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Letter to the Editor

A Novel Ca^{2+} Binding β Hairpin Loop Better Resembles Integrin Sequence Motifs Than the EF Hand

Putative Ca^{2+} binding sequence repeats in integrin α subunits have attracted great interest. Depending on the integrin, Ca^{2+} can either support ligand binding or antagonize Mg^{2+}-dependent ligand binding (Hynes, 1992). The putative Ca^{2+} binding sequences in integrins mimic those found in a structure known as the "EF hand" (Tuckwell et al., 1992) which is present in many Ca^{2+} binding proteins, including troponins, parvalbumin, and calmodulin (Fig.1). Therefore, the sequence repeats in integrins are widely referred to as EF hand–like. In EF hands, Ca^{2+} is bound in a loop between two α helices, with one α-helical residue contributing to the Ca^{2+} coordination (Strynadka and James, 1989) (Figure 2B).

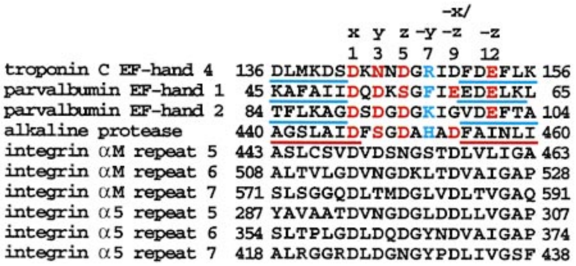
The integrin Ca^{2+} binding motifs are located in repeats of about 60 residues each in the N-terminal portion of the α subunit; the structure of these repeats is controversial. There are seven repeats in total; repeats 5–7 and also in some integrins repeat 4 each contain one Ca^{2+} binding sequence motif. Starting with the similarity to EF hands as a hypothesis, integrin repeats have been modeled as α-helical, EF hand–containing proteins (Stanley et al., 1994), and repeats 4–7 of α5 have been expressed in isolation and found to bind ligand and contain both α-helical and β sheet structure (Baneres et al., 1998). On the other hand, the repeats have been predicted to fold into seven β sheets that are arranged in a β propeller fold, where each Ca^{2+} binding motif is in a β hairpin loop between antiparallel β strands (Springer, 1997). Furthermore, work on intact integrins maps ligand binding to loops that are adjacent in the β propeller fold in repeats 2 and 3 and not to the cation binding repeats (Irie et al., 1995; Guerrero-Esteo et al., 1998; Tozer et al., 1999; Zhang et al., 1999; Puzon-McLaughlin et al., 2000).

Because of these controversies and the importance of the Ca^{2+} binding repeats in hypothesis-driven research on integrins, it is important to point out a heretofore overlooked, integrin-like Ca^{2+} binding site that indeed occurs in a β hairpin loop. We found this loop by examining Ca^{2+} binding structures in the Protein Data Bank; it is not listed among the Ca^{2+} binding motifs in PROSITE release 16. Although present in crystal structures of an alkaline protease from *Pseudomonas aeruginosa* (Baumann et al., 1993; Miyatake et al., 1995), this Ca^{2+} binding loop was not described in the original publications, which focused on a different type of Ca^{2+} binding motif present in multiple copies in the same protein. We compare the novel β hairpin loop and EF hand Ca^{2+} binding motifs structurally, and compare their sequence characteristics to those of integrins.

In the EF hand motif, the Ca^{2+} ion is coordinated by oxygen atoms in the side chains of residues 1, 3, 5, 9, and 12, and the carbonyl oxygen of residue 7 (Figures 1, 2B, and 2D). Residue 9 is often in a secondary coordination shell, where it holds a water molecule in position to form the primary coordination at the −x position. Residues 1–9 are in the loop between the α helices, and residue 12 is one turn into the following α helix (Strynadka and James, 1989). In a variant motif, observed in galactose binding protein (Vyas et al., 1987), the Ca^{2+} binding loop occurs between an α helix and a β strand (Figure 2C). The Ca^{2+} ion is coordinated by side chains of residues 1, 3, 5, and 9 and the main chain carbonyl of residue 7, but the role of residue 12 in the EF hand is substituted by an acidic side chain from a neighboring, parallel β strand.

