P C125 - Synthetic mimicry of conformationally defined protein binding sites through nonlinear and scaffolded peptides and peptide libraries

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Functional and binding sites of proteins are often not localized in short, continuous stretches of the amino acid sequence, but rather in sequentially distant fragments of the molecule, which are brought into spatial proximity by protein folding. Synthetic molecules aimed at mimicking such discontinuous, conformationally defined protein binding sites should therefore also be conformationally constrained and/or structurally discontinuous. The synthetic basis of this concept are nonlinear and scaffolded peptides and peptide combinatorial libraries, which, due to their molecular architecture, are more likely than linear, consecutive amino acid sequences, to adopt structures capable of mimicking discontinuous protein binding sites (Fig.1).
In an earlier study [1], a combinatorial library of scaffolded peptides, in which short peptide fragments were presented through a bicyclic scaffold in a discontinuous, nonlinear fashion, was generated and used for the de novo design of synthetic peptide receptors. We are now presenting a range of non-linear peptides, which have been designed based on the structure of the conformationally defined bindings sites of protein interaction domains (i.e., the WW and EVH1 domains), whose structures are well understood [2.3]. These peptides were found to mimic the binding specificities of the domain binding sites for their respective proline-rich, protein-derived, linear peptide ligands. Such studies exemplify the successful synthetic mimicry of protein-protein interactions through peptide-peptide interactions.

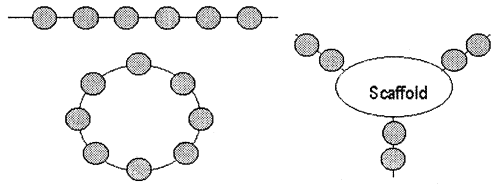

Fig. 1 - Linear and cyclic (left) vs. scaffolded (right) peptides, in which fragments are presented in a non-linear, discontinuous fashion.

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P C126 - A peptide template as an allosteric supramolecular catalyst for the cleavage of phosphate esters

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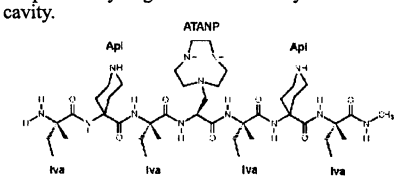
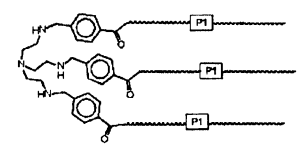
The heptapeptide methylamide H-Iva-Api-Iva-ATANP-Iva-Api-Iva-NHCH$_3$ (P1), where Iva is (S)-isovaline, Api is 4-amino-4-carboxypiperidine and ATANP is (S)-2-amino-3-[1-(1,4,7-triazacyclononane)]propanoic acid, was synthesized by solution methods. On the basis of a CD-NMR study it turns out that its conformation in aqueous solution is essentially that of a 3_{10}-helix. By connecting three copies of P1 to a tris(2-aminoethyl)amine (Tren) platform (T) via an aromatic spacer, a new peptide template [T(P1)3] was obtained. This molecule is able to bind up to four metal ions (Cu^{II} or Zn^{II}): one in the Tren subsite and three in the azacyclononane subunits. Binding of the metals to the Tren platform induces a change from an open to a closed conformation in which the three short, helical peptides are parallelely aligned with the azacyclononane units pointing inward within the pseudo-cavity.



P1



$T(P1)_3$

The T(P1)3 template shows a peculiar behaviour in the transphosphorylation of phosphate esters: the tetrazinc complex is a catalyst for the cleavage of 2-hydroxypropyl-p-nitrophenyl phosphate (HPNP), while the free ligand is a catalyst for the cleavage of an oligomeric RNA sequence with selectivity for pyrimidine bases. In the case of HPNP Zn^{II} acts as a positive allosteric effector by enhancing the catalytic efficiency of the system. In the case of the polyanionic substrate Zn^{II} switches off the activity, thus behaving as a negative allosteric regulator. We suggest that the opposite behaviour of the catalyst induced by Zn^{II} is associated with the conformational change of the Tren platform and, consequently, with a modification of the relative spatial disposition of the three linked peptides, that occurs upon binding of the metal ion.

P C127 - Probing structural requirements of fMLP receptor: an amphiphilic residue at position 1 of the tripeptide

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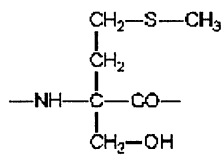
Human neutrophils, the major subset of circulating leukocytes, are phagocytic cells specialized in host defence against pathogens. They also represent the major cell type associated with acute inflammatory reactions. Various inflammatory mediators, including the N-formylmethionyl tripeptides derived from newly synthesized bacterial proteins, are capable of activating neutrophils *via* specific receptors. As for the prototypical tripeptide fMLP or its active methyl ester derivative (fMLP-OMe), it has been shown that, in particular, the Met residue at position 1 is optimal for binding to and for activation of the receptor, because of the side-chain, electron-rich sulphur atom that allows the peptide to interact with a restricted positive arm on the receptor.



HmMet

In this work we replaced the Met[1] residue of fMLP(OMe) with either L(S)- or D(R)-C^{α}-hydroxymethyl methionine (HmMet) [1]. This novel, amphiphilic α-amino acid belongs to the family of $C^{\alpha,\alpha}$-dialkylated glycines, which are useful replacements for protein amino acids in that they exhibit strong secondary structure-promoting effects and induce increased proteolytic stability in the resulting peptidomimetics.
The two diastereomeric f-L-HmMet-L-Leu-L-Phe-OMe and f-D-HmMet-L-Leu-L-Phe-OMe tripeptides were synthesized by solution methods and fully characterized. Their solution conformational preferences were assessed by use of the IR absorption and ^{1}H NMR techniques. Our biological tests highlight that this type of substitution does facilitate good chemotaxis, but only for the L-HmMet diastereomer. The role played by chirality, C^{α}-tetrasubstitution and amphiphilicity of the residue at position 1 of the fMLP(OMe) tripeptide chemoattractant will be discussed in comparison with the published results on other analogues in the same position.

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P C128 - Conformational preferences of peptides containing reverse-turn mimetic γ-lactams

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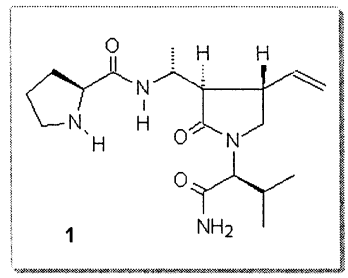
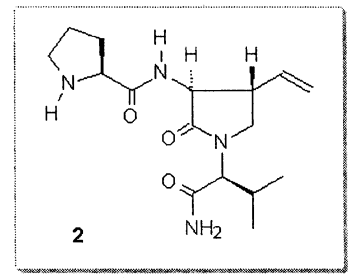
As a part of a research program directed towards the synthesis of conformationally restricted oligopeptides containing a γ-lactam moiety realised exploiting methods well suited in our laboratory, we devised to prepare compounds such as 1 and 2 in the enantiomerically pure form. Our experimental results were complemented by molecular modeling studies for a deeper insight into the conformational preferences of these compounds. In particular, we investigated their ability to mimic some properties of the corresponding natural peptides, such as their capability of forming β and/or γ-turns.





The conformational space was explored through Monte Carlo conformational search and molecular mechanics energy calculations of optimised structures were performed by using Amber all-atoms force field (AMBER*). These computational studies were carried out *in vacuo*, in chloroform and water by using the implicit GB/SA solvation model. The results were compared and an increase of the reverse turn mimic capability of the two peptidomimetics in $CHCl_3$ arised with respect to polar solvents such as water. In the latter solvent the conformers forming inverse-γ turns and β II' turns are far above the global minimum. This behaviour was ascribed to the preferred *inter*-molecular H bond formation instead of *intra*- in polar media. Moreover the behaviour of these systems *in vacuo* is very similar with that observed in chloroform since there are a number of low energy reverse-turn conformers.

