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Two-step chromatographic method for separation and purification of nerve growth factor from venom of Chinese cobra

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Abstract

By selecting the different combination schemes, a simple, fast and highly efficient method for separation and purification of nerve growth factor (NGF) from venom of Chinese cobra is reported in this paper. This purification process consists of a two-step chromatographic separation on DEAE-Sepharose F.F. anion-exchange medium followed by a Sephadex G-50 gel filtration. On reducing and non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), the nerve growth factor obtained with this process proved to be homogeneous and its molecular weight was separately estimated to be approximately 14.5 and 29.0 kD, which was consistent with that reported in literature; and on high performance size-exclusion chromatography and reversed-phase chromatography, its purity was about 99%. The yield of this purification method was 0.51% and the nerve growth factor obtained had the activity of eliciting neurite outgrowth from chick embryonic dorsal root ganglia. The optimum concentration of nerve growth factor was 5–100 ng/ml and the minimal concentration eliciting neurite outgrowth from chick embryonic dorsal root ganglia was 5.0 ng/ml.

Keywords: Chinese cobra venom; Nerve growth factor

1. Introduction

Nerve growth factor (NGF) is a protein necessary for the maintenance and development of sympathetic and sensory neurons [1]. It can not only promote the fission of neuron but also determine the growth direction of neurons. It also plays an important physiological role in central nervous system [2–4]. In addition, NGF can mediate an increase in choline acetyltransase activity in neonatal rat forebrain [5] and can prevent basal forebrain neuronal death after transection of fimbria formix [6]. These findings have led to a suggestion that therapy with NGF for neurological disorders, such as Alzheimer's disease, Parkinson's disease, etc., may be valuable [7,8] and treatment of some peripheral neuropathies, such as diabetes-associated polyneuropathy, with NGF may also be beneficial [9].

Abundant NGF exists in snake venom. To obtain purified NGF from snake venom, authors have used a variety of different methods. Hogue-Angeletti first used a four-step chromatographic method, including Sephadex G-100, DEAE cellulose, Sephadex G-75 and CM-Cellulose, to purify NGF from snake venom in 1968 [10,11]. Subsequently, they reported a two-step purification procedure consisting of a gel filtration step and a CM-cellulose column [12]. Furukawa and Hayashi isolated NGF from *Naja naja atra* venom by the method of ion exchange chromatography followed by a gel filtration chromatography in the same year [13]. Khamidov et al. purified NGF from cobra venom by gel filtration, ion exchange chromatography and preparative isoelectric focusing on thin layers of G-75 superfine Sephadex in 1985 [14]. Siigur et al. purified the NGF from *Vipera berus berus* venom by Sephadex G-100, DEAE Sephadex A-50 and chromatography focusing on PBE118 in 1986 [15]. Khamidov et al. purified NGF by PM-10 filter ultrafiltratin, TSK-gel medium, CM-cellulose and gel filtration on Sephadex G-75 in 1989 [16]. Smith et al. isolated NGF by the method of gel exclusion chromatography followed by a reversed-phase chromatography in 1992 [17]. The same year, Kilnion reported an Acti-Disk cartridge method [18]. The Acti-Disk cartridge contains a microporous plastic sheet composed of silica and polyvinyl chloride that can be activated with various ion exchange and affinity ligands. And Koyama

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et al. purified NGF from the venom of *Vipera russelli russelli* by Sephadex G-50, S-Sepharose and Blue Sepharose CL-6B chromatography [19]. In 1994, Horie et al. purified NGF from *Crotalus adamanteus* venom by gel filtration on a Sephadex G-100, ion exchange on DEAE-Cellulose, Blue Sepharose CL-6B and Monos column [20]. In 1995, Kostiza et al. separated NGF from *Naja naja atra* venom by using weak cation-exchange chromatography followed by reversed-phase chromatography [21]. Recently, Li et al. isolated NGF from Chinese cobra venom by Sephadex G-50, DEAE Cellulose D-52, CM CL-6B and a Superose 12 column in fast protein liquid chromatography [22]. Xu et al. purified NGF from *Naja naja atra* by ion exchange chromatography, followed by a gel filtration process in fast protein liquid chromatography [23].

