Conformations of Peptide Fragments Comprising the Amino-terminal, Central, and Carboxyl-terminal Regions of a Membrane-active Polypeptide

BUILD-UP OF SECONDARY STRUCTURE IN PARDAXIN*

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The conformations of synthetic peptides of different lengths corresponding to the amino-terminal, central, and carboxyl-terminal regions of pardaxin (GFFALIPKIISSPLFKTLLSLAVGSALSSSGEQE) have been studied by circular dichroism spectroscopy. The peptides GFFALIPKIISSPLF-OMe, GFFALIPKIISSPLFK-OMe corresponding to the amino-terminal region, as well as peptides KIISSPLFKTLLSLAV and IISSPLFKTLLSAA corresponding to the central region of the toxin have a marked tendency to adopt helical conformation. Ordered conformation is also discernible in the 8- and 7-residue peptides GFFALIPK and IISSPLF-OMe. Peptides corresponding to the central segments KTLLSLAV, LSASVGSAL, and the carboxyl-terminal segment SSSQGEQ, however, exhibit very little secondary structure. The peptide segments that adopt ordered conformation show similar conformation when present in the entire toxin as suggested by proton magnetic resonance data (Zagorski, M. G., Norman, D. G., Barrow, C. J., Iwashita, T., Tachibana, K., and Patel, D. J. (1991) Biochemistry 30, 8009–8017). The observation that peptide segments corresponding to the amino-terminal and central regions of the toxin adopt ordered conformations compared to the carboxyl-terminal segment in isolation as well as in the toxin, indicates a role for these regions in initiating and maintaining ordered conformation of pardaxin.

Pardaxin is a 33-residue peptide toxin present in the defense secretions of certain species of soles of the genus Parachirus (Primor and Zlotkin, 1976). The toxin has attracted attention due to its shark-repelling properties (Primor et al., 1978) as well as its ability to exhibit diverse pathological and pharmacological effects (Primor and Tu, 1980; Primor et al., 1980, 1983; 1984; Primor and Lazarovici, 1981; Primor, 1984; Pal et al., 1981; Primor and Zlotkin, 1975). The biological activities of pardaxin are presumed to arise from its ability to perturb membranes. Support for this assumption comes from the observation that the peptide is highly surface-active and interacts with model membranes of phosphatidylcholine and serine (Lelkes and Lazarovici, 1988). However, pardaxin does not have any primary structural similarity with other toxins.

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residues at the amino and carboxyl terminus exist predominantly in extended conformations. In an effort to determine the role of various regions of pardin in initiating and directing secondary structure, conformational analysis of synthetic peptides corresponding to amino-terminal, central, and carboxyl-terminal regions of the toxin by circular dichroism (CD) spectroscopy was undertaken, and the results are described in this paper.

**EXPERIMENTAL PROCEDURES**

**Synthesis of Peptides**—Peptides listed in Table I were synthesized by solid-phase and solution-phase methods. In solution-phase synthesis, dipeptide couplings were effected in dichloromethane (CH₂Cl₂) by dicyclohexylcarbodiimide. Tripeptides and longer peptides were synthesized in dimethylformamide with dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. In solid-phase synthesis, the peptide chain was built on a Merrifield resin using 7-butyloxycarbonyl chemistry, manually. The protocols employed were essentially as described for signal sequences (Reddy et al., 1985; Reddy and Nagaraj, 1986, 1989) and hydrophobic peptides like alamethicin (Nagaraj and Balaram, 1981a, 1981b, 1981c). All protected peptides were purified by column chromatography on silica gel (230-400 mesh, Merck, Germany) using mixtures of chloroform and methanol as eluants. The deprotected peptides 15P-OMe, Ac14P, and Ac15P were purified using fast performance liquid chromatography (FPLC), 1 using an analytical pepRPC (reverse phase, C18) column. The homogeneity of all peptides was confirmed by FPLC under similar conditions and quantitative amino acid analysis done on an LKB 4151 alpha plus amino acid analyzer. Details of the amino acid analysis are summarized in Table II.

**Circular Dichroism (CD) Studies**—Spectra were obtained on the JASCO J-20 spectrometer and John-Yvon Dichrograph V. The spectrometers were calibrated with (+)-10-camphorsulfonic acid. Cells of 0.1-cm path length were used, and the spectra were run at the ambient temperature of 22°C. δ values correspond to mean residue ellipticities.

