

A proposal for the structure of conotoxin – a potent antagonist of the nicotinic acetylcholine receptor

R.C. Hider

Department of Chemistry, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, England

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Conotoxin, a thirteen residue neurotoxic peptide, is demonstrated, by circular dichroism measurements, to possess a high content of α -helix. The location of this helix in the sequence is severely limited by the structural preferences of individual amino acids and by the positioning of the two cystine links. Comparisons are made between the reactive site of elapidae snake venom postsynaptic neurotoxins and the surface of the conotoxin molecule.

Conidae Conotoxin Neurotoxicity Circular dichroism Secondary structure

1. INTRODUCTION

The harpoon-like radula teeth of Conidae, a family of marine gastropods, are used to immobilise prey organisms, in particular fish [1–3]. Kohn et al. [4] demonstrated that a major action of the venom was interference with neuromuscular transmission, a finding subsequently supported by the work of Endean and Rudkin [5]. Isolation and partial characterisation of the components responsible for this activity were reported by Spence et al. [6] and Cruz et al. [7] both of whom purified peptide components from *Conus geographus* venom. The primary sequences of three homologous conotoxins were presented in 1981 [8]. These peptides contain between 13 and 15 amino acids including 4 half cystines (table 1). The assignment of the two cystine links has been achieved by chemical synthesis for 4 homologous conotoxins [9,10].

Synthetic conotoxins [9–12] possess full biological activity and hence the information for the correct folding of the peptide chain is present in the amino acid sequence. Thus conotoxins would appear to be suitable candidates for secondary structure prediction.

Similar studies with other disulphide-rich venom toxins have been remarkably successful, for in-

stance apamin [13,14], elapidae postsynaptically acting toxins [15,16] and phospholipase A₂ molecules [17]. This paper proposes a structure for the conotoxins on the basis of both circular dichroism (CD) measurements and an analysis of secondary structural tendencies.

2. METHODS

Circular dichroism measurements were made on a Jasco J40CS instrument, using 0.02- and 1-cm cells at 25°C. Toxin concentrations were determined using an estimated ϵ_m (280 nm) of 115. Molar ellipticities per residue are reported.

A modification [14] of Chou and Fasman's original method of secondary structure prediction [18], which uses the product of the parameters rather than the arithmetic mean to detect structural tendencies, was adopted. The parameters determined by Levitt [19] were used.

3. RESULTS AND DISCUSSION

The CD spectrum of conotoxin GI (1.75×10^{-4} M) in Tris-HCl (20 mM, pH 7.4) (fig.1) is consistent with a high α -helical content and is remarkably similar to that of apamin [20], which,

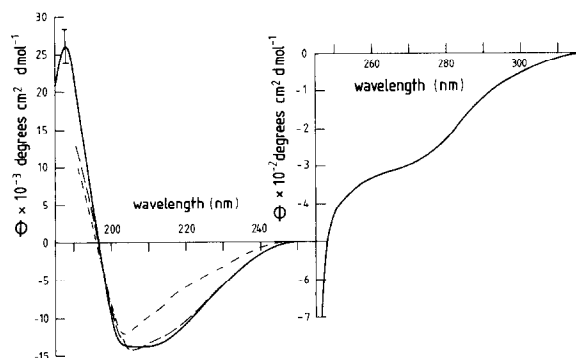


Fig.1. CD spectra of conotoxin GI. (—) Tris-HCl (20 mM, pH 7.4); (---) H₂O-HCl (pH 1.0); (- - - -) 90% aqueous trifluoroethanol. 185–250 nm (0.02 cm cell); 250–320 nm (1 cm cell).

despite possessing two cystine links, is α -helical [13,14,20,21]. The negative cotton effect for conotoxin in the region 200–220 nm is in agreement with previous CD studies [10]. The near UV region is dominated by contributions from the cystine and tyrosine functions. The dichroism at 280 nm is of similar magnitude and sign to that of apamin [22] and oxytocin [23], the former peptide possessing two cystine functions and the latter one cystine and

one tyrosine. By comparison with a series of computed spectra corresponding to a polypeptide chain in varying percentages of α -helix and random coil [24], it can be estimated that approx. 50% of conotoxin GI possesses α -helical conformation. Like apamin, the conformation of conotoxin remains relatively constant over a wide pH range (1.0–9.0), and in the presence of 90% trifluoroethanol (fig.1). This stability is probably dependent on the two disulphide links and hydrogen bonding between the backbone amide groups.

To locate the most probable site of the helical region in conotoxin, a secondary structure prediction study was undertaken for the 4 homologous toxins [23] (fig.2). Although some appreciable α -helical tendency is present in the N-termini of the 4 sequences, in particular for conotoxin GII, the conserved proline-5 renders such a structure improbable. A consistent α -helical prediction was obtained for the region 6–10 with an average $\langle P\alpha \rangle$ value of 1.03 for the 4 peptides. Although this value is not high, it is appreciably greater than the corresponding $\langle P\beta \rangle$ value, 0.82. As pointed out by Levitt [19], this average $\langle P\alpha \rangle$ corresponds to a value where only 40% of the residues are found experimentally to be α -helical. However, the average

