BASIC ENCEPHALITOGENIC PROTEIN: A SIMPLIFIED PURIFICATION ON SULPHOETHYL-SEPHADEX

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1. Introduction

Experimental allergic encephalomyelitis (EAE †) is an experimental autoimmune disease induced in laboratory animals by a single injection of brain or spinal cord tissue in complete Freund's adjuvant [1,2]. The disease is characterized clinically by loss in weight and paralysis of hind limbs and histologically by perivascular infiltration and demyelinization. The histological manifestations of EAE are similar to those observed in humans with multiple sclerosis. In recent years the active encephalitogenic component in the nervous tissue has been shown to be a basic protein which is a constituent of myelin, and various purification procedures of this basic encephalitogen (BE †) have been reported [3-6]. These procedures involve several purification steps on various chromatographic columns. We report herein a method of obtaining a purified protein by passage through one column of SE-Sephadex.

2. Materials and methods

Bovine spinal cord (BSC †) was obtained fresh from the abattoir and frozen as soon as possible after the slaughter of the animal. It was defatted and the basic proteins extracted according to the procedure of Roboz et al. [7]. A crude mixture of the proteins (300 mg) extracted from BSC was applied to a column of SE-Sephadex C-50 (12 × 1.6 cm) (Pharmacia, Uppsala), equilibrated with 0.05 M phosphate buffer, pH 7,5, containing 0.1 M NaCl and NaN_3 (1/5000 w/v) at room temperature. After the preliminary peak had been eluted, a logarithmic salt gradient was applied; 0.53 M NaCl and NaN_3 (1/5000 w/v) in 0.05 M phosphate buffer, pH 7.5 (100 ml), was added dropwise to the eluting buffer (100 ml). The flow rate of the column was 7 ml/hr and volumes of 1.3 ml were collected. The resultant fractions were pooled, dialyzed against de-ionized water for 24 hr, and then freeze-dried.

Acrylamide gel electrophoresis was carried out at pH 4.6 [8]. The gels were prepared in 4 M urea [9] and photopolymerized. The stock solution of acrylamide was kept over Amberlite monobed resin MB-1 (BDH). The electrical conductivities of the 8 M urea and acrylamide solutions were always less than 10 μmho before polymerization. The conditions of the electrophoretic run were those of Chao and Roboz-Einstein [9]. The proteins in the gels were precipitated by 12.5% TCA, stained with Coomassie brilliant blue R-250 (Mann Research Laboratories) [10], and allowed to destain overnight in 12.5% TCA [11]. All chemicals were analytical grade.

The molecular weight was determined in a Spinco model E ultracentrifuge from sedimentation and diffusion measurements, as described earlier [12], and by low speed sedimentation equilibrium using interference optics [13]. The amino acids were determined, after hydrolysis in 6 M HCl at 110° for 22 hr, on a Beckman Model 120B amino acid analyzer [14].

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† *Abbreviations:*
EAE, experimental allergic encephalomyelitis;
BE, basic encephalitogen;
SE, sulphoethyl;
BSC, bovine spinal cord;
TCA, trichloroacetic acid.

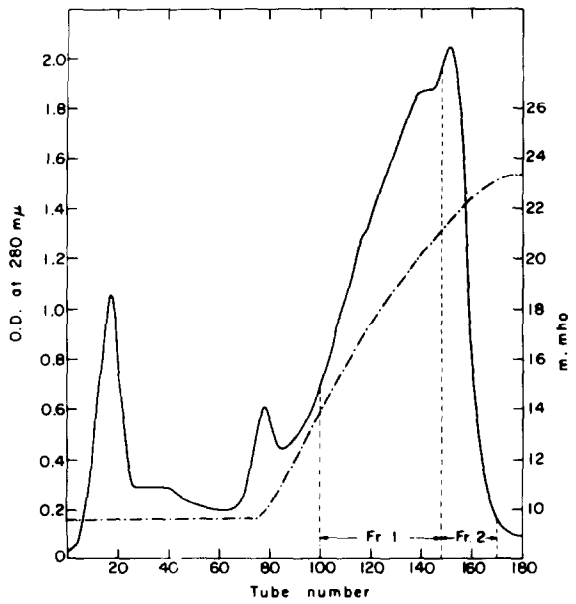

Fig. 1. Elution profile of crude BE (300 mg) on a column (12 X 1.6 cm) of SE-Sephadex C-50, equilibrated with 0.05 M phosphate buffer, pH 7.5, containing 0.1 M NaCl and NaN_3 (1/5000 w/v), and eluted by the application of a logarithmic gradient (100 ml in each flask) using 0.53 M NaCl in the same buffer. The gradient profile is shown by the broken curve.

Tryptophan was estimated according to Barman and Koshland [15].

Encephalitogenic activity was assayed by a single injection of the lyophilized protein in complete Freund's adjuvant into the hind foot pads of albino guinea pigs weighing more than 300 g each.

