SAXS STUDY OF STRUCTURE AND CONFORMATIONAL CHANGES OF CROTAMINE

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ABSTRACT The radius of gyration of crotamine is determined by the small angle x-ray scattering technique. Several molecular solutions have been studied to correct for concentration effects. The apparent molecular radius of gyration is also determined as a function of pH. An important change between pH 9.5 and 12.5 is attributed to a dominant effect of molecular aggregation.

INTRODUCTION

Crotamine is a neurotoxin isolated from the brazilian snake *Crotalus durissus terrificus*. It is a polypeptide toxin, strongly basic ($pH_i = 10.3$), with a molecular weight of 4,870 and it is composed of 42 residues of 15 common amino acids including six half-cystines. It has a very high lysine (nine residues) and low arginine (two residues) content. The NH_2-terminus is tyrosine and the COOH-terminus glycine. Crotamine has been sequenced by Laure (1975), who also localized the three cysteine bridges (Conti et al., 1980).

Recent laser Raman spectroscopy studies (Kawano et al., 1982), related to the determination of the secondary structure of crotamine, indicate that crotamine may contain β-sheet and α-helix structure with slight predominance of the former. By spectropolarimetric titration (Hampe et al., 1978), it was possible to detect conformational changes in crotamine at several pH values and temperatures. Three pH-isomers were identified: a neutral isomer (I) found at pH values between 4 and 8.5, corresponding to the native conformation, an acid isomer (II) occurring at pH < 2.0, and a basic isomer (III) found at pH >9.5.

This paper is a study of the overall structure of crotamine in aqueous solution using the small angle x-ray scattering (SAXS) technique under different concentration and pH conditions, at room temperature.

METHOD AND MATERIALS

From small-angle x-ray scattering data of very dilute solutions, the molecular radius of gyration, R, and volume, V, of proteins can be derived. If the incident x-ray beam has a "linear and infinite" cross-section (Guinier and Fournet, 1955), R and V are determined by means of the following equations

$$J(s) = J(0)\exp\left(-\tfrac{4}{3}\pi^2 Rs^2\right) \tag{1}$$

and

$$V = \frac{1}{\sqrt{3\pi}}\,\frac{J(0)\,R}{\int_0^\infty J(s)s\,ds}. \tag{2}$$

In Eqs. 1 and 2, $J(s)$ is the SAXS intensity as a function of the modulus of the scattering vector **s**. It is related to the scattering angle ϵ by $s = 2[\sin(\epsilon/2)]/\lambda$, where λ is the x-ray wavelength. Eq. 1 (Guinier law) strictly holds for pinhole collimation but it is also usually applied, as an approximation, for slit collimation.

In practice, the SAXS intensity from dilute solutions is very weak. The concentration effects of SAXS results can be eliminated by collecting the data from several molecular solutions and extrapolating the apparent structure parameters R and V to zero concentration (Glatter and Kratky, 1982). These extrapolations are usually performed when the parameters exhibit a linear dependence on concentration.

The pure crotamine used in the experiments was obtained from snake venom purified as described by Laure (1975). The protein solutions were placed in Lyndemann capillary tubes (1.0 mm diam.) (Mark-Röhrchen, West Berlin, Federal Republic of Germany) and kept at room temperature. The concentration of the solutions of pH 4.5 was varied from 10 to 15% by weight of protein, in order to apply the SAXS method, which corrects for finite dilution effects. The experiments at different pH were carried out on solutions with 10% of protein. We did not use in these measurements the method of variable concentraton because of the very limited quantity of available pure crotamine. Although SAXS studies on a single concentration of crotamine solution provide less precise structure parameters, their results are useful in the detection of conformational changes associated with pH variations. The pH values were adjusted by the addition of HCl or NaOH.

The SAXS curves at constant pH and different concentrations of crotamine were obtained by using a position sensitive detector (Tennelec Inc., Oak Ridge, TN). The scattering curves for constant concentration and different pH were obtained by means of a small-angle goniometer (Rigaku/USA Inc., Danvers, MA), a scintillation detector, and an automatic step-scanning device (Slaets and Craievich, 1976). CuKα-filtered radiation and slit collimation were used in both kinds of measurements. The scattering due to the solvent and capillary tube was subtracted from the total experimental scattering intensity. The resulting SAXS curves were normalized to equivalent sample absorption and thickness.

