STRUCTURAL PROPERTIES OF TOXIN II OF SEA ANEMONE *(ANEMONE SULCATA)* DETERMINED BY LASER RAMAN SPECTROSCOPY

B. PRESCOTT and G. J. THOMAS, Jr.
Department of Chemistry, Southeastern Massachusetts University, North Dartmouth, Massachusetts, 02747 USA

László BÉRESS
Institut für Meereskunde an der Universität Kiel, Kiel, West Germany

Gert WUNDERER
Institut für Klinische Chemie und Klinische Biochemie, der Universität München, München, West Germany

and

Anthony T. TU
Department of Biochemistry, Colorado State University, Fort Collins, Colorado, 80523 USA

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1. Introduction

The sea anemone, *Anemonia sulcata,* paralyses its prey by injecting potent neurotoxins into it [1]. Recently, Beress et al. [2] isolated from *Anemonia sulcata* three neurotoxic polypeptides (toxins I, II and III) and characterized each according to its gross amino-acid composition, molecular weight, N-terminus residue and molar absorptivity. Toxin II was the most easily purified [2] and its amino-acid composition and sequence have been under further investigation [3]. Toxin II is of particular interest since recent studies suggest that its effect is neurotoxic on some organisms (e.g., crayfish) but cardiotoxic on others (e.g., guinea pig) [4–7]. Because toxicological specificity may be related to the protein conformation, we have undertaken an examination of the laser Raman spectrum of toxin II to identify specific features of its conformational structure.

The Raman spectrum consists of a series of lines or frequencies, due to scattering by vibrations of specific molecular subgroups [8]. For proteins and polypeptides, Raman frequencies of both the peptidyl backbone and side-chain groups are revealed, and several of these have been shown to be sensitive to the molecular conformation or environment [9]. (A review is given in [10].) The structural information thus derived from Raman spectra of proteins is not easily obtained by other methods, particularly when water is employed as a solvent.

Recent applications of Raman spectroscopy include conformational studies of a cardiotoxin from venom of the Mojave rattlesnake [11] and of neurotoxins from the venoms of sea snakes [12,13]. Amino acid compositions of these toxins differ substantially from one another [14–17] and from the composition of sea anemone toxin II (table 1).

2. Experimental methods

The sea anemone, *Anemonia sulcata*, was collected from the Bay of Naples by L. Béress and co-workers (Institut für Meereskunde, Universität Kiel), and toxin II was extracted and purified according to previously described procedures [2]. Amino acid analysis was conducted by G. Wunderer.

Spectroscopic examination of toxin II was carried

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Table 1
Amino acid composition of sea anemone toxin II[a]

Amino acid residue	Number per molecule of toxin
Glycine	8
Cystine (1/2)	6
Serine	4
Proline	4
Tryptophan	3
Leucine	3
Lysine	3
Valine, Isoleucine	5[b]
Histidine	2
Aspartic acid	2
Asparagine	2
Threonine	2
Alanine	1
Arginine	1
Glutamine	1
Total	47

[a] From data of [2] and [3] and G. Wunderer (unpublished results).
[b] A microheterogeneity in the sequence prevents the number of residues of valine and isoleucine from being quoted separately as integers, although these values are determined to be 1.5 and 3.5 respectively.

out at Southeastern Massachusetts University. Raman spectra of aqueous (144 μg toxin/μl H_2O) and lyophilized samples were excited with the 514.5 nm line of an argon ion laser (Coherent Radiation, Model CR2) and were recorded on a Spex Industries, Ramalog spectrometer. Capillary cells of 1.0 mm inner diameter were filled to approximately 10 μl with aqueous samples. Lyophilized samples were mounted in a goniometer for positioning in the laser beam. The spectra were recorded with a slit width of 8–10 cm^{-1} and photographed for presentation below (figs.1 and 2).

