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Conformation of cyclic peptides. Principle concepts and the design of selectivity and superactivity in bioactive sequences by 'spatial screening'

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Abstract: The description of small cyclic peptide conformations can be simplified by substitution of the peptide bond for an olefinic structure, which then is converted into a single bond (for the *E* configuration) or a pseudo-CH_2-group (for *Z* olefins). The resulting cycloalkane conformations are related to the observed cyclic peptide structures. The individual conformation, however, is strongly influenced by the array of chirality in the peptide sequence. This can be used for a "spatial screening" of biologically active peptide sequences. The procedure is demonstrated on selective and highly active inhibitors for $\alpha_v\beta_3$ integrins with a strong potential for development into anticancer drugs.

Introduction

Peptides play an important role in many biologically relevant processes and are of outstanding interest in pharmaceutical research. Locking the active conformation in cyclic peptides can give superpotent analogues in matched cases (1). In addition conformational constraints provide the basis for receptor selectivity; often different receptors bind the same flexible substrate in different conformations. We will present a new way to design selectivity and superactivity by "spatial screening". However, firstly we want to discuss the conformations of small cyclic peptides based on conformations of cycloalkanes. This reduction (Fig. 1) is done in the following way:

The peptide bond is substituted by a carbon-carbon double bond (*E* or *Z*) yielding an olefin. According to Dunitz and Waser (2, 3), the conformational behavior can be further reduced by substitution of a *E* double bond by a chemical bond and of a *Z* double bond by a pseudo-CH_2-group. For example, 1,5-cyclooctadiene yields a rigid chair and a flexible boat conformation analogous to cyclohexane.

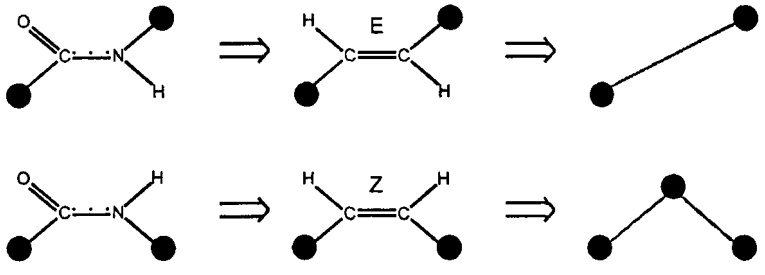


Fig. 1 Schematic description of the extension of the DUNITZ-WASER concept to peptides.

Conformation of Cyclic Peptides and Cycloalkanes

To verify this concept, cyclic peptide structures were taken from the Cambridge Structural Database (CSD) (4). Metal coordinated cyclic peptides were omitted.

Cyclic Tripeptides

The analogy of cyclic tripeptides containing three cis peptide bonds with cyclonona-1,4,7-triene and cyclohexane has been previously discussed (5, 6) and forms the basis for this work for the analysis of larger ring cyclic peptides.

Cyclic Tetrapeptides

Most cyclic tetrapeptides exhibit a cis-trans-cis-trans peptide bond configuration. Their "conversion into cycloalkanes" results in a cyclohexane ring, a methylene-ethylene-methylene-ethylene equivalent for the peptide bonds. Both chair and boat type conformations are found (Table 1). Typical examples are shown in Fig. 2 and Fig. 3.

Only one X-ray structure of an all-trans cyclic tetrapeptide has been reported, which corresponds the butterfly-like conformation of cyclobutane (Fig. 4) (9).

