Molecular Size of Nerve Growth Factor in Dilute Solution*

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Nerve growth factor (NGF) is a protein composed of two identical chains of mass 13,259. An analysis of the sedimentation equilibrium, sedimentation velocity, and gel filtration behavior of dilute solutions of NGF indicates the existence of a rapidly reversible monomer $\rightleftharpoons$ dimer equilibrium and that the association constant $K$ for the reaction at neutral pH is $9.4 \times 10^9$ M$^{-1}$. Reaction mixtures consist of equal concentrations of monomer and dimer at a total protein concentration as high as 1.4 g/ml, and at 1 ng/ml, monomer accounts for greater than 99% of the total. The latter concentration is 20 to 30 times that required for the biological activity of NGF. Several lines of evidence suggest that the dimerization reaction is highly stereospecific, although its biological significance is not known.

procedure employed (12). (The octapeptide sequence missing from some of the chains of 2.5 S NGF is not required for biological activity (13).)

During the course of studies on the biological function and molecular properties of the nerve growth factor secreted by cells in culture (6-8) and that present in serum, we observed that under certain conditions the gel filtration behavior of 2.5 S NGF was inconsistent with a molecular weight of 26,600. Consequently we have examined the gel filtration and sedimentation properties of the protein over a wide range of concentrations at neutral pH. As described below, the results indicate that aqueous solutions of NGF comprise a rapidly reversible monomer $\rightleftharpoons$ dimer equilibrium system and, further, that NGF exists largely as the monomer at concentrations considerably greater than those required to exhibit biological activity.

**EXPERIMENTAL PROCEDURE**

**Reagents**—Twice glass-distilled water was used for all solutions. NGF (2.5 S) was prepared by minor modifications (6) of the method of Bocchini and Angeletti (5). As judged by disc gel electrophoresis in three different solvent systems and by immunoelectrophoresis, these preparations are homogeneous (6). Purified NGF was stored at $-15^\circ$. All buffer components were reagent grade. Horse heart ferricytochrome c and bovine serum albumin were obtained from Sigma Chemical Co.; bovine chymotrypsigenin from Schwarz/Mann; blue dextran 2000 from Pharmacia; and $\text{H}_2\text{O}$ from New England Nuclear, Inc.

**Protein Concentrations**—Concentrations of NGF solutions were measured either spectrophotometrically (with extinction coefficient taken to be 1.39 ml/mg-cm at $\lambda = 280$ nm (14)) or by radioimmunoassay. Spectrophotometric measurements utilized a Zeiss PMQ II spectrophotometer. $^251$-NGF was prepared as previously described (8); it contained 0.6 g atom of iodine/mol of NGF. The immunosassay procedure is presented in detail elsewhere (8). Light of wavelength 412 nm was used to measure concentrations of cytochrome c.

**Sedimentation Studies**—A Beckman model E analytical ultracentrifuge equipped with electronic speed control, Rayleigh optics, and split beam photoelectric scanning absorption optics was used to study the sedimentation equilibrium and sedimentation velocity properties of NGF.
of NGF. The RTIC temperature control unit was frequently checked for calibration during these studies. The interference optical system of the instrument was aligned by the procedures described by Richards and Schachman (15), and the absorption optical system by the methods of Schachman et al. (16). To minimize stray light (absorption optics) within the vacuum chamber and to ensure satisfactory light pulse separation between solvent and sample sectors of a double sector cell, radially oriented masks were placed over both collimating and condensing lenses. For all sedimentation equilibrium work and for sedimentation velocity studies at \( \lambda = 280 \, \text{nm} \), aluminum-filled epon double sector cells with sapphire windows were used.