**Preparation of Peptide Samples for CD**—All peptide stock solutions were made up in MeOH. These stocks were subjected to quantitative amino acid analysis. Aliquots of peptide stock solution were transferred to acid-washed test tubes (3 ml) and dried using the Savant Speed-Vac. Appropriate spectroscopic grade solvent (hexafluorooisopropanol, methanol, or trifluoroethanol) was added up to a known concentration just before recording the spectrum. For studies with SDS micelles, the peptide stock aliquots were transferred to tubes that were then subjected to Savant Speed-Vac drying. Appropriate volumes and concentrations (above the critical micelle concentration of 8 mM) of SDS solution was then added prior to running the sample. All spectra were baseline-corrected. The CD spectra of the 14-16-residue peptides were analyzed for helical content according to the method of Taylor and Kaiser (Taylor and Kaiser, 1987). This method compares the experimental ellipticity at 222 nm to a theoretical value and has been routinely used to estimate helical structures in signal sequences and model peptides (Bruch and Giersch, 1990; Padmanabhan et al., 1990; Hoyt and Giersch, 1991; Keller et al., 1992).

1. The abbreviations used are: FPLC, fast protein liquid chromatography; HFIP, hexafluorooisopropanol; OMe, methyl ester; TFE, trifluoroethanol.
Conformation of Peptide Fragments Related to Pardaxin

### TABLE II

**Amino acid analysis of synthetic peptides corresponding to various segments of paradanin**

Theoretical values are in parentheses. S/T values not corrected for loss during hydrolysis.

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<th>Peptide</th>
<th>A</th>
<th>E</th>
<th>G</th>
<th>I</th>
<th>L</th>
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<td>0.91</td>
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<td>11P-OMe</td>
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<td>1.02</td>
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**FIG. 1. Spectra of 15P-OMe.** a, TFE (0.1 mg/ml); b, SDS micelles (0.1 mg/ml); c, lysophosphatidylcholine micelles (0.1 mg/ml).

nm band. The ~222 nm band intensity in either case is similar and corresponds to a high α-helical content of 67%. In contrast to the case of 15P-OMe and 16P-OMe, an extra lysine residue leads to a significant increase in percent helicity on going from 14P to AcK15P.

**KTLSSAV-OMe**—The spectra are shown in Fig. 8 and indicate that the peptide is unordered in all three organic solvents HFIP, MeOH, and TFE. The TFE spectrum has a weak positive ~220 nm and ~240 nm bands and a negative band at ~200 nm. Likewise, the SDS spectrum has a prominent minimum ~200 nm and a shoulder at ~225 nm. This would argue against the peptide adopting a strongly helical structure in this medium. It is, however, possible that the spectrum is indicative of β-turn formation, although the ~200 nm (−) band is weak compared to that exhibited by an ideal β-turn. Thus, the spectrum suggests a mixture of β-turn and random conformation for the peptide in SDS.

**LSA** **VGSAL-OMe**—The spectra in different solvent systems are shown in Fig. 9. These spectra are also characteristic of the unordered conformation. However, the spectrum in TFE suggests that a small fraction of the peptide exists in a β-turn conformation.

**11P-OMe**—The spectra are shown in Fig. 10. In MeOH and HFIP, the spectra indicate that the peptide is unordered. On going to TFE, the peptide has a tendency to adopt a helical structure. However, the low [θ] 222 value indicates only a small...
amount of helical structure, with the peptide largely adopting an unordered conformation. In SDS micelles, the spectrum has a more intense ~208 nm (−) band and an ~222 nm (−) shoulder, indicating higher helical content. Thus, 11P-OMe shows a marked tendency to adopt an α-helical conformation in media of increased hydrophobicity.

*Carboxyl-terminal Segment: SSSGEQE-OMe*

The spectra of this peptide have been obtained in HFIP, MeOH, TFE, and SDS micelles and are described in Fig. 11. The spectra in all solvent systems are archetypical "random" spectra, with a very weak ~230 nm (−) band and much stronger ~200 nm (−) band. The ~215 nm band may be
negative or positive, as has been observed with other spectra indicative of unordered conformation (Mattice and Harrison, 1975).

The CD spectra of 15P-OMe, 16P-OMe, 14P, and AcK15P were independent of peptide concentration in the range of 0.05 mg/ml to 0.2 mg/ml. The shorter peptides also did not show any concentration dependence.

**DISCUSSION**

An α-helical conformation appears to be a common structural motif in a large number of peptide toxins comprising 13–35 residues (Kaiser and Kezdy, 1987). The Pardaxin sequence is composed of amino acids which can occur in helical and β-sheet conformations. Analysis of Pardaxin’s primary sequence by the method of Chou and Fasman (Chou and Fasman, 1978) predicts 60% β-sheet, 20% α-helix, and 15% random coil, as shown below.

