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NERVE GROWTH FACTOR BINDS TO SERUM ALPHA-2-MACROGLOBULIN

Hans Ronne, Helena Anundi, Lars Rask, and Per A. Peterson

Department of Cell Research
Box 562
University of Uppsala
S-751 22 Uppsala, Sweden

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SUMMARY

Nerve growth factor labelled with ^{125}I and incubated with mouse serum became associated with a serum protein that eluted in a high molecular weight position on Sepharose 6B.The binding protein for the nerve growth factor was purified to homogeneity and characterized as the murine counterpart of the human α_2-macroglobulin. The nerve growth factor was quantitatively bound to highly purified mouse α_2-macroglobulin after a few hours of incubation. The interaction displayed little species specificity in as much as human and equine α_2-macroglobulin strongly bound murine nerve growth factor.

INTRODUCTION

Nerve growth factor (NGF) is a term used collectively for proteins derived from various species and cell lines that have the ability to sustain the survival in vitro of certain nerve cells of neural crest origin. This group of proteins often induces an extensive outgrowth of neurites from NGF-sensitive cells (1-3).

Early attempts to measure the amount of NGF in the sera of various species led to conflicting results (4-6) probably due to the fact that NGF interacts with serum proteins (7,8). Similar problems were encountered in this laboratory. An attempt to identify the interfering substance(s) was therefore initiated. In this communication we demonstrate that NGF binds to α_2-macroglobulin (α_2M).

MATERIALS AND METHODS

Antisera. An antiserum against the purified murine α-protein was raised in a rabbit. Rabbit antiserum against human α_2M was purchased from Behringwerke AG.

Proteins. Highly purified equine α_2M was kindly obtained from Dr. P. Altevogt. The NGF was prepared in the 2.5S form from adult male mouse submaxillary glands (9).

Electrophoretic methods. Polyacrylamide gel electrophoresis in SDS was a modification of the Laemmli procedure (10). Agarose gel electrophoresis

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(11) and preparative zone electrophoresis (12) were carried out as described.
Analytical gel chromatography. Molecular weights were determined by gel filtration on a Sepharose 4B column (135 x 2 cm) as described by Fish et al. (16). Stokes' radii were determined by chromatography on Sepharose 6B columns (95 x 1 cm), equilibrated with 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl and 0.02% sodium azide (subsequently referred to as Tris Buffer).
Labelling of proteins. Proteins were labelled with ^{125}I and ^{131}I by the Chloramine T method (17). A special protocol was used for NGF, since heavy labelling (10-100 μCi/μg) affected its binding to serum proteins. 0.1 mCi of radioactive iodine was added to 30 μg of NGF and tenfold more diluted Chloramine-T and sodium metabisulphite were employed. A specific activity of about 1 μCi/μg was obtained.
Binding of NGF. The NGF-binding capacity of various fractions was monitored by agarose gel electrophoresis. Aliqouts were incubated overnight with trace amounts of ^{125}I-NGF. Alone,or when incubated with for example, albumin,NGF displayed no or at most γ_2-mobility. Incubation of NGF with mouse serum, or with NGF-binding fractions thereof, increased the NGF mobility so that it appeared in the α-region (Figure 2).

All incubations of ^{125}I NGF with sera and proteins were performed at 4°C in Milian EET-23 400-μl capped plastic tubes.

RESULTS

Interaction of ^{125}I-labelled NGF with mouse serum proteins. Trace amounts of ^{125}I-labelled NGF[1] were incubated with 1-ml portions of mouse serum for various periods of time. The incubation mixtures were subjected to gel chromatography on columns of Sepharose 6B equilibrated with the Tris buffer. After 4 hours at 4°C most of the labelled NGF was eluted in a position close to that of marker β_2-microglobulin (mol.wt.12,000). However, some of the radioactivity occurred at an elution position corresponding to a Stokes' molecular radius of about 103 A. In contrast, after 8 hours of incubation most of the labelled NGF emerged from the column in the high molecular weight position (Fig.1).

The material representing the 103 A-peak was pooled, concentrated and subjected to sedimentation velocity ultracentrifugaion. The labelled NGF in the 103 A-peak behaved as a single, homogeneous component with a sedimentation constant of 20 S. This value together with the value for the Stokes' molecular radius suggested that the labelled NGF was associated with a serum component with an approximate molecular weight of 900,000 (18, 19).