EXPERIMENTAL RESULTS AND DISCUSSION

The first experimental results concern a set of SAXS curves from crotamine solutions of various concentrations at pH 4.5. The slope of the linear region of the plot in log J vs. s^2 scale (Guinier plot) leads to the apparent molecular radius of gyration for each concentration. The experimental values of R, which are plotted in Fig. 1, show a linear dependence on molecular concentration. By extrapolating the experimental values to zero concentration, the radius of gyration of the crotamine molecule was found to be R = 13.5 Å.

The parameter V is also concentration dependent. Fig. 1 shows the apparent values of V determined by means of Eq. 2 and the linear extrapolation yielding a volume V = 13,200 Å^3.

The next step was to obtain from the SAXS results some information on the overall shape of crotamine. Most of the water soluble proteins have a spheroidal (globular) shape. However, several studies of the atomic structure of other snake neurotoxins indicate a disk-like molecular overall shape (Tsernoglou and Petsko, 1976). We calculated the geometrical radius a of crotamine under the assumption of a spherical molecular shape [$a = (5/3)^{1/2} R$]. The associated volume, $V = (4/3)\pi a^3$, is equal to ~20,000 Å^3, which is much higher than that obtained from Eq. 2 (V = 13,200 Å^3). This discrepancy suggests that the crotamine molecule is not spherical. The absence of oscillations in the outer part of the experimental SAXS curves corroborates the suggestion of a nonspherical molecular shape (Glatter and Kratky, 1982). If the shape of the molecule is assumed to be ellipsoidal, its deviation from sphericity can be estimated by means of the geometrical equation

$$V = \frac{4}{3}\pi \nu R^3 \left(\frac{2+\nu^2}{5}\right)^{-3/2} \qquad (3)$$

where ν is the ratio a_3/a_1 of the two ellipsoid axis ($a_1 = a_2$). Substituting V and R in Eq. 3 by their experimental values, ν results in either 0.7 (oblate ellipsoid) and 1.5 (prolate ellipsoid). Thus, our experimental SAXS results are consistent with two (flattened and elongated) globular shapes. Although the asymptotic region of SAXS curves provides the molecular surface-to-volume ratio (Glatter and Kratky, 1982), the low accuracy of the experimental data at high angles did not allow us to use this ratio to decide between both ellipsoidal shapes.

The SAXS curves $J(s)$ at various pH are plotted in Fig. 2. The scattering curves in this figure can be approximated by two straight lines. This feature is expected from solu-

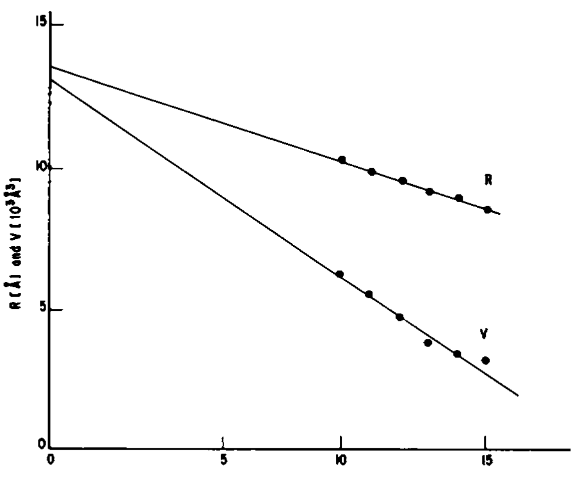

FIGURE 1 Extrapolations to obtain the molecular radius of gyration, R, and volume, V, at pH 4.5.

