Type II′ to Type I β-Turn Swap Changes Specificity for Integrins

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The integrins represent a large class of cellular adhesion proteins, many of which bind to the Arg-Gly-Asp (RGD) sequence.[1] A prototypical RGD-dependent integrin is GPIIbIIIa ($\alpha_{IIb}\beta_3$), which mediates the aggregation of platelets, and numerous cyclic RGD peptides have been designed to have high affinity for GPIIbIIIa. These compounds are effective antithrombotics[2] and have also served as starting points for the design of nonpeptide antagonists of this receptor.[3]

Recently the related integrin $\alpha_v\beta_3$ has been recognized as a potentially important pharmaceutical target, involved in neovascularization.[4] Inhibitors of this process could be of benefit for treating cancer, diabetic retinopathy, and macular degeneration.[5] $\alpha_v\beta_3$ has been hypothesized to recognize a conformation of RGD in which the Arg and Asp side chains are much closer than when bound to GPIIbIIIa.[2f] In this paper we provide strong support for this hypothesis and, additionally, show that the turn types incorporating the RGD sequence can play an important role in modulating this distance and hence the specificity.

Figure 1 illustrates the conformation of DMP728, **1**, a member of a family of peptides of sequence *cyclo*(D-Xxx-NMeArg-Gly-Asp-Mamb).[6] These peptides bind with extremely high selectivity and affinity to GPIIbIIIa (Table 1).[7] Both solution and solid state structures show a type II′ β-turn centered at the D-Xxx-NMeArg dipeptide.[8] Replacement of the D-Xxx-NMeArg dipeptide[9] with L-Ala-L-Arg resulted in a striking reversal in selectivity to favor binding to $\alpha_v\beta_3$ (**3** in Table 1). In cyclic peptides, a dipeptide consisting of a D-amino acid followed by an N^{α}-methylamino acid is a strong inducer of type II′ turns, while an L,L dipeptide in the same position favors a type I turn.[10] Thus, a change in turn type might modulate receptor specificity. To test this possibility, we prepared **4** and **5**, which contain the turn-stabilizing Aib and L-Pro in place of L-Ala.[2a,9] **4** and **5** are also highly potent and selective $\alpha_v\beta_3$ ligands (Table 1).

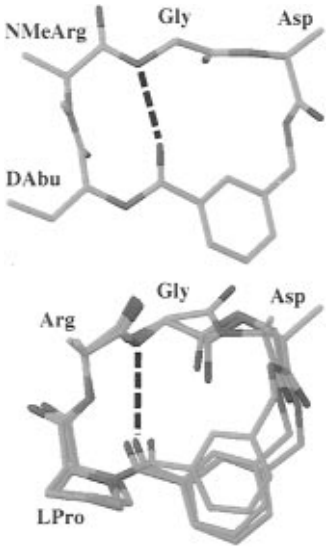

Figure 1. Top: Solution and solid state conformation of **1**, featuring a type II′ β-turn centered at D-Abu-NMeArg. Bottom: Centroids of conformational families of **5**, showing the type I β-turn centered at Pro-Arg. The NMeArg and Asp side chains have been truncated in both panels.

Table 1. Sequence, Activity and β-Turn Type for RGD-Containing Cyclic Peptides

compd	X-Xxx-Y-Yyy	GPIIbIIIa[a] (nM)	$\alpha_v\beta_3$[b] (nM)	ratio[c]	turn
1	D-Abu-NMeArg	2	500	250	II′
2	D-Ala-NMeArg	8	500	63	II′
3	L-Ala-Arg	18000	20	0.001	I
4	Aib-Arg	9000	36	0.004	I[d]
5	L-Pro-Arg	45000	13	0.0003	I

[a] IC_{50}, platelet aggregation.[7] [b] IC_{50}, Elisa.[7] [c] $\alpha_v\beta_3$ activity/GPIIbIIIa activity. [d] Assumed turn type.

The solution structure of **3** has been determined using NMR derived distance constraints with distance geometry and energy minimization (DG/EM).[8] Here we determine the structure of the most active $\alpha_v\beta_3$ ligand **5**.[11] Three low-energy conformers were found (Figure 1 and Table 2), each with a type I β-turn centered at Pro-Arg. The ϕ, ψ angles of **5** are closer to an ideal canonical type I turn than in the less active $\alpha_v\beta_3$ ligand, **3**.[8,10] The presence of a type I turn was supported by a low-temperature dependence for the Gly NH ($\Delta\delta/\Delta T = -3.0$ ppb/

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(6) NMeArg: *N*-Methylarginine. Mamb: *m*-(Aminomethyl)benzoic acid. D-Xxx: A small, aliphatic amino acid such as D-Ala (**2**), D-Pro, etc. Aib: α-Aminoisobutyric acid.

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(11) The NMR spectrum of **5** was completely assigned at 600 MHz using TOCSY[12] experiments in D_2O and H_2O/D_2O (90:10). Thirty-one NOEs were obtained from a 180 ms ROESY[13] experiment in H_2O/D_2O at 10 °C. An additional 230 anti-distance constraints were also used in the DG/EM calculations.[14] Time-averaged MD *in vacuo* were performed using GROMOS.[15] Starting conformations for the GROMOS calculations came from the results of the DG/EM calculations.