Primary sequence: GFFALIPKISSLFLKTLSSAVGSLSSSGEQL; secondary structure prediction: XXXXBBBT BBBBBBBBBBBBBBBBBCCCCCXXX. (Notations are: X, helix; B, β-sheet; T, β-turn; C, random coil.)

The appearance of the CD spectra of pardaxin in MeOH and in the presence of lipid vesicles as well as the mean residue ellipticity values in the 217–222 nm region (Shai et al., 1990) would suggest the presence of β-structure although it has been argued that the toxin adopts predominantly helical structure. Hence, a detailed study of the conformation of synthetic peptides corresponding to amino-terminal, central, and carboxyl-terminal regions of the toxin has been carried out in order to determine the importance of various segments of the toxin in initiating and maintaining ordered conformation.

The amino-terminal 15P-OMe and 16P-OMe exhibit α-
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**FIG. 7. Spectra of AcK15P.** a, TFE (0.1 mg/ml); b, SDS (0.05 mg/ml).

**FIG. 8. Spectra of KTLLSAV-OMe.** a, MeOH (0.35 mg/ml); b, HFIP (0.63 mg/ml), in units of [θ] × 10⁻²; c, TFE (0.62 mg/ml); d, SDS micelles (0.2 mg/ml).

**FIG. 9. Spectra of LSAVGSAL-OMe.** a, MeOH (0.5 mg/ml); b, HFIP (0.33 mg/ml); c, TFE (0.5 mg/ml); d, SDS micelles (0.3 mg/ml).
helix-type spectra in TFE and SDS, with crossovers at ~197–200 nm. The low intensity of the 207 nm and 222 nm bands indicate only ~25% helix, or alternately that the peptide is completely helical 25% of the time. Manning et al. (1988) have suggested that distortions of helical structure result in lower helical estimates due to a decrease in strength of the $n\pi^*$ transition and consequently result in the decrease in negative ellipticity at 222 nm. Also, the value of the negative ellipticity at 222 nm depends to a large extent on the length of the helix as well distortion in the helical structure (Manning et al., 1988). For example, an 11-residue helical peptide could have a $[\theta]_{222}$ of ~10,000 as compared to a value of ~30,000 for an infinite helix. A good crossover for 15P-OMe and 16P-OMe in spite of $[\theta]_{222}$ of ~10,000 strongly argues for an ordered conformation. The 8- and 7-residue peptides GFFALIPK-OMe and IISSPLF-OMe also have a tendency to adopt ordered structures, particularly in a hydrophobic environment.

The central fragments KTLLSAV-OMe and LSAVGSAL-OMe exhibit very little secondary structure although 11P-OMe has a capacity to adopt helical structure in hydrophobic environments. Thus, this relatively less hydrophobic central fragment exhibits weak secondary structural propensities in isolation. Its carboxyl-terminal neighboring sequence, SSSGEQE, does not adopt any secondary structure, and, thus, its continuation in the carboxyl-terminal direction as 18P (KTLLSAVGSALSSSGEQE) does not bring about change in the secondary structure of this segment.\(^2\) The amino-terminal IISSPLF-OMe, in contrast, does have a tendency to adopt helical structure, and it is likely that significant secondary structure may be induced in a longer peptide incorporating this peptide and 11P.

The central part of the toxin encompasses the sequences 14P and AcK15P. AcK15P is markedly more helical than 14P.

\(^2\) S. Thennarasu and R. Nagaraj, unpublished data.
Thus, the amino-terminal lysine exerts a strong structure-promoting influence on this peptide, and the peptide is largely helical in spite of a proline in the middle of the sequence. Whereas 15P-OMe and 16P-OMe have 2 prolines each, 14P and AcK15P have only 1 proline each. The proline residues can bring about distortions in a helical structure and thereby cause a decrease in the [θ] value, especially in 14P and 15P-OMe, the shorter of the two pairs. In all four peptides, 1 proline occurs in the middle of the sequence. The second proline in 15P-OMe and 16P-OMe is the 3rd or 4th residue from the carboxyl terminus. At the amino terminus of an ideal helix, the first three carbonyl groups are involved in hydrogen bonding, whereas the first three NH groups remain nonbonded. At the carboxyl terminus, the converse situation holds good, with the three NH groups hydrogen-bonded. Since proline cannot form a hydrogen bond, having this residue up to the third position at the carboxyl terminus is more detrimental to α-helical structure than having it at the amino terminus. Alternatively, the presence of a turn at the beginning could help stabilize a helix (Blagdon and Goodman, 1975).

