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Solution structure of drosomycin, the first inducible antifungal protein from insects

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Abstract

Drosomycin is the first antifungal protein characterized recently among the broad family of inducible peptides and proteins produced by insects to respond to bacterial or septic injuries. It is a small protein of 44 amino acid residues extracted from *Drosophila melanogaster* that exhibits a potent activity against filamentous fungi. Its three-dimensional structure in aqueous solution was determined using ^{1}H 2D NMR. This structure, involving an α-helix and a twisted three-stranded β-sheet, is stabilized by three disulfide bridges. The corresponding Cysteine Stabilized $\alpha\beta$ (CS$\alpha\beta$) motif, which was found in other defense proteins such as the antibacterial insect defensin A, short- and long-chain scorpion toxins, as well as in plant thionins and potent antifungal plant defensins, appears as remarkably persistent along evolution.

Keywords: antifungal protein; CS-alpha-beta motif; drosomycin; insect immunity

Insects respond to septic or bacterial injuries by the rapid and transient synthesis of a battery of potent antibacterial peptides and proteins (Hoffmann & Hetru, 1992; Hoffmann et al., 1996). These molecules are produced by the fat body, a functional analogue of the mammalian liver, and are secreted into the blood. They are mostly cationic peptides and proteins with a broad spectrum of activity against Gram-positive and Gram-negative bacteria. Since the initial characterization of cecropins in 1981 by Boman and associates (Steiner et al., 1981), more than 100 inducible antibacterial peptides and proteins have been isolated from various insect species. It has only recently become apparent that insects can also synthesize antifungal polypeptides in response to a septic injury (Fehlbaum et al., 1994, 1996; Levashina et al., 1995). Indeed, in addition to the production of several distinct antibacterial peptides, the fruitfly *Drosophila melanogaster* synthesizes a 44-residue protein with potent antifungal activity directed against filamentous fungi, but inactive against bacteria (Fehlbaum et al., 1994). This protein, named drosomycin, has eight cysteine residues engaged in four intramolecular disulfide bridges, a characteristic likely to confer to the molecule a highly compact structure and that certainly accounts for the remarkable resistance of drosomycin to proteases and to heat treatments. The small size of *Drosophila* precludes the isolation of sufficient quantities of drosomycin for NMR elucidation of its three-dimensional structure. We have recently expressed in *Saccharomyces cerevisiae* adequate amounts of recombinant protein, which was shown by various approaches to be identical to native drosomycin (Michaut et al., 1996). Here we describe the three-dimensional structure of the recombinant protein.

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Results and discussion

Structural features

Distance and angle restraints used to build a model of drosomycin structure were established from two-dimensional ^{1}H-NMR spectra recorded on a 3 mM solution of recombinant protein in water at pH 4.0 and 293 K. From the 200 structures generated with the DIANA program (Güntert et al., 1991), the 30 best, which target function ranges from 0.8 to 1.5 at the last step of calculations, were subjected to restrained simulated annealing calculations and then to restrained energy minimization with the X-PLOR program (Brünger, 1992). A set of 15 structures, with the fewest number of residual violations on distance restraints and internal geometry, was finally retained to represent the solution structure of drosomycin. Figure 1B shows that this family of structures is especially well-defined in the secondary structure regions mentioned below, where an average pairwise RMSD of 0.38 Å is found for C_α atoms (Table 1). The number of NOEs per residue collapses at the N- and the C-terminal residues and around Pro 10, Gly 27, and Pro 35