Although many purification methods of NGF from snake venom have been reported, all of them were complicated and time-consuming or could not be easily industrialized to obtain purified NGF. To find an effective method of separating and purifying NGF from venom of Chinese cobra, a combination of different chromatographic methods, such as Sephadex G-50, CM-Sepharose CL-6B, DEAE-Sepharose Fast Flow, Heparin Sepharose CL-6B, etc., was used in this paper. A simple, high efficiency and high-yield two-step chromatographic method, which includes a DEAE-Sepharose F.F. anion-exchange column followed a Sephadex G-50 column, was established for the separation and purification of NGF from venom of Chinese cobra.

2. Experimental

2.1. Materials and Instruments

DEAE-Sepharose Fast Flow, CM-Sepharose CL-6B, Sephadex G-50 (Middle), Sepharose CL-6B, CM-Cellulose 32, Heparin Sepharose CL-6B and Sephadex G 25 (Middle) were purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden). The venom of Chinese cobra (*Naja naja atra*) was obtained from Guang Zhou venom institute (Guang Zhou, P.R. China). Tris, bovine serum albumin and molecular weight marker proteins were from Sigma (St. Louis, MO, USA). DMEM medium was from Gibco BRL. All the other chemicals were of analytical grade or better.

Biologic LP chromatography system and Mini-protein II Electrophoresis Cell were obtained from Bio-Rad (USA). LC-10A high performance liquid chromatography, Diol-300 size-exclusion column and VP-ODS reversed-phase column were from Shimadzu corporation (Kyoto, Japan). Water Jacket CO_2 incubator was from Shellab (Oregon, USA). UV-9100 Spectrophotometer was from Rui Li Instrument Corporation (Peking, P.R. China).

2.2. Isolation and purification of NGF

Crude cobra venom 4.9 g was dissolved in 10 ml buffer A (50 mM Tris–HCl, pH 8.5), centrifuged at 12,000 rpm for 15 min. Then the soluble fraction was collected and loaded on the chromatographic column.

2.2.1. DEAE-Sepharose F.F. anion-exchange chromatography

In the first step, a DEAE-Sepharose F.F. chromatographic column (20 cm × 3.5 cm i.d.) was used to isolate and purify NGF from the venom of Chinese cobra. Buffer A was 50 mM Tris–HCl (pH 8.5), and buffer B was buffer A with the addition of 200 mM NaCl. About 10 ml soluble fraction derived in the above was loaded on the DEAE-Sepharose F.F. column after equilibration with buffer A, then the column was washed with the same buffer for 50 min and eluted with a linear gradient of 0–200 mM NaCl for 190 min. Finally, the column was eluted with 100% buffer B for 60 min. The flow-rate of mobile phase was 6.1 ml/min. UV absorption of the column effluent was monitored at 280 nm. Fractions were collected and the NGF activity of each faction was assayed.

2.2.2. Sephadex G-50 gel chromatography

The dimension of Sephadex G-50 column was 150 cm × 3.5 cm i.d. The buffer used was 20 mM PBS, pH 7.5 (buffer C). A 65 ml fraction with NGF activity obtained from DEAE-Sepharose F.F. column was loaded on Sephadex G-50 column after equilibration with buffer C and then the column was eluted with buffer C. The flow-rate of elution solution was 2.4 ml/min and the collected fractions were monitored at 280 nm.

2.3. SDS-PAGE

After each step of chromatographic purifications, the fractions including NGF were confirmed on reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The separating gel consisted of 13% polyacrylamide and the electrophoresis voltage was 105 V. The following proteins were used as molecular weight markers: phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and α-lactalbumin. Gels were stained with Coomassie Blue R-250.

The purified NGF was simultaneously analyzed with non-reducing SDS-PAGE. The electrophoresis conditions were the same as the reducing SDS-PAGE except the loading buffer without β-mercaptoethanol.

2.4. Analysis of high performance size-exclusion and reversed-phase columns

The fraction including NGF collected from Sephadex G-50 was simultaneously analyzed with a high performance size-exclusion column and a high performance octadecylsilyl reversed-phase column. Size-exclusion chromatography was performed on a Shim-pack Diol-300 gel column (25 mm × 7.9 mm i.d.) with a pre-column (10 mm × 4.6 mm i.d.), the elution solution was 20 mM

sodium phosphate buffer (pH 7.5) with the addition of 200 mM NaCl. Reversed-phase chromatography was performed on a Shim-pack VP-ODS column (15 mm × 4.6 mm i.d.) with a pre-column (10 mm × 4.6 mm i.d.). Elution buffer A was H_2O with the addition of 0.1% (v/v) trifluoroacetic acid, elution buffer B was acetonitrile with the addition of 0.1% (v/v) trifluoroacetic acid. The column was eluted with a linear gradient of acetonitrile ranging from 0 to 100% (v/v). Both the flow-rates of mobile phase were 1.0 ml/min, and UV absorptions of proteins were monitored at 280 nm.

