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Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N resonance assignments of the von Willebrand factor A3 domain

Noritaka Nishida[a,b], Mayumi Miyazawa[a], Hiromi Sumikawa[b,c], Masayoshi Sakakura[a], Nobuhisa Shimba[b,c], Hideo Takahashi[d], Hiroaki Terasawa[a], Ei-ichiro Suzuki[b,c] & Ichio Shimada[a,d,*]

[a]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan;* [b]*Japan Biological Information Research Center (JBIRC), Japan Biological Informatics Consortium (JBIC), Hatchobori, Chuo-ku, Tokyo, 104-0032, Japan;* [c]*Institute of Life Sciences, AJINOMOTO CO., Inc. Suzuki-cho, Kawasaki-ku, Kawasaki 210-8681, Japan;* [d]*Biological Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), Aomi, Koto-ku, Tokyo 135-0064, Japan*

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Biological context

von Willebrand factor (vWF) plays an essential role in platelet adhesion at the sites of vascular injury, by serving as a molecular bridge between the subendothelial collagen and the platelet membrane receptor, glycoproteins Ib/IX/V complex, under high-shear conditions. It is now well established that the collagen binding site is mainly included in the A3 domain (amino acids 920-1111 of vWF) of vWF (Sadler, 1998).

Sequences homologous to the A3 domain are found in a variety of proteins, and are categorized as von Willebrand factor A-type (VWA) domains (Colombatti and Bonaldo, 1991). Some VWA domains function as collagen-binding proteins, like the A3 domain of vWF. One of the best characterized VWA domains with collagen-binding activity is the α2-I domain from the α2-subunit of integrin. Recently, the crystal structure of the α2-I domain in complex with a collagen-like peptide revealed that the α2-I domain adopts a 'dinucleotide-binding' fold, with its central hydrophobic β strands flanked by amphipathic α helices on both sides, and that the collagen triple-helix is buried in the trench formed at the 'top' face of the domain, where the glutamate residue from the collagen-peptide is directly coordinated with the divalent cation at the so-called MIDAS (metal ion dependent adhesion site) motif (Emsley et al., 2000). The crystal structures of the A3 domain have already been determined (Bienkowska et al., 1997; Huizinga et al., 1997). Although the A3 domain shares a similar chain fold to that of the α2-I domain, the metal was not present, due to the imperfect formation of the MIDAS motif. Furthermore, point mutations introduced around the top face of the A3 domain did not disrupt its collagen-binding activity. Therefore, these results strongly suggest that the collagen-recognition mechanism of the A3 domain is different from that of the α2-I domain.

Here we report the backbone assignments of the A3 domain, as established by triple-resonance experiments. The assignments obtained from the present study will be used for the elucidation of the collagen-binding mode of the A3 domain by exploiting our recently developed NMR method, termed transferred cross saturation (Takahashi et al., 2000; Nakanishi et al., 2002).

Methods and experiments

The uniformly ^{13}C/^{15}N labeled A3 domain was produced by culturing *E. coli* BL21(DE3) cells, with the expression vector pET-42b, in M9 minimal media, containing 1 g/l of $^{15}NH_4Cl$ and 3 g/l of $^{13}C_6$-glucose. The uniformly ^{2}H/^{13}C/^{15}N labeled protein was produced in the same media prepared with 99% D_2O.

*To whom correspondence should be addressed. E-mail: shimada@iw-nmr.f.u-tokyo.ac.jp

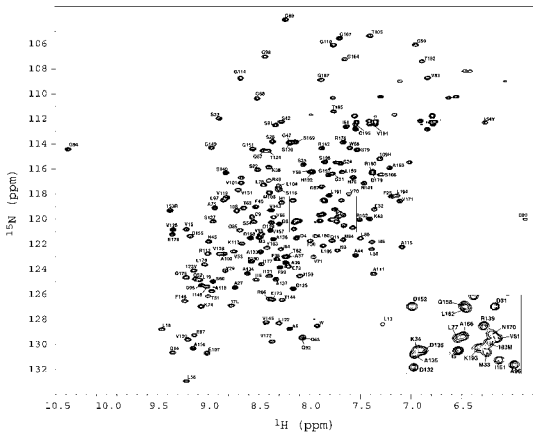

Figure 1. ^{1}H-^{15}N HSQC spectrum of the A3 domain at pH 6.0 and 310 K. Assignments of backbone amide peaks are shown by single-letter code and residue number. The cross peak originating from the indole NH of W68 is indicated by W.

Proteins were expressed as Glutathione S-transferase (GST) fusions and were purified with Glutathione Sepharose 4B agarose. The GST-tag was digested with Factor Xa, and the protein was purified by Mono-Q ion exchange chromatography. The purified protein contains the region corresponding to residues 920 to 1111 of vWF, with an additional five N-terminal residues (Gly-Ser-Met-Asp-Ileu) derived from the cloning vector. For NMR experiments, samples of 0.5–0.8 mM A3 domain in 10 mM phosphate buffer (pH 6.0), 100 mM NaCl, 0.05% NaN_3, and 10% D_2O were prepared.

NMR spectra were recorded at 37 °C on a Bruker DRX600 spectrometer equipped with a triple-resonance inverse probe with pulse field gradient units. The sequential assignment of the backbone resonances of the A3 domain was obtained based on the sets of triple-resonance experiments, HNCA, HN(CO)CA, CBCA(CO)NH, and deuterium decoupled-TROSY HNCACB. FIDs were processed using the program NMRPipe (Delaglio et al., 1995), and the data analysis was assisted by the software ANSIG (Kraulis, 1989). To facilitate the sequential assignment, several amino acid specific ^{15}N labelings of the amide-nitorogens were implemented. The ^{1}H chemical shifts were referenced to the methyl signal of DSS (0 ppm), while the ^{15}N and ^{13}C chemical shifts were referenced indirectly to the absolute frequency ratios $^{15}N/^{1}H = 0.101329118$ and $^{13}C/^{1}H = 0.251449530$.

Extent of assignments and data deposition

Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of the A3 domain. To avoid confusion, our amino acid numbering starts with G1-S2-M3-D4-I5 of the additional sequence, followed by A6, and terminates with G197, which correspond to A920 and G1111 in the native vWF protein, respectively. All of the resonances originating from the amide groups of the backbone were assigned in a residue-specific way, with the exception of the two N-terminal residual sequences and S189, due to line broadening. The chemical shifts of the amide groups for residues N69 and N188, whose resonances are missing in the triple resonance experiments, were assigned by using the amino acid selective ^{13}C/^{15}N-double labeling technique (Torchia et al., 1988).

The backbone resonance assignments of the A3 domain, including the $^{1}H_N$, ^{15}N, $^{13}C_\alpha$, and $^{13}C_\beta$ chemical shift values, have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the accession number 5456.

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