Are β-Amino Acids γ-Turn Mimetics? Exploring A New Design Principle for Bioactive Cyclopeptides

Frank Schumann, Annett Müller, Mario Koksch,[†] Gerhard Müller,[‡] and Norbert Sewald*

Faculty of Chemistry, University of Bielefeld Universitätsstrasse 25, D-33615 Bielefeld, Germany

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β-Peptides solely composed of β-amino acids display new types of conformations such as helical structures.[1] The question whether peptides with a mixed composition of both α- and β-amino acids display conformational preferences has only been addressed with few nonrepresentative examples, for example, peptides containing β-alanine[2] or modules exerting a strong conformational bias (e.g., proline, D-amino acids),[3] thus not revealing general design rules.

We report on the finding that incorporation of a distinct β-amino acid in cyclic peptides results in the stabilization of the overall secondary structure. Within all tetra- and pentapeptides examined by us, β-amino acid residues preferably occupy the central sequence position of a modified γ-turn conformation, in the following termed pseudo γ-turn (Ψγ-turn). The conformational bias of the β-amino acid residue employed even overrides the strong preference of a D-amino acid residue, commonly found in the $i + 1$ position of βII′-turns. According to the experimental conformational analysis of β-amino acid-containing peptides, the β-amino acids act as γ-turn mimetics. These findings might provide a new principle for the design of cyclopeptides with control of conformation.

For the structural design studies conducted in our group, we envisioned cyclic RGD peptides of known 3D structure and biological activity as conformational platforms, ideally suited for the systematic investigation of both structural and biological consequences of β-amino acid incorporation. The triad Arg-Gly-Asp (RGD) is known to be a universal cell recognition sequence binding to cell surface-exposed integrins, thus mediating cell−cell and cell−matrix interactions. The cyclic hexapeptide **1** described by Kessler et al. efficiently inhibits binding of fibrinogen to the integrin $\alpha_{IIb}\beta_3$ involved in thrombocyte aggregation, while the cyclic pentapeptide **2** prevents binding of vitronectin to the integrin $\alpha_V\beta_3$ playing a role in tumor cell adhesion, angiogenesis, and osteoporosis.[4] The selectivity profile of these antiadhesive cyclopeptides is rationalized by a mutually different presentation of the pharmacophoric groups. The RGD motif is found to occupy positions $i + 1$ to $i + 3$ of a β-turn in the $\alpha_{IIb}\beta_3$ selective hexapeptide **1** while it resides in positions i to $i + 2$ of a regular γ-turn in the $\alpha_V\beta_3$ selective pentapeptide **2**.

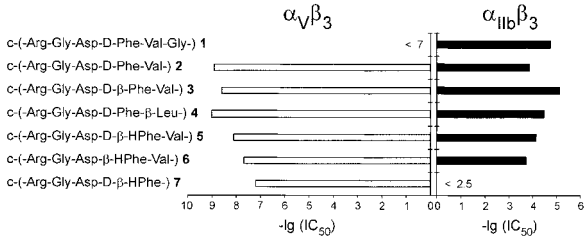

Figure 1. Biological activity of the peptides examined.

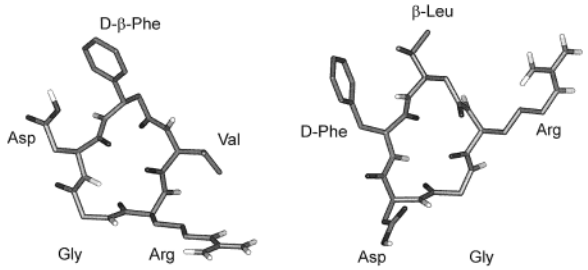

Figure 2. Solution conformation of **3** and **4** in DMSO-d_6. D-β-Phe and β-Leu induce a Ψγ-turn each (upper part of the molecule).