P C129 - Potent cyclic angiotensin II analogues confirm the ring cluster conformation: implications in the design of AT1 non-peptide antagonists

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Two Angiotensin II cyclic analogues: γ, ε – cyclo(3, 5) –[Sar1-Glu3-Lys5] ANG II and γ, ε – cyclo(3, 5) –[Sar1-Glu3-Lys5-Ile8] ANG II have been designed, synthesized and bioassayed in anesthetized rabbits in order to further refine structural ring cluster characteristics, important in receptor activation. Design was based on previous SAR studies, which show that Val3, Ile5 are not important for biological activity. The constrained cyclic analogues with a lactam amide bridge linking a Glu-Lys pair or the reverse Lys-Glu pair at positions 3 and 5 and Phe or Ile at position 8, were synthesized by solution procedure using the maximum protection strategy. Analogue 1 with Phe at position 8 was found to be a potent agonist while analogue 2 with Ile at position 8 was found to be an inhibitor of Angiotensin II. It appears that the aromatic ring cluster (Tyr-His-Phe) in agonist peptides is an essential stereo-electronic feature for Angiotensin II to exert its biological activity. A three-residue ring in the central X3-Tyr4-Ψ5 (X=Glu, Lys, Ψ=Lys, Glu) core, allows this cluster to exert its receptor action. Based on NMR spectroscopy coupled with computational analysis comparisons between ring cluster and AT1 antagonists, we have designed and synthesized by novel methods a non-peptide mimetic, 5-Methyl-2-hydroxymethyl-1-[[2'-[(N-triphenylmethyl)-tetrazol-5-yl] biphenyl-4-yl] methyl]-imidazole. This mimetic was found to be an active inhibitor of Angiotensin II induced hypertension when tested in the rat uterus assay and in anesthetized rabbits.

P C130 - Enantiopure N,N'-linked oligoureas as foldamers

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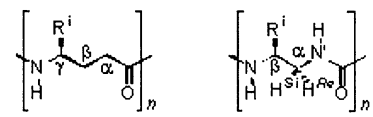
The functional diversity in proteins, although mediated by sophisticated tertiary and quaternary structures, relies on a small set of distinct secondary structural elements: i) sheets, ii) helices, and iii) turns. Unnatural oligomeric scaffolds, i.e. foldamers,[1,2] designed to reproduce or mimic these essential protein features, while retaining the chemical diversity of amino acid side chains have gained considerable interest in recent years. Work published by *Seebach*, *Gellman* and *Hanessian* has revealed that short chain peptides consisting exclusively of enantiopure β- or γ-amino acids with defined substitution patterns can form stable helical or pleated-sheet-type structures in solution and in the solid state. In particular, γ-peptides such as **A**, with L-amino acid-derived chirality centers form a right-handed (*P*)2.6_{14} helix of ca. 5 Å pitch with both ethane bonds in a (+)-*synclinal* conformation.[3,4] Enantiopure *N,N'*-linked oligoureas of general formula **B**, obtained by formal replacement of the C^{α} of γ-amino acid residues in **A** by a nitrogen atom have been originally described in 1995 by *Burgess* and coworkers and used as novel peptide backbone mimetics.[5] Although *N,N'*-linked oligoureas are readily accessible by solid-phase synthesis approaches using appropriate monomers, [5,6] their conformational preferences and their folding propensities have not been elucidated so far. We found that linear *N,N'*-linked oligoureas of general formula **B** adopt a stable 2.5 helical secondary structure in solution that is characterized by a pitch of approximately 5.1 Å pitch and by the simultaneous presence of 12- and 14-membered hydrogen bonded rings. [7] The structure is closely related to the (*P*)2.6_{14} helix of ca. 5 Å pitch of γ-peptides, [3] The knowledge of this three-dimensional structure is likely to be useful for the *de novo* design of oligoureas with controlled shape and defined biological activities.



A **B**

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P C131 - *De novo* design of artificial peroxidases

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We designed and synthesized single-chained 49-mer-polypeptides containing one histidine at position 19 and another at position 40-46 (βαβα(His-40) to βαβα(His-46), respectively) applying the amphiphilic α-helix and β-strand (baba) motifs. (Fig.) The iron(III)-5-(4-carboxyphenyl)-10,15,20-tritolylporphyrin was chosen to be connected on the side chain of ornithine residue at the hydrophobic side of the first β-segment. Two histidine residues were also placed at the hydrophobic faces of two α-helix segments to coordinate to the heme iron. [1]
Peptide synthesis was carried out by solid-phase-synthesis and stepwise segment elongation method in Boc chemistry. An oxidase sensitive dye, 10-N-methylcarbamoyl-3, 7-dimethyl-amino- 10H-phenothiazine (MCDP) was employed as a reductant to detect the rates of the consumption of peroxides by these peptides. Cumene hydroperoxide (CHP) and H_2O_2 were used as oxidants. The rate of MCDP oxidation was obtained by monitoring the increase in absorbance at 666 nm corresponding to the absorption of the generated methylene blue molecule (ε at 666 nm = 9.6×10^4 $M^{-1}cm^{-1}$). With increasing concentrations of oxidants, activities of these peptides were measured in aqueous solution (pH 7.2) at 25°C to determine the Michaelis-Menten parameters. Peptides βαβα(His-40), βαβα(His-42), and (βαβα(His-44), having their histidines in hydrophobic region of amphiphilic βαβα motifs, showed rather high MCDP oxidation activity in the presence of CHP.

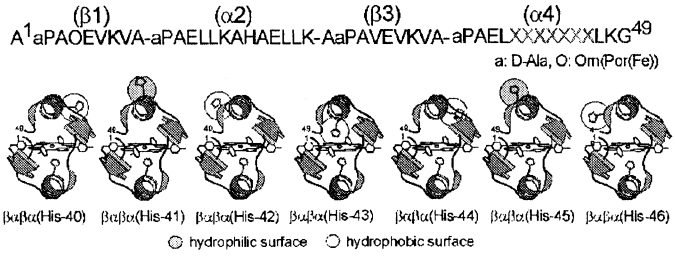

Fig.1 - His positions in possible baba-structure as designed. His may coordinate to Fe(III) with perturbation of the fourth a-helix segment.

P C132 - Novel peptide nucleic acids that contain pyrrolidine rings of various streoisomers

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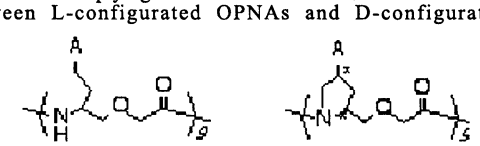
In our previous work, we have shown that an oligopeptide based on ether linkage in the main chain with side chain nucleo bases (L-OPNA). The most notable advantage of the L-OPNA is the very sharp melting curve of the L-OPNA-DNA hybrids and is its improved solubility, particularly for the purine-rich sequences. In this communication, we reported optimum side chain conformation of a novel OPNA that contain 4-adeninylprolinol units (Scheme 1). By restricting the side chain rotations and partly the main chain conformation, we will have information on the conformational requirements to achive stable hybridization with the complementary DNAs. Adenine nine-homooligomers of OPNAs (**o(A9)**)with four kinds of structural isomer (*cis*-L-, *cis*-D-, *trans*-L, and *trans*-D configurated ; **cL-o(A9)**, **cD-o(A9)**, **tL-o(A9)**, and **tD-o(A9)**, respectivery) were synthesized and hybridization of all complementary OPNA-DNA hybrids was investigated from the melting curves of UV absorption. The melting temperatures (*Tm*) were 30 °C for **tD-o(A9)-d(T9)**, 29 °C for **cD-o(A9)-d(T9)**, 23°C for **tL-o(A9)-d(T9)**, 34°C for **cL-o(A9)-d(T9)** and about 15 °C for the complementary oligonucleotide (**d(A9)-d(T9)**) hybrid, respectively, that is, the stability of **o(A9)-d(T9)** hybrids is as follows: **cL-o(A9)** > **tD-o(A9)** = **cD-o(A9)** > **tL-o(A9)**. Every **o(A9)-d(T9)** hybrid showed a very sharp transition, suggesting that the ether linkage in the main chain is responsible for the sharp melting curve. Presumably, the flexible poly(etheramide) main chain causes a large entropy loss and a large enthalpy stabilization when it forms duplex with DNA, and results in the sharp melting curves. Even if the side-chain rotations are restricterd by a pyrrolidine ring, the main chain of OPNA was retained flexibility. The sharp melting curve is very advantageous for the OPNA when they are applied as antisense drugs. L-configurated OPNA-DNA hybrids were different from the *Tm* value (34°C and 23°C), however, D-configurated OPNA-DNA hybrids were similar to the Tm value (30°C and 29°C). We are considered that these facts were implying that the minor differences from mode of binding to DNA between L-configurated OPNAs and D-configurated OPNAs.



