Determination of the binding epitope of RGD-peptidomimetics to $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrin-rich intact cells by NMR and computational studies

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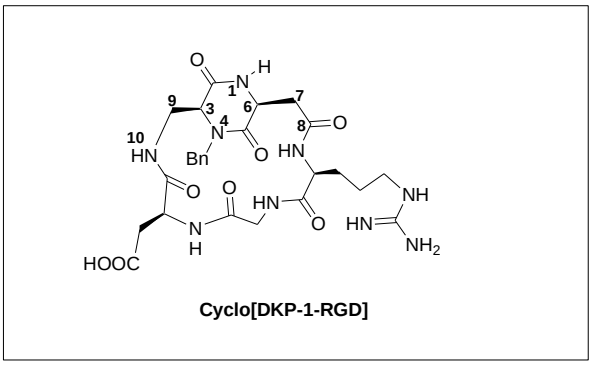
Supplementary information

NMR data

The spectra were performed on a Bruker Avance 600 MHz. Each sample was prepared in a 3 mm NMR tube with 0.6 mg of ligand and $6x10^6$ cells in 200 µL of 20 mM phosphate buffer containing additionally 157 mM NaCl, 6 mM KCl and 4 mM $CaCl_2$.

Compound 1



Cyclo[DKP-1-RGD]

Table 1. ^{1}H chemical shifts (δ in ppm) of compound **1** in buffer solution in presence of ECV cells suspension at T=298K and T=282K

Compound 1 / Protons	298 K (δ (ppm), multiplicity,J(Hz))	282 K (δ (ppm), multiplicity,J(Hz))
NH_Arg	8.45 (s br)	8.55 (s br)
αH-Arg	4.04 (s)	4.04 (s)
βH-Arg	1.82 -1.66 (m)	1.80-1.66 (m)
γH-Arg	1.62 (m)	1.61 (m)
δH-Arg	3.18 (s)	3.18 (dd,15.4-5.8Hz)
NH_guan	7.23 (br)	7.25 (s br)
NH_Gly	8.72 (br)	8.83 (t, 6.0Hz)
αH-Gly	4.03-3.68 (d, 20 Hz)	4.04-3.68 (d, 17.80 Hz)
NH_Asp	8.08 (d, 8.1 Hz)	8.13 (d, 4.31Hz)
αH-Asp	4.40 (br)	4.39 (s br)
βH-Asp	2.59 (dd, 15-4 Hz)-2.40 (dd, 15-4 Hz)	2.59 (dd,16.0-9.9 Hz)-2.40 (dd,16.0-9.9 Hz)
DKP_NH1	8.45 (s br)	8.55 (s br)
DKP_NH10	7.54 (s br)	7.57 (s br)
DKP_H9	3.95-3.50 (dd, 16-3 Hz)	3.95 (d, 18.0 Hz)-3.50 (d, 18.0 Hz)
DKP_H3	4.32 (br)	4.33 (s)
DKP_H7	3.16-2.86 (dd, 18.7-5.3 Hz)	3.16 (d,18.5Hz)-2.89 (dd,17.5-3.9 Hz)
DKP_H6	4.51 (s br)	4.51 (s)
CH_2-Bn	- (deleted by water saturation)	- (deleted by water saturation)
H-Ar	7.38-7.30	7.36-7.31

Compound 2

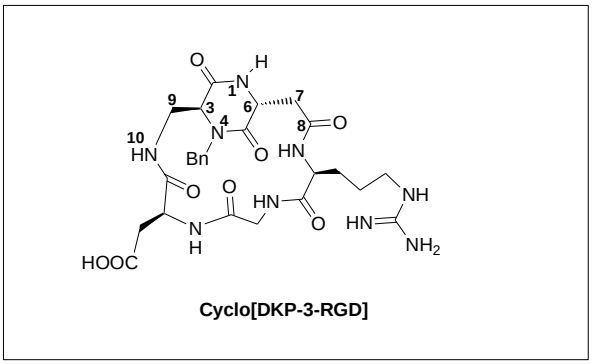


Table 2. ^{1}H chemical shifts (δ in ppm) of compound **2** in buffer solution in presence of ECV cells suspension at T=298K and T=282K

