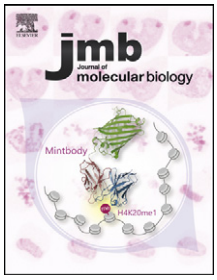

Interaction Analyses of the Integrin β2 Cytoplasmic Tail with the F3 FERM Domain of Talin and 14-3-3ζ Reveal a Ternary Complex with Phosphorylated Tail

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Abstract

Integrins, which are heterodimeric (α and β subunits) signal-transducer proteins, are essential for cell adhesion and migration. β cytosolic tails (β-CTs) of integrins interact with a number of cytosolic proteins including talin, Dok1, and 14-3-3ζ. The formation of multiprotein complexes with β-CTs is involved in the activation and regulation of integrins. The leukocyte-specific β2 integrins are essential for leukocyte trafficking, phagocytosis, antigen presentation, and proliferation. In this study, we examined the binding interactions between integrin β2-CT and T758-phosphorylated β2-CT with positive regulators talin and 14-3-3ζ and negative regulator Dok1. Residues of the F3 domain of talin belonging to the C-terminal helix, β-strand 5, and the adjacent loop were found to be involved in the binding interactions with β2-CT. The binding affinity between talin F3 and β2-CT was reduced when β2 T758 was phosphorylated, but this modification promoted 14-3-3ζ binding. However, we were able to detect stable ternary complex formation of T758-phosphorylated β2-CT, talin F3, and 14-3-3ζ that involved the repositioning of talin F3 on β2-CT. We showed that Dok1 binding to β2-CT was reduced in the presence of 14-3-3ζ and when β2 T758 was phosphorylated. Based on these data, we propose a sequential model of β2 integrin activation involving these molecules. Our study provides for the first time insights toward β2 integrin activation that involves a multiprotein complex.

Introduction

Integrins are transmembrane receptors that mediate cell–cell and cell–extracellular matrix adhesions [1]. Integrins are obligate heterodimers composed of non-covalently linked α and β subunits. Each subunit contains a large extracellular domain, a single-pass transmembrane domain, and a short cytoplasmic tail (CT) [1]. Integrin inside-out activation is a process by which the disruption of the interface between the membrane-proximal (MP) regions of the α and β subunits and the cytoplasmic tails leads to the separation of their transmembrane domains, followed by global conformational changes in the extracellular regions [2]. The integrin β-CTs, excluding β4 and β8, contain two highly conserved NPxY/F motifs. Both motifs are docking sites for cytoplasmic proteins that regulate integrin activation [3].

Talin-1 (henceforth referred to as talin) is a large cytoskeletal protein composed of a 4.1-ezrin-radixin-moesin (FERM)-containing head region and a long rod region [4]. Talin is a well-established positive regulator of integrin activation. It binds directly to the integrin β-CTs and disrupts the MP interface of the cytoplasmic tails [5]. The phosphotyrosine binding (PTB) fold in the F3 subdomain of talin binds to the MP NPxY/F motif of the integrin β-CT [6]. It has been reported that the phosphorylation state of the integrin β3 MP NPLY functions as a switch that regulates the binding of either talin or Dok1, a cytoplasmic negative regulator of integrin activation [7]. Recently, it has been demonstrated that an alternative phosphorylation switch involving two Ser residues flanking the integrin β2 MP NPLF motif served a similar function [8]. These data exemplify the relevance of integrin β tail post-translational modification(s) as a part of the mechanism by which fine-tuned regulation of integrin activation could be achieved.

The leukocyte-restricted integrin β2 subfamily contains four members that share a common β2

subunit, namely αLβ2, αMβ2, αXβ2, and αDβ2 [3]. The β2-CT contains a triplet Thr motif that lies in between its two NPxF motifs. The phosphorylation status of this triplet Thr motif modulates the association of the integrin β2-CT with cytoplasm proteins. 14-3-3 family of proteins is known to bind pSer/Thr-containing sequences, and they form dimers [9–11]. 14-3-3ζ binds the pTTT motif in the integrin β2-CT, and it serves as a positive regulator of β2 integrin-ligand binding [12,13]. Structural data revealed a 14-3-3ζ homodimer in complex with two pTTT-containing integrin β2 tail peptides, suggesting that 14-3-3ζ can positively regulate β2 integrins functions by promoting receptor clustering [14]. Notably, the same study also showed that the phosphorylation of Thr758 (the first residue of the triplet Thr) in the integrin β2-CT promotes 14-3-3ζ but inhibits filamin binding [14]. Phosphorylation of the triplet Thr in the integrin β7-CT has also been shown to reduce filamin binding, a negative regulator of integrin activation, but not talin binding [15]. Furthermore, β2-CT phosphorylation of residue S756 close to the NPLF754 and T^{758}TT motif recruits Dok1, a negative regulator of integrin activation [8]. Taken together, phosphorylation of the triplet Thr motif and adjacent Ser756 in the integrin β2-CT serves like a switch that regulates the docking of 14-3-3ζ, filamin A, and Dok1 with opposing functions with respect to integrin activation.