2.5. Protein concentrations

Protein concentrations were determined by the method of Bradford with BSA as a standard.

2.6. Assay of NGF biological activity

The NGF biological activity was detected by the method of eliciting neurite outgrowth from chick embryonic dorsal root ganglia [24]. The dorsal root ganglia dissected from 8-day-old chick embryos were incubated in the culture bottle with the rat tail collagen. The tissue culture medium consisted of TEMED and the fractions including NGF. Then the culture bottle was incubated for 24 h in CO_2 incubated with 5% CO_2 at 37 °C.

3. Results and discussion

3.1. Selection of separation methods

To identify an effective method to isolate and purify NGF from venom of Chinese cobra, as shown in Fig. 1, the following combinations of chromatographic methods were evaluated.

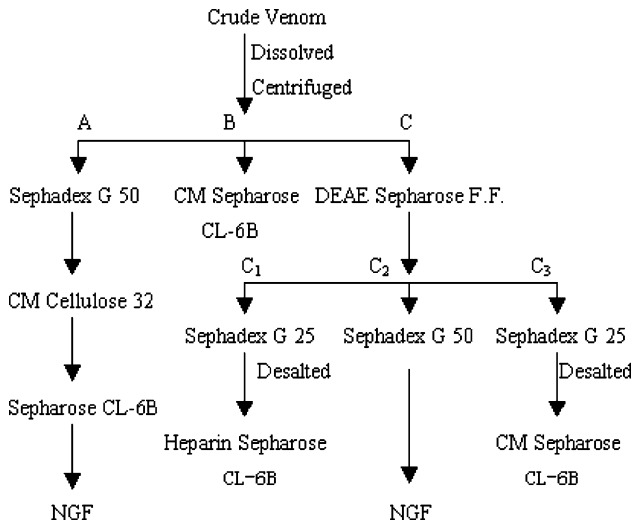

Fig. 1. The separation schemes of nerve growth factor from crude venom of Chinese cobra.

In Fig. 1, route A is a traditional purification method. It consists of a three-step chromatographic method of Sephadex G-50, CM-Cellulose 32 and Sepharose CL-6B. However, this method can usually be used only in lab-scale separation and cannot be applied in a large-scale process because a low-capacity and time-consuming Sephadex G-50 separation was used in its first step. In a typical separation program in ours laboratory, a combination of a 150 cm × 10 cm i.d. Sephadex G-50 column, a 100 cm × 5 cm i.d. CM-Cellulose 32 column and a 150 cm × 5 cm i.d. Sepharose CL-6B column were used and 5.0 g crude venom of Chinese cobra was applied. The whole separation process took about 137.5 h and 15 mg NGF, with about 96% purity, was obtained. In procedures B and C, a CM Sepharose CL-6B cation-exchange column (20 cm × 1.0 cm i.d.) and a DEAE-Sepharose F.F. anion-exchange column (20 cm × 3.5 cm i.d.) were separately used in the first step of the purification scheme. In CM Sepharose CL-6B cation-exchange chromatography, buffer A was 50 mM NaAc-HAc (pH 5.0) and buffer B was buffer A with the addition of 0.8 M NaCl. In DEAE-Sepharose F.F. anion-exchange chromatography, buffer A was 50 mM Tris–HCl (pH 8.5), and buffer B was buffer A with the addition of 200 mM NaCl. Both the elution modes used in cation-exchange chromatography and anion-exchange chromatography were: 0–50 min, buffer A 100% (buffer B 0%); 50–240 min, buffer B from 0 to 100% (buffer A from 100 to 0%); 240–300 min, buffer B 100% (buffer A 0%). The separation chromatograms for crude venom in CM Sepharose CL-6B and DEAE-Sepharose F.F. were shown in Figs. 2 and 3, respectively. From Fig. 2, it can be seen that at pH 5.0 most of proteins in crude venom exists in a positive form and can be adsorbed in CM Sepharose CL-6B column. Only a small fraction of proteins in crude venom exists in a negative form, they cannot be adsorbed by CM Sepharose CL-6B medium and flow out of the chromatographic column along with the solvent front. The biological activity detection shows that NGF exists in the

