THE COMPLEX TOXIC COMPONENTS OF THE RUSSELL'S VIPER VENOM

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Summary

Russell's viper venom has been fractionated on CM-cellulose, CM-Sephadex and Sephadex gel filtration and disc electrophoresis to obtain 4 toxins one of which is a glycoprotein with 2 subunits, one with 4 cationic subunits one of the subunits being dialysable, one with 3 cationic subunits and a dialysable low molecular weight minor toxin. The specific subunits of individual toxins were found to be necessary for their biological activities.

Master and Rao (1) had observed that the toxicity of the Russell's viper venom was found to be inactivated on starch gel electrophoresis which could be restored on the recombination of the electrophoretic fractions. This was attributed by them and later by Dimitrov and Kankonkar (2,3) to some co-factor which might be involved in the toxicity of this venom. The present report gives an explanation of that phenomena on the basis of the toxic components separated by column chromatography.

EXPERIMENTAL AND RESULTS: Sixty mg of lyophilized Russell's viper venom obtained from the Haffkine Institute, Bombay, India, was fractionated on a CM-cellulose (Whatman, U.K.) column (25 cm x 0.6 cm) using a linear pH gradient obtained with 0.05 M sodium acetate and 0.2 M HCl. The first of the two well separated

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peaks (designated as Peaks I and II) was further fractionated on a CM-Sephadex C-50 column under the same conditions. A homogeneous (4) toxin obtained was designated as T_1.

The second peak (II) of CM-cellulose chromatography on further fractionation on a Sephadex G-100 column (25 x 0.6 cm) on elution with distilled water yielded a toxin designated as T_2. On fractionation of the same peak II on a CM-Sephadex C-50 column and a pH gradient-elution with sodium acetate (0.05 M) against 0.5 M HCl, a homogeneous toxin designated as T_4 was obtained. (The Sephadex preparations were products of Pharmacia, Uppsala, Sweden.)

The homogeneity of the toxins was tested by disc electrophoresis for basic proteins as described by Panyim and Chalkley (4) and confirmed in molecular weight determinations by following the disc electrophoretic procedure described by Weber and Osborne (5) and adapted for basic proteins by Reisfied et al. (6) using crystalline albumin (Armour Pharmaceuticals, U.S.A.), pepsin (Metropolitan Drugs, India), trypsin (N.B.C., U.S.A.) and cytochrome C (Horse) Sigma Chemical Company, U.S.A. as standards of known molecular weights. The molecular weight of the dialysable toxin T_3 was determined by the Sephadex gel filtration method of Andrews (7).

Dialysis of the whole venom against distilled water and subsequent disc electrophoresis (6) of the lyophilized dialysate gave two well separated components; the slower moving being a toxin designated T_3 and the faster moving one proved to be a co-factor (C.T.) which is in fact the fourth subunit of T_2 without which T_2 is biologically inactive. Both these components (viz. T_3 and C.T.) were eluted separately from several gels with distilled water and obtained individually in homogeneous states.

The chemicals for disc electrophoresis were products of Serva, Heidelberg, Germany.

Glycoproteins after disc electrophoresis were visualized by the method of Zacharius et al. (8). Toxicity was determined by intravenous injections of

Table 1

Characteristics of the Isolated Toxins of the Russell's Viper Venom

Toxin	Molecular weight**	Characteristics	Toxicity* LD_{100}
T_1	55,000	glycoprotein with 2 subunits	216
T_2	16,000	basic protein with 4 subunits	173
T_3	Less than 4,000	dialysable basic polypeptide	400
T_4	13,000	basic protein with 3 subunits	246.5

*Toxicity = LD_{100} in μg of intravenously injected protein for six 20 g white mice in the case of T_1, T_2 and T_4. Toxicity for T_3 is its own weight in μg.
**See text.

the solutions of the toxins in physiological saline to white mice (20 g Kasauli strain) and observing the lethal effect for 24 hours. The minimum dilution to kill all the six animals in a set was the LD_{100}.

T_1, T_2 and T_4 further resolved into subunits (Table No. 1) on disc electrophoresis in the presence of 8 M urea. The subunits were found to be non-lethal to mice individually but on specific recombination their lethality was restored as tested by intravenous injections of phosphate buffered physiological saline (pH 7.3) eluates into white mice (18-20 g). The results are represented in Tables 1 and 2.

Discussion: The Russell's viper venom has been found to consist of a number of toxic proteins most of which have more than one non-lethal subunits which are necessary for their biological activity. As the individual toxins require the presence of all the specific subunits for their biological activities, in fractionation

Table 2

The Toxicity Experiments with Disc Electrophoretic Components

Component** injected	In presence + or absence - of 8 M urea	LD recovered/LD loaded per gel*
T_1	-	3/3
Subunit 1 of T_1	+	0/3
Subunit 2 of T_1	+	0/3
Subunits 1 + 2 of T_1	+	3/3
T_2 omitting C.T.	-	0/4
C.T.	-	0/4
T_2 including C.T.	-	3/4
The four subunits of T_2 individually	+	0/4 each time
All four subunits of T_2	+	3/4
T_2 omitting subunit 1	+	0/4
T_2 omitting subunit 2	+	0/4
T_2 omitting subunit 3	+	0/4
T_2 omitting subunit 4	+	0/4
Individual 3 subunits of T_2 + 150 μg C.T. to each 150 μg C.T.	+	0/4 each time non-toxic
The first three subunits of T_2 + 150 μg C.T.	+	3/4

*These experiments were repeated several times with appropriate controls.
**The buffered saline eluate (pH 7.3) of each disc electrophoretic components injected intravenously into 20 g mice. The lethal effect was observed for 24 hrs.
LD = Intravenous Lethal Dose for 20 g mice.
Subunits = The disc electrophoretic components in presence of 8 M urea.
C.T. = Co-toxin = the 4th subunit of T_2.
Similarly the toxicity of T_4 was found to be a combined effect of all 3 of its subunits.

procedures where the subunits get separated from one another, the individual components lose their toxicities which can be restored on the recombination of the specific separated subunits.

The highly lethal nature of the Russell's viper venom is thus the cumulative effect of several individual lethal toxins.

REFERENCES

1. Master, R. W. B and Rao, S. S. (1961) Jour. Biol. Chem. 236, 1986-1990.
2. Dimitrov, G. D. and Kankonkar, R. C. (1968) Toxicon 5, 213-221.
3. Dimitrov, G. D. and Kankonkar, R. C. (1968) Toxicon 5, 283-288.

4. Panyim, S. and Chalkley, R. (1969) Arch. Biochem. and Biophys. 130, 337-346.
5. Weber, K. and Osborne, M. (1969) Jour. Biol. Chem. 246, 4406-4412.
6. Reisfeld, R. A., Lewis, U. J. and Williams, D. E. (1962) Nature 195, 281-283.
7. Andrews, P. (1964) Biochem. J. 91, 222-233.
8. Zacharius, R. M., Zell, T. E., Morrison, J. H. and Woodlock, J. J. (1969) Anal. Biochem. 30, 148-152.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Jour. Biol. Chem. 193, 265-275.