A PROPOSAL FOR THE STRUCTURE OF APAMIN

R. C. HIDER and U. RAGNARSSON

Department of Chemistry, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ England and *Institute of Biochemistry, Biomedical Center, University of Uppsala, S-751 23, Uppsala, Sweden*

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1. Introduction

Apamin is an octadecapeptide neurotoxin present in bee venom. It acts both centrally and peripherally, binding specifically to a protein in rat forebrain with high affinity ($K_d \sim 10^{-11}$ M) [1] and acting as a potent noncompetitive inhibitor of α-adrenergic and purinergic agonists [2]. The amino acid sequence (fig.1) has been independently determined by two groups [3,4] and the positions of the disulphide bridges have been established [5]. Synthetic apamin [6–8] possesses full biological activity and hence the information for the correct folding of the peptide chain is contained in that chain and not in an apamin precursor. As a result, apamin is a suitable candidate for secondary structure prediction. This paper proposes a structure for apamin on the basis of such a study. The structure is capable of explaining all the available physico–chemical data of apamin and its derivatives, including its high degree of stability. The proposed conformation will facilitate structure–activity studies with this class of molecule.

2. Methods

A modification [9] of Chou and Fasman's original method [10] which uses the product of parameters rather than the arithmetic mean in order to detect structural tendencies has been successfully applied to snake venom toxins [9] and melittin [11]. Consequently we adopted this method for a similar analysis of apamin and some of its synthetic analogues. Location of β-turn potential was achieved using the data in [12].

3. Results and discussion

The circular dichroism spectrum of apamin [13] is consistent with a high α-helical content. Although β-turns of the gramicidin-*S* type produce similar CD spectra, the essential components of such a structure, glycine or D-amino acids [14,15] are not present in apamin. Thus of the two structural forms, the α-helix would appear to be the most likely contributor. The conformation of apamin is not greatly influenced by chemical modification of the side chains [13,16] and CD studies have shown that its conformation remains relatively constant both over a wide pH range and in the presence of organic solvents and 6 M guanidinium–HCl [13]. These studies indicate that the stability of apamin is largely dependent on the two disulphide links and hydrogen bonding between the backbone amide groups.

In order to locate the most probable site of the helical region in apamin we undertook a secondary structure prediction study. An extensive region of α-helix was predicted. However, a section of this region was also found to possess high β-sheet potential, although the average α-helical parameters were larger (fig.1) which is in agreement with the CD data. Five tetrapeptide sequences were located possessing high β-turn potential.

It is not possible to construct the 1–11 and 3–15 disulphide links when the entire 4–17 peptide segment is in an α-helical conformation. However, this crosslinking becomes possible when the α-helix is limited to either segment 8–17 or segment 4–12. The important ^{1}H NMR study of apamin backbone amide protons [18] is compatible with the former. The existence of an α-helical 8–17 segment permits