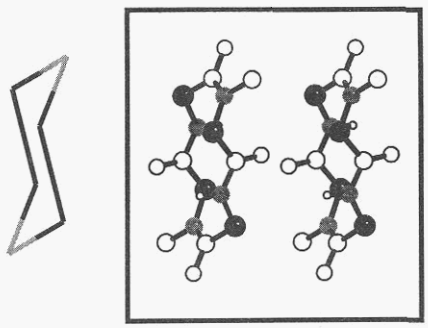


Figure 2

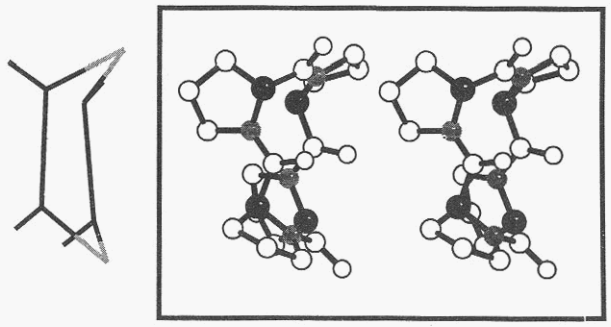


Figure 3

Fig. 2 A stereoplot of the crystal structure of cyclo(-Gly-Sar-Gly-Sar-) (7) is shown in the frame. α-Carbon atoms are given in solid black, nitrogen atoms are in gray. All other atoms are not specifically assigned. At the left hand side its "reduced" backbone conformation is depicted with the pseudoatoms in light lines.

Fig. 3 A stereoplot of the crystal structure of cyclo(-D-Pro-L-Pro-D-Pro-L-Pro-) (8) is shown in the frame. At the left hand side its "reduced" backbone conformation is depicted with the pseudoatoms in light lines.

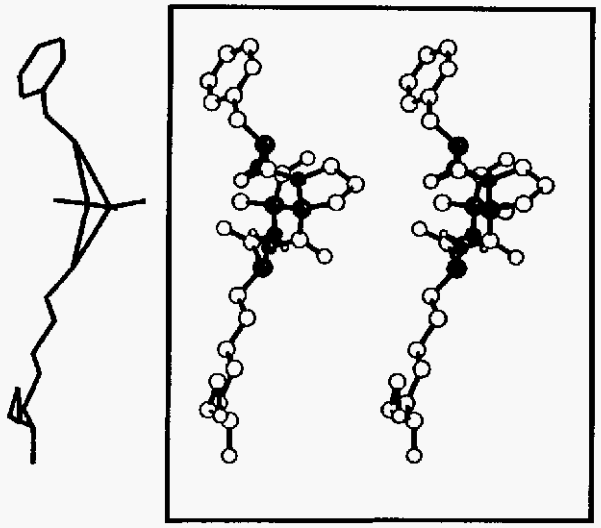


Fig. 4 A stereoplot of the crystal structure of cyclo(-Phe-D-Pro-Xxx-Aib-) (9) is shown in the frame. At the left hand side its "reduced" backbone conformation is depicted.

TABLE 1. Comparison of the cycloalkane-like conformations of different sized cyclic peptides taken from the CSD

cyclic oligomeric peptide	butane	pentane	hexane				total number
	butterfly	envelope	chair	boat	twisted boat	envelope/ planar	
tetra	1	1	6	4			12
penta		6	2				8
hexa			10	9	5	4	28

Cyclic Pentapeptides

Most cyclic pentapeptides prefer all-trans amide configurations. According to the DUNITZ-WASER concept, such structures can be reduced to a five-membered ring resembling cyclopentane. Most cyclic pentapeptide structures were found to correspond to the envelope conformation of cyclopentane. Cyclic pentapeptides containing one cis peptide bond exhibit a distorted cyclohexane structure.

Cyclic Hexapeptides

Cyclic hexapeptides normally adopt an all trans configuration about the peptide bond and prefer a conformation with two β-turns (10). In an idealised βII turn all four Cα-atoms lie within one plane whereas in the βI turn a twisted orientation results.

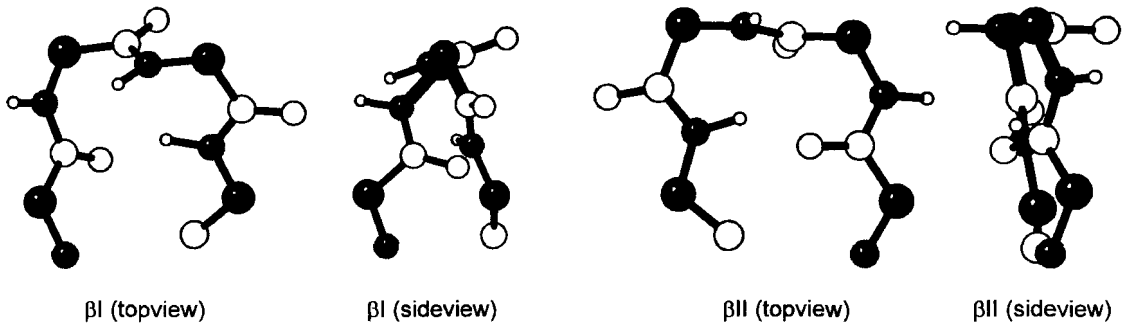


Fig. 5 Idealised βI and βII turns in two orientations.