3. Results and discussion

The purification method described here involves the passage of crude encephalitogen over one chromatographic column (fig. 1). As the elution profile shows a shoulder in the gradient portion, the material under the main peak was divided into two fractions, SE-1 and SE-2. The SE-2 fraction gives only one band by acrylamide gel electrophoresis, but occasionally shows signs of a slower moving protein, possibly an aggregate (fig. 2). By this method a yield of 60 to 70 mg of SE-2 BE is obtained from 300 mg of crude protein extract, and a further amount of pure product

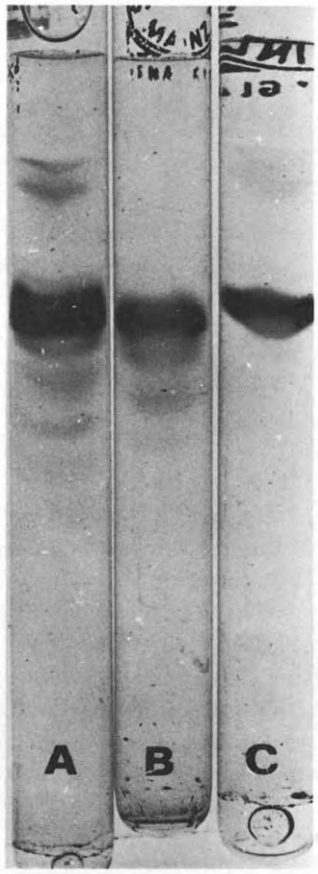

Fig. 2. Acrylamide gel electrophoresis at pH 4.6 in 15% gels containing 4 M urea: A, crude extract; B, SE-1; C, SE-2. (Anode is above)

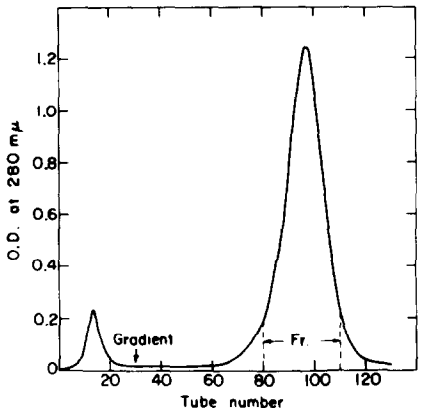

Fig. 3. Elution profile of SE-2 rechromatographed in identical conditions to those described in fig. 1.

can be obtained from a second chromatographic run of the shoulder fraction, SE-1, on the same column. When a sample of the SE-2 fraction is rerun over a similar SE-Sephadex column, there is a minimal amount of impurity which is eluted in the dead volume, and the main peak, eluted by the gradient, is sharp and symmetrical (fig. 3). The yield from this run is 75%.

The profile of elution before the onset of the gradient varies from one crude preparation to another, but that of the shoulder (SE-1) and peak (SE-2) has never been found to vary in numerous runs. Attempts to use wider columns, faster flow rates, higher pH values, or a lower experimental temperature, did not yield satisfactory results, and the elution outline after the onset of the gradient showed no shoulder, and was very similar to that observed when the protein was eluted off a column of Cellex-P [5].

The amino acid composition of the SE-2 fraction is comparable to that of the purified bovine BE obtained by other authors, and is given in table 1. The molecular weight calculated from the measurements of the intrinsic sedimentation and diffusion coefficients assuming a partial molar volume, $\bar{v} = 0.721$ [16], was 20, 480, whereas the value obtained by the low speed equilibrium method was 20,880. This value is higher than the most recent value published by Hashim and Eylar [17], but very similar to that observed by Chao and Roboz-Einstein [18]. The molecular weight calculated according to the amino acid composition is approximately 16,000.

The SE-2 BE (10 μg lyophilized material) caused clinical paralysis in 13/15 of the guinea pigs in the third week after challenge.

Acknowledgement

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Table 1
Comparison of the amino acid composition of different preparations of BSC-BE.

Amino acid	Nakao et al. [3][a]	Hashim and Eylar [11]	Carnegie et al. [4]	SE-2[b]
Lysine	11	13	12	12
Histidine	8	10	9	8
Arginine	15	17	14	16
Aspartic acid	9	10	10	10
Threonine	6	9	7	6
Serine	13	19	15	14
Glutamic acid	9	10	10	10
Proline	9	12	11	11
Glycine	21	24	20	21
Alanine	11	14	12	12
Valine	1	4	3	2
Methionine	2	2	2	2
Isoleucine	2	3	3	3
Leucine	8	10	9	9
Tyrosine	3	3	4	3
Phenylalanine	6	8	7	7
Tryptophan	-	1	1	1
TOTAL	134	169	149	145

[a] Calculated from values expressed in publication as moles/100 moles.
[b] Average from four independent determinations carried out on two batches assuming 10 aspartic acid residues.

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