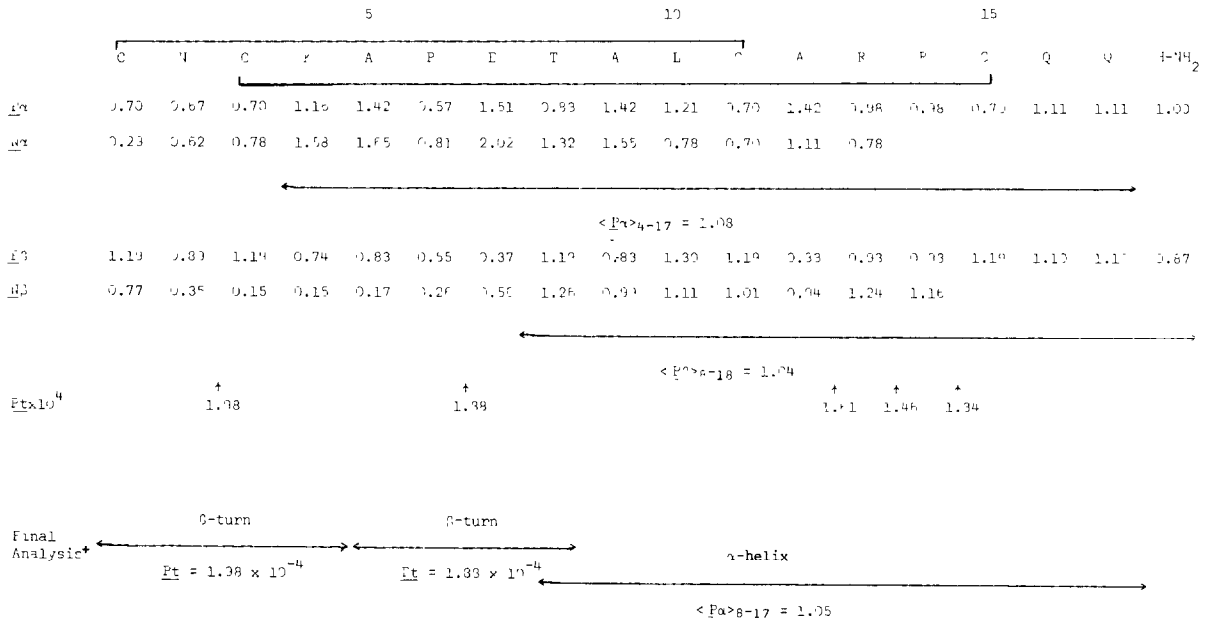

Fig.1. Sequence and conformational parameters of apamin. $P\alpha$, $\langle P\alpha \rangle$, $P\beta$ $\langle P\beta \rangle$ and Pt are defined in [17]. $N\alpha$ and $N\beta$ are defined in [9]. +Final analysis was made after consideration of CD [13] and NMR [18] spectra. The α-helical content of apamin was estimated to be 50%, using a value of $\theta_{190\ nm} = 7.7 \times 10^4$ deg. cm^2. dmol^{-1} [19].

the formation of two strongly predicted β-turns for the N-terminal octapeptide. Formation of these turns orientates the half cystines 1 and 3 with the half cystines 11 and 15, respectively (fig.2). The overall conformation is rigid, with the exception of the C-terminal dipeptide unit and is entirely consistent with the detailed NMR analysis [18]. Glutamate 7 is adjacent to the N-terminal amino function and on treatment with gadolinium nitrate the carboxylate function would be expected to be in the immediate environment of threonine-8 and alanine-9 which accounts for the intense gadolinium perturbation of these residues [18]. Furthermore the conformation shown in fig.2 offers an explanation for the NH deuterium exchange rates of the individual amide protons [18] as indicated in table 1. Indeed it is possible to assign the amide protons located in the centre of the helix to slower exchange rates than those located on the more exposed C-terminal end of the helix.

Specific removal of arginine-14 from N^{α},N^{ϵ}-lysyldiacetylapamin by trypsin treatment caused complete structural collapse of the apamin molecule [13]. Such modification would disrupt the rigid relative orientation of cystine-(1–11) and cystine-(3–15), which in the proposed structure (fig.2) is maintained by the α-helix.

The structure depicted in fig.2 is not in agreement with the statement that the dipeptide arginylarginine is a strong helix breaker [17]. However, no support for this statement was found in the larger protein data set [20], where it is clear that arginylarginine does not show antihelical tendancies.

Substitution of both arginine-13 and arginine-14 either by lysine residues [21] or ornithine residues [22] causes a dramatic loss in neurotoxic activity. It is unlikely that this change in activity results from a structural modification in the peptide, as the predicted parameters for the dilysine isotoxin are even more favourable for the proposed conformation, namely $\langle\langle P\alpha \rangle_{8-17} = 1.08$ and $\langle P\beta \rangle_{8-18} = 1.00$. In contrast substitution of only one arginine by lysine fails to induce a dramatic loss in neurotoxicity [16,21]. As a result of the predicted flexibility of the 17–18 dipeptide, it would be possible for a C-terminal carboxylate anion to form salt links with one or both of the arginine residues. C-Terminal amidation of apamin prevents such an interaction.