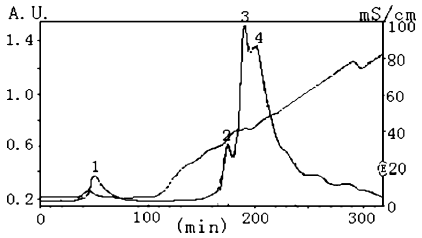

Fig. 2. Chromatogram of crude venom on CM-Sepharose CL-6B column. Sample: 0.5 g crude venom of Chinese cobra. Column: 20 cm × 1.0 cm i.d. Buffer A: 50 mM NaAc-HAc (pH 5.0); buffer B: buffer A plus 0.8 M NaCl. Flow-rate: 0.5 ml/min. The elution gradient see text. The left vertical axes: UV adsorption at 280 nm (AU). The right vertical axes: conductance of mobile phase (mS/cm). Symbols 1, 2, 3 and 4 separately denote the isolated chromatographic peaks.

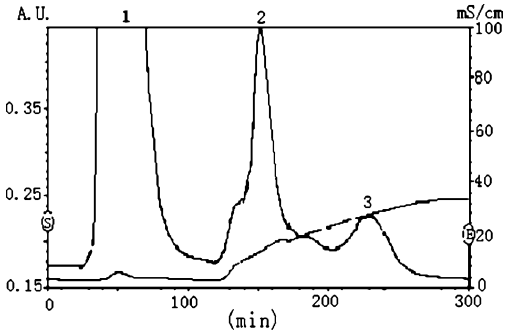

Fig. 3. Chromatogram of crude venom on DEAE-Sepharose F.F. column. Sample: 4.9 g crude venom of Chinese cobra. Column: 20 cm × 3.5 cm i.d. Buffer A: 50 mM Tris–HCl (pH 8.5); buffer B: buffer A plus 200 mM NaCl. Flow-rate: 6.1 ml/min. The elution gradient see text. The left vertical axes: UV adsorption at 280 nm (AU). The right vertical axes: conductance of mobile phase (mS/cm). Symbols 1, 2 and 3 separately denote the isolated chromatographic peaks.

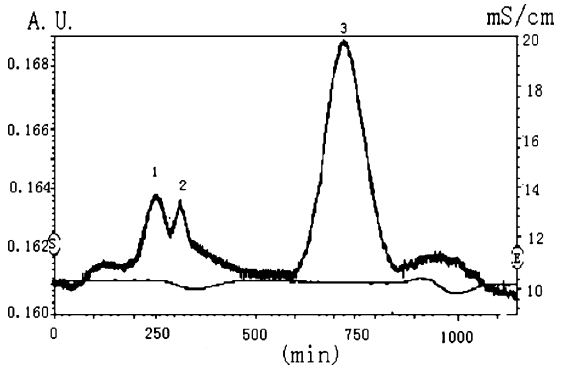

Fig. 4. Chromatogram of the NGF fraction on Sephadex G-50 column. Sample: 65 ml NGF fraction isolated from DEAE-Sepharose F.F. column. Column: 150 cm × 3.5 cm i.d. The elution buffer: 20 mM PBS (pH 7.5). Flow-rate: 2.4 ml/min. The left vertical axes: UV adsorption at 280 nm (AU). The right vertical axes: conductance of mobile phase (mS/cm). Symbols 1, 2 and 3 separately denote the isolated chromatographic peaks.

third and fourth peaks in Fig. 1. Therefore, NGF cannot be effectively separated with the other components in crude venom using CM Sepharose CL-6B medium. By contrast, most of proteins in crude venom, as shown in Fig. 3, can be eluted in cationic form using a DEAE-Sepharose F.F. column. And the biological activity detection shows that NGF exists only in the second peak of Fig. 3. Therefore, by using DEAE-Sepharose F.F. anion-exchange medium, most proteins can be removed from crude venom and NGF can be isolated in the second peak of Fig. 3. Thus, DEAE-Sepharose F.F. anion-exchange medium was adopted as the first step in the following purification procedures.