Isolation of the NGF-binding serum component. Mouse serum, 200 ml, was subjected to gel chromatography on a column (8 x 120 cm) of

[1] By NGF is henceforth meant mouse submaxillary gland nerve growth factor prepared in the 2.5S form. α_2M is α_2-macroglobulin; SDS is sodium dodecyl sulfate.

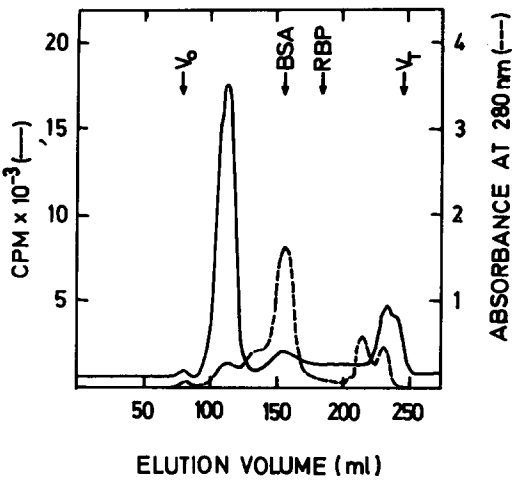

Fig. 1 Gel filtration of ^{125}I-labelled NGF in the presence of mouse serum. A Sepharose 6B column (1.5 x 140 cm), equilibrated with the Tris buffer, was eluted at 4 ml/h. A trace amount of ^{125}I-NGF was incubated for 8 hours at 4°C with 1 ml of mouse serum, prior to application to the column. ^{131}I-markers: bovine serum albumin and retinol-binding protein (69,000 (BSA) and 21,000 (RBP) mol.wt.).

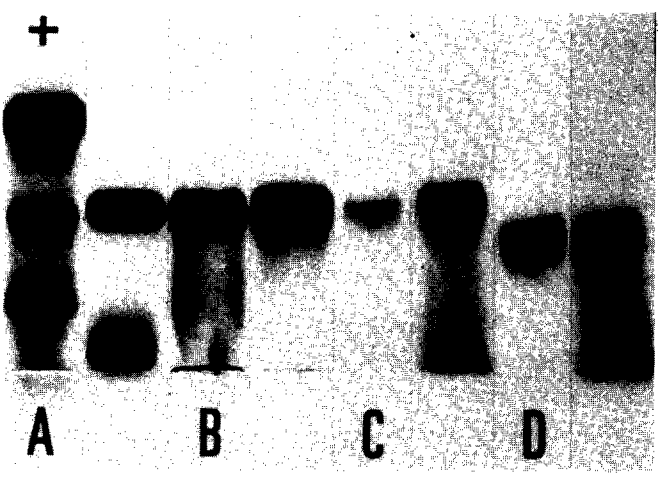

Fig. 2 NGF-binding assay. Aliquots to be tested were incubated with ^{125}I-NGF and then subjected to agarose gel electrophoresis. NGF was detected by autoradiography. A) Mouse serum. B) The void volume pool of mouse serum on Sephadex G-200. C) The purified murine α-protein. D) Purified equine α_2M. Protein to the left, autoradiographies to the right in each lane.

Sephadex G-200 equilibrated with the Tris buffer. The major peaks were pooled, concentrated, and assayed for binding of NGF. The NGF-binding activity was recovered in the void volume pool (Fig.2).

The NGF binding activity was further purified by preparative electrophoresis. A major protein zone was found in the α-region

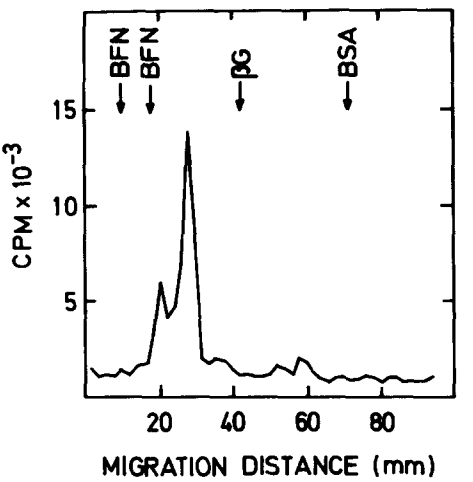

Fig. 3 SDS polyacrylamide gel electrophoresis under reducing conditions of the purified murine α-protein. The protein was labelled with ^{125}I, and applied to a 5% acrylamide gel. The gels were sliced and counted for radioactivity. ^{131}I-markers: bovine fibronectin, β-galactosidase and bovine serum albumin (220,000 (BFN) and 205,000 (BFN), 135,000 (βG) and 69,000 (BSA) mol.wt.).

but minor bands with albumin and β-to-γ-mobility were also evident. NGF-binding activity was confined to the protein with α-mobility. Therefore, this material was pooled and concentrated.