In the β hairpin motif, both the sequence and the backbone conformation of residues 1–7 are remarkably similar to those in the EF hand (Figures 1, 2A, and 2D). Interestingly, however, residue 9 shifts by several angstroms and delivers two carboxylate oxygen atoms to ligation position −z (Figure 2D). Thus, it fulfills the same function as residue 12 in the EF hand, as previously predicted for integrins (Oxvig and Springer, 1998). The resulting coordination of the Ca^{2+} ion is similar to the EF hand, including the water molecule often observed at position −x. However, this water is not coordinated by another residue as usually observed in the EF hand, and this may allow larger ions, such as Sr^{2+}, to bind to β hairpin loops with affinity comparable to Ca^{2+}, as found for the integrin Mac-1 but not for EF hands (Oxvig and Springer, 1998).

Thus, Ca^{2+} can be bound in a loop between two β strands, as well as in a structurally similar loop between two α helices or between an α helix and a β strand. Therefore, the conservation of Ca^{2+}-coordinating residues at positions 1, 3, 5, and 9 is insufficient to determine the type of Ca^{2+} binding structure a sequence assumes; however, we may ask whether there are other features of the Ca^{2+} binding β hairpin or EF hand motifs that resemble patterns in integrins. Like the Ca^{2+} binding β hairpin, integrins always lack the acidic residue at position 12 that is characteristic of EF hands (Figure 1).



		−x/ x y z −y −z −z 1 3 5 7 9 12	
troponin C EF-hand 4	136	DLMKDSDKNNDGRIDFDEFLK	156
parvalbumin EF-hand 1	45	KAFAIIDQDKSGFIEEDELKL	65
parvalbumin EF-hand 2	84	TFLKAGDSDGDGKIGVDEFTA	104
alkaline protease	440	AGSLAIDFSGDAHADFAINLI	460
integrin αM repeat 5	443	ASLCSVDVDSNGSTDLVLIGA	463
integrin αM repeat 6	508	ALTVLGDVNGDKLTDVAIGAP	528
integrin αM repeat 7	571	SLSGGQDLTMDGLVDLTVGAQ	591
integrin α5 repeat 5	287	YAVAATDVNGDGLDDLLVGAP	307
integrin α5 repeat 6	354	SLTPLGDLDQDGYNDVAIGAP	374
integrin α5 repeat 7	418	ALRGGRDLDGNGYPDLIVGSF	438

Figure 1. Sequence Alignment of Ca^{2+} Binding Motifs

Sequences are from troponin C (PDB code 5tnc), parvalbumin (5cpv), alkaline protease (1kap), and human integrins αM and α5. The α helices and β strands in the structures are underlined in blue and red, respectively. The residues that coordinate Ca^{2+} with side chain or carbonyl oxygens are shown in red and blue, respectively. Residues 1, 3, 5, 7, 9, and 12 in the Ca^{2+} binding loops are numbered, and in the line above is shown the position in the octahedral coordination shell.