3. Results and discussion

Raman spectra of sea anemone toxin II in aqeous solution and in the solid state are shown in figs.1 and 2, respectively. Because of the different instrumental conditions employed for the two different samples and because the baseline in fig.1 is influenced by the solvent, figs.1 and 2 are best compared after proper compensation for these effects, viz., with subtraction of the sloping background below 700 cm^{-1} and the broad shoulder near 1645 cm^{-1} in fig.1 (both due to H_2O), and with normalization of the Raman intensities to the 760 cm^{-1} line of tryptophan in each spectrum (a reasonable normalization procedure [18]). It is thus seen that the spectra are nearly identical to one another. Exceptions are the lines at 874, 985, 1059 and 1075 cm^{-1}, due to C–C and C–N stretching vibrations of side chains (see below), which are more intense in the spectrum of aqueous toxin than in the spectrum of the solid. Also, the lines at 1311, 1340 and 1449 cm^{-1}, due to C–H deformation vibrations of side chains, are more intense in the spectrum of the solid than in that of aqueous toxin.

Accordingly, it appears that the conformation-sensitive amide I and amide III frequencies, and therefore the secondary structure of toxin II are not significantly affected by removal of the aqueous solvent. However, the effect of the removal of the

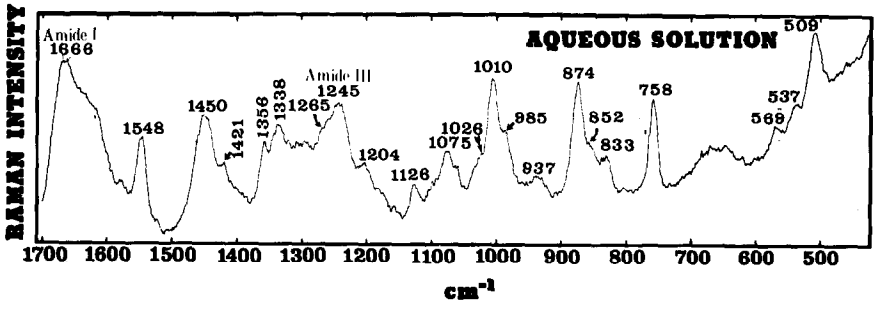

Fig.1. Raman spectrum at 32°C of sea anemone toxin II (400–1700 cm^{-1}), aqueous solution at pH 6.5, 144 μg toxin/μl H_2O. Conditions: excitation wavelength 514.5 nm; radiant power 300 mW; spectral slit width 10 cm^{-1}; scan speed 25 cm^{-1}/min; rise time 10 sec.

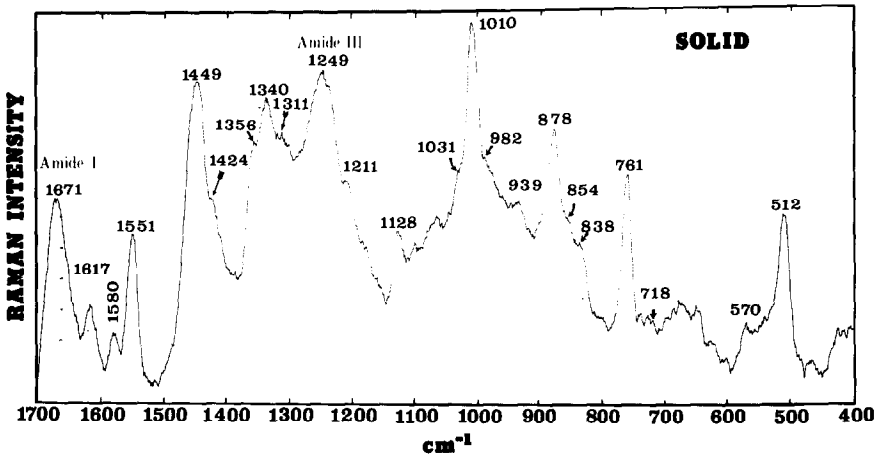

Fig.2. Raman spectrum at 32°C of sea anemone toxin II (400–1700 cm^{-1}), lyophilized powder. Conditions: as in fig.1 with the exception that the radiant power is 150 mW.

aqueous solvent on the side-chain frequencies is apparent.

