Integrin Ligands

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The Constrained Amino Acid β-Acc Confers Potency and Selectivity to Integrin Ligands**

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Interactions between the extracellular matrix and membrane proteins are of importance for cell adhesion, tissue formation, and transmembrane signaling processes. Among cell adhesion molecules, integrins occupy a highly prominent position. Many integrins, among them $\alpha_5\beta_1$ and $\alpha_V\beta_3$, recognize the tripeptide sequence -Arg-Gly-Asp- (RGD) in their ligands. The discovery of the role of the RGD sequence in cell–cell and cell–matrix interactions prompted the development of a broad variety of RGD peptides and peptidomimetics for potential therapeutic applications. Soluble derivatives of these compounds are able to competitively inhibit the interaction between an RGD-containing protein and its integrin counterpart, whereas immobilized RGD peptides support cell attachment, for example, to artificial implants.[1]

Integrins play a crucial role in numerous disorders. Integrin $\alpha_v\beta_3$ promotes angiogenesis, which is an essential event in proliferation and metastatis of human tumors, regulates adhesion of cancer cells, and participates in the progression of osteoporosis.[2] Although integrin $\alpha_5\beta_1$ is also involved in angiogenesis, it participates predominantly in various inflammatory disorders, for example, asthma and rheumatoid arthritis.[3] Therefore, the discovery of new peptide ligands displaying high activity and selectivity is a challenge for both biochemistry and medicinal chemistry.

The rational design of biologically active peptides involves prediction of their three-dimensional structure. Spatial screening of peptides and peptidomimetics is an important concept in this context.[4] It is applied in the search for an unknown active conformation of a recognition sequence present in a ligand. Spatial screening comprises the synthesis of a library of stereoisomeric cyclopeptides in which cyclization leads to restriction of the peptide conformation. A recognition epitope, for example, the RGD sequence, is combined with a secondary-structure-inducing element, for example, a D-amino acid,[4] an *N*-alkyl-amino acid,[5] or a β-amino acid.[6]

The cyclic pentapeptide *cyclo*-(-Arg-Gly-Asp-D-Phe-Val-) **1** was developed by Kessler and co-workers in a spatial screening approach as a very active and selective ligand of integrin $\alpha_V\beta_3$.[7,8] Replacement of Val by its *N*-methyl derivative led to the *cyclo*-(-Arg-Gly-Asp-D-Phe-*N*(Me)-Val-) peptide with enhanced biological activity and selectivity to the integrin $\alpha_V\beta_3$. The derivative displays an IC_{50} value in the subnanomolar range[9] and is being investigated under the name Cilengitide in clinical trials as a tumor therapeutic.[10,11]

The presence of β-amino acids stabilizes distinct overall conformations of cyclopeptides, eventually leading to entropy-driven improved receptor binding as long as the appropriate conformation is still accessible. If a single β-amino acid is incorporated into a cyclic pentapeptide, it preferably occupies the central position of a γ turn that is extended by one CH_2 group and hence called a pseudo-γ turn ($\Psi\gamma$).[6] The conformational bias of the β-amino acid may even exceed that of a D-amino acid residue. Thus, β-amino acids act as γ-turn mimetics.[6]

cis-β-Aminocyclopropanecarboxylic acid (β-Acc) derivatives[12–15] have improved the stabilization of the secondary structure of peptides[16] and have been used for the synthesis of neuropeptide Y analogues with a higher affinity for the receptor subtype Y_1.[17] β-Acc may be regarded as a chimera displaying structural features of a β-amino acid, with respect to conformational bias, and methyl aspartate, with respect to hydrogen-bond-acceptor capability.

Herein, we present the synthesis, evaluation of the biological activity, and structure determination of new ligands for integrin $\alpha_V\beta_3$. Pentapeptides *cyclo*-(-Arg-Gly-Asp-(+)-β-Acc-Val-) (**3**) and *cyclo*-(-Arg-Gly-Asp-(−)-β-Acc-Val-) (**4**) contain enantiomeric (+)- or (−)-β-Acc adjacent to the RGD motif. Cell-adhesion assays showed a high affinity of these new peptides. To explain their efficiency, structure analysis was performed to establish a structure–activity relationship. Linear peptides were assembled by solid-phase peptide synthesis by using the 9-fluorenylmethoxycarbonyl (Fmoc)/*t*Bu protection scheme.[18] β-Accs were introduced as the dipeptides shown in Figure 1.