Scheme 1

P C133 - Peptide mimics of subunit B of DNA-gyrase in interactions studies with coumarin drugs

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The coumarin antibiotics are potent inhibitors of DNA replication which target is the bacterial enzyme DNA gyrase. Gyrase is an ATP-dependent bacterial type II topoisomerase, able to introduce negative supercoils into covalently closed circular DNA. It is believed that coumarin drugs bind to the B subunit of gyrase and inhibit DNA supercoiling by blocking the ATPase activity. As the nature of the inhibition of these antibacterial agents is still object of many debates, we have synthesized a peptide mimic of subunit B of *Escherichia coli* DNA gyrase (AGYRB) and examined its interaction with novobiocin, DNA and ATP, for better to evaluate this inhibition. The model peptide AGYRB was built using the natural fragments 131-146 (coumarin resistance-determining region) and 753-770 (DNA interaction region), and a residue of -aminocaproic acid to connect them.

Ac-E^{131} L V I Q **R**^{136} E G K I H R Q I Y E^{146} – Z – I^{753} T X D P E S **R** R X L R V T V K D A^{770}-NH_2 (AGYRB)

Fluorescence and novobiocin affinity column were used to study the interaction of the AGYRB, DNA, coumarins and ATP. The fluorescence emission of AGYRB was remarkably quenched when novobiocin, coumermycin A1, DNA or ATP were added, indicating the AGYRB/coumarins, AGYRB/DNA and AGYRB/ATP interactions, partially confirmed by affinity chromatography and binding assays. The novobiocin and the ATP binds to the peptide mimic independently of the DNA presence, but do not at the same time, indicating overlapping (if not identical) binding sites, supporting the idea that coumarins are competitive with ATP, a result previously found with 43-KDa protein fragment of the DNA gyrase. No binding of the DNA with AGBICN (AGYRB analog without the C-terminal fragment 753-770) was found, indicating the importance of this region to the binding of the DNA. On the other hand, binding of the AGYRB to ATP and coumarins was reduced when mutation of Arg-136 to Leu-136 (AGYRBM) was introduced, a change previously found in the subunit B of DNA gyrase from several resistant clinical isolates of *Escherichia coli* (MIC ≥ 160 μg/ml). These results suggest that AGYRB interact with coumarins and ATP, possibly in the identical binding sites and the DNA have different binding site in the AGYRB, as DNA gyrase, evidencing that AGYRB is a good mimetic for subunit B of gyrase

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P C134 - Template-directed ligation enhanced by complementary interaction using nucleobase amino acids

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Complementary interaction plays an important role in natural biosystems. Therein, the formation of complementary hydrogen bonds between nucleobases is a simple and sophisticated system. To apply the complementary nucleobase interaction to the *de novo* peptide design, conjugation of peptides with nucleobases has been attempted using an artificial L-α-amino acid having a nucleobase unit in the side chain (nucleobase amino acid; NBA) [1,2]. The peptide-NBA conjugates will provide novel functional peptides with a tertiary structure accompanied by nucleobase complementarity. To explore the availability of nucleobase interaction in peptide-peptide recognition, we have introduced the nucleobase interaction into a peptide self-replication system. Template-directed peptide fragment ligation, which auto-catalytically produces the template, is based on the hydrophobic recognition generated from the coiled-coil formation [3,4]. The nucleobase pairs were incorporated at g-g' heptad positions in an antiparallel coiled-coil, and the self-replication reactions supported by the nucleobase interaction were examined. The incorporation of complementary nucleobase pairs at g-g' positions raised theÊreaction efficiency compared with that of peptides without nucleobases. It was suggested that the complementary base pairs at g-g' positions were effective for the peptide-peptide recognition. The positional preference of nucleobase pairs for the peptide-peptide recognition has been examined. The replication reaction was accelerated in the peptide system with complementary base pairs at g-g' positions rather than those at e-e' positions, suggesting that the complementary base pairs at g-g' positions in an antiparallel coiled-coil serves as an element for the peptide-peptide recognition. Furthermore, in a peptide ligase system where the peptide product is different from the ligase itself, the complementary nucleobase interaction significantly acted on the recognition of peptide substrates and the enhancement of the condensation reaction.

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P C135 - Design and synthesis of three-α-helix proteins that bind small ligands in a cavity

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Artificial receptor proteins that bind small ligands are attracting targets for designing a molecular sensor or an absorber of toxins. In order to design the artificial receptor proteins, it is necessary to understand what kinds of amino acids are effective for the ligand binding in a created cavity. As an appropriate scaffold for the receptor proteins, we have attempted to construct 3α-helix proteins possessing a cavity in a hydrophobic region [1]. In this study, various amino acids were introduced in the cavity to investigate the effect of amino acid side-chain on the ligand binding. The small library of 3α-proteins was constructed by combining a variety of two different helix segments. In order to estimate the ligand binding and selectivity, 1,8-ANS (1-anilinonaphthalene-8-sulfonic acid) and 2,6-ANS (2-anilinonaphthalene-6-sulfonic acid) were used as small ligands for the proteins.

Fluorescent changes of 1,8-ANS and 2,6-ANS with the proteins indicated that the cavity size of the proteins significantly affected the ligand binding. One of the proteins that has a Phe residue in the bottom of cavity showed selectivity for 2,6-ANS. NMR analyses suggested that the phenyl ring of Phe residue and the aromatic rings of 2,6-ANS were interacted. These results suggested that novel receptor proteins can be created by using 3α-helix proteins with appropriate amino acids in the cavity. Furthermore, another library of immobilized proteins on a solid-support was also constructed for the efficient detection of the ligand binding.

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P C136 - *De novo* design of peptides containing L-α-nucleobase amino acids and their recognition of hairpin RNA

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RNA-protein interaction plays an important role in the organism. In many cases, RNA-binding proteins interact with the specific sites of RNA, such as hairpin loops, internal loops and bulges. Construction of molecules that recognize a structured RNA specifically can supply the information of not only the RNA function in nature but also the drug design against RNA. Therefore, we have attempted the de novo design of peptides having L-α-amino acids with nucleobases (nucleobase amino acids; NBAs) [1], expecting that the nucleobases on the peptides interact specifically with RNA bases. In this study, we designed and synthesized α-helical NBA-peptides (13 AA) and evaluated the binding affinities and specificities to the P22 phage boxB RNA containing a GNRA-like tetra loop.

Binding analyses revealed that the binding affinities of the NBA-peptides were significantly dependent on the type and position of the NBAs. Especially, the peptide having three NBA units bound to the boxB RNA, stronger than the N peptide with the native sequence (17 AA), which is known to recognize the boxB RNA strongly and specifically. These results strongly indicate that the designing strategy using NBA units on the peptide is useful to construct the molecules capable of recognizing hairpin RNAs.

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P C137 - Design synthesis of SS-dimer and SS-hybrids based on Cyl-1 (cyclic tetrapeptide) as anti-cancer prodrugs.

