimicrobial peptides derived from naturally processed precursors is a topic of growing interest in relation to their therapeutic use. The structural features necessary for the activities of these new antibacterial and antifungal peptides are discussed in relation with the NMR analysis.

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NEW PEPTIDES FROM ACTINOMYCETES FOR PLANT PROTECTION

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During the course of screening program for new insecticidal and antifungal antibiotics using different test organisms, *Streptomycete* strains were found to produce a new original antibiotics - peptides in its culture broth. New *Streptomycetes* strains were isolated from soil samples, collected in different regions (Armenia, France, India). New biopreparations **Indocid, Octeberin, Chrizomal** on the base of these strains were prepared. The bioactive products from **Octeberin** *(Str. octemberanum var. nov.)* were chromatographed on Al_2O_3 column. Silica gel (_ 5/40 μ) preparative TLC, gel filtration on Sephadex G-25 and HELC gave biologically active compounds A and B. Compound A was found to demonstrate high contact activity against test - insects and to be low molecular weight protease (trypsin, chymotrypsin, papain, kallikrein) inhibitor (M.w. 1400). Molecular weight of component A was determine using gel filtration on Sephadex G-25. To determine trypsin inhibitor specifically N_α-benzoil-DL-arginine-p-nitroanilide and casein were used. Acidhydrolysis of component A in 6N hydrochloric acid at 110°C for 24 hours gave some products which were found to be treonin, prolin, glycin, alanin, valin, tirosin, phenylalanin. These data were obtained using Automatic amino acid analysis of the hydrolysate, thin layer chromatography, ion exchange chromatography on "Fixion" and infrared spectra. UV-spectrum of component A showed maximum at 280 nm. Main biologically active components of **Indocid** and **Chrizomal** were found to be original polypeptids.

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Pasteur Auditorium - Bioactive Peptides

IDENTIFICATION OF BIOLOGICALLY ACTIVE PEPTIDES IN LAMININ ALPHA CHAINS

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Lamininns are a family of extracellular matrix proteins with at least 12 different isoforms. They are hetertrimeric proteins consisting of genetically distinct α, β, and γ chains, and have diverse biological activities including promotion of cell adhesion, migration, neurite outgrowth, angiogenesis, tumor metastasis, and collagenase IV production. So far, five different α chains were identified. Our goal is to understand the biological role of laminin in normal development and disease processes. Our approach has been to localize cell adhesion sites on laminin using synthetic peptides. We hypothesize that there should be diverse active sites on laminin α chains that mediate various cellular processes. We have identified about 10 active sequences from the laminin α1 and α5 chains using a systematic screening for cell binding sites with 226 overlapping synthetic peptides, which covered the laminin α1 and α5 chains G-domain. Three peptides (AG-10, AG-32, and AG-73) from the laminin α1 chain showed cell attachment activities with cell-type specificities. Cell spreading on AG-10 and AG-32 was inhibited by β1 and α6 integrin antibodies. In contrast, cell adhesion and spreading on peptide AG-73 was not inhibited by integrin antibodies. The minimum active sequences of AG-10, AG-32 and AG-73 were determined to be SIYITRF, IAFQRN, and LQVQLSIR, respectively. AG-73 was found to cause metastases of B16-F10 mouse melanoma cells to the liver and to enhance lung colonization in mice. *In vitro*, the AG-73 peptide enhanced tumor cell adhesion, migration, invasion, and gelatinase production, and blocked laminin mediated cell migration. Additionally AG-73 promoted neurite outgrowth. Moreover, this peptide inhibited laminin mediated acinar-like development of a human submandibular salivary gland cell line. These sequences on laminin may be important biologically active sites. These active peptides may be useful for the study of the molecular mechanism of laminin-receptor interactions and laminin-mediated cellular signaling pathways. They are also useful for development of therapeutic reagents for tumor metastasis and angiogenesis.

