CLXXXII. THE MOLECULAR WEIGHT OF CROTOXIN

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SLOTTA & FRAENKEL-CONRAT [1938, 1] have succeeded in purifying and crystallizing the active substance in the venom from the rattlesnake (*Crotalus terrificus*). They showed that this snake poison is a protein, with a large sulphur content (4 %), and gave it the name crotoxin. As far as we know, this is the first snake poison ever isolated in a pure crystalline state. It was therefore of great interest to determine its molecular weight.

From analytical determinations of the methionine content of the protein molecule, Slotta & Forster [1938] conclude that the minimum molecular weight is 11,000. They state that it is very probable that the real size is three or six times this value.

We have determined the molecular weight of crotoxin by the ultracentrifugal methods developed in this laboratory as described by Svedberg [1937].

The partial specific volume of the crotoxin was found to be 0·704, which is a comparatively low value for a protein. Due to lack of material we were not able to make more than one determination (1 % protein in 4 % salt solution). However, the error should not exceed 2 %.

The sedimentation constant was determined in the ultracentrifuge at a speed of 70,000 r.p.m., corresponding to a centrifugal force of 350,000 *g*. The observations were made by the scale method of Lamm [1937]. All the solutions were prepared immediately before the experiments from a stock solution, containing 1 % crotoxin in 0·67 *M* NaCl. Table I gives the results.

Table I. *Sedimentation velocity determinations*

Crotoxin concentration %	Salts in solvent	Total salt molarity	*p*H	s_{20}*
1·0	NaCl	0·67	Not buffered	3·10
0·5	,,	0·67	,,	3·18
0·3	,,	0·2	,,	3·09
0·2	,,	0·2	,,	3·14
0·3	NaCl, HOAc	0·25	3·8	2·92
0·3	,,	0·25	3·8	2·95
0·3	NaCl, HOAc, NaOAc	0·25	4·4	2·92
0·3	,,	0·26	5·5	3·06
0·3	NaCl, KH_2PO_4, $Na_2B_4O_7$	0·23	8·7	3·21
0·3	NaCl, $Na_2B_4O_7$, Na_2CO_3	0·25	10·0	3·20

* Sedimentation constants are given in units of 10^{-13}.

The sedimentation constant is, within experimental error, independent of concentration. The average of the first four determinations is $s_{20}=3·13$. In acid solutions the sedimentation constant is a little lower, but in neutral and alkaline solutions it has about the same value. The sedimentation curves obtained are symmetrical and suggest a homogeneous substance.

The diffusion of crotoxin was measured according to Lamm's method [1937; see also Lamm & Polson, 1936]. The following results were obtained:

Table II. *Diffusion determinations*

Crotoxin concentration %	Salts in solvent	Total salt molarity	D_{20}*
1·0	NaCl	0·67	8·70
0·6	NaCl, Na_2HPO_4, KH_2PO_4	0·72	8·51
0·5	NaCl	0·67	8·55
0·25	NaCl	0·67	7·55

* Diffusion constants are given in units of 10^{-7}.

The average of the first three values is $D_{20}=8{\cdot}59$. The last value is probably not so accurate, owing to the low concentration. The diffusion curves are in all experiments very nearly normal distribution curves, indicating a homogeneous substance. The diffusion constant was calculated also from the spreading of the boundary in the first of the sedimentation experiments, which gave $D_{20}=9{\cdot}0$. Such a calculation cannot give the same accuracy as a static diffusion experiment, but its good agreement with the figures in Table II shows that crotoxin is a monodisperse protein.

Using the formula

$$M=\frac{RT\,s_{20}}{(1-V\rho)D_{20}}$$

a value of 30,000 is obtained for the molecular weight of crotoxin. The ratio of the molecular frictional constant to that of a spherical molecule of the same weight, f/f_0, is 1·2. Slotta's value of 11,000 for the minimum molecular weight is, within experimental errors, one-third of our value.

To check the value we have also made a sedimentation equilibrium determination. The formula used for calculation is

$$M=\frac{2RT\ln(c_2/c_1)}{(1-V\rho)\,\omega^2\,(x_2^2-x_1^2)}.$$

The speed of the centrifuge was 10,000 r.p.m. Two cells were used, placed at 5·0 and 5·7 cm. distance from the centre of rotation. They both gave the same average value of 30,500 for the molecular weight. There was no drift in the calculated values along the radius, showing that the substance is homogeneous. This is in agreement with the results of the diffusion measurements given above.

Slotta & Fraenkel-Conrat [1938, 2] have found that cysteine reduces the poison value (for definition see Slotta & Szyszka [1938]) of the crotoxin, probably due to its specific reduction power for protein-S—S-bonds. It was of interest to see whether it was possible to determine the particle size of the decomposition products. We therefore made an experiment according to Slotta's & Fraenkel-Conrat's experimental data. The only difference was that for ultracentrifugal determinations we needed a higher concentration of the protein than they used. We started with a 0·25 % solution of crotoxin; on standing with cysteine a precipitate was formed, which was probably cystine. The solution remaining was ultracentrifuged, and was found to contain very little high-molecular material, but consisted mainly of low-molecular products of variable, undeterminable size.

Crotoxin, which had been standing 3 days at 37° and *p*H 8·7, contained the usual high-molecular component (s_{20} was measured in two experiments, 3·39 and 3·08), but in addition decomposition products of low molecular weight were

present. It is of interest to note that Slotta has found a decrease in the poison value by this treatment.

It is therefore obvious that the poison activity is associated with the unchanged protein, as already pointed out by Slotta, and the decomposition products have no, or only a very small, activity, as is observed also with the decomposition products of enzymes and other active proteins.

Summary

1. The partial specific volume of crotoxin was determined to be $V=0{\cdot}704$.

2. The sedimentation constant of crotoxin was found to be independent of concentration and of *p*H within the range 5·5–10·0. Its value was 3·13.

3. The diffusion constant was found to be 8·59.

4. Crotoxin behaved in sedimentation and diffusion as a homogeneous substance, and the molecular weight was calculated to be 30,000. The frictional ratio is 1·2.

5. Sedimentation equilibrium measurements gave the value 30,500 for the molecular weight, in good agreement with the preceding value.

6. The molecule is split into smaller, inactive substances by treatment with cysteine or by warming in alkaline solution.

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