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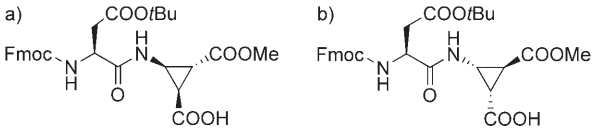

Figure 1. The dipeptide building blocks Fmoc-Asp(O*t*Bu)-(+)-β-Acc-OH (a) and Fmoc-Asp(O*t*Bu)-(−)-β-Acc-OH (b) that were incorporated in the cyclic RGD peptides.

The biological activity of peptides **1**–**4** was evaluated in cell-adhesion assays with two cancer cell lines. Adhesion of the K562 and WM115 cells to their ligands fibronectin and vitronectin is predominantly mediated by integrins $\alpha_5\beta_1$ and $\alpha_V\beta_3$, respectively.[3] The ability of the RGD peptides **3** and **4** to inhibit the adhesion of the K562 and WM115 cells to their ligands was compared with the previously described peptides *cyclo*-(-Arg-Gly-Asp-D-Phe-Val-) (**1**)[4] and *cyclo*-(-Arg-Gly-Asp-D-Phe-Val-β-Ala-) (**2**)[3] as references (Table 1).

Table 1: IC_{50} values of the cyclic peptides as determined by cell-adhesion assays with K562 and WM115 cells.

Peptide	WM115, $\alpha_v\beta_3$ IC_{50}[a] [μM]	K562, $\alpha_5\beta_1$ IC_{50}[a] [μM]	Ratio K562/WM115[b]
1	0.2 (0.06)	18.5 (6.4)	92.5
2	1.4 (0.40)	9.1 (5.3)	6.5
3	0.02 (0.002)	1.5 (0.5)	75.0
4	0.6 (0.23)	1.8 (0.7)	3.0

[a] The standard deviation is given in brackets. [b] Ratio of the IC_{50} values of the cyclopeptides from the tests with K562 and WM115.

Reference peptide **1** inhibited adhesion of WM115 cells to vitronectin with $IC_{50} = 0.2$ μM. This value is in good agreement with literature data on the inhibitory ability of this peptide as estimated by Aumailley et al.[7] Peptide **3**, containing (+)-β-Acc, displayed a tenfold higher affinity ($IC_{50} =$ 20 nM) than **1**. The inhibition effect of the diastereomeric Acc peptide **4** was approximately comparable to that of peptide **1**. Hexapeptide **2** displayed a rather moderate influence on WM115 cell adhesion to vitronectin ($IC_{50} =$ 1.4 μM).

Integrin $\alpha_5\beta_1$ mediated cell adhesion of K562 cells to fibronectin was nearly equally inhibited by peptides **3** and **4**, with IC_{50} values of 1.5 μM and 1.8 μM, respectively. In this assay, peptides **3** and **4** were approximately five- to sixfold more active than the reference peptide **2**, which in turn was about twice as active as peptide **1**. Peptides **3** and **4** had a larger influence on the interaction between vitronectin and integrin $\alpha_V\beta_3$ than on that between fibronectin and integrin $\alpha_5\beta_1$. The ratio of the IC_{50} values in both tests (K562/WM115) should give a rough estimate for the selectivity of the peptides between integrins $\alpha_5\beta_1$ and $\alpha_V\beta_3$. Peptides **1** and **3** showed a higher selectivity towards integrin $\alpha_V\beta_3$.[3] The significantly increased affinity of the RGD peptide **3** to integrin $\alpha_V\beta_3$ is interpreted to be a consequence of the introduction of the rigid β-Acc derivative. Furthermore, diastereomer **3** is the most active ligand of the integrin $\alpha_V\beta_3$ investigated so far.

Distance restraints derived from NOESY spectra were applied in distance geometry calculations that led to different structures clustered according to similarity in their backbone torsion angles.[19] For both peptides **3** and **4**, one major cluster comprising more than 95 % of the structures was obtained and used as a starting structure for restrained molecular dynamics (MD) calculations. Torsion-angle clustering of the observed conformers gave one major cluster that contained more than 87 % of the conformations observed during the trajectory for **3**. Peptide **4** gave two clusters, the major one being populated during 80 % of the trajectory. The central structures of the major clusters of both peptides are shown in Figure 2. These structures were used as starting structures in free MD calculations. Although the backbone of both peptides **3** and **4** remained rather rigid around the β-Acc residue, it showed relatively high dynamics in the RGD sequence, which is known for cyclic pentapeptides.[5] Therefore, the conformational analysis discussed herein is based on the structures obtained by restrained MD simulations as these are derived from experimental distance values.