For sedimentation velocity studies utilizing light of wavelength 230 to 220 nm and very low concentrations of NGF, the procedures described by Schachman and Edelstein (17) were employed as follows to maximize the low light levels striking the photocathode. With a milling machine, the center ribs of two double sector centerpiece were removed, thus creating a much wider sector. One cell was filled with solvent and the other with an NGF solution, and the two cells were placed 180° apart in an An-D rotor. With this arrangement, the slit opening at the photocathode could now be lengthened maximally (about 1 cm). A multiplier assembly (Rockman) was used to keep track of the light pulses from sample and solvent cells and the rotor reference hole provided the reference distance from the axis of rotation. In this way, we have been able to measure the sedimentation coefficient of NGF at initial concentrations as low as 6 \( \mu \text{g/ml} \). Before every run, the sapphire windows as well as the collimating and condensing lenses were soaked briefly in a concentrated solution of sulfuric acid/chromic acid to remove oil deposits.

**Gel Filtration Studies**—A column (1.0 \( \times \) 22.0 cm) of Sephadex G-75 was used to measure the weight-average partition coefficient \( (c_w) \) of NGF as a function of initial protein concentration applied to the column. The procedures used were those described by Henne and Ackers (19). The column was then thoroughly equilibrated with solvent at room temperature (about 24°), and then a sufficient volume of NGF solution to establish a concentration plateau was applied to the column at a flow rate of 7.5 ml/hour. Fractions (0.25 ml) were collected in preweighed glass test tubes (8 x 75 mm), and the weight of the tube solution to establish a concentration plateau was applied to the column at a flow rate of 7.5 ml/hour. Fractions (0.25 ml) were collected in preweighed glass test tubes (8 x 75 mm), and the weight of the tube content was determined at each fraction. The average molecular weight \( (\bar{M}_w) \) of NGF was determined by radioimmunoassay (8). Solutions of cytochrome \( c \) and chymotrypsinogen were used to establish the partition coefficients for these proteins, and the void \( (V_0) \) and internal \( (V_i) \) column volumes were measured with blue dextran and \( ^3\text{H}_2\text{O} \), respectively.

**RESULTS**

In early gel filtration studies (using Sephadex G-200), we consistently observed that NGF emerged from the column at a position nearly indistinguishable from the internal column volume, i.e., the elution volume was appreciably greater than would be expected for a globular protein of molecular weight 26,600. At first, this behavior was attributed to the possibility that NGF was undergoing adsorption chromatography on Sephadex, since the protein is known to bind to a variety of surfaces including glass and plastic. However, as shown below, this is not the explanation for the retarded elution position.

Fig. 1 illustrates a zonal elution profile of NGF on Sephadex G-75. The column was equilibrated with 0.1 M potassium phosphate, pH 7.0, containing 1 mg/ml of bovine serum albumin, and it was loaded with 100 \( \mu \text{l} \) of a solution containing 10 mg/ml of cytochrome \( c \), 50 mg/ml of chymotrypsinogen, and 22 \( \mu \text{g/ml} \) of NGF. Values for \( V_o \) and \( V_i \) were determined in a separate column run, since NGF binds strongly to blue dextran and emerges with it at \( V_o \). As shown in Fig. 1, NGF emerges after chymotrypsinogen and before cytochrome \( c \) (the large peak near the front of the column represents aggregated bovine chymotrypsinogen and probably arises from the high concentration of this marker protein applied). For purposes of column calibration, the molecular weights of chymotrypsinogen and cytochrome \( c \) have been taken to be 25,000 and 12,400, respectively.

![Graph of sedimentation equilibrium studies](http://www.jbc.org/)

**Fig. 1.** Zonal Sephadex G-75 gel filtration profile of NGF. A solution (100 \( \mu \text{l} \)) containing 50 mg/ml of bovine chymotrypsinogen, 10 mg/ml of cytochrome \( c \), and 22 \( \mu \text{g/ml} \) of NGF dissolved in 0.1 M potassium phosphate, pH 7.0, containing 1 mg/ml of bovine serum albumin was applied to a column (1.0 \( \times \) 22 cm) of Sephadex G-75 equilibrated with the same solvent at room temperature. Flow rate, 7.5 ml/hour. Chymotrypsinogen and cytochrome \( c \) concentrations were measured by absorbance at 280 nm and 412 nm, respectively. NGF concentrations were measured by radioimmunoassay. \( V_o \) (with blue dextran) and \( V_i \) (with \( ^3\text{H}_2\text{O} \)) were determined from a second column run. ---, chymotrypsinogen; O---O, NGF; ---, cytochrome \( c \).