The carboxyl-terminal SSSGEQE has also been shown to be important in toxin activity (Shai et al., 1998), with a 4000-fold decrease of channel-forming ability of the 1–26-residue stretch of Pardaxin. The importance of charge at the carboxyl terminus and the presence of a proteinaceous channel has been emphasized (Greene and Anderson, 1991). It has, however, been established that the negative charges of SSSGEQE are not important for Pardaxin activity (Shai et al., 1990). A transmembrane channel also requires optimized energy wells at the entrances of a channel (Anderson, 1989), and it is possible that SSSGEQE plays a structural role in the toxin’s channel-forming abilities. The carboxyl-terminal SSSGEQE is random under all the conditions examined. This peptide is coupled to the LSAGVSGAL fragment in the native toxin. The latter fragment also does not exhibit strong secondary structural propensities. The peptide KTLLSAGVSGALSSGEQE corresponding to carboxyl-terminal portion of the toxin has been shown to have weak helical propensities when examined by CD in TFE. In the larger multihelix proteinaceous channels, it has been proposed that β-sheets or smaller helices may be present at the center of the helix cluster, hidden from the hydrophobic lipid tails (Lodish, 1988). Experimental evidence for such a motif has been obtained for a potassium channel (Yool and Schwarz, 1991). However, no such arrangement has been proposed or demonstrated for a bundle of helices formed by the aggregation of short independent peptides. The carboxyl-terminal SSSGEQE could conceivably be visualized in such a superstructure in the membrane although our circular dichroism and NMR studies (Zagorski et al., 1991) indicate the absence of its adopting any secondary structure (in isolation or as part of the entire toxin). Thus, the reason for the important role of this heptapeptide in toxin action must await determination of the toxin structure in membranes.

Although the ellipticities of pardaxin in SDS micelles had been reported as [θ]192 and [θ]222 ~ −15,000 degrees cm²/dmol (Thompson et al., 1986), circular dichroism studies of the toxin in other media have been reported only recently (Shai et al., 1990). The spectrum in water [θ]192 ~ −3,000 degrees cm²/dmol, [θ]222 ~ −6,000 degrees cm²/dmol, and crossover ~ 202 nm) is indicative of ~30% α-helix, 30% β-sheet, and 40% random coil (Greenfield and Fasman, 1969). The spectrum in MeOH, however, is characterized by a single minimum at ~220 nm ([θ]192 ~ −10,000 degrees cm²/dmol, crossover ~ 208 nm) indicating 60–80% β-sheet content. The spectrum in the presence of soybean lecithin vesicles is indicative of a mixture of α-helix and β-sheet, with a minimum at 222 nm ([θ]222 ~ −15,000 degrees cm²/dmol), a shoulder at 215 nm ([θ]215 ~ −12,000 degrees cm²/dmol), and a crossover ~ 205 nm. Although the authors have interpreted the activities of pardaxin and its analogs in terms of changes in α-helical content, the MeOH and lecithin vesicle spectra clearly indicate significant β-sheet content. Zagorski et al. (1991) have determined the structure of pardaxin in 1:1 trifluoroethanol-water, using two-dimensional NMR techniques. Based on the pattern of interresidue NOE connectivities involving NH, C=H, CαH, and C=H protons, they have identified 5 structurally distinct peptide segments: 1–5, 6–11, 12–13, 14–26, and 27–33. Segment 6–26 is proposed to have a helix-bend-helix conformation with the bend centered at residues 12 and 13. A random structure has been proposed for the carboxyl-terminal end (residues 27–33). Although the 1–5 region is not as ordered as the subsequent segments, the NMR data suggest the presence of some ordering of the side chains. However, the structure of pardaxin has not been determined in the membrane by high resolution techniques like NMR. While it is conceivable that the conformational behavior of the various segments of the toxin in solution may have limited relevance to their structure in the toxin in the membrane, our results presented in this paper and the conclusions from the NMR study (Zagorski et al., 1991) indicate that elements of secondary structure like helical conformations are present in short peptides that correspond to helical regions in the toxin in solution. Also, the carboxyl-terminal fragment SSSGEQE has no strong secondary structural propensities in isolation as well as when part of the entire toxin. Thus, amino-terminal and central regions are likely to have an important role in initiating and maintaining helical conformation of pardaxin. The investigations by Dyson et al. (1992) suggest that elements of secondary structure like reverse turns, nascent helix, and ordered helical conformations are abundant in short peptide fragments corresponding to helical regions of proteins. These structures are presumed to play an important role in folding of helical proteins. Our study suggests that short segments of amphipathic, membrane-active, helical peptides composed of 25–35 residues have strong propensities to occur in helical conformations, particularly in apolar solvents, and are likely to have an important role in initiating and maintaining helical conformation of the peptide.

Acknowledgment — We thank V. M. Dhople for amino acid analysis.

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