Specific conformational properties of the toxin which can be deduced from the spectra of figs.1 and 2 are as follows. The peptidyl backbone is deficient in ordered secondary structures of the α-helical and antiparallel β-sheet types. The α-helical structure is expected to give an amide I frequency near 1650 cm^{-1} and an amide III frequency near 1275 cm^{-1}, the former intense, while the β-structure is expected to give an amide I frequency near 1665 cm^{-1} and an amide III frequency near 1235 cm^{-1}, both intense. On the other hand, a disordered (so-called 'random-coil') structure is expected to give an amide I frequency near 1670 cm^{-1} and an amide III frequency near 1245 cm^{-1}, again both intense [9]. Thus, by these criteria, figs.1 and 2 indicate a peptidyl backbone that is predominantly disordered.

The weak shoulder near 1265 cm^{-1} in fig.1 is most probably due to the two histidine residues in each molecule of the toxin [2,3] (table 1). However, the possibility of a small amount of α-helical structure in aqueous toxin II cannot be ruled out. This feature is not evident in the spectrum of the solid (fig.2). Raman scattering near 938 cm^{-1} (figs.1 and 2) has also been identified with disordered and α-helical chain conformations [19,20].

It is interesting to note that the envelope of Raman scattering of toxin II in the amide I and III regions (1650–1700 cm^{-1} and 1225–1300 cm^{-1}, respectively) is closely similar to that reported for chemically and irreversibly denatured lysozyme and ascribed to a random-coil conformation [18,21]. The use of the term 'random-coil', requires some qualification in view of the foregoing.

In aqueous solution the intensity of the line at 509 cm^{-1} (512 cm^{-1} in the solid), due to S–S stretching vibrations, indicates that all three disulfide linkages of the toxin [2] are intact in the samples investigated. Moreover, from studies of model compounds [22,23], it can be concluded that the position of the Raman line at 509 cm^{-1} indicates a gauche–gauche–gauche configuration for each of the C–C–S–S–C–C networks. Therefore, the 'disordered' secondary structure of toxin II, referred to above, does not imply a statistical random-coil in which the chain is free to assume any orientation, but should be regarded as a structure in which the average configuration of peptidyl groups differs markedly from that normally occuring in either α-helical or β-sheet structures. Again, we note that irreversibly (heat) denatured lysozyme also exhibits amide frequencies characteristic of a disordered backbone, yet with most, if not all, of its disulfide linkages intact [18].

The three tryptophan residues (of a total of 47) in each molecule of the toxin (table 1) dominate much of the Raman spectrum. Lines observed near 538, 570, 710, 760, 876, 1010, 1338, 1356, 1423,

1550, 1580 and 1618 cm^{-1} are due in whole or in part to vibrations associated with tryptophan ring [24]. The sharpness of the 1356 cm^{-1} line (fig.1) indicates that the tryptophan residues are probably "buried" and not exposed to the solvent [18,25]. The two histidine residues contribute mainly to the Raman lines near 623, 985, 1204 and 1265 cm^{-1}. Glycine, the most abundant amino acid in the toxin, is assigned the line near 1030 cm^{-1} which appears as a shoulder to the 1010 cm^{-1} line of tryptophan.

The remaining Raman lines in the spectra are assigned to C–C stretching (800–950 cm^{-1}), C–N stretching (1050–1200 cm^{-1}) and C–H deformation (1300–1500 cm^{-1}) vibrations of the side chains*. The absence of any intense lines due to phenylalanine or tyrosine residues confirms amino-acid analyses on toxin II which reveal that neither of these is an amino acid constituent ([2,3] and table 1).

When the present results are compared with those obtained on other toxins [11–13], many structural dissimilarities are apparent. Neurotoxins from sea snake venoms [12,13] have been shown to contain large amounts of β-structure, while the cardiotoxin of Mojave rattlesnake venom [11] is predominantly α-helical. The toxins thus far examined therefore display a remarkable diversity in their conformational structures. Clearly, more toxins will have to be investigated before any definite conclusions can be made relating their conformational and toxicological properties. Nevertheless, it is tempting to speculate that the disordered structure detected here for the sea anemone toxin II may also be a stable conformation in vivo. It is feasible that such a chain conformation could provide the flexibility needed for the polypeptide to function either as a neurotoxin or as a cardiotoxin.

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*A complete list of the observed Raman frequencies and assignments for toxin II is obtainable on request from the first authors.

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