In the next purification steps, three separation schemes, C_1,C_2 and C_3, to follow the first step were evaluated. The affinity and electrostatic interactions between NGF and Heparin Sepharose CL-6B medium in scheme C_1, the electrostatic interaction between NGF and CM Sepharose CL-6B in scheme C_3 and the size-exclusion effect for NGF in Sephadex G-50 in scheme C_2, were used to separate and purify NGF from crude venom, respectively. The mobile phase of affinity column (20 cm × 1.0 cm i.d) was Tris–HCl, including NaCl (pH 8.5) buffer, and the column was pulse eluted with 0, 0.25, 0.5, 0.75, 1.0, 1.5 M NaCl. The chromatographic conditions of scheme C_3 were same as procedure B. The size-exclusion column (150 cm × 1.3 cm i.d) in scheme C_2 was washed by 20 mM PBS (pH 7.5). The results show that schemes C_1 and C_3 not only have to adopt an additional desalting step but also cannot obtain the NGF with high purity, and a subsequent isolation step must also be used. In contrast, in scheme C_2, we can not only obtain the NGF with high-purity using only Sephadex G-50 column, but also the total separation time only was 26 h, which is one-fifth of traditional method. Therefore, scheme C_2 was selected as the one to separate and purify NGF from crude venom.

3.2. Separation of NGF from crude venom

3.2.1. DEAE-Sepharose F.F. column

Fig. 3 illustrated the separation chromatogram of crude venom in DEAE-Sepharose F.F. column. It shows that on DEAE-Sepharose F.F. anion-exchange medium, most of proteins and peptides in crude venom are eluted in cationic forms, which are poorly retained on this column (peak 1), while the other components included in crude venom are mainly eluted in peaks 2 and 3. Only the second peak exhibits NGF activity. Its SDS-PAGE is displayed in lane 4 of Fig. 5. Now that most of the proteins and peptides, which do not exhibit NGF activity, are not retained by the DEAE-Sepharose F.F. column, the followed separation steps is lessened.

3.2.2. Sephadex G-50 column

The separation chromatogram of the fraction including NGF activity, which is isolated from DEAE-Sepharose F.F. column, on Sephadex G-50 column is shown in Fig. 4.

Fig. 4 shows that the fraction with NGF activity obtained from DEAE-Sepharose F.F. can be separated into three major peaks on Sephadex G-50. After pooling, the biology activity of each fraction was assayed. The results showed that the first and second peaks collected were the proteins with high molecular weight and no NGF biological activity, while the third peak was confirmed to have NGF biological activity. Their SDS-PAGE are separately shown in lanes 1 and 2 in Fig. 5.

3.3. SDS-PAGE of the collected fractions

The SDS-PAGE of the fractions collected from DEAE-Sepharose F.F. and Sephadex G-50 columns is shown in Fig. 5. It is known that the molecular weight of NGF in SDS-PAGE is about 14.5 kD. Combining the biological activity results and the SDS-PAGE of the collected fraction of crude venom eluted from the DEAE-Sepharose F.F. column,

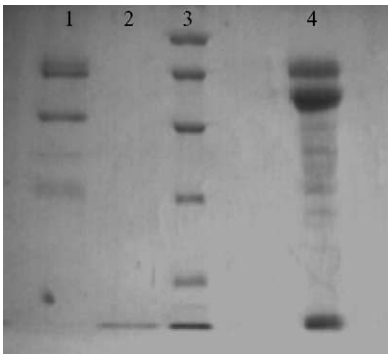

Fig. 5. SDS-PAGE of the fractions collected from DEAE-Sepharose F.F. and Sephadex G-50. Lane 1: mixture of peaks 1 and 2 of Fig. 4; Lane 2: peak 3 in Fig. 4; Lane 3: molecular weight marker (from bottom to top: 14.4, 20.1, 31.0, 43.0, 66.2 and 97.4 kD, respectively); Lane 4: peak 2 of Fig. 3.

it can be seen that NGF was included in peak 2 of Fig. 3. Because the peaks 1 and 2 in Fig. 4 cannot be effectively separated on Sephadex G-50 column, SDS-PAGE of their mixture was carried out. The SDS-PAGE results indicate that NGF is not included in the first and second peaks eluted from the Sephadex G-50 column. However, the SDS-PAGE of peak 3 in Fig. 4, as shown in lane 2 in Fig. 5, shows a single protein band with a molecular weight of about 14.5 kD, corresponding to the reported molecular weight of NGF [23]. Therefore, in view of both the SDS-PAGE and the biological activity results, it can be concluded that the peak 3 collected from Sephadex G-50 is the pure NGF from crude venom of Chinese cobra. The reducing and non-reducing SDS-PAGE of the NGF collected from Sephadex G-50 were simultaneously carried out. As shown in lane 1 of Fig. 6, a single band is also observed in SDS-PAGE without β-mercaptoethanol. And by comparing the mobility of band 1 with those of marker proteins, the molecular weight of NGF collected from Sephadex G-50 is estimated to be about 29.0 kD, which is about twice that on reducing SDS-PAGE. This result is consistent with the conjecture that NGF consists two peptide chains, whose molecular weights are nearly identical [25].