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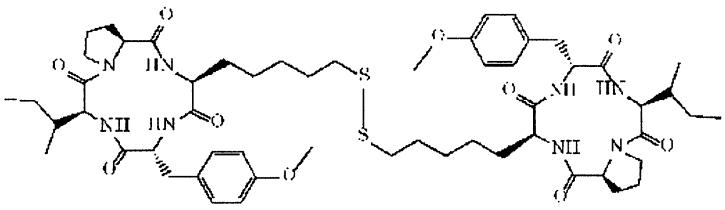
Histone deacetylases (HDACs) are deeply involved in the regulation of the cell cycle. The inhibition of HDAC by natural and antificial molecules causes hyperacetylation of core histones, thereby activating the transcription of genes such as *p21* encoding a CDK inhibitor. We designed cyclic tetrapeptides containing 2-amino-7-mercaptoheptanoic acid instead of L-Aoe ((2S,9S)-2-amino-8-oxo-9,10-epoxydecanoic acid) in Cyl-1. The sulfhydryl group was expected to coordinate to the zinc atom in HDAC to inhibit the hydrolytic activity. The corresponding homodimers (SS-dimer, Figure) and SS-hybrids with smaller mercaptans were also designed and synthesized. They are potent in activating the *p21* promoter suggesting to be promising drug candidates for cancer therapy.



A SS-dimer analog of Cyl-1.

P C138 - Are the β-isomeric peptides stable secondary structures similar to the α-peptide ones?

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Unnatural oligomers that adopt specific, compact conformations have a wide range of potential applications. This kind of oligomer (known as foldamers) with well defined secondary structure preferences (i.e. helices, sheets, turns) could be used to create new types of tertiary structures, useful aim to built macromolecules with functional properties. Short foldamers (less than 10 aa) that display specific conformations may have medicinal applications such as the disruption of protein-protein interactions or antibiotic activity. Several methods have been developed for the synthesis of different unnatural oligomers and a few of them have been shown to adopt well-defined secondary structures in solution. Short β-amino acid oligomers (β-peptides) have recently been shown to exhibit residue-controlled secondary structure and have remarkable stability to proteases. Usually these studies were carried out with β-peptides composed of homologated β-amino acids.
In the present work, we study the secondary structure of different β-peptides composed of isomeric β-amino acids. The goal is to determine if the structural pattern of these peptides is similar to their α-peptide analogues under different experimental conditions, in order to use them as mimetics of α-sequences in the synthesis of new materials. We will discuss the synthesis of isomeric β-peptides and their structural characterization by circular dichroism and NMR.

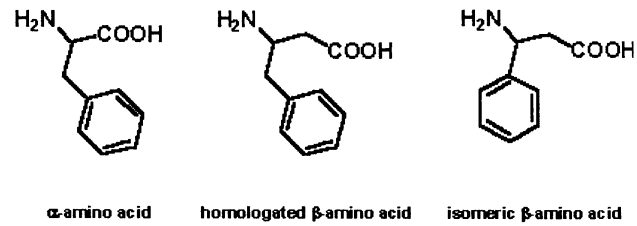

Fig 1.- Amino acid structures

P C139 - A fully automated software strategy for *de novo* sequencing of whole LC-MS/MS datasets

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Large quantities of MSMS spectra can be acquired and database-searched in an automated manner, allowing rapid identification of large numbers of protein samples. A challenge remains, however, in that many of the spectra acquired do not provide matches when searched against known protein sequences. The nature of database searching is such that only peptides that match exactly those within the databank will be identified. Consequently, many good quality spectra of novel peptides or of those containing a single amino acid substitution remain unmatched. Currently the solution to this would be to extract these spectra manually from the data set, derive some degree of peptide sequence and performed further database searching. This, however, can be time consuming. With the introduction of an automated computer sequencing algorithm, MS/MS spectra can be identified by direct derivation of the novel peptide sequence. Here the intelligent batch-submission of a number of MSMS spectra is introduced, such that, upon completion of a database search, all unmatched and poor-scoring spectra are automatically de novo sequenced. The results are integrated in a single Java interface, from which novel peptide sequences can undergo homology searching through submission to BLAST.

P C140 - Dendritic poly(L-lysine)s combining clusters of zinc(II)-porphyrins and methyl viologens for a photoinduced electron transfer system

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We introduced clusters of Zn(II)-porphyrins and methyl viologens to dendritic poly(L-lysine)s in different manners (Figure). The synthesis of (Zn8/MV16)K14 and (Zn8/MV8)K14 were carried out according to previous reports [1, 2]. Both the dendrimers showed about six times more efficient fluorescence quenching in comparison with the unbound porphyrin/methyl viologen system. Furthermore, the purple colored dendrimer solution turned brilliant blue on continuous irradiation of the Soret band of Zn(II)-porphyrin in (Zn8/MV16)K14 and (Zn8/MV8)K14 in the presence of triethanolamine indicating the generation of methyl viologen radical ion.

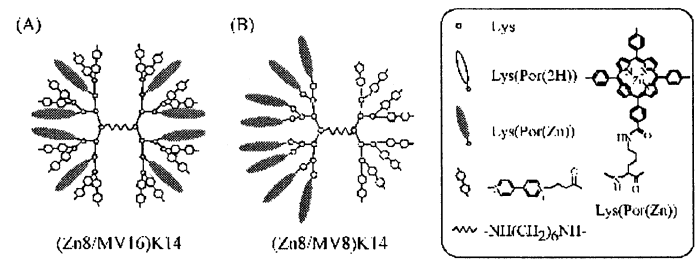

Illustration of dendritic poly(L-lysine)s combining clusters of Zn(II)-porphyrins and methyl viologens in mixed (A) and separated (B) manners.

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P C141 - On the biochemical basis of autism

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There is much evidence to suggest that levels of serotonin are perturbed in autism and related disorders. [1,2] Serotonin is formed in a two-step sequence from tryptophan by the enzymes *tryptophan hydroxylase* (TPH) and *aromatic amino acid decarboxylase* (AAAD).
HPLC analysis of the urine of autistic subjects indicated the presence of an unidentified peptoid component in greatly increased concentrations. Spectroscopic techniques and an independent synthesis were used to identify this peak as corresponding to indolyl-3-acryloylglycine (IAG), [3] presumed to be an aberrant metabolite in the tryptophan pathway.
We have also examined the TPH reaction to determine if it can be inhibited by various exogenous and endogenous factors, which could lead to variation in the levels of serotonin. We found that pesticides, such as lindane, permethrin, diazinon and malathion, had no significant effect upon this enzymic reaction. Several indole-based putative tryptophan metabolites were evaluated for their inhibition and were also found to have no significant inhibitory effect. However, the tryptophan metabolites kynurenate and xanthurenate were found to be inhibitory to TPH. We have preliminary results suggesting a possible link between certain pesticides and increased levels of these tryptophan metabolites.

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P C142 - Components of tissue-specific peptide pools: contribution to regulation of cell number

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Screening of > 100 endogenous fragments of functional proteins (components of tissue-specific peptide pools) isolated from different tissues has demonstrated that 77% of them are active. Among the active peptides 72% suppress cell proliferation, 17% stimulated proliferation and 11% exhibit both effects. I.e., the tissue-specific peptides contribute to the negative regulation of cell number, rather than to growth stimulation. As no reliable ability to affect cell differentiation and adhesion has been shown, we considered that the main function of pool components at cellular level is regulation of cell number. The predominant part (80%) of the inhibitors belong to structural families of β-globin (32-41) fragments (hemorphins) and to β-actin (75-90) and (68-77) segments. Tissue extracts contain also 3-4-membered peptides enriched with acidic amino acid residues, their effects being similar to those of hemoglobin and β-actin fragments. The inhibitors exhibit high antiproliferative effect in tumor cells and are several fold less active in normal cells. They induce reversible arrest of cell proliferation and resistance to further treatment with the same compound. The ability to reduce tumor growth *in vivo* points to the involvement of pool components in antitumor defense of the organism. All proliferative peptides belong to 2 families of α-globin fragments corresponding to (106-141) and (134-141) segments. Proliferative peptides support proliferation of tumor and normal cells in serum deprivation conditions and stimulate cell proliferation in the presence of growth factors. The effects of proliferative peptides are more pronounced at stress conditions (deficiency of growth factors or low cell density). The 3 group is represented by the families of peptides corresponding to (1-32) and (12-25) segments of α-globin. The peptides exhibit both effects in concentration dependent manner. To model the combined effect of pool components we studied the interactions between different peptides *in vitro*. The effects of inhibitory peptides, hemorphins and β-actin fragments in the case of combined application are not additive and lead to reversible cross-resistance to further application. Since VV-hemorphin–5 (valorphin) and β-globin-(137-141) (neokytorphin) are the most abundant representatives of pool components in the tissues, we studied the combined effect of the peptides in tumor cells. The proliferative effect was detected when neokyotorphin concentration was 100-fold higher than that of valorphin. In equal ratio, corresponding to that in the organism at normal conditions, the former peptide induced 2-fold reduction of growth inhibitory effect of valorphin. I.e., the rate of cell proliferation can be directly regulated by the change of ratio of these peptides. We believe that interactions between most abundant peptides reflect the overall effect of pool components in tissue cells. Bearing in mind the domination of growth inhibitors among the pool components, we consider that at normal conditions these peptides control excessive cell proliferation. In the case of deviation from the norm (tissue damage, lack of growth factors, etc.) proliferative peptides could contribute to tissue regeneration.