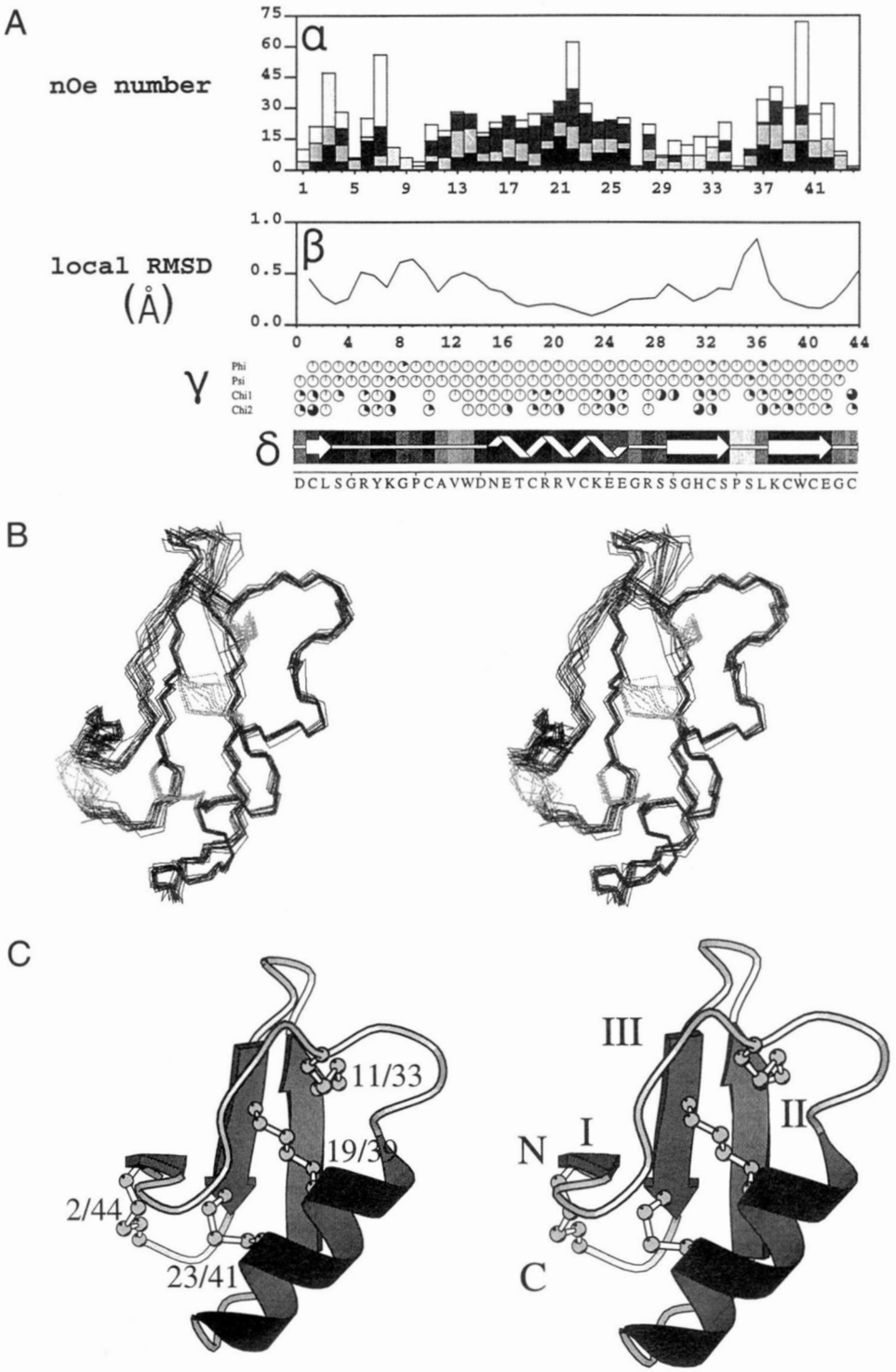

Fig. 1. Structural data for the 15 selected NMR structures of drosomycin. **A:** From top down, plots as a function of the residue number of (α) the number of upper distance constraints used in the final structure calculations [intraresidual (dark), sequential (light shadowed), medium- (dark shadowed), and long-range (very light shadowed)]; (β) RMSDs from the average structure for the backbone heavy atoms N, C_α, C′, and O, obtained by fitting Cα atoms in the secondary structure elements; and (γ) ϕ, ψ, χ_1, and χ_2 angular circular variances defined as: $CV_\theta = 1 - \sqrt{\{\sum(\cos\theta_i)^2 + \sum(\sin\theta_i)^2\}}/n$, where θ is a torsion angle and n the number of θ values. The black part of the dial is proportional to the circular variance (drawn with PROCHECK, Laskowski, 1993). (δ) Schematic representation of the secondary structure elements of drosomycin with the detail of accessibility [buried residues in black, accessible ones in white, drawn with PROCHECK (Laskowski, 1993)]. **B:** Stereo view of 15 of the best structures of drosomycin, superimposed over the C_α atoms in secondary structure element. This superposition shows a low variability of the backbone even in the region outside secondary structures. Disulfide bridges are light shadowed. The 23/41 one exhibits a well defined conformation. **C:** Schematic stereo view of the backbone of drosomycin with the detail of the four disulfide bridges, drawn with MOLSCRIPT (Kraulis, 1991). The α-helix and the twisted three-stranded β-sheet defining the CS$\alpha\beta$ motif are clearly displayed.

Table 1. *Structural statistics and residual restraint violations of the 15 structures that represent the solution structure of drosomycin (average value, min, max)*

RMSD pairwise (Å)	
Global	1.55 (1.19, 1.94)
Cα	0.56 (0.26, 0.96)
Cα (secondary structure)	0.38 (0.19, 0.63)
RMSD/mean structure (Å)	
Global	1.06 (0.92, 1.26)
Cα	0.38 (0.25, 0.66)
Cα (secondary structure)	0.26 (0.18, 0.40)
Ramachandran plot	
Most favored regions	78.3 (74.3, 85.7)
Additionally allowed regions	19.6 (14.3, 22.9)
Violations of NOE restraints	
Number > 0.4 Å	0.4 (0, 1)
Number > 0.3 Å	2.1 (2, 3)
Number > 0.2 Å	5.2 (3, 8)
Violations of bond length	
Number > 0.05 Å	6.1 (3, 10)
Violations of bond angles	
Number > 10°	0.6 (0, 2)
Energies (kcal/mol)	
Etot	−472 (−415, −506)
EvdW	−166 (−153, −177)
Eelect	−683 (−642, −729)

[a]The secondary structure is defined as 2–4, 16–26, 30–34, and 38–42. The Ramachandran plot was calculated with PROCHECK (Laskowski, 1993).

residues (Fig. 1A). The final structures having 78% of residues lying in the most favored regions of a Φ,Ψ Ramachandran plot and about 20% lying in the additionally allowed regions are in excellent agreement with the experimental restraints and present good internal geometry (Table 1).