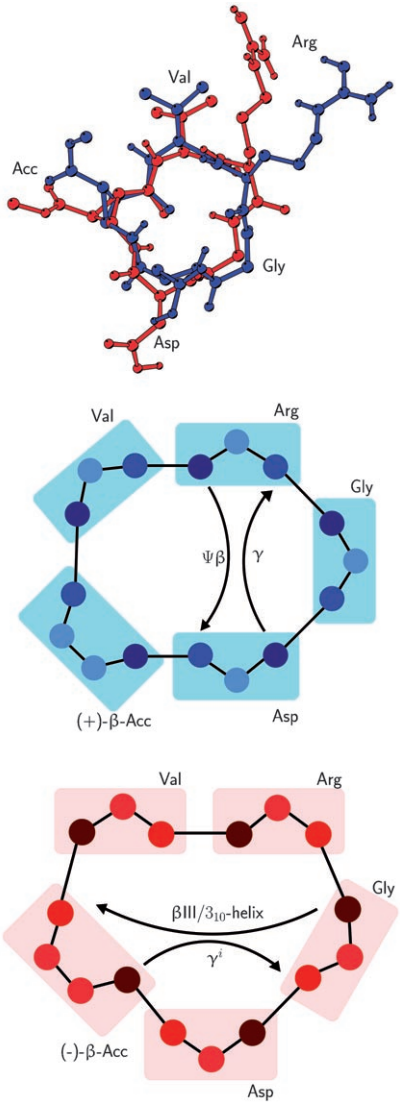

Figure 2. Overlay (top) and schematic representation (middle and bottom) of the structures of peptides **3** (blue) and **4** (red) as obtained by restrained MD calculations based on experimental distance information. Ψβ = pseudo-β turn. The arrows indicate the hydrogen bonds.

The distance between C^{β} atoms of Asp and Arg determines whether the RGD sequence is stretched or nonstretched. It has been shown previously that the RGD motif in peptide ligands of integrin $\alpha_V\beta_3$ is required to adopt a nonstretched conformation with a distance between the C^{β} atoms of Arg and Asp of about 650 pm.[10] As D-amino acids prefer the $i+1$ position of βII′ turns, the RGD sequence of cyclic pentapeptides like *cyclo*-(-Arg-Gly-Asp-D-Phe-Val-) adopts a nonstretched conformation with glycine in the central position of a γ turn, resulting in highly active integrin $\alpha_V\beta_3$ ligands with a distance of 668 pm between C^{β} atoms of Arg and Asp.[4,20] In another previously investigated peptide, *cyclo*-(-Arg-Gly-Asp-Phe-D-Val-), aspartic acid is found in the central position of a γ turn with an Arg to Asp C^{β} atom distance of 905 pm.[20] Therefore, the RGD sequence is more stretched, which accounts for the peptide being a somewhat less-active and less-specific ligand of integrin $\alpha_V\beta_3$. Reference peptide **2** is also characterized by an elongated conformation of the RGD sequence with a distance of 930 pm between C^{β} atoms of Arg and Asp, which correlates well with the lower affinity toward integrin $\alpha_V\beta_3$.[3]

With incorporated β-Accs, peptides **3** and **4** contain conformationally highly restrained β-amino acids. In the structure of peptide **3**, glycine is in the central position of a γ turn as judged by the torsion angles. Owing to the restrained cyclopropane system, the μ angle in (+)-β-Acc has to be close to zero. (+)-β-Acc occupies the $i+1$ position of a pseudo-β turn in **3**. In **4**, however, Asp lies in the central position of a γ^{i} turn. The structure has torsion angles like a 3_{10} helix between the amino acids (−)-β-Acc and Gly, thus adopting a conformation formerly known as the βIII turn with valine in the $i+1$ and arginine in the $i+2$ position.

For peptide **3**, the distance between the C^{β} atoms of Asp and Arg is 706 pm in the central structure of the major cluster. For peptide **4**, it is considerably longer (826 pm) although it fluctuates during the trajectory. However, for the majority of the structures of peptide **3**, this distance lies between 600 and 800 pm, and for peptide **4** between 700 and 900 pm. The distance between the C^{α} atoms of Arg and Asp is also shorter in peptide **3** than in peptide **4**. While it varies from about 525 to 650 pm during the trajectory in peptide **3**, most structures adopt values from 600 to 700 pm in peptide **4**. This indicates a more stretched conformation of the RGD sequence for peptide **4** than for peptide **3**. In **3**, the β and γ turns are at the same positions as in the highly active reference peptide *cyclo*-(-Arg-Gly-Asp-D-Phe-Val-) although the type of β turn differs between the two peptides. In contrast to **3**, **4** is similar to *cyclo*-(-Arg-Gly-Asp-Phe-D-Val-), displaying a more stretched RGD sequence like in **2**.