P C143 - The recognition mechanism in enteroviral $3C^{pro}$/RNA interactions: role of the RNA structure

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3. Institut fur Virologie, Friederich-Schiller Universitaet - Jena - Germany

Picornaviruses are a large family of small RNA viruses including the enterovirus group, a large family containing several human and animal pathogens. The 5'-nontranslated region of enteroviruses contains RNA structural elements which serve in the initiation of cap-independent translation and in the initiation of positive strand RNA synthesis. In enteroviruses, a 5'-terminal cloverleaf(CL)-like secondary structure is essential for virus viability and is part of a ribonucleoprotein complex involving the viral 3CD proteinase [1]. On the ground of an accurate knowledge of the molecular mechanisms involved it is conceivable to develop new antiviral compounds, due to the conservation of this recognition process among different enteroviruses. Here, we investigated the interaction of the 3C proteinase ($3C^{pro}$) component of the 3CD proteinase with the CL RNA from coxsackievirus B3 (CVB3). Using electrophoretic mobility shift assay (EMSA) we demonstrate that recombinant proteolytically active $3C^{pro}$ of CVB3 forms a stable complex with in vitro transcribed CL RNA of poliovirus (PV)1, bovine enterovirus (BEV)1 and human rhinovirus (HRV)2. The subdomain D of each of these cloverleaf RNAs is capped by a four-nucleotide loop (D-loop). EMSAs demonstrate that RNA representing the stemloop D of the CL are sufficient for complex formation with $3C^{pro}$. The apparent dissociation constants of the RNA:$3C^{pro}$ complexes range from 2 to 10 micromolar. The HRV14 CL, which has three nucleotides in the D-loop, does not bind CVB3 $3C^{pro}$. Insertion of a fourth nucleotide renders this RNA into a $3C^{pro}$-binding molecule. The second CL of BEV has a D-loop with five nucleotides. While deletion of a nucleotide in this D-loop does not lead to $3C^{pro}$ binding, a G:C to C:G mutation at nucleotide position 162:184 leads to a stable $3C^{pro}$ binding. These findings suggest that the CL:$3C^{pro}$ interaction is not dependent upon the D-loop sequence, but rather upon the loop conformation and a second site adjacent to an internal symmetric bulge region of stemloop D. Biochemical, Circular Dichroism and NMR data obtained using an in vitro transcribed version of a portion of CVB3 CL containing the D-loop confirmed this finding will be presented.

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P C144 - Evidence for the presence of a secondary binding site between HIV-MN-gp120 PND and the CD4 CDR3 region

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The entry of human immunodeficiency virus (HIV) into cells requires the interaction of the viral exterior envelope glycoprotein, gp120, with the CD4 glycoprotein; a chemokine receptor (CXCR4 or CCR5) on the cell surface is also involved. The CD4 binding site on gp120 molecule is represented by a depression formed at the interface of the three principal parts of the protein named outer domain, inner domain and bridging sheet. Residues in contact are concentrated from 25 to 64 in CD4, but they are spread over six segments in gp120. Several studies about gp120 determinants of coreceptor utilization have shown that many mutations in gp120 alter tropism. The gp120 V3 loop (residues 303-338) has long been recognized to be the primary determinant of viral tropism, and it has now been shown that substitution of just single amino acids within it, may result in a switch in coreceptor utilization. The current interpretation of these data is that an interaction must occur between the V3 region and the different coreceptors. However, to date, no attempt has allowed a clear cut demonstration of a physical interaction between the V3 peptides and the cognate coreceptor. We have previously demonstrated that a synthetic peptide reproducing the PND (Principal Neutralizing Domain, residues 308-330) of the HIV-1-MN strain (named DB3) is able to bind CD4 molecule at the V1/V2 domain site, identified by the monoclonal antibodies MT-151 and B66, and enhances HIV-1 induced syncytia formation and infection in $CD4^+$ target cells. Peptides that were designed from the PND sequences of other viral strains exhibited less or no infection enhancement, and these differences paralleled a different affinity for CD4 binding. Other studies demonstrated that the biological effect was due to both the up-regulation of CD4 and a greater gp120 affinity induced by the peptide. Point-mutation investigations revealed that $Phe^{15} \rightarrow$ Ile, $Tyr^{16} \rightarrow$Ile and $His^{8} \rightarrow$D-His modifications completely abrogate the activity of the peptide; $Lys^{3} \rightarrow$Asn and $Arg^{6} \rightarrow$Asn analogues had a lower enhancing effect whereas $Lys^{19} \rightarrow$ Asn analogue showed the strongest enhancement of HIV-1 infection. In order to add confirmatory evidence to the hypothesis of the existence of a specific interaction between the V3 loop of gp120 and the CD4 antigen and to individuate the exact binding site of DB3 on CD4, we have designed a photo affinity labeling experiment. Photo affinity labeling of biological receptors for ligands, such as peptide or proteins, is a most valuable mean of receptor identification and isolation. In this approach, a chemically stable but photolabile group is conjugated to a potent ligand. Then the receptor-ligand complex is subjected to photolysis to generate highly reactive carbenes or nitrenes that may establish a covalent bond between the receptor and the ligand. Accordingly two DB3 analogues carrying two different photo affinity markers (N_3- or NO_2) in the side-chain of the first residue (Phe) have been synthesized. Incubation of each analogue with a soluble form of CD4 (sCD4), composed by the first four domains of the molecule, and subsequent irradiation, have produced the formation of a DB3-CD4 covalent complex as determined by MALDI mass analysis. Characterization of the covalent complex by enzymatic digestion and fragments analysis has permitted the identification of HIV-1-MN gp120 PND binding site on sCD4 in the 63-77 sequence.

P C145 - Thermal and guanidine hydrochloride-induced denaturation of human cystatin C

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Cystatins are natural protein inhibitors of cysteine proteases, proteolytic enzymes involved in many intracellular and extracellular processes of physiological importance. The inhibitors structurally constitute a single superfamily, which can be divided to three families. Human cystatin C belongs to the family 2 cystatins and is a widely distributed basic protein composed of 120 amino acids.
Wild-type human cystatin C and its variant L68Q are directly involved in pathological fibrils formation leading to haemorrhage, dementia and eventually death of persons suffering from cerebral amyloid angiopathy. We have investigated the thermal and the chemical (guanidine hydrochloride-induced) denaturation of native cystatin C in order to understand its folding/refolding processes and accompanying self-association and aggregation events. Studies performed with tryptophan fluorescence, calorimetry, circular dichroism and Fourier transform infrared spectroscopy revealed that neither chemical nor thermal denaturation processes of cystatin C are simple two-state events. The intermediate form, probably the dimer, existed in both of them. Our studies also demonstrated that the β-structural motif was directly involved in the formation of this intermediate form, what confirmed the results obtained in crystallographic studies.