This communication gives an indication of the

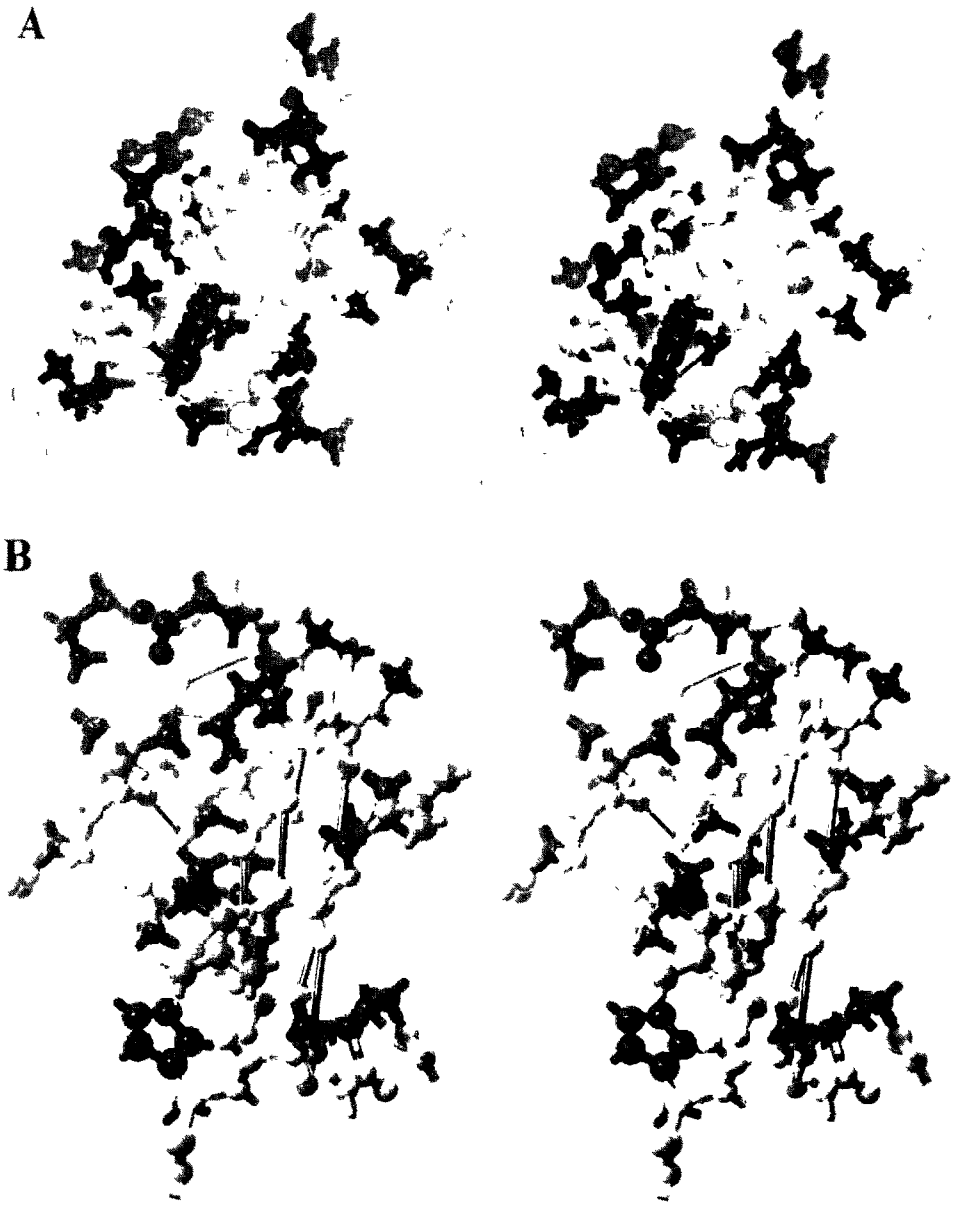

Fig.2. Stereophotographs of proposed apamin structure built with Nicholson models. The polypeptide backbone forms a cylindrical framework, l = 17 A°, $\leq \sim 5$ A°. (A) Normal to the α-helix, viewed from the C-terminal. (B) Parallel to the α-helix, showing the two N-terminal β-turns.

Table 1
Correlation between NH deuterium exchange rates [18] and the environment of NH function in proposed apamin structure

Amide NH	NH-deuterium exchange rates ($t_{1/2}$ (h))	Position in postulated structure
Cystine-1	–	–
Aspargine-2	< 0.1	Not H bonded and fully exposed
Cystine-3	0.2	Not H bonded and fully exposed
Lysine-4	4	H bonded in β-turn
Alanine-5	11	Not H bonded but shielded from solvent
Proline-6	–	–
Glutamate-7	0.1	Not H bonded and fully exposed
Threonine-8	4	H bonded in β-turn[a]
Alanine-9	0.1	Not H bonded and fully exposed
Leucine-10	0.5	Not H bonded and fully exposed
Cystine-11	26	Not H bonded but shielded from solvent[a]
Alanine-12	26	H bonded in α-helix
Arginine-13	>100	H bonded in α-helix (centre of helical region)
Arginine-14	20	H bonded in α-helix
Cystine-15	14	H bonded in α-helix
Glutamine-16	11	H bonded in α-helix
Glutamine-17	2.7	Loosely H bonded in α-helix
Histidine-18	< 0.1	Not H bonded and fully exposed

[a] A minor modification of the structure illustrated in fig 2 involves rotation of the amide group connecting glutamate-7 and threonine-8. Such rotation would disrupt the 5–8 β-turn hydrogen bond but would simultaneously result in the extension of the α-helix by forming a new hydrogen bond between the half cystine-11 amide proton and the carbonyl oxygen of glutamate-7. Such a modification would not require any other associated conformation changes

Although the H/D exchange study was run at pH 2.9, the CD spectrum remains constant over pH 2–7 [18] indicating no large conformational change occurs on acidifying the medium

potential of secondary structure analysis, when coupled with 300 MHz ^{1}H NMR and CD spectroscopy, for peptide structure elucidation. Further work involving related bee venom peptides is in progress.

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