We performed a systematic β-amino acid scan on the cyclic model peptides with permutational replacement of one α-amino acid by the corresponding β-analogue.[5] The development of methodology for on-resin cyclization was a precondition for parallel synthesis of cyclic peptides.[6] The peptides modified by β-amino acids are active integrin antagonists ($\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$).[7] The cyclic pentapeptide **3** inhibits thrombocyte aggregation ($\alpha_{IIb}\beta_3$) with an IC_{50} value comparable to that of the known hexapeptide **1** (Figure 1). The RGD sequence in **3** is found in positions $i + 1$ to $i + 3$ of a β-turn (βII′-type characteristics) according to the conformational analysis employing 2D NMR, distance geometry, and restrained molecular dynamics simulations (Figure 2). The temperature gradient of the chemical shift of Asp H^N ($\Delta\delta/\Delta T = -0.9$ ppb/K) hints toward involvement in a hydrogen bond. Several characteristic NOE effects (e.g., strong: Arg H^α/Arg H^N, Gly H^N/Asp H^N; medium: Arg H^α/Gly H^N) are found. The β-amino acid residue occurs in the central position of a tight reverse turn closely related to a γ-turn conformation, the only difference being the insertion of a single carbon atom into the peptide backbone (Ψγ-turn).

The mutual superposition of the solution-derived conformations of **3** and **1** reveals a satisfactory overlay of the peptide backbones and of the $C^\alpha \rightarrow C^\beta$ vectors which may explain the comparable biological activities of both peptides. These results suggest that the β-amino acid acts as a turn mimetic, adopting a Ψγ-turn conformation.

Pentapeptide **4** demonstrates the full extent of the structural bias exerted by a single β-amino acid residue on a cyclic pentapeptide. Two inducers of secondary structure, a β-amino acid and a D-amino acid are arranged in a noncooperative manner.

* Corresponding author. Telephone: (+49)521-1062051. Fax: (+49)521-1068094. E-mail: norbert.sewald@uni-bielefeld.de.

[†] Universitätsklinikum Leipzig AöR, Medizinische Klinik und Poliklinik I, Fachbereich Kardangiologie, Johannisallee 32, D-04103 Leipzig.

[‡] Bayer AG, Zentrale Forschung, ZF-WFM, Q 18, D-51368 Leverkusen.

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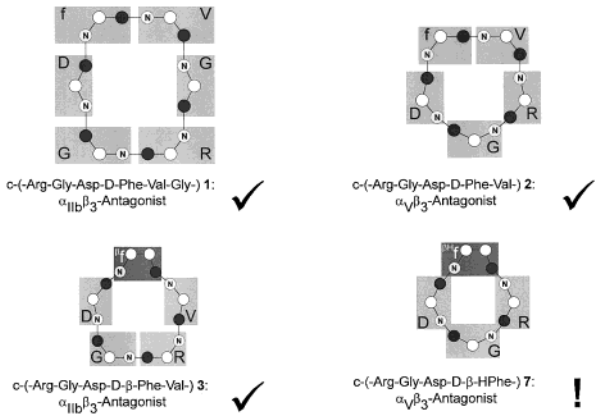

Figure 3. Rational design of an $\alpha_V\beta_3$ antagonist by incorporation of a β-amino acid as a $\Psi\gamma$-turn inducer.

While the latter usually prefers position $i + 1$ in a βII′-turn, it is found in position $i + 3$ in **4**. β-Leucine on the other hand is again placed in the central position of a $\Psi\gamma$-turn (Figure 2). Flat temperature gradients are observed for D-Phe H^N and Arg H^N. Again, strong (Gly $H^{\alpha,pro-R}$/Gly H^N) and medium (Asp H^α/Asp H^N, Gly $H^{\alpha,pro-R}$/Asp H^N, Asp H^N/D-Phe H^N, Asp H^α/D-Phe H^N) NOE effects are observed. A slight deviation from the ideal torsion angles of a βII′-turn is due to partial γ^i-turn formation around Asp. We conclude that a β-amino acid may possess a higher conformational bias than a D-amino acid. The formal replacement of D-Phe-Val-Gly in **1** by D-β-Phe-Val (β-turn $\rightarrow$ $\Psi\gamma$-turn) obviously does not induce a significant change within the pharmacophoric RGD region; **1** and **3** also display comparable affinity toward $\alpha_{IIb}\beta_3$. Given the results obtained on **3** and **4**, a β-amino acid residue stabilizes a $\Psi\gamma$-turn conformation in a cyclic peptide (Figure 2).