OC 58

CALCITONIN DERIVED CARRIER PEPTIDES ARE NOVEL TOOLS FOR SELECTIVE DRUG DELIVERY

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Biopolymers, such as peptides, proteins, oligonucleotides or plasmid DNA, offer great potential for the treatment of human diseases. However, their therapeutic use is often hampered by poor cellular uptake and membrane permeation. Delivery concepts to overcome such problems are frequently limited by a narrow range of applicability, low efficiency, antigenicity, or even cytotoxicity. Improved drug delivery across biological membranes, particularly epithelia and endothelia, has thus been a long-term aim of both companies and scientists. As a novel approach for controlled cellular drug delivery into and across biological barriers, we invented the use of bioconjugates composed of a membrane-translocating peptide sequence derived from human calcitonin (hCT) as a carrier peptide[1]. Recently we demonstrated that hCT and its C-terminal fragment hCT (9-32) are internalized by the nasal epithelium and various cell lines via an endocytotic pathway[1]. The internalization of hCT appears to be mediated by supramolecular self-assembly of hCT in the lipid membrane, induced by intermolecular β-sheets. To enlighten the underlying principles of uptake, we are now performed a systematic study of the minimally required amino acid sequence by (i) truncations, and (ii) a systematic variation of the amino acid sequence of hCT. Based on the results of both uptake studies and structural analysis of a series of hCT-derived peptides, we investigate the translocation of the derived carrier sequences in order to elucidate the essential requirements for internalization.[1] *Schmidt, M. C., et al. Translocation of human calcitonin in respiratory nasal epithelium is associated with self-assembly in lipid membrane. Biochemistry 1998, 37, 16582-16590.*

OC 59

Inhibition of the ubiquitin-proteasome pathway in Alzheimer Disease

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Alzheimer disease is the most common cause of dementia in the elderly. While several genetic defects have been identified in patients with a family history of this disease, the majority of cases involve individuals with no known genetic predisposition. A mutant form of ubiquitin, termed Ub^{+1}, has been selectively observed in the brains of Alzheimer patients, including those with nonfamilial Alzheimer disease, but it has been unclear why Ub^{+1}, expression should be deleterious. Here we show that Ub^{+1}, is an efficient substrate for polyubiquitination *in vitro* and in transfected human cells. The resulting polyubiquitin chains are refractory to disassembly by deubiquitinating enzymes, and potently inhibit the degradation of a polyubiquitinated substrate by purified 26S proteasomes. Thus, expression of Ub^{+1}, in ageing brain could result in dominant negative inhibition of the ubiquitin-proteasome pathway, leading to neuropathologic consequences.

Lectures and Oral Communications

OC 60 Einstein Auditorium - Peptides Conjugates - Ligation OC 61

DIASTEREOSELECTIVE SYNTHESIS OF α-HYDROXYESTERS A ROUTE TO A NEW CLASS OF *C*-LINKED GLYCOPEPTIDES

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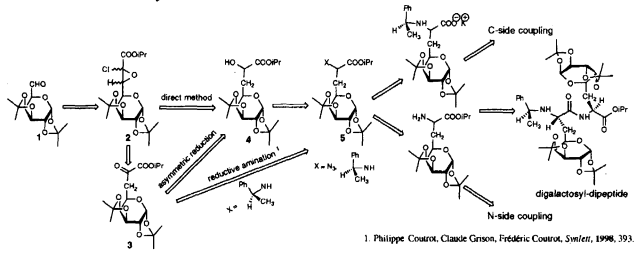
In many natural glycoproteins, carbohydrate moieties are bound to the peptide by a *N*- or *O*-glycosidic linkage *via* a serine, a threonine or an asparagine residue. As a result, many chemists are interested in the synthesis of *N*- or *O*-glycopeptides, which are analogs of natural products. However, glycopeptides having such a glycosidic bond are unstable towards base, acid, or enzymes. It follows that the domain of *C*-glycopeptides has increased during the last years especially because these compounds constitute the nearest analogues of *N*- and *O*-glycopeptides with a more resistant C-C anomeric bond. Other glycosyl peptides linked with a C-C bond in another sugar position have been described. All these unnatural products have a great biological interest, especially because glucidic moiety prevents the peptide against enzymatic degradation and induces a conformational restraint on the peptide.