Although the partition coefficient of NGF corresponding to a given concentration cannot be determined from the data of Fig. 1 (since a plateau is not established), it will be seen later in this section that the elution volume \( (V_o) \) of the protein can be varied at will by changing the loading concentration \( (c_w) \).

**Sedimentation Equilibrium Studies**—Following these preliminary studies with Sephadex, equilibrium sedimentation experiments were carried out using both the absorption and interference optical systems. These runs were performed at rotor velocities in the range 36,000 to 44,000 rpm and initial NGF concentrations between 50 and 200 \( \mu \text{g/ml} \). Equilibrium scanner traces or photographs were taken after 24 hours of operation and point by point number-, weight-, and z-average molecular weights were calculated with the computer program of Koak and Yphantis (19). The partial specific volume of NGF was taken to be 0.716 ml/g as calculated from the amino acid composition (14). In all cases, regardless of rotor velocity or \( c_o \), plots of \( \ln c(x) \) versus \( x^2 \) \( (x = \text{distance from center of rotation}) \) displayed upward curvature which was most striking at the lower protein concentrations. While this feature could be explained by heterogeneity of the NGF solutions due to contamination by other proteins, several lines of evidence demonstrate that this is not the case. First, the sedimentation coefficient of NGF has been determined over a wide range of concentrations (4 to 15 mg/ml), where only a single boundary was observed with \( x_{2w} \) invariant with concentration (5). Second, sedimentation equilibrium studies of NGF with \( c_o \) near 1 mg/ml yield linear plots of \( \ln c \) versus \( x^2 \) and molecular weight values close to that expected for a protein composed of two chains of mass 13,300 (20). Third, the gel electrophoretic and immunoelectrophoretic results in our laboratory (6) demonstrate a single component in NGF solutions prepared as described by Bocchini and Angeletti (5). Consequently, all available information pointed to the existence of a monomer = dimer equilibrium, with significant dissociation of the dimer at concentrations somewhere below 50 \( \mu \text{g/ml} \).
All attempts to study the reaction by sedimentation equilibrium failed. While reasonably reliable point by point molecular weight averages could be calculated, the data were too imprecise to permit calculation of point by point association constants. The reason for this probably stems from the relatively large association constant for the reaction (see below), coupled with the instability of the absorption scanning system to yield sufficiently accurate molecular weight values at the low protein concentrations where dissociation is occurring. However, we have been able to use a procedure suggested by Teller (21) to analyze the stoichiometry of the reaction. Fig. 2 presents a plot of

\[ n = \frac{M_r(x)}{M_f} \left( \frac{M_r(x) - M_f}{M_f(x) - M_r} \right) \tag{1} \]

versus \( c(x) \), where \( n \) is the degree of association for a rapid reaction of the kind \( n(\text{monomer}) \rightarrow (\text{polymer}) \), \( M_r \) is the molecular weight of the monomer, and \( M_r(x) \) and \( M_f(x) \) are the weight- and number-average molecular weights, respectively, throughout the cell. As shown in Fig. 2, a monomer = dimer reaction is indicated.

### Sedimentation Velocity Studies

As shown by Gilbert and by Gilbert and Gilbert (22, 23), the existence of a monomer = dimer equilibrium in which the rates of association-dissociation are much faster than the rate of separation should give rise to a single sedimenting boundary in the ultracentrifuge. Further, the weight-average sedimentation coefficient can be used to estimate the association constant. The relevant equations are

\[ s_w = \frac{s^0 + s^0 c_c}{c_c} \tag{2} \]

\[ c_c = c_1 + c_2 \tag{3} \]

\[ K = \left( \frac{c_1}{c_c} \right)^{-1} \tag{4} \]

where \( s_w \) is the weight-average sedimentation coefficient; \( c_1 \) and \( c_2 \) are the concentrations of monomer and dimer, respectively; \( c_0 \) is the total protein concentration; and \( K \) is the association constant for the reaction. Actually, the sedimentation coefficients above contain a nonideality term of the kind

\[ s_w = s^0 (1 - k c_c) \tag{5} \]

where \( s^0 \) is the sedimentation coefficient at infinite dilution, and \( k \) is a constant. However, since the studies presented here were carried out at very low values of \( c_0 \), where concentration-dependent effects would be expected to be negligible, we have equated \( s^0 \) and \( s^0 \) All values of \( s_w \) were corrected to a standard state of water at 20°C.