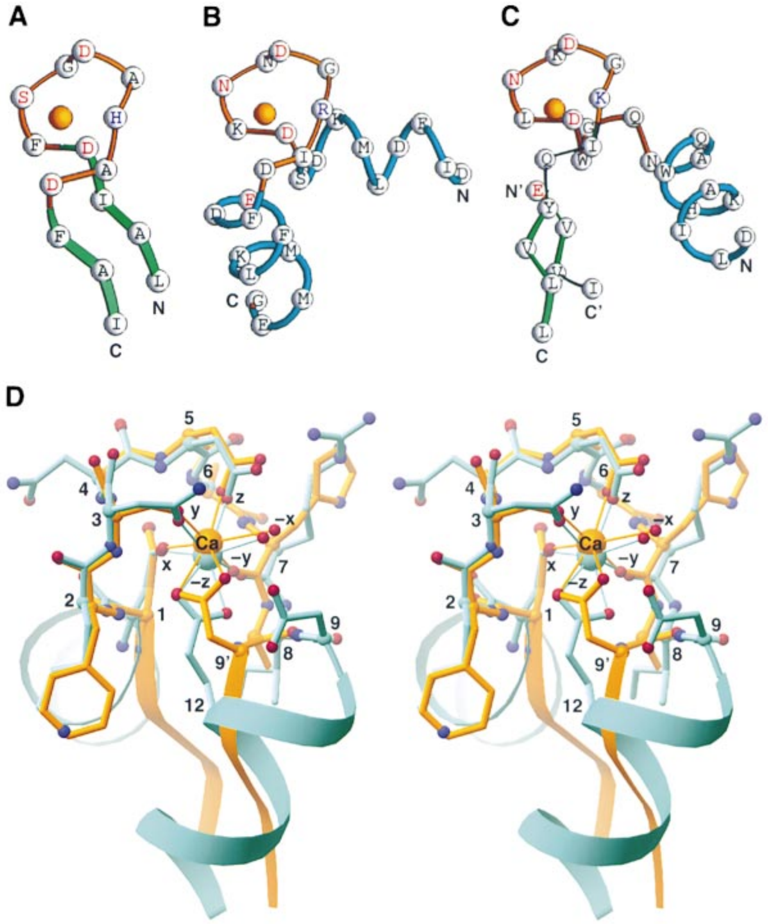


Figure 2. Structure of the Ca^{2+} Binding Motifs

(A–C) The Ca^{2+} binding β hairpin in alkaline protease (A, residues 443–457 from 1kap), EF hand 4 in troponin C (B, residues 133–160 from 5tnc), and helix-turn-strand motif in galactose binding protein (C, residues 121–146 and 205–208 from 3gbp). The α helices, β strands, and loops are in cyan, green, and yellow, respectively. The sequences are labeled on the corresponding Cα atoms, with the Ca^{2+}-coordinating residues colored as in Figure 1. The orientation of the Ca^{2+} binding loops is the same in all structures. (D) Stereodiagram of a superposition of the two Ca^{2+} binding motifs shown in (A) and (B). The ribbons, bonds, coordinations, Cα atoms, and Ca^{2+} ion are in yellow for the Ca^{2+} binding β hairpin or green for the EF hand. Residues 1–9, the water molecule at position −x, and residue 12 in the EF hand are shown as ball-and-stick, with oxygen and nitrogen atoms in red and blue, respectively. Coordinations to the Ca^{2+} ions are shown as thin bonds. The view is rotated ~90° about a vertical axis relative to (A) and (B). Figure prepared with RIBBONS (Carson, 1997).

Residues 10–12 are hydrophobic in integrins, but not in EF hands. The residues preceding and following the Ca^{2+} binding loop in integrins are predicted to be β strands by a variety of computational techniques (Tuckwell et al., 1994; Irie et al., 1995; Springer, 1997), whereas these are amphipathic α helices in EF hands. Residue 9 has the important function of forming two primary coordinations to Ca^{2+} in the β hairpin loop, whereas it usually forms one secondary coordination or no coordination at all in EF hands (Figure 1). Notably, residue 9 is Asp in 96% of integrins, and Asp, Asn, or Glu in 100% of integrins, whereas the corresponding values in EF hands are only 34% and 51%, respectively (Oxvig and Springer, 1998). Residue 8 is a hydrophobic anchor in EF hand proteins that is buried in the interface formed by pairwise association of EF hand motifs (Strynadka and James, 1989). This residue is hydrophobic in 100% of EF hands, but in only 15% of integrin Ca^{2+} binding repeats (Oxvig and Springer, 1998) (Figure 1). Residue 2 of the EF hand loop is generally exposed to solvent. It is only hydrophobic in 28% of EF hands, but is hydrophobic in 100% of integrin Ca^{2+} binding loops (Oxvig and Springer, 1998) (Figure 1). These critical differences suggest that integrins do not have an overall fold that resembles EF hand proteins. Furthermore, the characteristics of integrin Ca^{2+} binding motifs better resemble those of the Ca^{2+} binding β hairpin loop than the EF hand.

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