Compound 2 / Protons	298 K (δ (ppm), multiplicity,J(Hz))	282 K (δ (ppm), multiplicity,J(Hz))
NH_Arg	8.74 (d, 8.4 Hz)	8.83 (d, 8.5 Hz)
αH-Arg	4.30 (br)	4.30 (q, 9.1 Hz)
βH-Arg	1.93-1.74 (m)	1.92-1.74 (m)
γH-Arg	1.60 (m)	1.61 (m)
δH-Arg	3.19 (br)	3.19 (m)
NH_guan	7.16 (br)	7.20 (t, 5.4 Hz)
NH_Gly	8.11 (s br)	8.15 (dd,4.6-7.8 Hz)
αH-Gly	4.24(dd, 15.5-7.1 Hz)-3.65 (d, 15.5 Hz)	4.24 (dd, 18.0-7.9 Hz)-3.65(dd, 18.0-3.6 Hz)
NH_Asp	8.07 (s br)	8.09 (d, 8.3 Hz)
αH-Asp	4.45	4.71 (s br)
βH-Asp	2.59(dd, 16.2-6.5 Hz)-2.56 (dd, 16.2-6.5 Hz)	2.59 (dd, 16.5-6.4Hz)-2.56 (dd, 16.5-6.4Hz)
DKP_NH1	8.11 (s br)	8.18 (s)
DKP_NH10	8.18 (t, 6.5 Hz)	8.30 (t, 6.8Hz)
DKP_H9	3.97(dd, 17.5-7.8 Hz)-3.57 (dd, 17.5-7.8 Hz)	3.97 (dd, 15.7-7.3 Hz)-3.58(m)
DKP_H3	4.05	4.05 (s br)
DKP_H7	2.81(dd, 14.2-8.4 Hz)-2.68 (dd, 14.2-8.4 Hz)	2.82 (dd, 13.9-8.5 Hz)-2.69 (dd, 13.9-8.5 Hz)
DKP_H6	- (deleted by water saturation)	- (deleted by water saturation)
CH_2-Bn	5.10(d, 16.2 Hz)-4.10 (d, 16.2 Hz)	5.12 (d,17.1Hz)-4.10 (d, 17.0Hz)
H-Ar	7.41-7.28	7.38-7.29

Compound 3

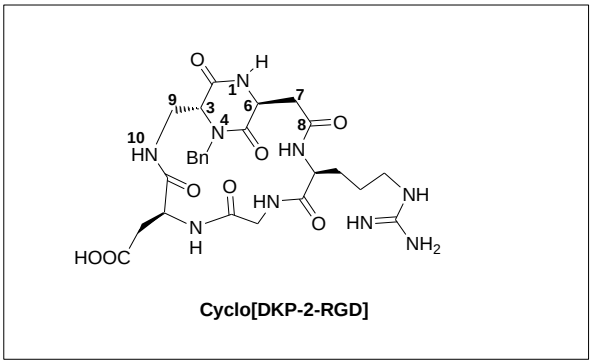


Table 3. ^{1}H chemical shifts (δ in ppm) of compound **3** in buffer solution in presence of ECV cells suspension at T=298K and T=282K

Compound 3 / Protons	298 K (δ (ppm), multiplicity,J(Hz))	282 K (δ (ppm), multiplicity,J(Hz))
NH_Arg	8.62 (s br)	8.72 (d,7.1Hz)
αH-Arg	4.19 (m)	4.18 (m)
βH-Arg	1.80-1.70 (m)	1.79-1.70 (m)
γH-Arg	1.63 (m)	1.64 (m)
δH-Arg	3.15 (t, 6.2 Hz)	3.15 (m)
NH_guan	7.12 (s br)	7.18 (s br)
NH_Gly	8.18 (s br)	8.26 (d, 10.0 Hz)
αH-Gly	4.09-3.50 (m)	4.10 (m) -3.48 (d, 18.8Hz)
NH_Asp	8.27 (s br)	8.40 (d, 8.5Hz)
αH-Asp	- (deleted by water saturation)	4.63 (m)
βH-Asp	2.58 (dd, 15.9-7.5 Hz)-2.50 (dd, 15.9-7.5 Hz)	2.60 (dd, 15.4-6.8 Hz)-2.49 (dd, 15.4-6.8 Hz)
DKP_NH1	8.60 (s br)	8.50 (s)
DKP_NH10	8.62 (s br)	8.79 (m)
DKP_H9	4.24 (br)-3.32	3.32 (m)
DKP_H3	4.29 (br)	4.33 (dd, 17.10-6.8 Hz)
DKP_H7	2.90 (dd, 15.7- 6.8 Hz)-2.38 (d, 15.7 Hz)	2.87 (dd,14.5-10.3Hz)-2.39 (d,14.5 Hz)
DKP_H6	4.60 (br)	4.65
CH_2-Bn	5.03(d, 16.56 Hz)-4.10	- (deleted by water saturation)
H-Ar	7.38-7.28	7.41-7.33

