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Structural studies of α5β1 integrin ectodomain

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Introduction

Integrins are cell adhesion receptors that transmit bidirectional signals across the plasma membrane and link the extracellular environment to the actin cytoskeleton [1]. All integrins are non-covalently linked heterodimeric molecules consists of one α and one β subunit. α5β1 integrin is a prototypic integrin that functions as receptor for the extracellular matrix protein fibronectin. Despite of the extensive studies of the interaction between integrin and fibronectin, the exact structural information between them has not been determined yet.

We report here the crystal structure of α5β1 integrin ectodomain in complex with inhibitory anti-β1 monoclonal antibody, SG/19 [2]. We further obtained the crystal structure of the ternary complex of α5β1 integrin-SG/19-RGD peptide by soaking method. These results provide structural insight for the specific ligand recognition by intergrin.

Experimental Procedure

The recombinant α5β1 integrin ectodomain was expressed using CHO-lec3.2.8.1 cells. The soluble α5β1 integrin was purified from culture supernatant by ammonium sulfate precipitation and affinity chromatography for basic tail. SG/19 IgG was purified from mouse hybridoma cell culture supernatant using protein-A column. Then SG/19 Fab fragment was generated by papain proteolysis using immobilized papain sepharose and further purified by pritein-A column. α5β1 integrin ectodomain in complex with SG/19 Fab was purified by gel filtration chromatography. Purified protein was finally concentrated up to 6 mg/ml for crystallization.

Crystallization was performed by hanging drop vapor diffusion method. The best crystals were obtained under the condition of 0.1 M Bis-tris (pH 6.5), 20 % PEG8000 at 293 K. The reproducibility of the crystals was greatly improved by the addition of microseed crystals.

Synchrotron diffraction data was collected at BL-17A at Photon Factory. The data was processed and scaled using HKL2000 program suite [3].

Phase determination was performed by molecular replacement method using the program MOLREP [4]. Model reconstruction was conducted manually with COOT [5]. Crystallographic refinement was performed using the program REFMAC5 [6]. The validation of the refined structure was assessed using the program MOLPROBITY [7].

Results and Discussion

Overall structure was refined at 2.9 Å resolution. The structure contains β-propeller and thigh domains of α5 subunit and the plexin/semaphorin/integrin (PSI), hybrid, and βA domain of β1, and SG/19 Fab (Figure 1). Two α5β1 integrin /Fab complexes were contained in one asymmetric unit, which were essentially identical except for subtle difference in inter domain angles. The allosteric inhibitory antibody SG/19 makes extensive interaction with the long loop of hybrid domain and prevents the swing-out movement. We purified α5β1 integrin in the physiological conditions (in the presence of calcium and magnesium ions) and thus identified three metal ions on the top of βA domain in β1 subunit. Based on the coordination geometry and the similarity to β3, we assigned one magnesium and two calcium ions.

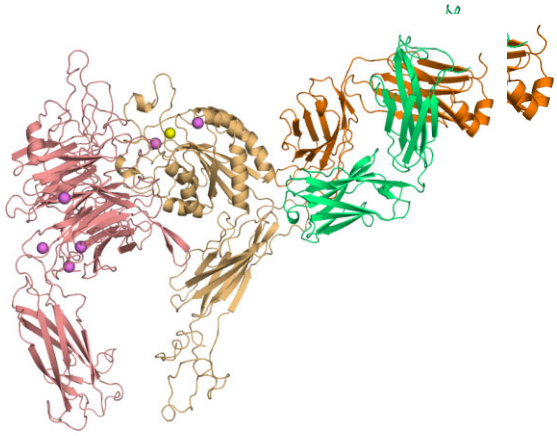

Figure 1 Overall structure of α5β1 integrin SG/19 complex.

References

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