In summary, we have synthesized two new diastereomeric cyclic peptides with the new rigid building block β-Acc in opposite absolute configurations. The induced conformational restraint led to enhanced biological activity towards integrin $\alpha_V\beta_3$. The more active diastereomer *cyclo*-(-Arg-Gly-Asp-(+)-β-Acc-Val-) **3** inhibited binding of vitronectin to integrin $\alpha_V\beta_3$ with an IC_{50} value of 20 nM and displayed a tenfold higher activity than the reference peptide *cyclo*-(-Arg-Gly-Asp-D-Phe-Val-) **1**. Structural analysis has shown that the RGD sequence in **3** occupies a γ turn with the centrally positioned glycine. This finding, in combination with the biological tests, confirms the previously known structure–activity relationship, which indicates that the more active peptide towards $\alpha_v\beta_3$ is the one with the bent RGD sequence.

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[1] M. Kantlehner, D. Finsinger, J. Meyer, P. Schaffner, A. Jonczyk, B. Diefenbach, B. Nies, H. Kessler, *Angew. Chem.* **1999**, *111*, 587 – 590; *Angew. Chem. Int. Ed.* **1999**, *38*, 560 – 562.
[2] A. Meyer, J. Auernheimer, A. Modlinger, H. Kessler, *Curr. Pharm. Des.* **2006**, *12*, 2723 – 2747.
[3] D. Zimmermann, E. Guthöhrlein, M. Malešević, K. Sewald, L. Wobbe, C. Heggemann, N. Sewald, *ChemBioChem* **2005**, *6*, 272 – 276.
[4] R. Haubner, D. Finsinger, H. Kessler, *Angew. Chem.* **1997**, *109*, 1440 – 1456; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374 – 1389.
[5] J. Chatterjee, D. Mierke, H. Kessler, *J. Am. Chem. Soc.* **2006**, *128*, 15164 – 15172.
[6] F. Schumann, A. Müller, M. Koksch, G. Müller, N. Sewald, *J. Am. Chem. Soc.* **2000**, *122*, 12009 – 12010.
[7] M. Aumailley, M. Gurrath, G. Müller, J. Calvete, R. Timpl, H. Kessler, *FEBS Lett.* **1991**, *291*, 50 – 54.
[8] M. Gurrath, G. Müller, H. Kessler, M. Aumailley, R. Timpl, *Eur. J. Biochem.* **1992**, *210*, 911 – 921.
[9] M. A. Dechantsreiter, E. Planker, B. Mathä, E. Lohof, G. Hölzemann, A. Jonczyk, S. L. Goodman, H. Kessler, *J. Med. Chem.* **1999**, *42*, 3033 – 3040.
[10] E. Lohof, E. Planker, C. Mang, F. Burkhart, M. A. Dechantsreiter, R. Haubner, H.-J. Wester, M. Schwaiger, G. Hölzemann, S. L. Goodman, H. Kessler, *Angew. Chem.* **2000**, *112*, 2868 – 2871; *Angew. Chem. Int. Ed.* **2000**, *39*, 2761 – 2764.
[11] G. C. Tucker, *Curr. Oncol. Rep.* **2006**, *8*, 96 – 103.
[12] C. Bubert, C. Cabrele, O. Reiser, *Synlett* **1997**, 827 – 829.
[13] R. Beumer, C. Bubert, C. Cabrele, O. Vielhauer, M. Pietzsch, O. Reiser, *J. Org. Chem.* **2000**, *65*, 8960 – 8969.
[14] R. Beumer, O. Reiser, *Tetrahedron* **2001**, *57*, 6497 – 6503.
[15] C. Zorn, F. Gnad, S. Salmen, T. Herpin, O. Reiser, *Tetrahedron Lett.* **2001**, *42*, 7049 – 7053.
[16] S. De Pol, C. Zorn, O. Zerbe, O. Reiser, *Angew. Chem.* **2004**, *116*, 517 – 520; *Angew. Chem. Int. Ed.* **2004**, *43*, 511 – 514.
[17] N. Koglin, C. Zorn, R. Beumer, C. Cabrele, C. Bubert, N. Sewald, O. Reiser, A. G. Beck-Sickinger, *Angew. Chem.* **2003**, *115*, 212 – 215; *Angew. Chem. Int. Ed.* **2003**, *42*, 202 – 205.
[18] M. Malešević, U. Strijowski, D. Bächle, N. Sewald, *J. Biotechnol.* **2004**, *112*, 73 – 77.
[19] "Effiziente Konformationsanalyse von Peptiden": E. W. Guthöhrlein, PhD Thesis, Bielefeld University, **2006**.
[20] G. Müller, M. Gurrath, H. Kessler, *J. Comput.-Aided Mol. Des.* **1994**, *8*, 709 – 730.