Table 4. Observed significative NOE contacts of compound **1-2-3** in buffer solution in presence of ECV cells suspension

Compounds / Protons	**Compound 1**	**Compound 2**	**Compound 3**
NH-Arg	DKP-H7	NH-Gly, DKP-H7	DKP-H7
αH-Arg			
βH-Arg			
γH-Arg			
δH-Arg			
NH_Guan			
NH_Gly	NH_Asp, γH-Arg	NH-Arg, αH-Arg	
αH-Gly	NH-Asp		NH-Asp
NH_Asp	αH-Gly	αH-Gly	αH-Gly
αH-Asp	NH-Asp, βH-Asp		
βH-Asp		DKP-NH10	
DKP_NH1			
DKP_NH10	βH-Asp	αH-Asp, βH-Asp	αH-Asp
DKP_H9	DKP-NH10		
DKP_H3			
DKP_H7	NH-Arg	NH-Arg	NH-Arg
DKP_H6			
CH_2-Bn			
H-Ar			

Computational methods

Docking calculations were performed by using Glide[1] (Grid-based Ligand Docking with Energetics) through the Maestro graphical interface of the Schrödinger suite of programs (http://www.schrodinger.com).

Integrin $\alpha_V\beta_3$ preparation

The crystal structure of the extracellular segment of integrin $\alpha_v\beta_3$ in complex with the cyclic pentapeptide ligand Cilengitide (PDB entry 1L5G)[2] was used for docking studies. Docking was performed only on the globular head of the integrin because the headgroup of integrin has been identified in the X-ray structure as the ligand-binding region. The protein structure was setup for docking as follows. The protein was truncated to residue sequences 41–342 for chain α and 114–347 for chain β. Due to a lack of parameters, the Mn^{2+} ions in the experimental protein structure were modeled by replacing them with Ca^{2+} ions. The resulting structure was prepared by using the Protein Preparation Wizard of the graphical user interface Maestro and the OPLSAA force field.

Integrin $\alpha_{IIb}\beta_3$ preparation

The recently solved crystal structure of the integrin $\alpha_{IIb}\beta_3$ headpiece bound to the cyclic heptapeptide Eptifibatide (PDB entry code 2VDN)[3] was used for docking studies. Docking was performed only on the globular head of the integrin because the headgroup of integrin has been identified in the X-ray structure as the ligand-binding region. The protein structure was setup for docking as follows. The protein was truncated to residue sequences 18-452 for chain α and 108-352 for chain β. The coordination shell of metal cation belonging to MIDAS (metal-ion-dependent adhesion site) and ADMIDAS (adjacent to MIDAS) in the β chain was completed on the basis of the integrin $\alpha_{IIb}\beta_3$ X-ray structures[3] by adding two and one water molecules, respectively. The Mg^{2+} ion at MIDAS in the experimental protein structure was modeled by replacing it with a Ca^{2+} ion. The resulting structure was prepared using the Protein Preparation Wizard of the graphical user interface Maestro and the OPLSAA force field.

Docking calculations

The automated docking calculations were performed using Glide (Grid-based Ligand Docking with Energetics)[1] within the framework of Impact version 4.5 in a standard precision mode (SP). The grid generation step started from the X-ray structures of $\alpha_v\beta_3$ complexed with Cilengitide and of $\alpha_{IIb}\beta_3$ complexed with Eptifibatide, prepared as described in the protein setup section. The center of the grid enclosing box was defined by the center of the bound ligand, as described in the original PDB entry. The enclosing box dimensions, which are automatically deduced from the ligand size, fit the entire active site. For the docking step, the size of the bounding box for placing the ligand center was set to 12 Å. No further modifications were applied to the default settings. The GlideScore function was used to select 20 poses for each ligand. The Glide program was initially tested for its ability to reproduce the crystallized binding geometries of Cilengitide in $\alpha_v\beta_3$ integrin,[2] and of Eptifibatide, Tirofiban and L739758 in $\alpha_{IIb}\beta_3$ integrin.[3] The program was successful in reproducing the experimentally determined binding modes of these compounds, as they correspond to the best-scored poses.

1. Glide, version 4.5, 2007, Schrödinger, LLC, New York, NY (USA).
2. J.-P. Xiong, T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S. L. Goodman, M. A. Arnaout, Science, 2002, 296, 151-155.
3. T. A. Springer, J. Zhu, T. Xiao, J. Cell Biol. 2008, 182, 791-800.