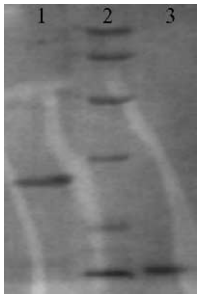

Fig. 6. Reducing and non-reducing SDS-PAGE of NGF. Lane 1: non-reducing SDS-PAGE of NGF; Lane 2: molecular weight marker (from bottom to top: 14.4, 20.1, 31.0, 43.0, 66.2 and 97.4 kD, respectively); Lane 3: reducing SDS-PAGE of NGF.

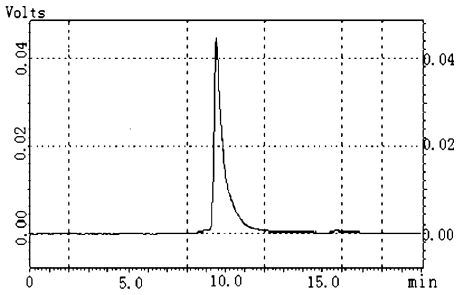

Fig. 7. The chromatogram of NGF on Shimadzu Diol-300. Sample: 15 μl peak 3 collected from Sephadex G-50. Column: 25 mm × 7.9 mm i.d. The elution buffer: 20 mM PBS (pH 7.5) containing 200 mM NaCl. Flow-rate: 1 ml/min. UV detection at 280 nm (V).

3.4. Analysis of size-exclusion column and reversed-phase column

At the same time, the peak 3 collected from Sephadex G-50 was also analyzed with a Shimadzu Diol-300 size-exclusion column and a Shim-pack VP-ODS reversed-phase column. On Diol-300 column, it was found that the peak 3 collected from Sephadex G-50 had a main peak and its purity is about 99.4%. And by calibration analysis with molecular weight markers, the molecular weight of the peak 3 collected from Sephadex G-50 is about 29.0 kD, this result is consistent with that in non-reducing SDS-PAGE; on Shim-pack VP-ODS column, the peak 3 collected from Sephadex G-50 also had a main peak and its purity is about 99.2%. The chromatogram of the peak 3 collected from Sephadex G-50 on Shimadzu Diol-300 and Shim-pack VP-ODS columns are separately shown in Figs. 7 and 8.

3.5. The results of purification

Table 1 summarized the results of NGF purification by using DEAE-Sepharose F.F. and Sephadex G-50 two-step chromatographic method. Table 2 compared the results of NGF purification presented in this paper with those reported in literatures.

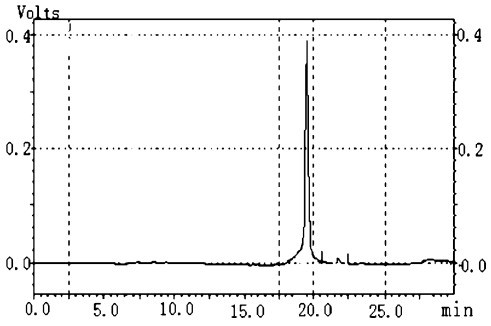

Fig. 8. The chromatogram of NGF on Shimadzu VP-ODS. Sample: 50 μl peak 3 collected from Sephadex G-50. Column: 15 mm × 4.6 mm i.d. Buffer A: H_2O plus 0.1% (v/v) TFA; buffer B: acetonitrile plus 0.1% (v/v) TFA; linear gradient: 0–30 min, acetonitrile from 0 to 100% (v/v). Flow-rate: 1 ml/min. UV detection at 280 nm (V).

