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3D-STRUCTURE DETERMINATION OF FLAVORIDIN IN SOLUTION: NEW COMPUTATIONAL STRATEGY FOR DISULFIDE-BRIDGE MAPPING

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Flavoridin, a protein with 70 amino acids from the venom of *Trimeresurus gramineus*, is a very potent inhibitor of blood platelet aggregation. The protein contains a local Arg-Gly-Asp (RGD) sequence at position 49 to 51. This primary sequence element is known to inhibit fibrinogen binding by a specific interaction with the integrin-type platelet receptor GPIIb/IIIa. By now, a rather large family of homologous RGD containing snake toxins have been sequenced. Most of these proteins contain $n=12$ cysteines which are all linked by disulfide bridges. Previous biochemical studies, however, have so far not revealed the native pattern of the individual cysteine pairings.

The ^1H NMR spectrum of Flavoridin was almost fully assigned in aqueous solution by conventional 2D ^1H NMR methods (2QF-COSY, clean TOCSY, 2Q-spectroscopy, NOESY). The 3D-structure calculation with distance geometry methods (DIANA) proceeded in several rounds:

(1) The global fold of Flavoridin was calculated from the collected set of NOE distance constraints (#666) and dihedral angle constraints obtained from vicinal coupling constants (#88) but *without* the use of any disulfide bridge constraints.
(2) The interatomic C^β-C^β distances between all possible pairs $[n(n-1)/2]$ of cysteines were measured in a set of 20 converged distance geometry structures. A probability weight w_{ij} ($0 \le w_{ij} \le 1$) is assigned to each individual

cysteine pair according to a gaussian shaped distribution function. When the average crystallographic C^β-C^β distance of a cystine disulfide bridge is matched, the weight $w_{ij} = 1$.
(3) All combinatorial patterns of 6 disulfide-bridges involving the 12 cysteines in Flavoridin were calculated and the 6 individual weights, w_{ij}, for each pattern were multiplied. Only patterns with individual $w_{ij} \ge 0.3$ were considered. On this objective basis, a single Cys-Cys pairing pattern could unambiguously be determined. The method was validated with known protein crystal structures of Cys-rich proteins. Also the experimentally observed NOE's between $C_i^\alpha H/C_j^\beta H$ and/or C_i^β/C_j^β of Cys residues, which commonly are taken as direct evidence for Cys-Cys disulfide links, do agree with the computationally evaluated Cys-Cys pattern.
(4) In a second step of the structure calculation, the Cys-Cys pairing was used as additional input for the distance geometry program. Finally, the 50 best structures were refined by energy minimization.

Our structural results show that the polypeptide backbone is folded in two domain-like structures composed of 8 turns and stabilised by 6 cystine cross-links. The conformation of the Arg-Gly-Asp (RGD) sequence is located in an extended loop structure exposed at the tip of a so called hairpin, which is rather flexible.