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P C146 - Angiogenesis: thrombin and thrombin-related proteins in focus

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The ability of thrombin, activated protein C and other coagulation factors to stimulate angiogenesis have been recently described [1-3]. Although these enzymes interact with many proteins and mode of interactions between them is complex, a correct analysis of the inter-domain and protein-protein interactions can provide basis for rational design of the novel interaction-based inhibitors.
Recent data have revealed the involvement at least three distinct pathways of thrombin-induced angiogenesis: thrombin receptor (PAR1), integrin α(v)-β(3) and matrix metalloproteinase 2 (MMP2). To gain insights into molecular features of angiogenesis, we sought to investigate the potential interactions between thrombin and other proteins of the coagulation system, their inhibitors and receptors (PAR1, thrombospondin-1, GP1b α, thrombomodulin). Anti-angiogenic substances, which are derived from several parent proteins by proteolysis (angiostatin, endostatin, tumstatin, heparin-binding fragment of fibronectin, etc.) were also analyzed for searching of the potential interaction sites. Our study based on the wide advances have occurred in the area of the protein's function and structure, which were used for comparison of some functionally related proteins by manual and visual methods. Besides, we report that a few thrombin and PAR1 fragments were synthesized by the solid phase method using Fmoc-strategy. On the basis of these studies, three potentially active sites in the PAR1's amino-terminal domain were suggested for proteolysis by MMP-2, cellular adhesion and binding to "heparin-like" proteins. In one's turn, we speculated that one of the thrombin's angiotensin-like motifs, TALPs [4] might serve as the secondary site of thrombin-PAR1 recognition and an essential factor of the effective cleavage of PAR1 *in vivo*. Because of thrombin's multiple effects on endothelium through the activation of different receptors and intracellular-signaling pathways, this bio-regulator could be considered as an angiogenesis mediator rather than as a simple coagulation factor. The data obtained on the protein's analysis allow us to reveal several structural motifs, which might affect some of the thrombin-induced steps in the angiogenic cascade and the initiation of matrix remodeling.

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P C147 - Cyclic non-RGD analogues: design, synthesis and antithrombotic properties

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The crucial step of the thrombus formation is believed to be the binding of fibrinogen (Fg) to the activated GPIIb/IIIa receptor on the platelets surface [1]. It has been shown that two copies of the Arg-Gly-Asp (RGD) sequence (95-97 and 572-574) of the fibrinogen α chain contribute to the recognition event [2]. Inhibition of the platelets aggregation is achieved in various ways, one of which is based on the RGD sequence through which the binding of Fg to the receptor is taking place. A great effort has been made to synthesize RGD containing peptides and non-peptide small molecules that could exhibit anti-aggregatory properties suitable for using as antithrombotic agents. A large number of conformational studies on RGD analogues have contributed a lot to the design and development of strong inhibitors. In our previous studies [3] we have shown that the (S,S) CDC sequence induces favorable, for the expression of the biological activity, orientation of the side chains of (S,S)XCDCY, where X is a basic amino acid and Y is Arg or -NH_2. In this study we designed, synthesized and studied the antithrombotic activity of several RGD analogues that contain the (S,S) CRC sequence: (S,S)Ac-Arg-Trp-Asp-Cys-Arg-Cys-NH_2, (S,S)Ac-Arg-Ser-Asp-Cys-Arg-Cys-NH_2, (S,S) Ac-Arg-Val-Asp-Cys-Arg-Cys-NH_2. Preliminary studies of these analogues, as well as of analogues which contain the (S,S) CDC sequence, were tested for their ability to inhibit specifically the Fg binding.

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P C148 - Synthesis, structural characterisation and epitope elucidation of the cytoplasmic domain of alzheimer's amyloid precursor protein (APP)

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APP is the precursor protein of amyloid-ß42 peptide, the major component of amyloid plaques that characterize Alzheimer's disease (AD). Cleavage of APP to amyloidogenic Aß involves a complex metabolic pathway mediated by intracellular targeting sequences the cytoplasmic tail, hence knowledge of the cytoplasmic domain of APP is important for understanding amyloid formation amyloid plaque formation in AD at the molecular level. Syntheses of C-terminal APP peptides were carried out on an Abimed EPS 221 synthesizer using the Fmoc strategy with a Novasyn TGA resin. The purified peptides were characterized by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS; Bruker 7T-ApexII) which provided ultrahigh mass resolution and mass determination accuracy (1 ppm). Fragmentation in nanoelectrospray-FT-ICR spectra using collision-induced dissociation (SORI-CID) in the ICR cell yielded fragments to cover almost the entire sequence and to provide detailed structure information. A monoclonal antibody (C-P767/"Jonas" mAb) against C-terminal APP has been shown to be a valuable tool for studying the intracellular metabolism of APP. For the identification of the epitope structure the mass spectrometric epitope excision method by limited proteolytic digestion of the intact antigen-antibody complex was employed which yielded the epitope to within a defined C-terminal oligopeptide sequence (APP737-748). This epitope is presently used in the molecular elucidation of intracellular degradation pathways of intact APP and recombinant APP protein fragments.

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P C149 - Peptide-based generation of subunit-specific antibodies against ion channel receptors

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The strychnine-sensitive glycine receptor (GlyR), a member of the ligand-gated ion channel family, mediates inhibitory synaptic transmission in the mammalian spinal cord and brain stem. Homologous to the other members of the receptor family, GlyR subunits possess a large extracellular N-terminal domain followed by four transmembrane regions (TM 1-4) and a short extracellular C-terminal portion. GlyRs are comprized of five membrane spanning subunits forming a central ion pore delineated by the transmembrane segment TM 2 of each monomer. Opening of the receptor channel follows agonist binding, leading to hyperpolarization of the cell due to influx of chloride ions. Four ligand-binding α-subunits (α1-4) and one structural β-subunit have been identified so far. The developmental regulation of glycine receptor subunit expression in the CNS has only been shown on the mRNA level. On the protein level the available antibody mAb4a is widely used for GlyR analysis, binding to an epitope in the N-terminal extracellular domain of all known α-subunits. In contrast to expression data obtained in RT-PCR experiments, the subunit-specific analysis of temporal and regional distribution of GlyRs on the protein level was limited due to lack of subunit-specific antibodies. The subunit-specific analysis of GlyRs is not restricted to fundamental research, as defects in the GlyR system are associated with human disorders. For example, hyperekplexia (startle disease stiff baby syndrome, STHE) is a hereditary neurological disorder characterized by an exaggerated startle response and infantile muscle hypertonia, which is caused by defects in GlyR α1 subunit. In order to detect specific GlyR subunits on the protein level, we started to generate antibodies against these subunits. In contrast to immunization using intact proteins, we applied the well-described strategy of peptide-based generation of polyclonal antibodies. The selection criteria for peptide design were based on two general requirements: On one hand, the antibodies had to be subunit-specific and on the other hand, a good immune-response had to be ensured. In detail, we searched the peptide sequence of the glycine receptor α2 and α3 subunits for stretches of up to 20 amino acids that exhibited the greatest diversity among all known GlyR subunits. The probability of selected sequences to elicit an immune-response (antigenicity) was estimated by combining values for hydrophilicity, surface probability, flexibility, as well as the secondary structure predictions.
Following peptide synthesis, the integrity and purity of the synthesized peptides were verified by HPLC and MALDI-TOF-MS analysis. Peptides were coupled to Keyhole Limpet Hemocyanin (KLH) and conjugates were used for immunization of rabbits and mice. Polyclonal antibodies were isolated from blood. Western Blot analysis showed successful generation of antibodies recognizing their target subunit. Antigen binding could be competed by using the antigenic peptide, indicating sequence specificity. These peptide-derived antibodies were suitable for both, Western blotting and immunohistochemical detection.

