SECONDARY STRUCTURE OF SEA ANEMONE TOXINS

Circular dichroism, infrared spectroscopy and Chou-Fasman calculations

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1. Introduction

Characteristic features of polypeptide toxins isolated from various species of sea anemone are low M_r, high content of hydrophobic amino acid residues, strong basicity and occurrence of 3 disulfide linkages in the molecule [1]. As physiological specificity of toxins may be related to their conformational states different methods were used to study spatial arrangements of toxin II from *Anemonia sulcata* (ATX-II) and anthopleurin A (AP-A) from *Anthopleura xanthogrammica* [2–5]. Preliminary data on the conformational stability of toxin I from *Radianthus macrodactylus** (RTX-I) appeared in [6]. Here a detailed investigation of circular dichroism (CD) spectra and infrared (IR) spectrum of RTX-I has been performed and an attempt made to generalize the conformational data obtained by spectral methods [2–6] and modified Chou-Fasman method [7] for all toxins considered in [1].

2. Experimental

RTX-I was isolated as in [8]. The carboxymethylated RTX-I was obtained as in [9]. CD spectra were recorded on a Dichrograph III (Jobin-Yvon) in the region of 185–250 nm in aqueous solutions at 1 mg toxin/ml. IR spectra were recorded on a Perkin-Elmer 180 spectrophotometer in matched CaF_2 window cells of 0.078 mm thickness at 10 mg RTX-I/ml in 0.1 M NaCl solution in D_2O. For toxins with the known amino acid sequences [1] the modified Chou-Fasman method [7] was used with Levitt conformational parameters built up on an extended basis of the data [10]. To predict β-turns we have calculated the product $N_t = P_{t1} \cdot P_{t2} \cdot P_{t3} \cdot P_{t4}$ making use of the Chou-Fasman data [11].

3. Results

3.1. *Circular dichroism*

CD spectra of proteins in the peptide region (185–250) are directly related to their secondary structure [12]. CD spectrum of RTX-I aqueous solution (fig.1) has a positive band (186 nm) and a negative band (201 nm) of nearly equal amplitudes both due to the $\pi-\pi^*$ amide chromophore transitions. The $n-\pi^*$ peptide transition in the RTX-I CD spectrum is observed as a shoulder at ~ 217 nm. A weak positive CD band at 232 nm may be assigned to transitions of tyrosine and/or tryptopan residues by analogy with CD spectra of snake toxins [13]. CD spectrum of the carboxymethylated toxin I (cmRTX-I) is also shown in fig.1. It has a negative maximum at 196 nm and a shoulder at ~220 nm. The absence of the 232 nm band is indicative of a change in the environment of aromatic amino acid residues on reduction of the disulfide linkages in the native RTX-I. The comparison of the obtained CD spectrum of RTX-I with that of AP-A [3] brings out only slight differences in wavelengths and intensities of the CD maxima which confirms once again the assumption of a close relationship between the spatial structure of RTX-I and the other sea anemone toxins [6].

As 'infinite' polypeptide models were used to analyse the secondary structure of proteins from CD spectra [12], some difficulties arise when attempting to determine small segments of the regular structures of short-chain proteins. In [14] model spectra of the

* This sea anemone was defined in [6] as *Homostichantus duerdemi*, but more accurate analysis has shown it to be *Radianthus macrodactylus*