The α-protein appeared highly purified as determined by agarose gel electrophoresis and SDS polyacrylamide gel electrophoresis under non-reducing conditions. Moreover, the α-protein appeared homogeneous by gel chromatography on a column of Sepharose 6B equilibrated with the Tris buffer. In immunoelectrophoresis, the α-protein gave rise to a single precipitin arc when examined against a polyvalent rabbit anti-mouse serum protein serum.

Characterization and identification of the purified α-protein. The isolated α-protein displayed the same size on gel chromatography as the NGF-containing material in mouse serum. This suggested that the isolated protein represented the intact NGF-binding component. On gel chromatography in 6M guanidine hydrochloride the α-protein behaved as a protein with an approximate molecular weight of 400,000. A similar molecular weight was obtained by SDS gel electrophoresis under non-reducing conditions. These results suggested that the α-protein was dissociated into half-molecules under denaturing conditions. When subjected to SDS gel electrophoresis under reducing conditions the α-protein resolved into a major component of about 180,000 mol.wt. and a minor 200,000 dalton species (Fig. 3).

These molecular weight data are compatible with those published for α_2-macroglobulin (see ref. 20-25). To demonstrate unambiguously that the isolated murine α-protein is the analogue of the human α_2M immunoprecipitation analyses were carried out. The purified mouse protein, labelled with ^{125}I, was quantitatively precipitated not only by rabbit antibodies raised against the α-protein but by rabbit antibodies against human α_2M as well. Moreover, the rabbit antibodies against the mouse α-protein precipitated highly purified equine α_2M. As normal rabbit serum did not react measurably with the ^{125}I-labelled mouse α-protein, it appeared reasonable to conclude that the isolated protein was the murine equivalent of α_2M[2].

The purified murine and equine α_2M bound ^{125}I-labelled NGF similarly as proteins in normal mouse and human serum, as revealed by agarose gel electrophoresis (Fig. 2) and gel filtration. At about 10 mg/ml murine α_2M bound most of the ^{125}I-labelled NGF in less than 2 hours at 4°C. Longer incubation periods were required for quantitative binding when the concentration of the α_2M was diminished, but binding was still detectable with hundred-fold dilutions of the physiological serum concentration of this protein.

DISCUSSION

This study shows that NGF binds to an α-protein in mouse serum and the present analyses clearly demonstrated that the NGF-binding protein is murine α_2M. The binding of peptide hormones and growth factors to α_2M has previously been reported. Insulin (26, 27) and human growth hormone (28) have been implicated in such interactions. However, subsequent reports challenged the specificity of these exceptionally slow binding-processes (29, 30).

The rate of binding of NGF to α_2M is, however, of another order of magnitude. At physiological concentrations of α_2M, binding was quantitative within a few hours of incubation at 4°C. That this binding may be of physiological importance was shown by intravenous administration of ^{125}I-NGF to mice. Immunoreactive, labelled NGF bound to α_2M occurred in serum (unpublished observations). There-

[2] The rodent protein has a higher electrophoretic mobility than the human counterpart, and has been referred to as α_1-macroglobulin. To avoid confusion, the protein is here called α_2-macroglobulin, whether of human, equine or murine origin.

fore, it seems reasonable to conclude that endogeneous NGF, when available in the plasma compartment, becomes associated with α_2M. This would most probably prolong the half-life of the serum NGF, which due to its small size should be rapidly filtered and catabolized in the kidney (see ref. 31).

In this study NGF was only found to interact with α_2M, and its possible binding to other serum proteins escaped detection. That other types of interactions might occur cannot be ruled out by the present data, as fast-equilibrium interactions would not have been recorded by the techniques employed. However, the binding of NGF to α_2M alone suffices to explain the disturbances noted by Suda et al.(8) in the one-site radioimmunoassay. The concentration in rat serum of the α_2M-homologue,α_1M, is reported to be about 9 mg/ml (32). A binding of NGF on a two-to-one molecular basis, which is reasonable to assume in view of the occurrence of two non-covalently linked half-molecules of α_2M, would then provide a maximal binding capacity of about 0.5 mg of NGF per ml of rat serum. This is, indeed, the value obtained for rat serum by Suda et al. (8). Further studies, now under way, are aimed at elucidating the stochiometry of the binding reaction and its biological implications.

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