The reported communication is relative to the study of an original synthetic route to new *C*-galactosyl-α-aminoacid chiron, where the α chiral carbone of the aminoacid is directely linked to the carbone 6 of an hexose.



1. Philippe Coutrot, Claude Grison, Frédéric Coutrot, *Synlett*, 1998, 393.

The preparation of optically pure chiron **5** is based on the asymmetric synthesis of α-hydroxyester **4** by diastereoselective reduction of α-ketoester **3** or directly from glycidic ester **2**. The chemoselective deprotections of the *N*- and *C*- extremities of this aminoacid chiron, followed by the study of its coupling, at *N*- or *C*-end of a peptidic chain are also reported.

PREPARATION AND BIOLOGICAL CHARACTERISTICS OF PEPTIDE-LIPOSOME CONJUGATES FOR DRUG TARGETING

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We have shown previously that immunoliposomes composed of 85-nm liposomes sterically stabilized with 2-kDa polyethylene glycol, which are linked to a monoclonal antibody (directed to the rat transferrin receptor) and loaded with drugs such as digoxin, daunomycin or hypericin, can be used successfully to target the blood-brain barrier (Huwyler et al, Proc. Natl. Acad. Sci. USA, 1996, 93: 14164; J. Pharmacol. Exp. Ther. 1997, 282: 1541; Mol. Pharmacology, submitted). In the present study, we have extended this drug delivery system to peptide receptor-mediated drug targeting. To this end, we have synthesized thiolated peptides, such as a Cys-containing fragment of [Nle^4, D-Phe^7]-α-MSH, and we have prepared maleimide-containing PEGylated liposomes of defined size which contain doxorubicin as anticancer drug. Different routes of formation of drug-loaded peptide-liposome conjugates were studied, either by using unloaded or preloaded liposomes to attach the peptide or by preparing PEGylated phospholipid-peptide monomers followed by formation/loading of the liposomes. Biological testing in vitro of the MSH-liposome conjugates showed that this drug delivery system binds specifically to melanocortin-1 receptors on melanoma cells and that it induces a marked intracellular signal and hence melanogenic response. Apparently, the liposomes are internalized and they induce a strong inhibition of tumour cell proliferation. These successful in vitro experiments are currently being extended to in vivo testing and preliminary data with an animal model will soon be available which will either confirm or dismiss the clinical perspectives of this tool of drug targeting.

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DESIGN AND SYNTHESIS OF AMINO ACID- OR PEPTIDE-MODIFIED RUTHENIUM TRIS(BIPYRIDINE) COMPLEXES FOR PHOTORESPONSIVE MOLECULAR DEVICES

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Recent development in laser technology has made it possible to react molecules in small domains. In such circumstances, photoresponsive molecules possessing attributes such as molecular recognition potential and catalytic activity are desired. One promising strategy is to functionalize an inherently photoreactive molecule. Ruthenium tris(bipyridine) complexes which can efficiently sensitize photochemical electron transfer (ET) processes are an excellent candidates for such functionalization. We specifically designed and synthesized ruthenium complexes of 2,2'-bipyridine, possessing L-amino acids or peptide residues at the 4,4'-positions. We further investigated the effect of modified peptides on the ET rates by using these ruthenium complexes with various peptides. The distance dependency of the ET rates was found to be smaller for oligo-phenylalanine complexes than oligo-leucine complexes. It implies that long-distant electron transfer is possible through peptides possessing π-electron systems in the side chains.