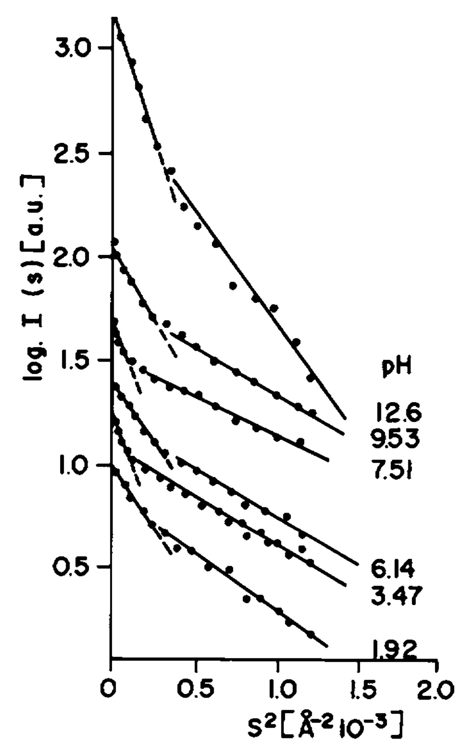

FIGURE 2 SAXS for different pH and constant concentration (c = 10%). The curves were vertically displaced for clarity.

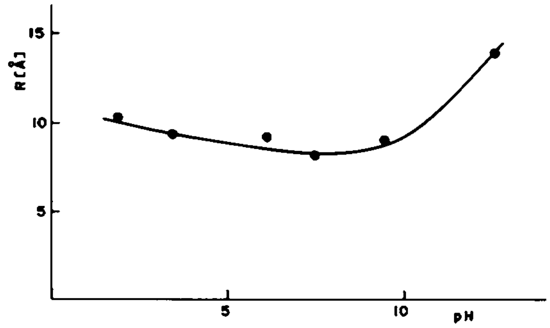

FIGURE 3 Radius of gyration of monomers as a function of pH.

TABLE I
APPARENT RADII OF GYRATION OF CROTAMINE MONOMERS AND AGGREGATES AT DIFFERENT pH*

pH	R_1[Å]	R_2[Å]
1.92	10.3	15
3.47	9.3	19
6.14	9.2	14
7.51	8.1	19
9.53	9.0	15
12.6	13.9	23

*Molecular concentration $c = 10\%$.

tions containing two kinds of particles with different radii of gyration R_1 and R_2. The two different values of R obtained for every solution at different pH are given in Table I. These (apparent) radii of gyration are not corrected for effects of finite concentration.

An important change of R_1 is observed for pH varying from 9.5 to 12.5. The sudden increase of the radius of gyration of the particles is a result of the dominant effect of molecular aggregation, which is the expected behavior for the solution close to its isoelectric point ($pH_i = 10.3$), leading then to a system without monomers. The SAXS result at pH 12.5 is consistent with a mixture of dimers and more complex molecular aggregates.

The second radius of gyration (R_2), deduced from the SAXS results (Table I), must be considered as a rather crude approximation, because of the narrow domain of validity of the Guinier law in the very small angle portion of the scattering curves (Fig. 2). Nevertheless, the SAXS results bring clear evidence of the heterogeneity of the macromolecular solution. The experimental values of Table I indicate that, from pH 2 to 10, the average values of R_1 (monomers) and R_2 (multimers) are 9.2 and 16.4 Å, respectively. The ratio of these two radii of gyration is $(R_2/R_1) = 1.78$. The geometrical calculation of the ratio R_2/R_1 for the simplest model of a dimer (two tangent spherical monomers) leads to $(R_2/R_1) = 1.78$, a value in close agreement with the experimental average ratio. Consequently, the experimental SAXS data, at pH ranging from 2 to 10, are consistent with the presence of a mixture of monomers and dimers with apparent radius of gyration of ~9 and 16 Å, respectively (see Fig. 3).

CONCLUSIONS

This small-angle x-ray scattering study of crotamine solutions provides the radius of gyration ($R = 13.5$ Å) of the molecule. The experimental results are consistent with a flattened or elongated (nonspherical) molecular shape. The overall shape of crotamine is probably not very different from that of the previously studied snake neurotoxins. Our SAXS results are not accurate enough to confirm this hypothesis by methods of direct comparison among the experimental curve and those curves theoretically predicted from different models (Glatter and Kratky, 1982).

This SAXS study shows the coexistence of isolated molecules and aggregates in the different solutions at various pH. At low pH (pH < 9), slight conformational variations in the molecule occur, leading to a more spheroidal shape for pH values approaching 9. At higher pH (pH 12.5), the high radius of gyration suggests a dominant aggregation process.

The availability of a larger amount of pure crotamine would allow SAXS measurements for a wider range of concentrations, which would provide more accurate information on the structure of the monomer and on the nature of aggregates, and more quantitative details of the molecular conformational variations.

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