P C150 - Prevention of experimental autoimmune encephalomyelitis by a MHC class II nonapeptide ligand identified with combinatorial peptide chemistry

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Inbred rat strains are commonly used for the induction of various experimental autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) or various arthritis models. Susceptibility or resistance in many of these models is associated with the MHC (RT1) class II due to the key role of these molecules in triggering CD4+ T cells by presenting restricted sets of peptides to the T cell receptor. But in spite of the widespread usage of rat models little is known about the structural characteristics of rat MHC class II molecules. The purpose of this study was the elucidation of the peptide binding pattern of a RT1.D molecule and the design of new high affinity ligands for this MHC molecule. Subsequently the capacity of these ligands to prevent induction of autoimmunity should be investigated. We were especially interested in the RT1.Dn molecule, the DR-like molecule of LEW.1N and BN rats since EAE can be induced in these rat strains with the extracellular domain of myelin-oligodendrocyte-glycoprotein (MOG 1-125) and the MOG derived peptide, MOG 91-108 [1]. Combinatorial acetylated nonapeptide amide collections were used in a competitive fluorescent ELISA to create a complete activity pattern containing favourable and unfavourable amino acid residues for binding to the rat DR-like MHC molecule RT1.Dn. By combining amino acids defined as favourable in sequence positions 1 to 9 several individual acetylated nonapeptide amides were created which revealed high affinity for the RT1.Dn allele. One of these high affinity binders (Ac-FWFLDNAPL-NH2) successfully inhibited experimental autoimmune encephalomyelitis after co-immunization with MOG 1-125 or with its encephalitogenic core peptide MOG 91-108 (SDEGGYTCFFRDHSYQEE) in LEW.1N (RT1n) rats. These results demonstrate that it is possible to prevent EAE with a newly designed RT1.Dn nonapeptide ligand structurally totally unrelated to the encephalitogen and indicate that naturally presented self-unrelated high affinity MHC class II ligands could be important for the control of autoimmunity in general.

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P C151 - New peptide-based biopreparations for plant protection

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During the course of screening program for new insecticidal, nemathicidal, acaricidal 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 2900 soil samples, collected in different regions: Italy, France, India, West Siberia, Armenia, Kazakhstan, Poland, Kalifornia (USA), Viet-Nam, Antarctic. Some of these strains producing biologically active peptides with insecticidal, nemathicidal, acaricidal and fungicidal activities and protease inhibitors are studied in details. New biopreparations for plant protection Octeberin-M, Indocid-55 and Indocid-56, Chrizomal-R21 and Alirin S on the base of these strains were prepared. This paper discusses their fermentation production, isolation, structure peculiarity and biological properties.
The bioactive products from Octeberin-M *(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 HPLC gave biologically active components I, II and III, possessing high contact activity against test – insect and nemathodes and suppressing growth of phytopathogenic fungi and bacteria. Acid hydrolysis of component I in 6N hydrochloric acid at 110°C for 24 hours gave some products which were found to be Thr, Pro, Gly, Ala, Val, Tyr, Phe and component II - Pro, Tyr, Val, Gly. A comparison of amino acid composition, molecular mass and biologically activity of inhibitors with substances described in literature indicates on its original structure. New biopreparations Indocid-55 and Indocid-56 were found as new high effective insecticides against large group of pests: whitefly, aphid, trips, nematode. The major component of Indocid-56 is high active against yeast and fungi, nonactive against Gram-negative and Gram-positive bacteria. According to analysis of IR, NMR-spectra and comparison of physic-chemical and biological properties with polypeptides described in literature Indocid-56 was classified as related to peptid lactones group (type micamicin-B) an original substance.
New biopreparations Chryzomal-R21 and Alirin-B are produced by *Streptomyces chrysomallus-VIZR* and *Bacillus subtilis-VIZR* respectively. Alirin-B is complex of major ninhydrin positive and tryene components possessed of fungicidal activity against different phytopathogenic fungi. Data of amino acid analysis of products of hydrolysis, NMR and IR spectroscopy corroborated its peptide nature, it was classified as belonging to the antibiotic peptide lactone type threonine.

P C152 - Biofunctionalisation of different surface types via $\alpha_v\beta_3$-integrin selective cyclic Arg-Gly-Asp peptides

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The cyclic pentapeptide (RGDfV) is a highly potent and $\alpha_v\beta_3$-selective integrin antagonist.[1] Substitution of valin by lysin results in only a small decrease in the IC_{50}. The highly active $\alpha_v\beta_3$- and $\alpha_v\beta_5$-integrin selective c(RGDfK) was functionalized via lysin sidechain with different linkers. The linkers consists of different anchors and spacers. This way c(RGDfK) can be bound covalently or via strong ionic interaction to diverse surface types to obtain new bioactive materials.[2] In a pilot study we bound the thiolpeptide 1 to maleinimide functionalised BSA and studied the adhesion of different osteoblast cultures.

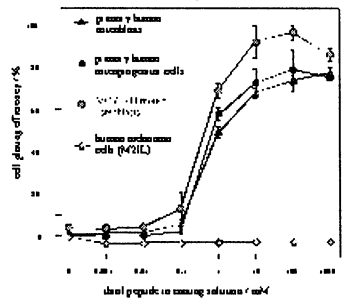

Fig.1 - Dependence of the cell plating effeciency of different osteoblast cultures on the concentration of the thiolpeptide 1. M21L cell which do not express $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors do not bind to the BSA surface.

c(RGDfK) coated polymethylmethacrylat-(PMMA)-surfaces effectively bind to murine osteoblasts as well as human osteoblasts *in vitro* when a minimum distance between surface and RGD-sequence is provided.[3] In contrast to osteoblasts in cell suspension, surface bound osteoblasts show no apoptosis but proliferation. Stimulation of cell adhesion by peptide-coated PMMA was shown in *in vivo* studies in rabbits.[4] We will present a kit of different spacer and anchor units for surface functionalisation with c(RGDfK) and c(RGDfE) or the inactive analouges c(RADfX).

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P C153 - Synthesis of peptides-based bioorganic-inorganic materials.

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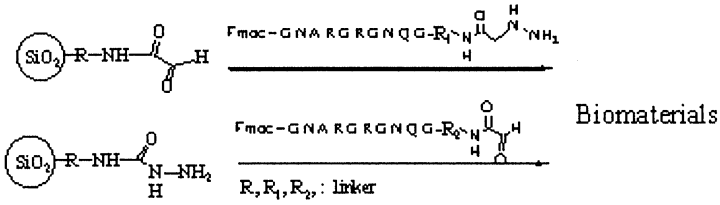
The field of biomaterials has been the subject of important researches in the last few decades, and developments in scopes such as biocatalysts, biosensors, and diagnostics are in progress.[1] In this context, selective methods for the covalent attachment of biomolecules to the surface of inorganic solids are highly needed. We present here the syntheses of functionalised silicas by using the sol-gel procedure [2] or the grafting of organosilanes on silica particles, for applications in diagnostics. The particles were functionalised by semicarbazide or α-oxo aldehyde groups which allow the chemoselective anchoring of biomolecules modified by hydrazine or α-oxo aldehyde moieties respectively. The reactivity, the selective bonding and the non specific interactions of peptides with the functionalized particles will be discussed.



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P C154 - Small peptide dendrimers with antimicrobial properties

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Dendrimers are highly ordered, hyperbranched polymers with number of potential applications. In medicine, dendrimers are useful as an antibody mimics, drug transport vectors, anticancer agents, et cetera. One of the major advantages of dendrimers is the opportunity of multiplication of active elements on the sphere. Macromolecules build from the lysine residue are one of the most popular peptidic dendrimers. The functionalisation of the dendrimer is performed through attachment of the active elements to the last generation of the growing polymer. The bacteria membrane is charged differently than mammalian cell. This difference is recognized by peptides of mammalian endogenous host-defense system. All natural antibacterial peptides are multicharged, containing several copies of basic amino acids, arginine or lysine. Therefore, we predicted that multicharged dendrimers might have also high affinity to bacterial membranes. To perform structure-activity studies, a small library of peptide dendrimers have been synthesized and tested for antimicrobial properties. The obtained results indicate that most potent antimicrobial properties contain two multiplicated elements, basic amino or guanidino groups and aromatic (lipophylic) components.