Conotoxin		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MI	G	R	C	C	H	P	A	C	G	K	N	Y	S	C		
GI		E	C	C	N	P	A	C	G	R	H	Y	S	C		
GIA		E	C	C	N	P	A	C	G	R	H	Y	S	C	G	K
GII		E	C	C	H	P	A	C	G	K	H	F	S	C		
α -helical tendency π^S_α	MI	0.81	0.72	0.97	0.97	0.49	0.47	0.84	0.47	0.35	0.68	-	-	-	-	
								$\langle P_\alpha \rangle = 1.00$								
	GI	0.80	0.72	0.72	0.45	0.48	0.94	0.65	0.48	0.95	-	-	-	-		
								$\langle P_\alpha \rangle = 1.03$								
	GIA	0.80	0.72	0.72	0.45	0.48	0.94	0.65	0.48	0.95	0.55	0.53	-	-	-	-
								$\langle P_\alpha \rangle = 1.03$								
	GII	1.08	0.97	0.97	0.49	0.47	1.13	0.94	0.70	1.38						
								$\langle P_\alpha \rangle = 1.07$								
Mean $\langle P_\alpha \rangle_{6-10} = 1.03$																

Fig.2. Conotoxin sequences and α -helical predictions [15,19]. π^5_α , product of $P\alpha$ values [19] of 5 adjacent residues. $\langle P\alpha \rangle$, average of $P\alpha$ values [18]. Sequences taken from McIntosh et al. [25].

$\langle P\beta \rangle$ value corresponds to a value where only 20% of the residues are found to possess a β -extended conformation. Significantly, if the parameters derived by Geisow and Roberts for α -helical rich proteins [26] are used, then the $\langle P\alpha \rangle$ values increase to 1.08. When this segment adopts an α -helical conformation, it is possible to form the 2-7 and 3-13 disulphide links. Indeed, systematic variation of the Ramachandran angles of the remaining residues of the conotoxin GI sequence generated a structure which is consistent with CD spectral properties of conotoxin (fig.3). This structure, which is currently being subjected to energy minimisation studies, offers an explanation for the conserved glycine-8, as the N-terminal peptide folds back along the surface of the α -helix adjacent to residue 8 (fig.3B). Recently, it has been demonstrated that the synthetic alanyl-8 isotoxin possesses a marked reduction in activity [27]. Thus glycine, normally a strong helix breaker, cannot be replaced without distorting the proposed native conformation of the toxin. In a similar fashion, substitution of proline-5, by glycine, yields a non-toxic peptide [27]. The proline, which is positioned at the N-terminal end of the α -helix (fig.3B), is probably essential for the close orientation of cysteine residues 3 and 13, during the folding process. Significantly, proline residues are positioned in analogous positions in the bee venom toxins apamin, mast cell degranulating peptide and tertiapin [28].

It has been established that conotoxins GI and GII compete with the binding of α -[125 I]bungarotoxin to the acetylcholine receptor present on mouse diaphragm end plates [29], and are at least 10-times more potent than the well characterised alkaloid antagonist d-tubocurarine. An extensive structure-activity study has been reported for the elapidae neurotoxins, including α -bungarotoxin, and a reactive site of these molecules has been identified [16]. It is now possible to compare the tentative 3D structure of the conotoxins with this reactive site [11]. In the 4 conotoxins there are two positive centres at positions 1 and 9 separated by approx. 14-15 Å, a similar distance to that between two of the conserved basic residues in the elapidae toxins, which are believed to be essential for interaction with the nicotinic receptor [15,16]. Furthermore, between the two basic residues on conotoxins GI and GII

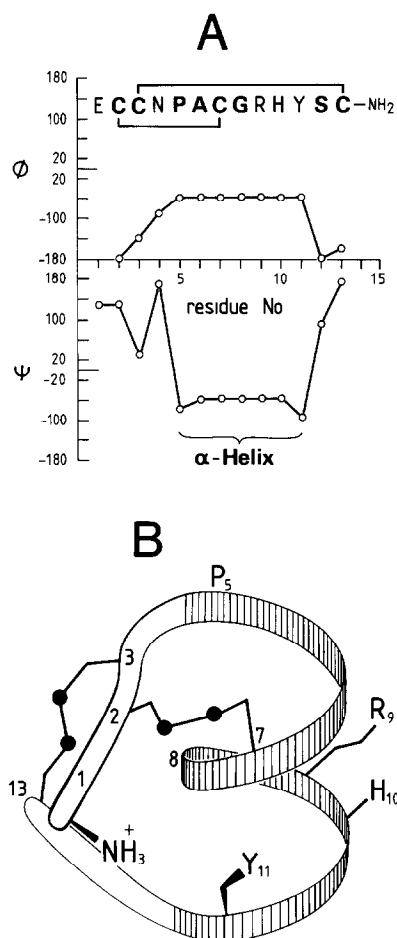


Fig.3. Structure of conotoxin GI. (A) Ramachandran angles of the refined structure built with Nicholson models. (B) Schematic representation of the structure, indicating the relative positioning of the disulphide bonds with respect to the α -helix. The proposed reactive site consists of residues 1, 9, 10 and 11.

are two aromatic side chains (fig.3B). Precisely the same arrangement occurs with the elapidae toxins [16] and dicationic alkaloid nicotinic antagonists [30,31]. Thus the interactive region of the conotoxins is probably centred on residues 1, 9, 10 and 11; significantly, replacement of L-tyrosine-11 with D-tyrosine leads to total loss of activity [27]. Presumably glutamate-1 is not essential for activity as glycylarginine replaces this residue in conotoxin M1. Although histidine-10 exchanges for asparagine-4 in M1, histidine-4 is orientated to the same side of the molecule as tyrosine-11. As cono-

toxins are amenable to chemical synthesis, specifically designed isotoxins could prove useful in the further characterisation of the 3D structure of the nicotinic receptor.

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