On the basis of this assumption, we extended our studies on the design of cyclic tetrapeptides containing a β-amino acid as $\Psi\gamma$-turn inducers (Figure 3). Again, a highly active and selective cyclic RGD peptide of defined 3D structure could be used as a strategic platform to test our hypothesis. Within c-(-Arg-Gly-Asp-D-Phe-Val-) **2** the regular γ-turn, formed by the recognition tripeptide RGD, was identified to be crucial for the high affinity toward the integrin $\alpha_V\beta_3$, as shown by the NMR-derived solution conformation. Consequently, the structural replacement of the dipeptide sequence D-Phe-Val in **2** by the β-amino acid D-β-HPhe yielded the cyclic tetrapeptide **7**. And indeed, **7** possesses nanomolar affinity to the isolated integrin $\alpha_V\beta_3$ ($IC_{50} = 63$ nM) with a remarkable selectivity [IC_{50} ($\alpha_{IIb}\beta_3$) > 300 μM]. The results from experimental structure determination by means of 2D NMR and molecular dynamics simulations confirm our initial hypothesis. Asp H^N displays remarkable temperature gradients: $\Delta\delta/\Delta T$ (DMSO-d_6) = +0.8 ppb/K; $\Delta\delta/\Delta T$ (H_2O/D_2O) = −3.1 ppb/K. Again, strong NOE effects (Arg H^N/Asp H^N, Gly H^N/Arg H^α; Gly H^N/Gly $H^{\alpha,pro-R}$) are found.

As anticipated, the β-amino acid is found in the central position of the $\Psi\gamma$-turn, thus inducing the required regulary γ-turn for the opposite peptide portion (Figure 4). This arrangement is comparable to that of **2**; the conformations of **2** and **7** within the pharmacophoric regions are in good agreement. The rationally designed cyclic tetrapeptide **7** is a selective nanomolar $\alpha_V\beta_3$ antagonist. The high activity and pronounced selectivity render **7** a potential lead structure which is under further optimization in our group.

The overlay of **7** and **2** clearly demonstrates the structural resemblance of the peptide backbone and of the vectors $C^\alpha \rightarrow C^\beta$

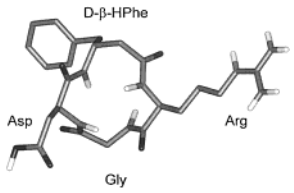

Figure 4. Solution conformation of **7** in DMSO-d_6. D-β-HPhe induces a $\Psi\gamma$-turn (upper part of the molecule); the RGD sequence forms a complementary γ-turn (Gly: $\phi = 97°$, $\psi = -54°$).

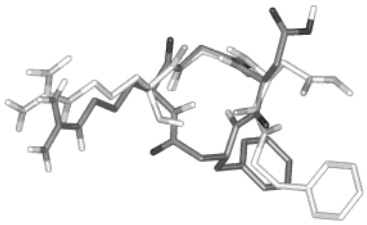

Figure 5. Overlay of the solution conformations of the cyclic pentapeptide c-(-Arg-Gly-Asp-D-Phe-Val-) **2** (light; only Arg-Gly-Asp-D-Phe displayed) and of the cyclic tetrapeptide c-(-Arg-Gly-Asp-D-β-HPhe-) **7**.

of Arg and Asp, respectively (Figure 5). The smaller ring size of **7** (13-membered ring) in comparison to **2** (15-membered ring) might account for the decreased (60-fold) affinity. Improper arrangement of the phenyl ring or of the Phe H^N group might result in a weaker hydrophobic interaction or a weaker hydrogen bond between peptide and integrin. The relevance of peptide structures based on NMR studies in DMSO is often questioned because of the nonphysiological conditions. We examined the conformational behavior of **7** in water by NMR and could not find any significant differences in the NOE constraints and backbone torsion angles.

Hence, the targeted application of β-amino acids in the design of cyclic peptides allows the control of the peptide backbone conformation such that these non-native building blocks clearly prefer to adopt $\Psi\gamma$-turns. These findings can generally be utilized in the de novo design of biologically active cyclopeptides, since the spatial orientation of side chains exposing potential pharmacophoric groups can be pre-defined by incorporation of tailor-made β-amino acids in appropriate sequential positions. The controlled employment of β-amino acids together with their structural preferences undoubtedly enriches the methodological toolbox which is currently used for a stepwise reduction of native peptidic character of lead sequences toward peptidomimetic lead compounds within pharmaceutical research.

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Supporting Information Available: A table with IC_{50} values and details of the conformational analysis by NMR and molecular modeling of the peptides described (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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