Note to the Editor

Optical rotatory dispersion of crotamine: effect of denaturants

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The amino-acid sequence of crotamine, a toxin found in the venom of the south Brazillian snake *Crotalus durissus terrificus* was recently determined by Laure[1]. It is a miniprotein with 42 amino-acid residues and a minimum molecular weight of about 5000. This molecule contains among others, 6 half-Cys, 2 Trp, 1 Tyr and 2 Phe residues which can contribute to the behaviour of the optical rotatory dispersion measured in the ultraviolet region of the spectra.

The present communication reports studies on the ultra-violet o.r.d. of crotamine and the effect of denaturants on the protein. The pure protein used in the experiments was obtained from the snake venom purified on a column of SP-Sephadex G-25 with a gradient of 0.5 to 3.0 M NaCl in 0.05 M ammonium formate, pH 3.2, after gel filtration in Sephadex G-75 with 0.05 M ammonium formate buffer pH 3.4. The purified material showed only a single component by disc electrophoresis in polyacrylamide gel[2].

O.r.d. measurements of the pure toxin, were carried out in a Fica Spectropol I Spectropolarimeter with a thermostatized cell and the reduced mean residue rotation values, $[m']$, were expressed in degrees $cm^2/dmol$.

The infra-red spectrum of crotamine was obtained with a Perkin-Elmer Model 225 Spectrophotometer in a pressed potassium bromide disc.

Figure 1 shows the o.r.d. curve of native crotamine dissolved in unbuffered distilled water pH 5.5 over a wavelength range of 197 to 325 nm. This anomalous spectrum is due to several Cotton effects caused by the

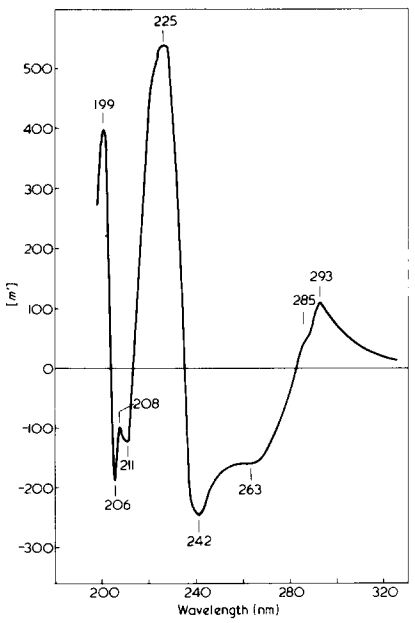

Figure 1 Optical rotatory dispersion of crotamine dissolved in unbuffered distilled water pH 5.5 at 25°C. The $[m']$ values from 325 to 237 nm are the average of those obtained for 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 mg/ml of protein using a 0.1 dm quartz cell, and those below 237 nm were obtained from 0.025 and 0.050 mg/ml of protein concentration using a 0.05 dm quartz cell

protein chromophores. The positive band found between 283 to 325 nm shows a peak at 293 nm which is related to the Trp residue. Besides the peak, a shoulder at 285 nm appears to the Tyr Cotton effect[3]. The very asymmetric negative band from 240 to 283 nm has two troughs centred at 263 and 242 nm. The first trough, indicates the participation of Phe[3], and disulphide bridges[4] in the anomalous o.r.d. spectrum of crotamine. The other one, can be related to β-II structure[5]. It is very difficult, if not impossible to determine which chromophores of the toxin are responsible for each Cotton effect found below 283 nm, mainly below 235 nm through the o.r.d. spectrum. Nevertheless, the presence of disulphide bridges can perturb some of the other Cotton effects of the protein[6].

Evidence of the presence of β-structure was obtained through the infra-red spectrum of the protein as shown in *Figure 2*. The peaks found at 1625 cm^{-1} of amide-I band and at 1533 cm^{-1} of amide-II band can be regarded as due to the presence of this structure in the molecule[7,8]. The $[m']$ values of +400 at 199 nm shown in *Figure 1*, and of −1460 at 232 nm of *Figure 3*, are not enough to postulate significant helical content in this macromolecule, despite the fact that sodium dodecyl sulphate at the concentration of 6.5×10^{-3} M changes the very anomalous o.r.d. curve to one characteristic of helical like structure with a trough at 232 nm (*Figure 3*, curve A).

The effect of chemical denaturant urea at a concentration of 8 M on the crotamine spectrum is shown in *Figure*

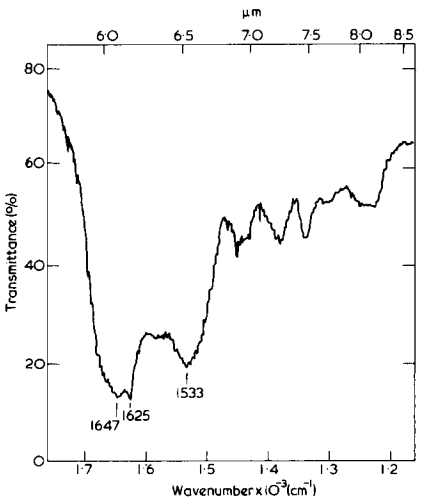

Figure 2 Infra-red spectrum of crotamine measured from 1.16×10^{-3} to 1.76×10^{-3} cm^{-1}. The spectrum was obtained using a pressed disc of 4 mg of protein with 200 mg of potassium bromide

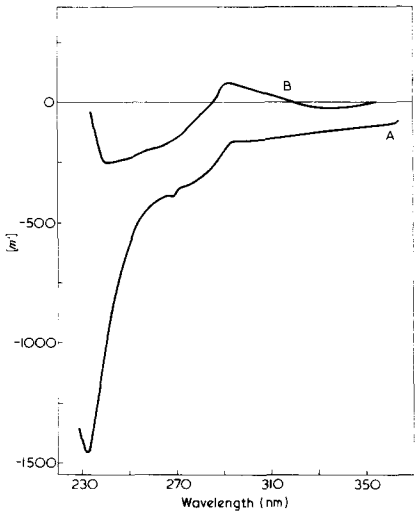

Figure 3 **Effect of denaturants on the conformation of crotamine. O.r.d. measurements of crotamine solution with a concentration of 1 mg/ml in 6.5 × 10^{-3} M sodium dodecyl sulphate were carried out between 230 and 360 nm (curve A) and of the same concentration of protein in 0.1 M acetate buffer pH 5.5 containing 8.0 M urea, between 230 and 350 nm (curve B)**

3, curve B. There are no significant changes in the spectrum from 235 to 350 nm which indicates a high resistance of the protein molecule to urea denaturation. The o.r.d. spectrum remained the same, even when a solution of crotamine in 0.1 M acetate buffer pH 5.5 containing 8 M urea was left at room temperature for twenty days and then heated at 60°C for two hours. However, the protein must have significant tertiary structure, since the reduction of the disulphide bridges by 2-mercaptoethanol in the presence of 8 M urea in 0.1 M tris-HCl buffer pH 8.6 abolishes the anomalous o.r.d. spectrum of the molecule between 250 and 320 nm.

The high resistance to conformational modifications of crotamine in the presence of concentrated urea solution combined with a very low intrinsic viscosity of 1.2 ml/g at pH 5.5, indicates that at tertiary structure level, the miniprotein is maintained very compact having an axial ratio close to the unity.

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