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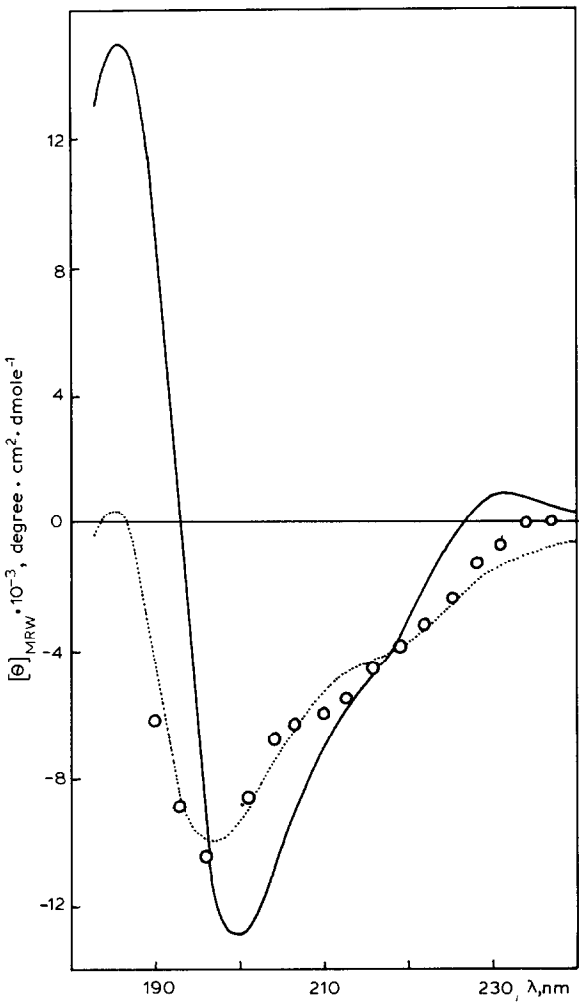

Fig.1. CD spectra of native (——) and carboxymethylated (. . .) RTX-I in aqueous solutions. (○) Theoretical spectrum of sea anemone toxin calculated on the basis of model CD spectra [14] and data of modified Chou-Fasman method [7].

α-helices had a limited number of peptide units. Making use of those data and the results obtained by the Chou-Fasman method (table 1) we have calculated the CD spectrum of a sea anemone toxin which contains the 6-residues α-helical segment (14%), β-structure (36%) and unordered form (50%). The results obtained agree well with the spectrum of cmRTX-I but differ considerably from that of native RTX-I (fig.1). This difference is presumably due to an occurrence of several β-turns in the sea anemone toxins [3], model spectra of which were not available in [14].

3.2. *Infrared spectroscopy*

IR studies of polypeptides and proteins conducted systematically in [15–18] enabled us to obtain quantitative data concerning their secondary structure. A graphical analysis of the absorption bands in the amide I region of the RTX-I IR spectrum (in 0.1 M NaCl/D_2O solution) is shown in fig.2. The experimental contour (fig.2a (1)) has a maximum at 1636 cm^{-1} and a shoulder at ~1650 cm^{-1} which are indicative of the β-structure and the unordered form in the RTX-1 [15]. To obtain the peptide absorption (curve (2)) we have subtracted the absorption of the amino acid side chains *(R)* from the experimental contour; the *R* curve has been calculated in according to the known amino acid composition of RTX-I [8] and the data from [16]. The contour thus obtained (fig.2b) has been consequently decomposed into components due to the β-structure (1630 and 1660 cm^{-1}) and unordered form (1654 cm^{-1}) absorptions. Amounts of these forms (21–25% and 45–50%, respectively) have been estimated from the band integral intensities [15]. The splitting of the β-form

Table 1
Secondary structure of sea anemone toxins calculated by modified Chou-Fasman method [7]

Toxin[a]	α-Helix	β-Structure	β-Turns
ATX-I	4–9	18–27,36–43	10–13,27–30
ATX-II	4–9 (31–37)[b]	17–26,37–44	10–13,27–30
AP-A	2–9 (33–41)	16–24,38–45	10–13,25–28,29–32
AP-C	4–9 (31–37)	17–25,37–44	10–13,27–30
Mean content	14%	36%	19%

[a] *Abbreviations:* ATX-I and ATX-II, toxins I and II from *Anemonia sulcata;* AP-A, anthopleurin A from *Anthopleura xanthogrammica;* AP-C, antopleurin C from *Anthopleura elegantissima*