Mechanistically, the dissociation of filamin A from the integrin β-CT, followed by the docking of positive regulators such as 14-3-3ζ and talin, is well rationalized and supported by available data. However, it remains to be clarified if 14-3-3ζ, talin, and Dok1 with binding sites that are close to each other on integrin β2-CT can form ternary complexes or if their interactions with the integrin β2-CT are mutually exclusive. There is also limited information on how the phosphorylation of the triplet Thr in the integrin β2-CT regulates the binding of 14-3-3ζ in the presence of talin and vice versa. In this work, we set out to understand the interactions among talin F3 domain, 14-3-3ζ, and Dok1 with β2-CT. Our results showed that talin F3 domain exhibits different affinities for β2-CT and pTβ2-CT by employing residues located at the C-terminal long helix, β-strand 5, and the adjoining loop region. Ternary complex formation of 14-3-3ζ, talin F3 domain, and pTβ2-CT was examined. Based on the docked structure of the ternary complex, the MP helix of pTβ2-CT interacts with the talin F3 domain, and its C-terminal region containing residue pT758 occupies the canonical binding pocket of 14-3-3ζ. These results provide important insights for multiprotein complex formation between β2-CT and its interacting partners that are relevant to the activation mechanism of β2 integrins.

Results

The F3 domain of talin interacted with β2-CT using the canonical binding pocket

Backbone ^{15}N–^{1}H correlations of talin F3 were assigned by combined analyses of 3D HNCACB and CBCA(CO)NH spectra. A series of ^{15}N–^{1}H HSQC spectra of F3 were acquired in the presence of different concentrations and molar ratios, 1:0, 1:0.4, 1:0.8, 1:1.2, 1:1.6, 1:2, 1:3, 1:4, and 1:5, of synthetic β2-CT. Table 1 provides a list of synthetic β2-CTs used in this study. ^{15}N–^{1}H HSQC spectra overlay of talin F3 in free solution (blue contour) and in the presence of β2-CT at 1:1 (red contour) and 1:3 (brown contour) molar ratios is shown (Fig. 1a). ^{15}N–^{1}H HSQC spectra of talin F3 showed chemical shift changes and also the broadening of resonances that suggest binding interactions. Residues of talin F3 located at the central segment (e.g., E350, W351, L353, T354, and N355) and a continuous stretch of C-terminal region (e.g., A^{389}QLIAGY^{395}) exhibited pronounced chemical shift changes in the presence of β2-CT (Fig. 1b). Additionally, ^{15}N–^{1}H cross-peaks of a number of residues at the central region of talin F3 were broadened due to its binding to β2-CT (Fig. 1b). An apparent dissociation constant (K_d) value of 243.8 μM of talin F3/β2-CT complex was estimated from the chemical shift changes of talin F3 (Fig. 1c and Table 2). The β2-CT-induced chemical shift changes (>0.04 ppm) and resonance broadening effect were mapped onto the 3D structure of talin F3 (Fig. 1d). The 3D

Table 1. Primary structures of synthetic β2-CT and β2-CT peptide fragments used in this study

Name	Residues	Sequence
β2-CT	724–769	K-A-L-I-H-L-S-D-L-R-E-Y-R-R-F-E-K-E-K-L-K-S-Q-W-N-N-D-N-P-L-F-K-S-A-T-T-T-V-M-N-P-K-F-A-E-S
pTβ2-CT	724–769	K-A-L-I-H-L-S-D-L-R-E-Y-R-R-F-E-K-E-K-L-K-S-Q-W-N-N-D-N-P-L-F-K-S-A-**pT**-T-T-V-M-N-P-K-F-A-E-S
pTDS20	751–769	D-N-P-L-F-K-S-A-**pT**-T-T-V-M-N-P-K-F-A-E-S
KS33	724–757	K-A-L-I-H-L-S-D-L-R-E-Y-R-R-F-E-K-E-K-L-K-S-Q-W-N-N-D-N-P-L-F-K-S
pTβ2WA	724–769	K-A-L-I-H-L-S-D-L-R-E-Y-R-R-F-E-K-E-K-L-K-S-Q-**A**-N-N-D-N-P-L-F-K-S-A-**pT**-T-T-V-M-N-P-K-F-A-E-S
pTβ2FA	724–769	K-A-L-I-H-L-S-D-L-R-E-Y-R-R-F-E-K-E-K-L-K-S-Q-W-N-N-D-N-P-L-**A**-K-S-A-**pT**-T-T-V-M-N-P-K-F-A-E-S
pSpTβ2-CT	724–769	K-A-L-I-H-L-S-D-L-R-E-Y-R-R-F-E-K-E-K-L-K-S-Q-W-N-N-D-N-P-L-**A**-K-**pS**-A-**pT**-T-T-V-M-N-P-K-F-A-E-S

Phosphorylated residues and Ala replacements are set in boldface.