FEBS Letters 584 (2010) 2237–2241

FEBS Letters

journal homepage: www.FEBSLetters.org

The domain structure of talin: Residues 1815–1973 form a five-helix bundle containing a cryptic vinculin-binding site

Benjamin T. Goult [a], Alexandre R. Gingras [a], Neil Bate [a], Igor L. Barsukov [b], David R. Critchley [a], Gordon C.K. Roberts [a,*]

[a] Department of Biochemistry, University of Leicester, Lancaster Road, Leicester, LE1 9HN, UK
[b] School of Biological Sciences, University of Liverpool, Crown Street, Liverpool, L69 7ZB, UK

ARTICLE INFO

Article history:
Received 3 February 2010
Revised 24 March 2010
Accepted 8 April 2010
Available online 20 April 2010

Edited by Judit Ovádi

Keywords:
Talin
Vinculin
NMR
Domain structure
Helical bundle

ABSTRACT

Talin is a large flexible rod-shaped protein that activates the integrin family of cell adhesion molecules and couples them to cytoskeletal actin. Its rod region consists of a series of helical bundles. Here we show that residues 1815–1973 form a 5-helix bundle, with a topology unique to talin which is optimally suited for formation of a long rod such as talin. This is much more stable than the 4-helix (1843–1973) domain described earlier and as a result its vinculin binding sequence is inaccessible to vinculin at room temperature, with implications for the overall mechanism of the talin-vinculin interaction.

Structured summary:
MINT-7722300, MINT-7760951: *Talin-1* (uniprotkb:P26039) and *Vinculin* (uniprotkb:P12003) *bind* (MI:0407) by *molecular sieving* (MI:0071)

1. Introduction

Talin is a large cytoskeletal protein (2541 amino acid residues) that activates the integrin family of cell adhesion molecules and couples them to cytoskeletal actin [1]. It is composed of an N-terminal globular head (~50 kDa) that interacts with the cytoplasmic tails of β-integrins [2], linked to an extended flexible rod (~220 kDa) comprising 62 helices organized into a series of helical bundles arranged like beads on a string [3] (Fig. 1A). The rod contains a second integrin binding site [4], numerous putative binding sites for the cytoskeletal protein vinculin [3], at least two actin binding sites [5,6], and a C-terminal helix required for assembly of talin dimers [5,7]. Talin exists in both an extended and a compact auto-inhibited form, and a domain in the talin rod (residues 1655–1822) interacts with the talin head rendering it unable to bind integrin tails [8].

While it is clear that the talin rod consists of a series of helical bundles the definition of the boundaries between these domains has not been straightforward. The first structures of the talin rod revealed a 5-helix bundle packed against a 4-helix bundle (residues 482–789) [9], and two 4-helix bundles (residues 755–889 and 1843–1973) [10,11]. Among helical bundle domains, 4-helix bundles are among the most frequently observed, with an up-down-up-down topology such as that seen in talin being particularly common (structural classification of proteins (SCOP) ID 47161: http://scop.mrc-lmb.cam.ac.uk/scop/data/scop.b.b.dg.html; [12]). However, our subsequent work showed that the domains towards the C-terminal end of the rod, spanning residues 1974–2482, are in fact 5-helix bundles [4,5,8], and this led us to re-examine the boundaries of the preceding domain. Here we show that this domain is actually a 5-helix bundle (residues 1815–1973) rather than a 4-helix bundle, and that the extra helix dramatically enhances the stability of the domain and renders the vinculin-binding site contained therein cryptic.

2. Materials and methods

2.1. Expression of recombinant talin polypeptides

The regions encoding murine talin1 residues 1788–1973 (6h), 1815–1973 (5h) and 1843–1973 (4h) were synthesized by PCR using a mouse talin1 cDNA as template, and cloned into the expression vector pet-151TOPO (Invitrogen). Talin polypeptides

Abbreviations: HSQC, heteronuclear single quantum coherence; SCOP, structural classification of proteins; VBS, vinculin-binding site

* Corresponding author. Fax: +44 116 229 7018.
E-mail address: gcr@le.ac.uk (G.C.K. Roberts).