The secondary structure elements defined by PROCHECK (Laskowski, 1993) are a short α-helix encompassing residues 16–26 and twisted β-sheet structure formed by a very short strand I (2–4) and by two more extended strands II (30–34) and III (38–42) (Fig. 1C). The long fragment (5–15) between the first strand and the helix is fastened to strand II by the 11/33 disulfide bridge and involves three β-turns: a first one, including Ser 4 to Tyr 7 residues, is of type IV in the majority of structures; the two other consecutive turns, 12–15 (type I) and 13–16 (type IV), can be regarded as forming a distorted helical turn, initiating the α-helix. This helix is followed by a tight turn, starting with Gly 27, whose conformation will be discussed below. The β-turn 34–37 linking strand II and III is of type IV in the majority of structures.

Among the two disulfide bridges connecting the α-helix to β-strand III, the 23/41 one adopts a well-defined right-handed conformation with an average χ_{ss} angle equal to +89 ± 5° and shows very low circular variances of χ_1 and χ_2 angles of both Cys residues, whereas three conformers are observed for the 19/39 bridge. The 11/33 bridge connecting the fragment (5–15) to strand II exhibits two unequally populated conformers with χ_{ss} angles equal to −141 ± 7° (in 10 structures from 15) and to +88 ± 3°, respectively, and a locked χ_1 angle of Cys 11. The conformation of the 2/44 bridge joining the N and C terminus of the polypeptide chain is ill-defined, which is reflected by the mobility of the N-terminal Asp 1 residue. Two of these bridges are partly (23/41) or totally (19/39) buried in the core of the molecule.

According to the low values of Φ,Ψ circular variances (Fig. 1A), the overall structure of drosomycin is remarkably rigid as far as it exhibits only small regions of moderate variability. One of these regions corresponds to the Lys 8–Gly 9 doublet preceding a rigid sequence, including Pro 10–Cys 11 and two consecutive β-turns. Another one involves Ser 36 and Leu 37 residues, which oscillate between two conformations in the β-turn linking strands II and III. Most amide protons, even outside secondary structures, are slowly exchanging with the solvent (data not shown), which confirms the structure rigidity and suggests a high compacity of the molecule.

Comparison with other proteins

The global fold of drosomycin bears resemblance with those of various small proteins exhibiting different biological activities, such as an antibacterial insect defensin A (Cornet et al., 1995), short- and long-chain scorpion toxins (Dauplais et al., 1995; Landon et al., 1996), plant thionins (Bruix et al., 1993, 1995; Nitti et al., 1995), and potent antifungal plant defensins (Terras et al., 1993; Broekaert et al., 1995). In spite of a low level of sequence homology, they contain a common structural motif, which we named Cysteine Stabilized αβ-motif (CSαβ motif) (Cornet et al., 1995), insofar as it involves an α-helix including an invariant sequence (Cys-Xxx-Xxx-Xxx-Cys) linked by two disulfide bridges to the last strand of a β-structure, which includes another invariant sequence (Cys-Xxx-Cys).

A comparison is made between drosomycin and insect defensin A, an antibacterial 40-residue protein extracted from the fleshfly *Phormia terranovae* (Lambert et al., 1989; Cornet et al., 1995). Although the level of sequence identity is relatively low, these two antimicrobial defense molecules of insects share significant aspects in their three-dimensional structures. Only eight residues are common to both proteins: these are two glycines (Gly 27 and Gly 31 in drosomycin, and Gly 24 and Gly 28 in defensin A) and six cysteines, which form the two disulfide bridges of the CSαβ motif and the bridge numbered 11/33 in drosomycin and 3/30 in defensin A. The main difference is located in the N-terminal fragments preceding the α-helix: in drosomycin, the loop inserted between Cys 11 and Cys 19 is shorter than the corresponding Cys 3–Cys 16 fragment in defensin A, by five residues. The additional 1–10 sequence present in drosomycin introduces a third β-strand and is fastened by a fourth disulfide bridge (2/44), bringing the C terminus near the N terminus. Similar features (four disulfide bridges, three-stranded β-sheet) are found in plant thionins (Bruix et al., 1993, 1995) and in long-chain scorpion toxins, like LqqIII (Landon et al., 1997). The N-terminal fragments of the two insect proteins have opposite positions with respect to the CSαβ motif (Fig. 2). Another important difference between drosomycin and defensin A concerns the α-helix. In drosomycin, its N end is extended by two residues when compared to defensin A, and two pairs of charged residues, Arg 20–Arg 21 and Lys 24–Glu 25, replace the two hydrophobic doublets, Ala 17–Ala 18 and Leu 21–Leu 22, found in defensin A. A Gly residue is present at the C end of this helix in both proteins, allowing the formation of a three-residue tight turn, which exhibits an $\alpha_R\beta\alpha_R$ and an $\alpha_R\beta\gamma_L$ conformation (Efimov, 1986; Sibanda et al., 1989) in drosomycin and in defensin A, respectively.

