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Letter to the Editor: Sequential assignment and secondary structure of saratin, an inhibitor of von Willebrand factor-dependent platelet adhesion to collagen

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Biological context

Collagen plays an important structural role in the extra-cellular matrix of tissues. Under normal circumstances it is not exposed to flowing blood. Upon injury of the vessel wall, collagen is exposed to flowing blood and its constituents leading to activation and release of a variety of pro-aggregatory and mitotic factors propagating aggregation and thrombosis.

The adhesion of platelets to the injured arterial wall is mediated by von Willebrand factor (vWF), which binds to collagen (for review see Sixma et al., 1997). As a consequence the collagen-bound vWF then gathers platelets, leading in the end to platelet activation (for review see Sadler, 1998). Thus vWF could act as a bridge between collagen and platelets and is a prerequisite for platelet adhesion. This process in itself may only be temporary, however, requiring additional, direct interactions between collagen and other receptors on the platelet surface in order to facilitate permanent platelet adhesion, activation and aggregation (Sixma et al., 1997). Such collagen receptors on platelets are known to include, but may not be limited to GP VI, GP Ia/IIa ($alpha_2beta_1$), to a lesser extent GP IV (CD36) (Moroi and Jung, 1997) and perhaps p65 (Chiang et al., 1997). In addition, vWF and fibrinogen facilitate cross-linking and further activation of platelets via GP IIb/IIIa receptor binding (Kulkarni et al., 2000), providing stability and strength for the developing thrombus.

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Saratin is isolated from the saliva of the leech *Hirudo medicinalis* and inhibits the binding of vWF to arterial wall collagens and thus may have potential as an inhibitor of platelet-mediated events resulting from arterial injury. Saratin consists of 103 amino acids and contains three disulfide bridges.

Methods and results

Saratin was isolated from *Hansenula polymorpha* and purified by chromatography as will be described elsewhere. For NMR experiments unlabeled, ^{15}N-labeled or ^{13}C-, ^{15}N- double labeled saratin were dissolved to a final protein concentration of 1.5–1.7 mM in 10 mM sodium phosphate buffer, pH 7.5 containing 50 mM NaCl, 0.1 mM DSS and either 95% ^1H_2O/5% ^2H_2O or 99.5% ^2H_2O. Data were recorded at 298 K. NMR spectra were recorded on a Bruker DRX500 spectrometer, only a ^{15}N seperated NOESY was recorded at 800 MHz on a Bruker DRX800. Data were typically acquired with 1024 data points in the direct proton dimension and with 128 data points in ^{13}C using constant time evolution and States-TPPI acquisition (Marion and Wüthrich, 1983) and 64 data points in ^{15}N with echo-antiecho type selection (Schleucher et al., 1994). Linear prediction (Barkhuijsen et al., 1985) in the indirect dimensions resulted in a spectral resolution of 4 Hz/data point in ^{1}H, 23 Hz/data point in ^{13}C and 36 Hz/data point in ^{15}N. ^{1}H NMR data were referenced to the ^{1}H resonance frequency of the methyl groups in 4,4-dimethyl-4-silapentane sulphonic acid (DSS), ^{13}C and ^{15}N resonances were

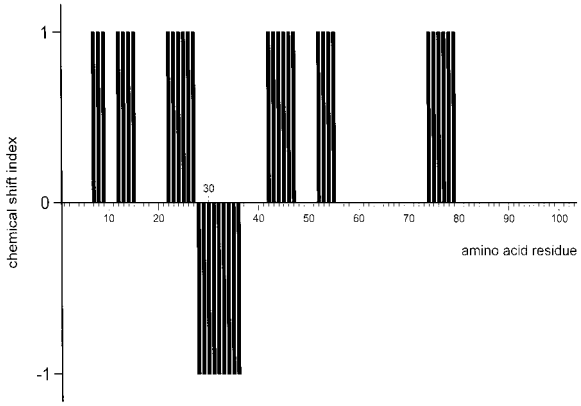

Figure 1. CSI-plot of saratin. Using the chemical shifts of the H^{α}, C^{α}, C^{β} and C' resonances the chemical shift index (Wishart and Sykes 1994) was calculated with the program CSI v. 1.1 (courtesy of Sykes et al., http://www.pence.ualberta.ca/ftp). Positive values are indicative for β-strands, negative values for α-helices.

referenced indirectly by multiplying the proton frequency by 0.25144953 for ^{13}C and 0.101329118 for ^{15}N (Wishart et al. 1995; Markley et al., 1998).

Sequential backbone assignment was accomplished utilizing the spectra HNCO, HNCA, CBCA (CO)NH, HBHA(CO)NH and ^{1}H-^{15}N-NOESY HSQC spectra, side chain assignments were done using the HCCH-TOCSY and the 2D NOESY/TOCSY experiments in $^{2}H_2O$. Data were processed in XWIN-NMR (Bruker, Karlsruhe) and evaluated in AURELIA (Bruker) (Neidig et al., 1995).

Extent of assignments and data deposition

Over 96% of the backbone carbonyl-carbons, amide-nitrogens, amide-protons, α-carbons, α-protons, β-carbons and β-protons were assigned as well as over 95% of the side chain protons and carbons (except the ^{13}C shifts of the aromatic rings). The chemical shift values have been deposited on the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 4973. That all six cysteine residues of saratin are involved in disulfide bridges is confirmed by β-carbon chemical shifts around 41 ppm. This shift is typical for cystine residues compared to 28 ppm for cyteines (Wishart et al., 1994). The chemical shift values of the carbonyl-carbons, α-protons, α-carbons and β-carbons were used for the secondary structure prediction with the Chemical Shift Index (Wishart and Sykes, 1994) and indicate the secondary structure elements as depicted in Figure 1. From these data six β-strands – Trp7-Phe9 (β1), Asn12-Tyr15 (β2), Phe22-Asp27 (β3), Tyr42-Asp47 (β4), Glu52-Tyr55 (β5), Phe74-Leu79 (β6) – and one helix – Leu28-Phe36 (α1) – can be predicted. The C-terminus is probably unstructured. Preliminary structure calculations indicate, that β1 and β2 form a common β-strand including a β-bulge and that the five strands are composed in an anti parallel manner.

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