Hence, a cyclic hexapeptide containing two βI turns adopts a perfect chair conformation (Fig. 6), while the presence of two βII turns results in a boat like conformation. In reality, due to minor variations in βI and βII turns found in cyclohexapeptides, some distortion is nearly always seen.

Discussion

The overall conformation of small cyclic peptides can be rationalized via "reduction" to cycloalkane conformations. However, due to the long distance between the α-carbon atoms, the amino acid sidechains do not have the same steric interaction as in small cycloalkanes. Thus more or less distorted conformations result. Moreover, the boat type conformations are not so destabilized as in cyclohexane itself. The strongest difference results from steric hindrance with the carbonyl oxygen of an amino acid avoiding 1,3-allylic strain (12) with the sidechain of the previous residue.

In general, the conformation of the cyclic hexapeptide is strongly influenced by the chirality of the amino acids (13). This behavior can be used for conformational design purposes.

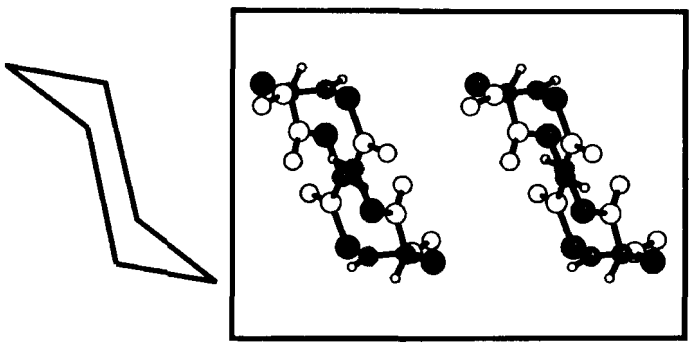


Fig. 6

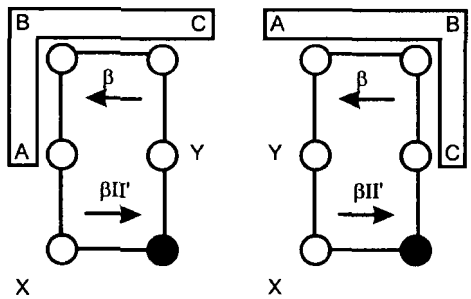


Fig. 7

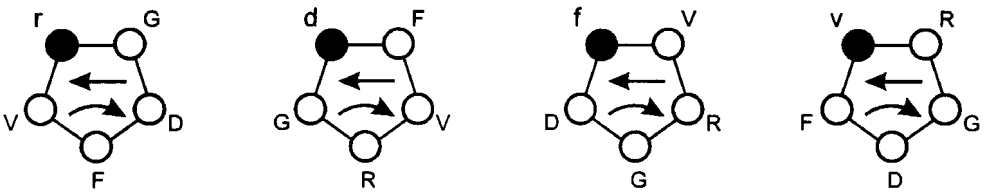
Fig. 6 A stereoplot of the crystal structure of cyclo(-Gly-Gly-Gly-Gly-Gly-Gly-) (11) is shown in the frame. At the left hand side its "reduced" backbone conformation is depicted.

Fig. 7 Conformations of cyclic hexapeptides. The filled circle corresponds to the D-configurated amino acid. The βII' turn on the southern side can be used to force the amino acid sequence A-B-C into the desired position. Arrows indicate intramolecular hydrogen bonding.

Conformational Design by "spatial screening"

There is a wealth of accumulated knowledge about the predictability of detailed conformations in cyclic peptides. This experience can be used to design a specific amino acid sequence into a desired conformation (14). For example, a D-amino acid in a cyclic penta- or hexapeptide strongly prefers the i+1 position of a βII′ turn. Together with the knowledge of the β, β turn pattern of cyclic hexapeptides, this fact can be applied to force a sequence of amino acids into a specific position of a β turn on the other side of the ring (Fig. 6).