Furthermore, the structure of the ruthenium complexes were examined. The chiral ligands are expected to endow the ruthenium complexes with chiral recognition or asymmetric catalysis properties. However, the chiral auxiliary in the ligands makes the whole molecular chirality more complicated, as ruthenium tris(bipyridine) is inherent chirality at the metal center (Δ and Λ). We solved this issue by separating diastereomers with chiral HPLC. Each diastereomer, L-Δ and L-Λ, has different stability and characteristics. Thus, the circular dichroism (CD) spectra of this diastereomer pair are appreciably different from each other at shorter wavelengths ($\lambda <$ 300 nm), giving *non-mirror image* patterns, but are almost mirror-images at $\lambda >$ 300 nm. The molecular mechanics calculations for the leucine complex indicated that the L-Λ form is more stable than the L-Δ. Further conformational examinations revealed that the L-Λ is most stabilized when the carbonyl oxygen of the bipyridinecarboxamide is oriented outward, while the alternative inward orientation is less stable. The knowledge obtained in this study should lead us to the design of more sophisticated photoresponsive molecules, mimicking the biological electron transfer in proteins.

SYNTHESIS AND CHARACTERIZATION OF A 64 AA CARBOPROTEIN BY CHEMOSELECTIVE LIGATION

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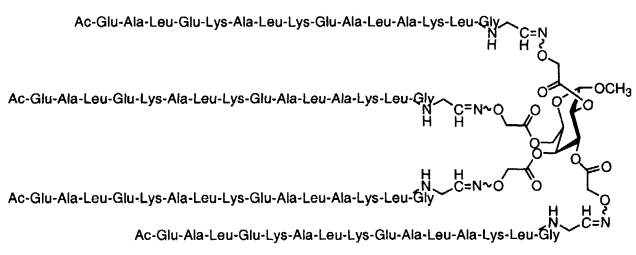
Carbopeptides are a novel class of chimeric compounds in which peptides are assembled on a carbohydrate template [1,2]. In a previous report we described the chemoselective ligation of a *C*-terminal heptapeptide aldehyde to a tetra-aminooxy functionalized α-D-galactopyranoside template to form a 28 AA model carbopeptide. Here we report on a significant extension of this strategy to the synthesis of a 64 AA *carboprotein* by reaction of four copies of an amphiphilic *C*-terminal hexadecapeptide aldehyde, prepared by a BAL handle strategy, to a tetra-functionalized α-D-galactopyranoside template to form a potential 4-α-helix bundle. Furthermore, we have studied the synthesis of a hydrazino functionalized template and its use in chemoselective ligations. Studies of the carboprotein and model compounds by CD and NMR spectroscopy are in progress.



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INTERACTION OF RANTES WITH GLYCOSAMINOGLYCANS AND CCR5, RECEPTORS FOR HIV-1 ENTRY.

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Chemokine receptor CCR5 is an essential co-receptor for the cellular entry of most primary HIV-1 strains, and the chemokine RANTES (regulated on activation normal T cell expressed and secreted), a natural ligand for this receptor, shows the highest potency in suppressing replication of HIV-1 *in vitro*. Positively charged residues of chemokines have been implicated in binding both CCR5 and glycosaminoglycans (GAG), such as heparin and chondroitin sulfates, present at the surface of different cells. Interestingly, interaction with GAG has been reported to significantly influence receptor binding properties and antiviral potency (and in some situations even promote infection) of native RANTES or more potent synthetic derivatives (e.g. AOP-RANTES). By using an optimized protocol of Fmoc-chemistry, in conjunction with the use of specific orthogonal protecting groups, we synthesized a number of RANTES derivatives, acetylated at every basic residue, with a single, double and triple modification. The effect of chemical modification of relevant basic residues of RANTES on GAG binding has been then evaluated by surface plasmon resonance technology, CCR5 binding by competition assays and activation by aequorin luminescence measurements. Obtained results allowed to map the sites responsible for CCR5 binding and activation on overlapping RANTES regions, while the GAG binding sites were identified on structural regions opposite to receptor binding. Interestingly, a RANTES derivative completely devoid of GAG affinity, showed unchanged receptor affinity and antiviral potency. Our results suggest that it is possible to discriminate between GAG and receptor affinity, and produce new RANTES analogs, able to suppress infection by primary viral isolates (mostly, CCR5-using), without the enhancement of T-tropic HIV-1 strains replication, a main limitation for therapeutic usage of chemokine derivatives.