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Note

Single-step fractionation of *Vipera russelli* venom
A sensitive fluorimetric method to study the elution profile

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Snake venom is a complex mixture of proteins and polypeptides. Some of the components are enzymes. Extensive studies of the components present in the venom of various snakes have been made[1–6]. A major problem of such studies is the isolation of the components in a pure state. Early studies[7–11] employed multistep procedures to fractionate snake venom. William and Esnouf[12] separated Russel's viper venom on DEAE-cellulose. Recently, Achyuthan *et al.*[13] developed a single-step separation procedure for several protein constituents of venom of the Indian cobra (*Naja naja*).

In this paper we report an ion-exchange chromatographic procedure which resolves the complex Russel's viper venom into twelve components in a singlestep. We also describe a fluorimetric method to screen the elution profile, to replace the tedious protein estimations.

EXPERIMENTAL

Commercial samples of Russel's viper (*Vipera russelli*) venom (Batch No. 759) were obtained from V. P. Chest Institute (New Delhi, India). CM-Sephadex C-25 (4.5 mequiv./g) was obtained from Sigma (St. Louis, MO, U.S.A.). Folin's phenol reagent was purchased from Centron Research Laboratories (Bombay, India).

The colorimetric measurements were made using a Bausch and Lomb Spectronic-20. The fluorimetric measurements were made with an Aminco-Bowman spectrophotofluorimeter. All chemicals and reagents used were of analytical grade.

Russel's viper venom was fractionated on a CM-Sephadex C-25 column (80 × 0.8 cm). A 50-mg amount of protein dissolved in the starting buffer was loaded. The column was eluted using phosphate buffers of various molarities and pH values, as given in Fig. 1. This column can be loaded with up to 100 mg venom protein. Fractionation was done at room temperature.

Fluorimetric screening of fractions was carried out by excitation at 288 nm and emission at 368 nm. Protein estimation was done by Miller's modification of Lowry's method[14].

RESULTS

By a single-step ion exchange/molecular sieving on CM-Sephadex C-25,

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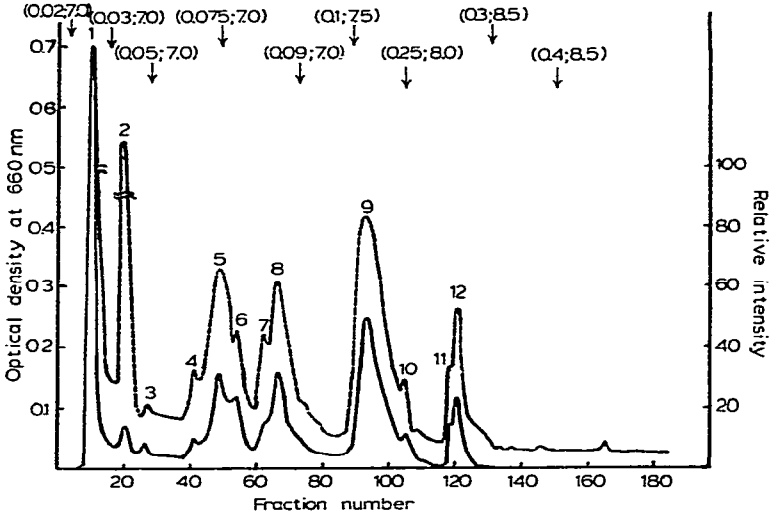

Fig. 1. CM-Sephadex C-25 column chromatography of Russel's viper venom (Batch No. 759). Loading: 50 mg in 1.0 ml of 0.02 *M* phosphate buffer (pH 7.0). Column: 80 × 0.8 cm. Flow-rate: 20 ml/h. Fraction volume: 2 ml. Temperature: room temperature. Elution was carried out stepwise with phosphate buffers of molarities and pH values as indicated. Recovery: 86%. ——, Protein quantity; – – –, fluorescence emission.

Russel's viper venom has been separated into twelve components, as shown by both colorimetric and fluorimetric screening. The two sets of measurements give similar elution profiles as shown in Fig. 1. Total recovery from the column is 86%. The yields and carbohydrate contents (expressed as percent glucose units) of the different components are given in Table I.

TABLE I

YIELDS AND CARBOHYDRATE CONTENTS OF DIFFERENT FRACTIONS OF RUSSEL'S VIPER VENOM

Proteins were estimated by Miller's modification[14] taking bovine serum albumin as standard. Carbohydrate was determined with phenol–sulphuric acid[15].