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P C155 - Discovery and structure-activity relationship of angiogenic peptides

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Successful tissue regeneration requires cell-adhesion and angiogenesis, as well as cell proliferation and differentiation. We have found several peptide fragments of laminin and osteopontin possessing both adhesion and angiogenic activities from morphological studies together with cell adhesion and cell motility assays using transformed rat lung endothelial cells. Among these peptides a heptapeptide SVVYGLR (designated angiogenic peptide, AGP) showed the most significant angiogenic activity, which was confirmed by tubular formation, polarity and formation of both tight junctions and microvilli. In addition an *in vivo* assay was performed with the disc-angiogenesis-system (DAS) [1]. The adhesion activity of the AGP has already been reported but not its angiogenic properties [2]. Indeed, VEGF [3] induced neovascularization moderately although the present AGP showed remarkable angiogenesis. An Ala-scan was performed and related peptides were prepared by the SPPS and submitted for DAS assay. The results indicated that the residues located in the middle section of AGP are important while both N- and C-termini are not crucial. Thus immobilization at both ends of the peptide has no influence on its actions. Since biomimetic, biodegradable and biocompatible materials for hard tissue reconstruction (such as teeth and bone) have been highlighted as improving the quality of life in an aging society, we have been attempted to generate improved biomaterials, in which functional peptides were immobilized onto tissue related proteins [4, 5]. In this context oligopeptides have significant advantages regarding safety, metabolism and structural diversity capabilities. These peptides bound to haptens were conjugated onto allergen free gelatin and used clinically.

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P C156 - Laminin peptides maintain activity when conjugated to a chitosan membrane

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Laminin-1, a major component of the basement membrane matrix, has diverse biological activities. Previously, we identified 20 biologically active sequences on laminin-1 using a large set of peptides [1-5]. Here, we have focused on the active peptides and investigated their potential for tissue engineering and therapeutic applications. We utilized a chitosan membrane as a mechanical support for the peptides and tested for biological activity. Chitosan is biodegradable and has been used as a biomedical material. Chitosan membrane alone adheres to tissues but does not show cell attachment. We covalently conjugated four biologically active peptides from the laminin-1 molecule (A99: AGTFALRGDGNPQ, AG73: RKRLQVQLSIRT, A13: RQVFQVAYIIIKA, and C16: KAFDITYVRLKF) on chitosan membranes and tested their biological activities using various cells. The peptide-conjugated chitosan membranes promoted cell attachment and spreading in a cell-type specific manner. Morphological differences were also observed with these peptide-chitosan membranes. Well-organized actin fibers were observed on the A99-chitosan membrane while the AG73-chitosan membrane promoted filopodia-induced activity. Cell morphology on the A13- and C16-membranes was between that of A99 and AG73. Cell attachment on the A99-chitosan membrane was inhibited by EDTA but not by heparin, and cell attachment on the AG73-, A13-, and C16-chitosan membranes was inhibited by heparin but not by EDTA. These results suggest that the A99-chitosan membrane interacted with an integrin and the AG73-membrane promoted proteoglycan-mediated cell attachment. Furthermore, only the AG73-chitosan membrane was found to promote neurite outgrowth with PC12 rat pheochromocytoma cells. The synergistic effects of cell attachment and spreading were observed when A99 and AG73 were conjugated together to a chitosan membrane. Taken together, these data suggest that cell adhesive peptides on the membranes are active with cell-type specificity and have a potential ability to serve as bio-adhesives for tissue repair and engineering.

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P C157 - Microarrays with α-helical peptides for protein detection using a FRET technique

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In proteomics studies it is essential to construct an easy and effective protein-detection system. Small peptides can be easily designed and synthesized with suitable secondary structures. Hence they may be useful as capturing agents for detecting proteins. In this study, we attempted to detect proteins using designed α-helical peptides with a FRET function. Furthermore, we constructed α-helical peptide libraries with various amino acid substitutions in order to detect a variety of proteins. To establish the synthetic and detection methods, we selected a model peptide, LK2, which is a cationic amphiphilic α-helical peptide that binds to calmodulin [1]. The designed peptides based on LK2 were synthesized according to the standard Fmoc-chemistry on Rink resin. Fluorescent probes were introduced onto the peptides on the resin. The changes in the fluorescence spectra of the peptides with various fluorophores were examined by the addition of calmodulin in aqueous solution. In comparison to the peptides with a single probe, a relatively high response was observed by FRET between two probes at both termini. It is also found that fluorescein was an effective fluorophore in this system because of its high fluorescence intensity. In addition, the target protein was detectable with the peptides immobilized on a solid support such as a glass plate. A remarkable increase in the fluorescence intensity was observed upon addition of the protein. Subsequently, α-helical peptide libraries with various charges and/or hydrophobicities were constructed for peptide microarrays. When the target protein was added, peptides showed different responses depending on their sequences. We have attempted to detect various proteins with peptide microarrays using these libraries.

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P C158 - The obtaining of bioactive peptide material from αs- and β-caseins

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The casein proteins of milk are precursors of many bioactive peptides, which can act as antihypertensive, antitrombotic, immunomodulative, antimicrobial, mineral binding, opioid agonistic and antagonistic substances as well as they possess many other activities [1]. It is known that some bioactive casein peptides can be absorbed and delivered to their targets by the blood circulation [2,3]. These peptides may be generated either after milk or milk products ingestion by digestive enzymes *in vivo* or by action of milk-clotting enzymes or proteases of lactic acid bacteria *in vitro*. Only few successful studies have shown the production of casein bioactive peptides in fermented milks [4]. In order to increase the biological value of fermented milk products it is necessary to exam the milk-clotting proteases and strains of lactic-acid bacteria to generate the peptides with physiological activities. We propose the scheme (fig.1) for proteolysis of casein's fractions. This scheme allows obtaining of the peptide material concentrate, which may be tested for different kinds of bioactivities. Previously we investigated the peptides obtaining from casein's fractions under the action of proteolytic system of lactococci. In this study we used the lactic acid bacteria and milk-clotting enzymes. The proteolysis was carried out in conditions similar to fermented milk products making. The oligopeptide materials (up to 1500 Da) were obtained after proteolysis of αs_1- or β-caseins by the biomass of *Lactococcus lactis* subsp. *lactis* strains or by lactococci in combination with pepsin or fromase. The samples of the obtained peptide materials have shown the bioactivity with the different grades as antihypertensive agents *in vivo* after the chronic administration into the rats with renovascular hypertension and as the inhibitor of angiotensin-converting enzyme (ACE) *in vitro*.

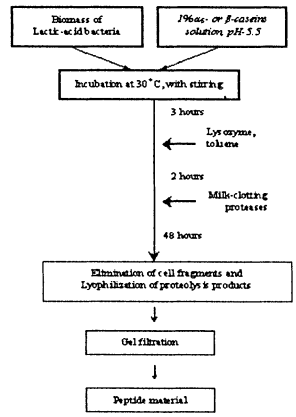

Figure 1. The scheme for peptide material obtaining from products of proteolysis of αs- and β-caseins

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P C159 - EVOblue - Its application in HTS assays

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EVOblue® 30 is a fluorescent dye developed for the labeling of biologically active molecules like small organic molecules, peptides, proteins, oligonucleotides, and antibodies. EVOblue® dyes are based on the laser dye family of oxazines and are optimized for an excitation wavelength of 635 nm and an emission around 670 nm - both allowing the use of common laser and filter sets. These dyes are stable towards acids and bases and their spectroscopic properties reveal a high chemical stability, and suitability for high performance fluorescence applications. Their small size and hydrophilic structure are advantageous for the use in labeling reactions. Several biologically active molecules were labeled with EVOblue® dyes without influencing their biological activity. These conjugates have been successfully used for assay development and HTS campaigns using a miniaturized 1 µl format. The readout is based on single molecule detection using FIDA (fluorescence intensity distribution analysis), fluorescence lifetime and 2D FIDA anisotropy.