[b] Second eventual α-helical region is shown in brackets

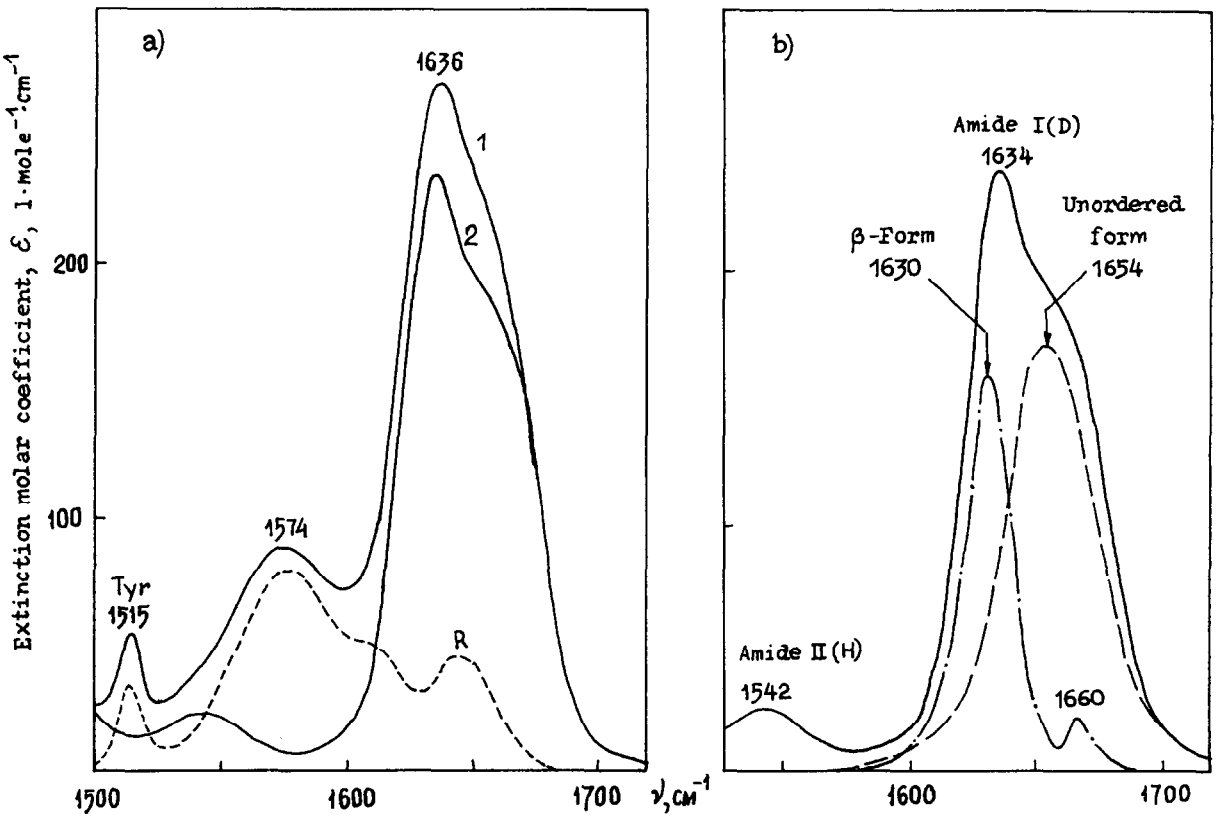

Fig.2. Graphical analysis of IR spectrum of RTX-I in 0.1 M $NaCl/D_2O$ solutions: (a) experimental spectrum (1) and contour of peptide absorption (2) obtained by subtraction of amino acid side chain absorption (R) from (1); (b) absorption bands of β-form (1630 and 1660 cm^{-1}) and unordered form (1654 cm^{-1}).

components which is equal to 30 cm^{-1} corresponds to an occurrence of 2 chains in the anti-parallel β-pleated sheet [17]. An eventual contribution of the α-helix to the peptide absorption is masked by the unordered form band [18] which makes difficult to estimate the content of the α-helical form. The obtained results on the ordered structure content may be underestimated due to distortion of the peptide unit geometry at the ends of the α- and β-regions.