Fraction	*Yield (%)*	*Carbohydrate content (% glucose units)*
1	21.02	2.02
2	4.38	8.95
3	1.26	19.30
4	1.56	6.67
5	11.40	1.20
6	6.12	3.56
7	2.50	4.99
8	9.00	1.04
9	22.40	0
10	1.50	0
11	1.32	4.73
12	3.92	5.00

DISCUSSION

Earlier, multistep procedures were used in the separation of venom components. *Bangarus caeruleus* venom was separated by Cassian and Changeaux[16] using CM-Sephadex, QAE-Sephadex, Bio-Rex 70, Sephadex G-50 and Bio-Gel P-30 chromatography. Four steps were required to purify *Agkistrodon acutus* venom protein Ac_1, involving molecular sieving and ion exchangers[17]. A coagulant protein from the venom of Russel's viper was purified by Yoshiko *et al.*[18] using Sephadex G-50, DEAE-cellulose and Sephadex G-200 column chromatographic techniques.

There are few reports on the single-step purification of venom components. CM-cellulose was employed for the purification of *Naja naja* venom by Larsen and Wolff[19], while Sleegers *et al.*[20] used SE-Sephadex C-25 for the fractionation of Formosan cobra (*Naja naja atra*) venom. William and Esnouf[12] used DEAE-cellulose with a buffer gradient for the separation of Russel's viper venom. *Agkistrodon bilineatus* venom has been separated into thirteen fractions on DEAE-Sephadex A-50 using ammonium acetate as buffer[21]. Finally, Achyuthan *et al.*[13] separated *Naja naja* venom on a CM-Sephadex C-25 column.

We have developed a highly reproducible column chromatographic procedure employing CM-Sephadex C-25 for the separation of Russel's viper venom. This procedure gives twelve fractions in a single step, and could possibly be scaled up for larger loads with columns of greater dimensions.

The fluorimetric analysis with excitation at 288 nm and emission at 368 nm used to study the elution profile has distinct advantages. For example, the laborious procedure of protein estimation and its inherent wastage can be avoided. The fluorimetric method is also highly sensitive compared to both the UV absorption method and protein estimation. These advantages may be exploited to separate smaller amounts of the complex venom protein mixture without any material loss. This type of screening is highly suitable for studying trace amounts of protein(s) fractions.

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REFERENCES

1 C. C. Yang, in P. Rosenberg (Editor), *Toxins: Animal, Plant and Microbial*, Pergamon, London, 1978, p. 261.
2 A. T. Tu, *Venoms, Their Chemistry and Molecular Biology*, Wiley, Chichester, 1977, p. 1.
3 C. C. Yang, *Toxicon*, 12 (1974) 1.
4 A. T. Tu, *Annu. Rev. Biochem.*, 42 (1973) 235.
5 C. Y. Lee, *Annu. Rev. Pharmacol.*, 12 (1972) 265.
6 S. A. Minton, *Clin. Toxicol.*, 3 (1970) 343.
7 R. A. Shipolini, G. S. Bailey and B. E. C. Banks, *Eur. J. Biochem.*, 42 (1974) 203.
8 L. Fryklund and D. Eaker, *Biochemistry*, 12 (1973) 661.
9 F. Miranda, C. Kupeyan, H. Rochat, C. Rochat and S. Lissitzky, *Eur. J. Biochem.*, 17 (1970) 477.
10 K. Narita and C. Y. Lee, *Biochem. Biophys. Res. Commun.*, 41 (1970) 339.

11 J. A. Vick, H. P. Ciuchta, C. Broomfield and B. T. Currie, *Toxicon*, 3 (1966) 237.
12 W. J. William and M. P. Esnouf, *Biochem. J.*, 84 (1962) 52.
13 K. E. Achyuthan, K. Ranganatha Rao and L. K. Ramachandran, *Indian J. Biochem. Biophys.*, 17 (1980) 228.
14 G. L. Miller, *Anal. Chem.*, 31 (1959) 964.
15 M. Dubois, K. A. Killes, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, 28 (1956) 350.
16 B. Cassian and J.-P. Changeaux, *Eur. J. Biochem.*, 74(1) (1977) 31.
17 N. Toshiaki, H. Sugihara and T. Tanaka, *Yakugaku Zasshi*, 97 (1977) 507.
18 F. Yoshiko, Y. Matsunaga and K. Hayashi, *Biochim. Biophys. Acta*, 453(1) (1976) 48.
19 P. R. Larsen and J. Wolff, *Biochem. Pharmacol.*, 17 (1968) 503.
20 J. Slegers, J. Simon, L. Brisbois, J. Peeters and L. Gillo, *J. Chromatogr.*, 36 (1968) 241.
21 E. M. Bronson, B. D. Johnson and D. H. Sifford, *Amer. J. Trop Med. Hyg.*, 27 (1978) 2 part 1, 365.