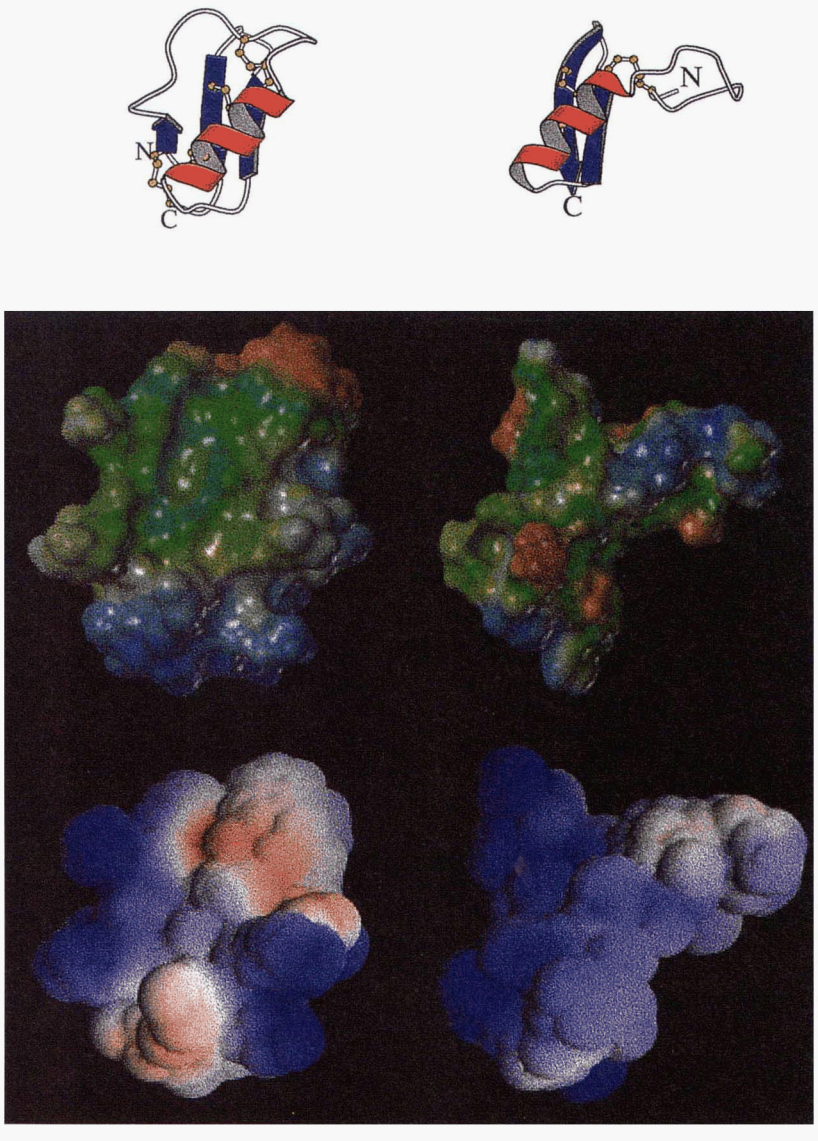

Fig. 2. Comparison of hydrophobic and electrostatic potential maps of drosomycin (left) and defensin A (right). Top: Schematic representation of the backbone showing the orientation of the molecule (drawn with MOLSCRIPT, Kraulis, 1991). Middle: Hydrophobic potential surface calculated with the MOLCAD option (Ghose et al., 1988; Heiden et al., 1993) of SYBYL software at the Connoly surface. Hydrophobic and hydrophilic surfaces are displayed in brown and blue, respectively, whereas green represents an intermediary hydrophobicity. Bottom: Electrostatic potentials calculated with the GRASP (Nicholls et al., 1991) program at the accessible surface. Positive and negative surfaces are displayed in blue and red, respectively.

In defensin A, small hydrophobic patches distributed within the polar residues delineate on the surface of the protein a mosaic structure (Cornet et al., 1995). The helix of the CS$\alpha\beta$ motif possesses an amphipathic character and positively charged side chains are located on two regions of the molecule (Lys 33 in one location and Arg 23, Arg 25, and Arg 39 in another one). The electrostatic potential surface is mainly positive (Fig. 2). The only negative spot corresponds to the Asp 4 side chain (data not shown). In contrast, calculations of the hydrophobic potential map of drosomycin reveal a quite different organization of hydrophobic and hydrophilic residues: Pro 10, Ala 12, Val 13, Trp 14, Pro 35, and Leu 37 residues form a well-defined hydrophobic cluster on one pole of its structure, whereas the opposite pole is clearly hydrophilic. On the electrostatic potential map, three negative patches (Asp 15/Glu 17, Glu 25/Glu 26, and Glu 42) appear clearly within a globally positive surface (Fig. 2).