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Table 2. ϕ and ψ Angles for the RGD Cyclic Peptides

	x-Xxx		NMeArg/Arg		$\beta-\beta^a$	twist[b]
family	ϕ	ψ	ϕ	ψ	(Å)	(deg)
1[c]	52	−112	−117	55	9.6	−30
II′ β-turn	60	−120	−80	0		−3
5A	−70	−24	−116	44	6.3	36
5B	−63	−22	−102	52	8.8	57
5C	−62	−27	−121	47	6.6	36
I β-turn	−60	−30	−90	0		49
6[d]	−58	−46	−128	73	6.7	18

[a] Distance between Cβ's of NMeArg/Arg and Asp. [b] Virtual dihedral angle.[19] [c] Data from ref 8. [d] Data from ref 2g.

K).[16] The conformers of **3** and **5** are highly similar with a 1.1 Å RMSD for the L-Xxx-Arg-Gly-Asp backbone atoms and 0.5 Å RMSD for the dipeptide spanning the turn. We also used time-averaged molecular dynamics[17] to study **5**. Conformations similar to those from the DG/EM calculations in the turn region were obtained.[18]

Thus, all evidence indicates that **5** adopts a type I turn (Table 2), showing that a switch of turn type from II′ to I accompanies a change in receptor specificity. A major difference between type I and II′ turns is their twist.[19] The twist of **5** ranges from 36° to 57° with an average of 43°, while the corresponding twist of **1** is −30° (Table 2). By comparison, the ideal twist for a type I turn is 49° and for a type II′ turn is −3°. The more negative value for **1** results from steric interactions between the *N*α-methyl group (which is essential for high GPIIbIIIa affinity) and the Arg side chain.

The consequence of these different twists is that the Arg side chain of **3** and **5** is raised above the plane formed by the backbone into a pseudoaxial position, while the NMeArg side chain in **1** adopts a pseudoequatorial conformation (Figure 2). Thus, the vectorial and spatial relationships of the Arg and Asp side chains differ between specific $\alpha_v\beta_3$ and GPIIbIIIa ligands, with a closer approach for the $\alpha_v\beta_3$ antagonists **3** and **5** (Figure 2 and Table 2).

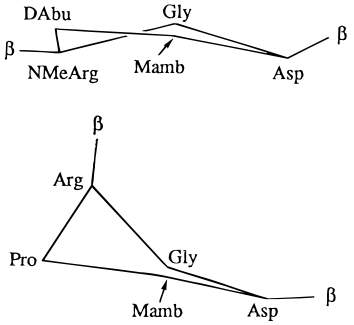

Figure 2. Cα backbone traces of **1** (top) and the centroid of family **5B** (bottom) showing their different twists. The Mamb C-terminal aromatic carbon is used as the Cα. The different orientations of the NMeArg/Arg Cα−Cβ bond vector are discussed in the text.

To further characterize the conformation of $\alpha_v\beta_3$ antagonists we compared the low-energy conformers of **3** and **5** with an $\alpha_v\beta_3$-specific compound: *cyclo*(Arg-Gly-Asp-D-Phe-Val) (**6**, IC_{50} = 50 nM from in-house $\alpha_v\beta_3$ Elisa).[2b,g,20] One conformer each of **3** and **5** overlays well with the solution conformation of **6** (Table 2) with RMSDs of 0.7 and 0.9 Å, respectively. Each displays a distorted type I β-turn centered at the Xxx-Arg dipeptide.[21] Thus a type I turn again appears to be important for orienting the Arg side chain for tight and selective binding to the receptor. It is likely that this type I turn is not necessary for binding, but instead simply serves as a convenient scaffolding for positioning the essential functional groups in proper juxtaposition. If we assume that the solution conformations observed in these two previous studies[2g,8] correspond to the integrin-bound conformation, then these conformations will serve as convenient starting points for the design of nonpeptide antagonists of $\alpha_v\beta_3$.

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Supporting Information Available: Assay methods, integrin purification, tables of NMR data, and complete dihedral angles (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(18) Analysis of the trajectories showed that hydrogen bonds appear between the Mamb C═O and Gly NH in >50% of the conformers from each trajectory. This hydrogen bond is consistent with a type I β-turn. A hydrogen bond also occurs at >40% between the Gly C═O and the Mamb NH, producing a γ-turn centered at Asp. The most frequently occurring hydrogen bond (>75%) in two trajectories is between the Asp NH and the Arg C═O, producing a γ-turn centered on Gly.
(19) Twist is the virtual dihedral angle formed by the four α-carbons of the turn.
(20) IC_{50}s of 20−150 nM have been reported fo **6**.[2f]
(21) Although Müller *et al.*[2b] have emphasized the presence of a type II′ turn centered at the D-Phe-Val dipeptide, visual inspection of their structure suggested the coexistence of a distorted type I turn centered at Val-Arg. Indeed, a model of **6** built with published dihedral angles confirms this finding.[2g]