0014-5793/$36.00 © 2010 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2010.04.028

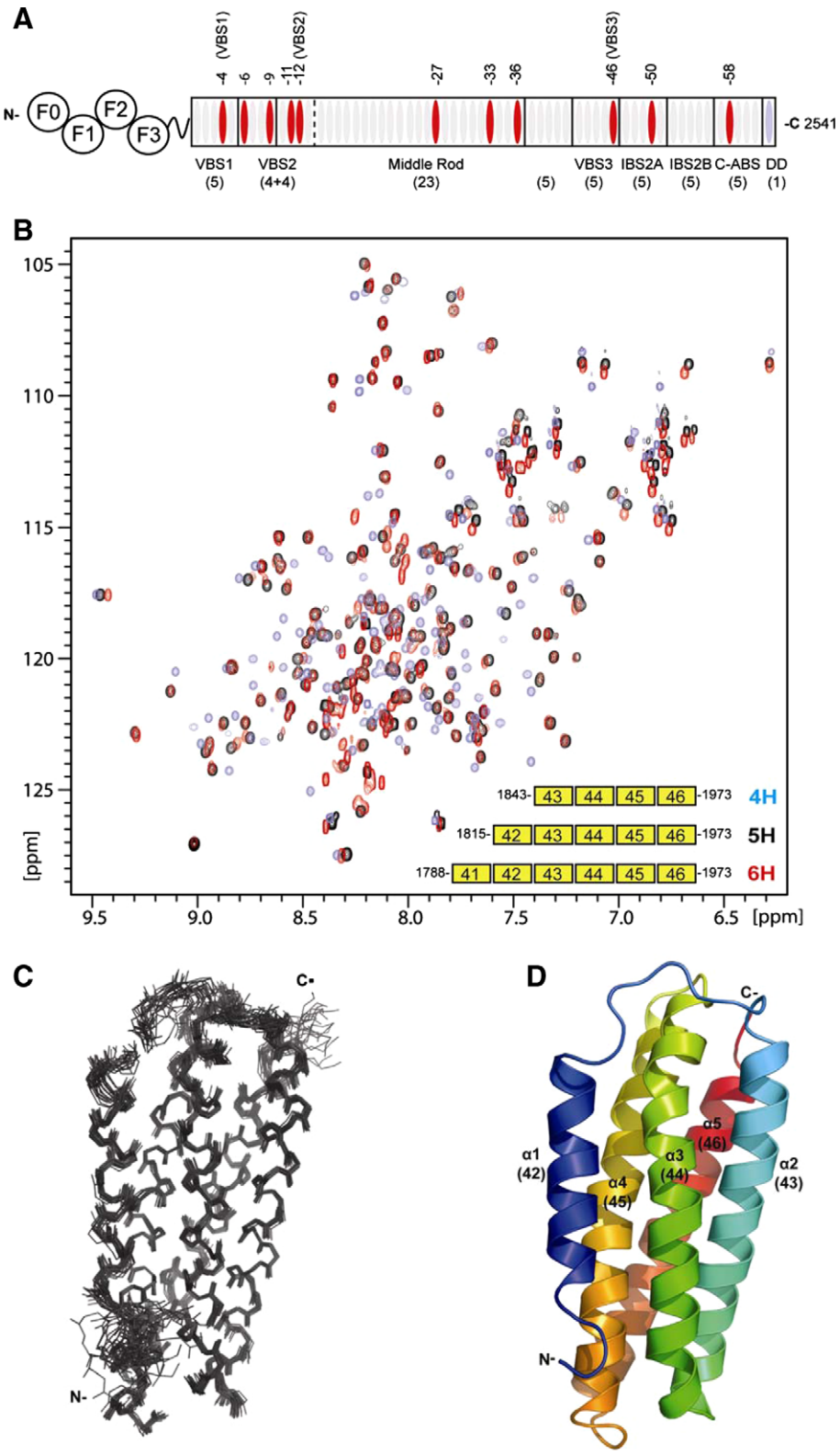

Fig. 1. Characterisation of the talin polypeptides containing VBS3. (A) Schematic diagram of the talin molecule. The rod contains 62 predicted α-helices (ovals); the ~11 vinculin-binding sites (VBS) are shown in red. (B) Superimposition of the 2D [^{1}H, ^{15}N]-heteronuclear single quantum coherence spectra of talin 1843–1973 (blue), 1815–1973 (black) and 1788–1973 (red). (Inset: Schematic of the constructs tested; the numbering corresponds to the helix number within the whole talin rod.) (C) Superimposition of the 20 lowest energy structures of talin 1815–1973 consistent with the NMR data. Only the structured region, 1820–1973, is shown, not the disordered N-terminus. (D) Ribbon drawing of a representative low-energy structure showing the overall topology of the 5-helix bundle.

were expressed in *Escherichia coli* BL21 STAR (DE3) cultured in M9 minimal media containing $^{15}NH_4Cl$ and/or ^{13}C-glucose. His-tagged talin polypeptides were purified by nickel-affinity chromatography. The His-tag was removed by cleavage with AcTEV protease