The structural similarities between drosomycin, plant defensins, and thionins are even more striking than those with insect defensins (Fig. 3). The degree of sequence identity is 36% between drosomycin and the plant defensin Rs-AFP1 extracted from the seeds of radish *Raphanus sativus* (Terras et al., 1992, 1993; Fant et al., 1997), and is 39% and 41% between drosomycin and the γ-1P and γ-1H thionins, extracted from wheat and barley, respectively (Bruix et al., 1993). Plant defensins, which are 5 kDa cationic proteins, inhibit the growth of a broad range of filamentous fungi (Terras et al., 1993), and consequently participate in the plant host defense, whereas thionins exert toxicity in various biological systems by destroying membranes (Bohlmann, 1994). A prelimi-

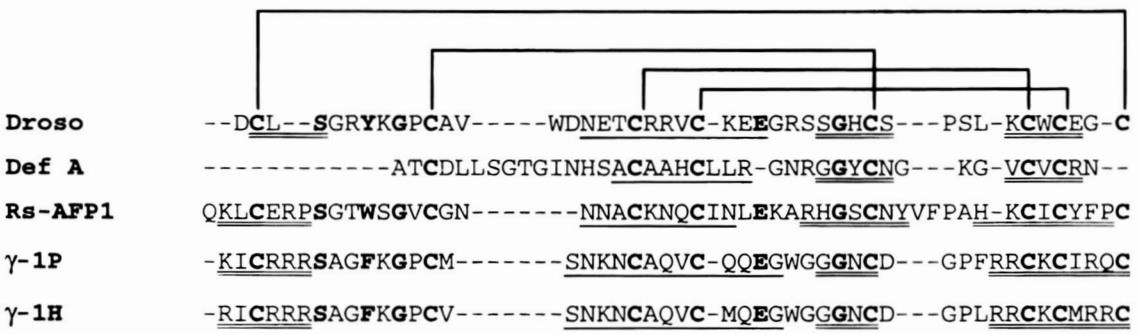

Fig. 3. Comparison of amino acid sequences of the antifungal drosomycin from *D. melanogaster* (Fehlbaum et al., 1994), the antibacterial defensin A from *P. terranovae* (Cornet et al., 1995), the antifungal Rs-AFP1 from the seeds of radish *R. sativus* (Terras et al., 1993), the γ-1P thionin from wheat, and the γ-1H thionin from barley (Bruix et al., 1993). α-Helix and β-strands are underlined by single and double lines, respectively.

nary description of the global fold of Rs-AFP1 (Fant et al., 1997) indicates the presence of a CSαβ motif with the characteristic Cys residue positions and a three-stranded β-structure. The coordinates of the NMR structures of the γ-1P and γ-1H thionins are available (Bruix et al., 1993).

Drosomycin, plant defensins, and γ-thionins share a consensus sequence including eight cysteine residues that form a conserved array of four disulfide bridges (Fig. 3). Four other residues are strictly conserved: Ser 4, Gly 9, Glu 26, and Gly 31 (drosomycin numbering), whereas Tyr 7 is replaced by a Trp residue in plant defensins and a Phe residue in γ-thionins. In drosomycin structures, Ser 4 side-chain hydroxyl group is located in the core of the protein and is buried in a hydrophobic cluster involving residues Val 22, Cys 23, Cys 39, and Cys 41. The hydroxyl hydrogen proton points toward a recurrent small cavity, with average dimension of 37 $Å^3$ and large enough to contain an internal water molecule. The strict conservation of this amino acid among all the primary sequences of related proteins suggest an important structural role. Tyr 7 in drosomycin, or Phe 10 in thionins, points toward the molecule and caps the 11/33 and 19/39 (drosomycin numbering) disulfide bridges (Fig. 4). Gly 9 is found in all primary sequences of plant defensins and thionins. The Ramachandran plots of drosomycin structure and thionins structures show peculiar values of ϕ and ψ angles. These angles are confined in a narrow region around 120° and 180°, respectively, which is only populated by Gly residues for steric hindrance reasons. Glu 26 points toward the solvent and is possibly involved in the interaction between drosomycin and the receptor. The conservation of Gly 31, located on strand II, can be explained by the very close contact between the helix and the sheet within the CSαβ motif.