Often it is not possible to know beforehand which is the most active conformation. For these cases we have developed the concept of "spatial screening", which we will demonstrate with the example of RGD peptides. Cyclic penta- and hexapeptides were used, but we will concentrate on pentapeptides here.
Cyclic pentapeptides containing one D- and four L-amino acids prefer a conformation with a βII' turn containing the D-amino acid in the i+1 position. A loop on the other side often involves a γ turn.
In an effort to force the βII' turn configuration at different positions, we synthesised four RGDFV peptides in which only one amino acid has the D-configuration (15, 16) (Fig. 8).



	c(rGDFV)	c(RGdFV)	c(RGDfV)	c(RGDFv)
HBL-100	>120	>120	0.1	30

Fig. 8 Conformations of cyclic pentapeptides containing the RGDFV sequence. The filled circle (small letter) corresponds to the D-amino acid. The biological activities (IC_{50} in μM) are the inhibition of HBL-100 (mammary epithelia cell lines) cell adhesion to vitronectin (16). A small number indicates a higher activity.

Here it is not possible to discuss each example in detail. The biological activities and selectivities of the four peptides vary dramatically although the constitution of the peptides is identical.

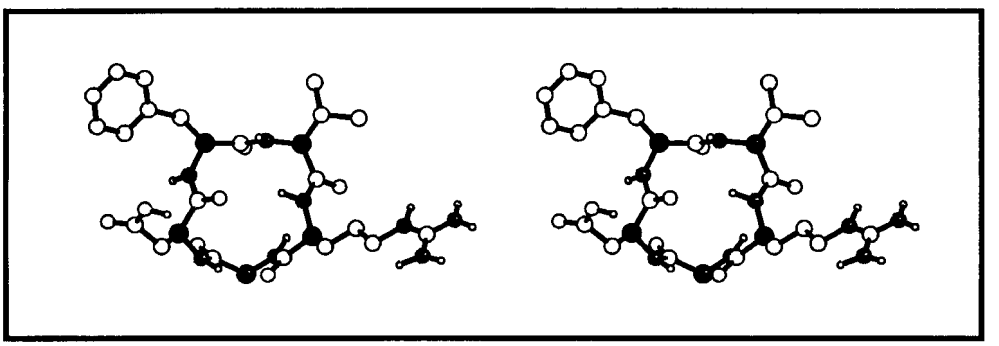


Fig. 9 A stereoplot of the NMR structure of cyclo(-Arg-Gly-Asp-D-Phe-Val-) is shown.

The most interesting observation was made with cyclo(-Arg-Gly-Asp-D-Phe-Val-) (Fig. 9), which is an extremely potent inhibitor of human epithelian cancer cellular adhesion (15, 17, 18, 19). Cell-cell and cell-matrix adhesion is caused by binding of integrin receptors to RGD containing proteins (20). There are many different integrin receptors, but cancer cells express high amounts of the $\alpha_v\beta_3$ receptor. It could be demonstrated that the above mentioned cyclic peptide is a specific inhibitor for this receptor, whereas platelet aggregation ($\alpha_{IIb}\beta_3$ receptor) is only weakly inhibited (selectivity !).
Recently, it was found that inhibitors of $\alpha_v\beta_3$ also repress angiogenesis (growth of blood vessels into tumor) and induces apoptosis of human tumor cell lines implanted in chick chorioallantoic membranes (21). Thus, the use of these peptides may lead to a new cancer therapy based on starving the tumors of their vital blood supply.

Summary
It was shown that conformational constraints of peptide structures can induce conformations in an often predictable way (14). If the bioactive conformation is not known, the spatial orientation of pharmacophoric groups on a distinct backbone conformation can be systematically screened. The procedure involves a shift of one (or more) D-amino acid(s) around a distinct scaffold. The functional groups of the sidechains and their neighborhood are retained but their spatial arrangement can be adjusted. If one of these conformations matches the bound conformation (i.e. the conformation at its biological receptor) superactivity can be expected. In addition, the constraints often prevent binding to related receptor subtypes resulting in higher selectivity. This procedure is applied to design a potent lead structure for an anticancer drug.
Today efficient screening strategies are being developed, especially using substance libraries. They may result in new lead structures which have to be refined by medicinal chemistry strategies. However, often the oligomeric components are flexible and the spatial orientation of the pharmacophoric groups is not known. The technique presented here provides the next step in the direction to useful drugs.

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