Table 1
Purification of NGF from crude venom of Chinese cobra

Fraction	Total protein (mg)	Protein concentration (mg/ml)	Yield (%)	Increases in purity
Crude venom	4900	490	100	–
DEAE-Sepharose F.F	82	0.189	1.67	59.8
Sephadex G-50	25	0.015	0.51	196

Table 2
Comparison of NGF purification between the method presented in this paper and those reported in literatures

References	Snake venom	Purification schemes	Yield (%)	Purity (%)	Times (h)
[12]	*Naja naja*	Sephadex G-50 + dialysis + CM-cellulose	0.15	–	>310
[13]	*Naja naja atra*	Sephadex G-50 + lyophilization + CM-cellulose + Sepharose 6B	0.50	–	>150
[15]	*Naja naja*	Sephadex G-100 + DEAE Sephadex A-50 + chromatography focusing	0.41	–	>170
[21]	*Naja naja atra*	CM-Sepharose + lyophilization + ProRPC	0.2–0.5	94.7	>27
[23]	*Naja naja atra*	SP-Sephadex C-25 + lyophilization, dialysis + Sephadex G-100 + resource S	–	–	>140
This method	*Naja naja atra*	DEAE-Sepharose F.F. + Sephadex G-50	0.51	99.2	26

By using DEAE-Sepharose F.F. and Sephadex G-50 two-step chromatographic method, the time for isolating NGF from venom of Chinese cobra is about 26 h, the purity of NGF obtained is about 99% in high-performance liquid chromatography, and the yield of NGF is about 0.51%. Therefore, the purification time of this method is much less than those reported in literatures (the isolation times in literatures presented in Table 1 are only the chromatographic purification times and do not include the lyophilization and dialysis times), the purity of NGF obtained is higher than those reported in literatures (the majority of purities in literatures presented in Table 1 are electrophoresis purity except reference [21]), and the yield of NGF is more than or not less than, at least, those reported in literatures.

The increases in purity in each purification step are shown simultaneously in Table 1.

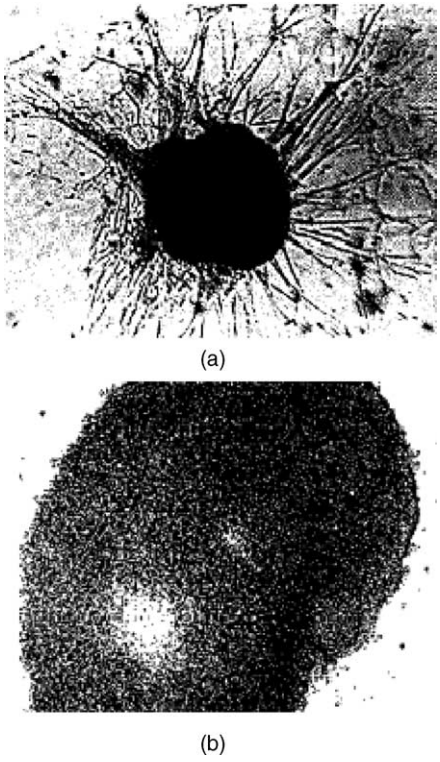

Fig. 9. The effect of NGF on 8-day-old chick dorsal root ganglia. (a) Microphotograph of ganglia at optimum concentration of NGF (10 ng/ml); (b) negative control, no NGF.

3.6. Assay of biological activity

After isolation using DEAE-Sepharose F.F. and Sephadex G-50 columns, the NGF biological activity of each fraction collected was assayed. The results showed that the second peak in DEAE-Sepharose F.F. column in Fig. 3 and the third one in Sephadex G-50 in Fig. 4 exhibit NGF activity. The optimum concentration of NGF is 5–100 ng/ml and the minimal concentration of eliciting neurite outgrowth from chick embryonic dorsal root ganglia is 5.0 ng/ml. Fig. 9 shows the effect of NGF on 8-day-old chick dorsal root ganglia.

4. Conclusion

Nerve growth factor is a unique growth factor which has the ability to stimulate sensory neuron growth. However, only a limited quantity can, so far, be obtained in disease treatment for the traditional purification method can not be industrialized. In the two-step chromatographic method presented in this paper, DEAE-Sepharose F.F. anion-exchange medium with high adsorption capability was used in the first step. Therefore, a large volume of crude venom of Chinese cobra can be separated and purified at one time and the low loading of other separation schemes reported in literatures can be overcome. And at the same time, because only two-step chromatography, DEAE-Sepharose F.F. and Sephadex G-50, was used to isolate and purify NGF from

crude venom and the other methods, such as lyophilization and dialysis, were not included in it, this purification method can be more easily industrialized to obtain NGF in large-scale than the traditional methods. This two-step chromatographic method was more efficient than the reported ones. Thus, the purification time can be shortened, the purity of NGF obtained can be raised, and even the loss of NGF can also be reduced.

Acknowledgements

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