A striking feature that emerges from the comparison of proteins exhibiting a CSαβ motif is the great variety of activities that appear to result from the variations in amino acid sequences. For example, considering scorpion toxins, charybdotoxin (Bontems et al., 1991) acts on mammalian K^+ channels, whereas LqqIII (Kopeyan et al., 1993) induces changes in the permeability of both

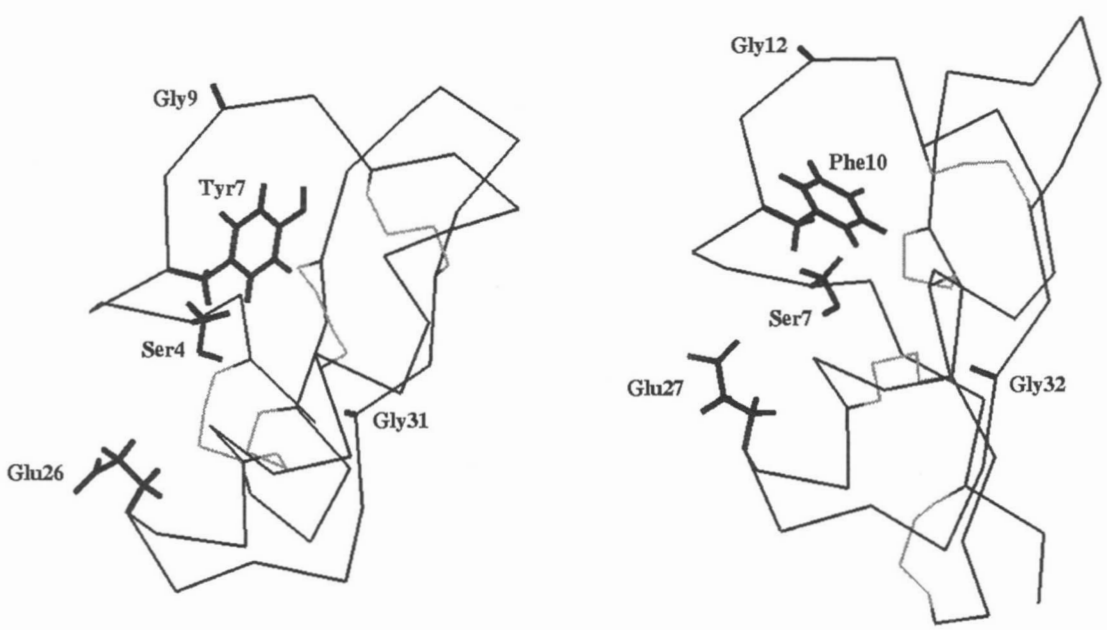

Fig. 4. Cα trace of drosomycin (left) and γ1-H structures (right) with a representation of the conserved residues.

mammalian and insect Na^+ channels. The structure of the latter protein, which we have established recently (Landon et al., 1997), differs from that of drosomycin by the presence of a C-terminal loop and by longer strands in the three-stranded β-sheet.

In conclusion, our study provides the first structural determination of an inducible antifungal protein that plays an essential role in the host defense of *Drosophila* (Lemaitre et al., 1995). Although very few residues are conserved between the antifungal drosomycin from *D. melanogaster* and the antibacterial defensin A from *Phormia terranovae*, these two defense proteins adopt the same CS$\alpha\beta$ architecture. Moreover, we show that, in addition to amino acid sequence similarities (Fehlbaum et al., 1994), drosomycin shares the same structural scaffold with plant antifungal proteins. This organization extends over the whole molecule and comprises a three-stranded β-sheet and an α-helix stabilized by disulfide bridges in the same array in both molecules. It is tempting to speculate that drosomycin and plant defensins have evolved from a common ancestor molecule that predated the separation of plants and animals. The high degree of conservation of these molecules, shown by the structural data, underlines the role of antimicrobial proteins in the host defense of eukaryotes.

Finally, we point out that these molecules, although complex, are amenable to chemical synthesis as illustrated for charybdotoxin (Vita et al., 1995). This field of research therefore has obvious potential for the design of new antibiotics. In particular, engineering of peptides, in which the basic structural motif is decorated with appropriate residues, might lead to molecules with well-targeted activity against selected microorganisms.