3.3. *Chou-Fasman calculations*

The statistical Chou-Fasman method [19] was successfully applied for prediction of the secondary structure of proteins. Moreover, this method as shown in [20] gives more accurate results for smaller and thermally stable proteins to which sea anemone toxins belong [6]. We have used the modified Chou-Fasman method [7] to predict the α-helix, β-structure and β-turns in 4 sea anemone toxins with the known primary structure [1] (table 1). The calculations show that 1 α-helical, 2 β-strand regions and 2 (or 3) β-turns may be proposed for the toxins studied. A second predicted α-helical region between the residues 31–37 (ATX-II and AP-C) or 33–41 (AP-A) may be regarded as an artifact because the analysis of spectral data on ATX-II [2] and AP-A [3] and our results do not confirm a high content of the α-helix in the sea anemone toxins.

These results indicate a similarity of the overall conformation of investigated toxins, which could explain their identical physiological activity and similar spectral properties [2–6]. The data of Chou-Fasman method and analysis of the IR spectrum of RTX-I have allowed us to suggest a model for spatial arrangement of the sea anemone toxins with anti-parallel packing of 2 predicted β-strands. For example, the tertiary structure of the AP-A is shown in fig.3, for which the greatest amount of spectral data is available [3,4].

4. Discussion

In pioneer Raman spectroscopic studies of ATX-II [2], it was concluded that only the unordered form was predominant in its molecule. A study of anthopleurin A by Raman spectroscopy showed [3] that

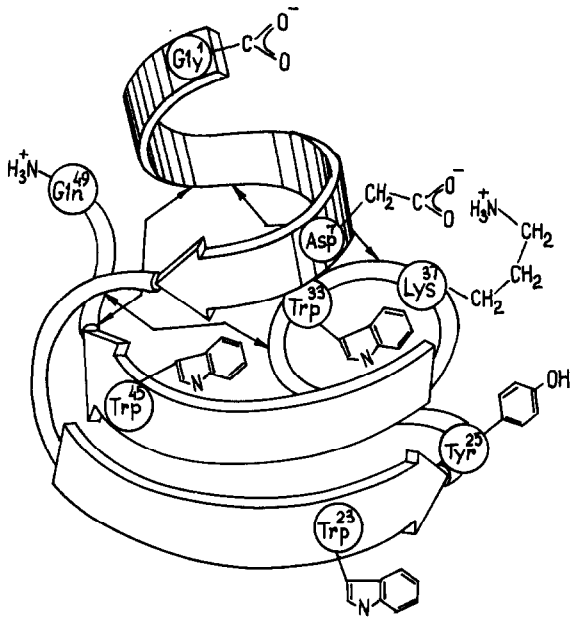

Fig.3. Probable model of spatial arrangement of AP-A built up according to data of modified Chou-Fasman method [7]. α-Helical region is shown as spiral band and β-structure as anti-parallel arrows. Disulfide bridges are shown as zig-zigged arrows [3].

its secondary structure contained α-helix (21%), β-structure (22%) and unordered form (57%). These results have been confirmed by our calculation of the AP-A secondary structure (table 1) by the modified Chou-Fasman method (16%, 34% and 50%, respectively). Analysis of the CD spectrum of AP-A suggests a probable occurrence of the β-structure and β-turns in its molecule [3] while the similarity between CD spectra of AP-A and RTX-I confirms a close relationship of the secondary structure of RTX-I and all formerly investigated sea anemone toxins.

A model of the spatial arrangement of AP-A (fig.3) proposed by us on the basis of the modified Chou-Fasman method agrees with the results of an NMR and Raman spectroscopy study of AP-A. As shown in [3,4] AP-A in solution has a roughly spherical shape with the Tyr-25 and Trp-23 residues exposed and the Trp-33 and Trp-45 residues buried for the solvent. The close located Asp-7 and Lys-37 residues form an intramolecular ionic hydrogen bond (fig.3). This part of the molecule, with its surrounding residues was assumed [5] to be 'an active site' of the AP-A and ATX-II toxins.

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