Materials and methods

Recombinant drosomycin from *D. melanogaster* was produced by a strain of *S. cerevisae* and purified according to procedures described previously (Michaut et al., 1996). Structures were determined from two-dimensional ^{1}H-NMR spectra recorded at 293 K on a Bruker AMX-500 spectrometer equipped with three axis gradient coils. ^{1}H DQF- and TQF-COSY, TOCSY, and NOESY spectra were assigned according classical procedures including spin-system identification, sequential assignment, and secondary structure identification (Wüthrich, 1986). Exchange kinetics of amide protons were estimated with 1D and 2D spectra recorded at 277 K and 293 K, after dissolution in D_2O. Interproton distance restraints were derived from NOE intensities (mixing time 120 ms) and torsion angle restraints from analysis of the NOEs and from the $^3J_{NH\text{-}C\alpha H}$ coupling constants determined with the INFIT program (Szyperski et al., 1992) of XEASY (Bartels et al., 1995). The 30 best structures from the 200 generated with the DIANA program (Güntert et al., 1991; Güntert & Wüthrich, 1991) were refined with a simulated annealing/energy minimization standard protocol using the X-PLOR computer program (Brünger, 1992). The system was submitted to molecular dynamics simulations at 1,000 K, cooled down at 300 K, and energy minimized. The final 512 NMR restraints set (from which values redundant with the covalent geometry had been eliminated by DIANA) consisted of 104 intraresidue, 144 sequential, 113 medium-range ($2 \leq |i - j| \leq 5$) and 151 long-range ($|i - j| > 5$) upper bond restraints deduced from NOEs, 12 distance restraints corresponding to the four disulfide bridges, and 84 backbone and 78 side-chain dihedral angle restraints. Only four restraints were introduced in the first calculations to form the two hydrogen bonds between Cys 2 and Cys 41. Thirteen hydrogen bonds proposed by the DIANA program, located in regular secondary structure elements and in accordance with the amide hydrogen exchange data, were added during further steps of calculations. A total of 22 stereospecific assignments, including 17 β-methylene pairs, were made using GLOMSA (Güntert et al., 1991). The 15 final structures used for analysis were selected on the basis of the number of residual violations of internal geometry and distance restraints. The coordinates of these 15 structures representing drosomycin have been deposited in the Brookhaven Protein Data Bank (accession code 1MYN).

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References

Bartels C, Xia TE, Billeter M, Güntert P, Wüthrich K. 1995. The program XEASY for computer-supported NMR spectral analysis of biological macromolecules. *J Biomol NMR 5*:1–10.

Bohlmann H. 1994. The role of thionins in plant protection. *Crit Rev Plant Sci 13*:1–16.

Bontems F, Roumestand C, Boyot P, Gilquin B, Doljansky Y, Ménez A, Toma F. 1991. Three-dimensional structure of natural charybdotoxin in aqueous solution by ^{1}H-NMR. *Eur J Biochem 196*:19–28.

Broekaert WF, Terras FRG, Cammue BPA, Osborn RW. 1995. Plant defensins: Novel antimicrobial peptides as components of the host defense system. *Plant Physiol 108*:1353–1358.

Bruix M, Gonzalez C, Santoro J, Soriano F, Rocher A, Mendez E, Rico M. 1995. ^{1}H-NMR studies on the structure of a new thionin from barley endosperm. *Biopolymers 36*:751–763.

Bruix M, Jiménez MA, Santoro J, Gonzáles C, Colilla FJ, Méndez E, Rico M. 1993. Solution structure of γ1-H and γ1-P thionins from barley and wheat endosperm determined by ^{1}H-NMR: A structural motif common to toxic arthropod proteins. *Biochemistry 32*:715–724.

Brünger AT. 1992. *XPLOR version 3.1: A system for crystallography and NMR*. New Haven, Connecticut: Yale University.

Cornet B, Bonmatin JM, Hetru C, Hoffmann JA, Ptak M, Vovelle F. 1995. Refined three-dimensional solution structure of insect defensin A. *Structure 3*:435–448.

Dauplais M, Gilquin B, Possani LD, Gurrola-Briones G, Roumestand C, Ménez A. 1995. Determination of the three-dimensional solution structure of noxiustoxin: Analysis of structural differences with related short-chain scorpion toxins. *Biochemistry 34*:16563–16573.

Efimov AV. 1986. Standard conformations of a polypeptide chain in irregular protein regions. *Mol Biol 20*:250–260.

Fant F, Vranken WF, Martins JC, Borremans FAM. 1997. Solution conformation of *Raphanus sativus* antifungal protein 1 (Rs-AFP1) by ^{1}H nuclear magnetic resonance. Resonance assignment, secondary structure and global fold. *Bull Soc Chim Belg 106*:51–57.

Fehlbaum P, Bulet P, Chernysh S, Briand JP, Roussel JP, Letellier L, Hetru C, Hoffmann JA. 1996. Structure–activity analysis of thanatin, a 21 residue inducible insect defense peptide with sequence homology to frog skin antimicrobial peptides. *Proc Natl Acad Sci USA 93*:1221–1225.

Fehlbaum P, Bulet P, Michaut L, Lagueux M, Broekaert WF, Hetru C, Hoffmann JA. 1994. Insect immunity. Septic injury of *Drosophila* induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides. *J Biol Chem 269*:33159– 33163.

Ghose AK, Pritchett A, Crippen GM. 1988. Atomic physicochemical parameters for three dimensional structure directed quantitative structure–activity relationships III: Modeling hydrophobic interactions. *J Comp Chem 9*:80–90.

Güntert P, Braun W, Wüthrich K. 1991. Efficient computation of three-dimensional protein structures in solution from NMR data using the program DIANA and the supporting programs CALIBA, HABAS and GLOMSA. *J Mol Biol 217*:517–530.

Güntert P, Wüthrich K. 1991. Improved efficiency of protein structure calculations from NMR data using the program DIANA with redundant dihedral angle constraints. *J Biomol NMR 1*:447–456.

Heiden W, Moeckel G, Brickmann J. 1993. A new approach to analysis and display of local lipophilicity/hydrophilicity mapped on molecular surfaces. *J Comput-Aided Mol Design 7*:503–514.

Hoffmann JA, Hetru C. 1992. Insect defensins: Inducible antibacterial peptides. *Immunol Today 13*:411–415.

Hoffmann JA, Reichhart JM, Hetru C. 1996. Innate immunity in higher insects. *Curr Opin Immunol 8*:8–13.

Kopeyan C, Mansuelle P, Martin-Eauclaire MF, Rochat H, Miranda F. 1993. Characterization of toxin III of the scorpion *Leiurus quinquestriatus quinquestriatus*: A new type of alpha-toxin highly toxic both to mammals and insects. *Natural Toxins 1*:308–312.

Kraulis PJ. 1991. MOLSCRIPT: A program to produce both detailed and schematic plots of protein structures. *J Appl Crystallogr 24*:946–950.

Lambert J, Keppi E, Dimarcq JL, Wicker C, Reichhart JM, Dunbar B, Lepage P, Van Dorsselaer A, Hoffmann JA, Fothergill J, Hoffmann D. 1989. Insect immunity: Isolation from immune blood of the dipterian *Phormia terranovae* of two insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. *Proc Natl Acad Sci USA 86*:262–266.

Landon C, Cornet B, Bonmatin JM, Kopeyan C, Rochat H, Vovelle F, Ptak M. 1996. ^{1}H-NMR-derived secondary structure and the overall fold of the potent anti-mammal and anti-insect toxin III from the scorpion *Leiurus quinquestriatus quinquestriatus*. *Eur J Biochem 236*:395–404.

Landon C, Sodano P, Cornet B, Bonmatin JM, Kopeyan C, Rochat H, Vovelle F, Ptak M. 1997. Refined solution structure of the anti-mammal and anti-insect LqqIII scorpion toxin: Comparison with other scorpion toxins. *Proteins Struct Funct Genet*. Forthcoming.

Laskowski RA. 1993. PROCHECK: A program to check the stereochemical quality of protein structures. *J Appl Crystallogr 26*:283–291.

Lemaitre B, Kromer-Metzer E, Michaut L, Nicolas E, Meister M, Georgel P, Reichhart JM, Hoffmann JA. 1995. A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the *Drosophila* host defense. *Proc Natl Acad Sci USA 92*:9465–9469.

Levashina EA, Ohresser S, Bulet P, Reichhart JM, Hetru C, Hoffmann JA. 1995. Metchnikowin, a novel immune-inducible proline-rich peptide from *Drosophila* with antibacterial and antifungal properties. *Eur J Biochem 233*:694–700.

Michaut L, Fehlbaum P, Moniate M, Van Dorsselaer A, Reichhart J-M, Bulet P. 1996. Expression in yeast of active recombinant drosomycin, an antifungal peptide from *Drosophila* and secondary structure determination. *FEBS Lett 395*:6–10.

Nicholls A, Sharp K, Honig B. 1991. Protein folding and association: Insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins Struct Funct Genet 11*:281–296.

Nitti G, Orru S, Bloch C, Morhy L, Marino G, Pucci P. 1995. Amino acid sequence and disulphide-bridge pattern of three γ-thionins from *Sorghum bicolor*. *Eur J Biochem 228*:250–256.

Sibanda BL, Blundell TL, Thornton JM. 1989. Conformation of β-hairpins in protein structures. A systematic classification with applications to modelling by homology, electron density fitting and protein engineering. *J Mol Biol 206*:759–777.

Steiner H, Hultmark D, Engström A, Bennich H, Boman HG. 1981. Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature 292*:246–248.

Szyperski T, Güntert P, Otting G, Wüthrich K. 1992. Determination of scalar coupling constants by inverse Fourier transformation of in-phase multiplets. *J Magn Reson 99*:552–560.

Terras FRG, Schoofs HME, De Boll MFC, Van Leuven F, Rees SB, Vanderleyden J, Cammue BPA, Broekaert WF. 1992. Analysis of two novel classes of plant antifungal proteins from radish (*Raphanus sativus* L.) seeds. *J Biol Chem 267*:15301–15309.

Terras FRG, Torrenkens S, Van Leuven F, Osborn RW, Vanderleyden J, Cammue BPA, Broekaert WF. 1993. A new family of basic cysteine-rich plant antifungal proteins from Brassicaceae species. *FEBS Lett 316*:233–240.

Vita C, Roumestand C, Toma F, Ménez A. 1995. Scorpion toxins as natural scaffolds for protein engineering. *Proc Natl Acad Sci USA 92*:6404–6408.

Wüthrich K. 1986. *NMR of proteins and nucleic acids*. New York: Wiley Interscience.