Table I presents values of \( s_{w,0} \) as a function of \( c_0 \) which were obtained with the photoelectric scanning system, and Fig. 3 presents three typical traces of an experiment with \( c_0 = 6.2 \mu \text{g/ml} \) and light of wavelength = 220 nm. As shown in Fig. 3, only a single boundary is detected, although Table I reveals that the sedimentation coefficient decreases with decreasing \( c_0 \).

In order to calculate values of \( K \), it is necessary to know the sedimentation coefficients for monomer and dimer (\( s_1 \) and \( s_2 \)). As noted earlier, Bocchini and Angeletti (5) have measured \( s_{w,0} \) for NGF over a range of concentrations (4 to 15 mg/ml) with a mean value of 4.23 S. The value \( s_{w,0} = 2.38 \) S for \( c_0 = 1.2 \mu \text{g/ml} \) (Table I) is close to this, and thus we have taken \( s_2 \) to be 2.4 S. Since we are unable to measure \( s_1 \) directly, it must be calculated. If we make the reasonable assumption that the frictional coefficients and partial specific volumes of monomer and dimer are closely similar, then

\[ \left( s_1 \right) = \left( \frac{M_f}{M_r} \right)^{1/2} \left( s_2 \right)^{-1} \tag{6} \]

and \( s_1 = 1.52 \) S with \( M_f = 13,300 \) and \( M_r = 26,600 \) (11). The values of \( K \) presented in Table I were calculated based upon these assumptions, and, over the range 6.2 to 82 \( \mu \text{g/ml} \), the values are closely similar.

In addition to the usual methods for measuring \( s \), we have taken advantage of a procedure originally described by Tiselius et al. (24) to try to obtain values of \( s \) at \( c_0 < 6 \mu \text{g/ml} \). For this purpose, a fixed partition separation cell with a perforated separation plate was used (Beckman Instruments, Inc.) It can be shown (24) that the sedimentation coefficient of a macromolecule can be determined by measuring its net transport across any given plane in the plateau region so long as the plateau

### Table I

<table>
<thead>
<tr>
<th>Method</th>
<th>Wave-</th>
<th>( c_0 )</th>
<th>( s_{w,0} ) ( s^0 ) ( K )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedimentation (scanner)</td>
<td>295</td>
<td>1200</td>
<td>2.38 ± 0.04</td>
</tr>
<tr>
<td>290</td>
<td>82</td>
<td>2.33 ± 0.04</td>
<td>9.1</td>
</tr>
<tr>
<td>230</td>
<td>59</td>
<td>2.32 ± 0.04</td>
<td>9.9</td>
</tr>
<tr>
<td>220</td>
<td>9.0</td>
<td>2.28 ± 0.06</td>
<td>8.7</td>
</tr>
<tr>
<td>220</td>
<td>6.2</td>
<td>2.14 ± 0.03</td>
<td>0.9</td>
</tr>
<tr>
<td>220</td>
<td>6.2</td>
<td>2.05 ± 0.20</td>
<td>3.9</td>
</tr>
<tr>
<td>Sedimentation (transport method)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Gel filtration | | | | 3.6 | 0.456 | 7.1 |
| | | | | 0.0615 | 0.510 | 22.0 |

* Values represent \( s_{w,0} \) ± S. D. of the regression coefficient calculated by least squares analysis of plots of \( \ln s \) versus time.
To test this method with our instrument, the sedimentation coefficient of cytochrome c was measured simultaneously by the transport method and by the scanner at a rotor velocity of 60,000 rpm and temperature of 25°C. A piece of Whatman No. 50 filter paper was cut to fit the separation partition, and the cell was loaded with a 1.0 mg/ml solution of cytochrome c dissolved in 0.1 M potassium phosphate, pH 7.0, containing 1 mg/ml of bovine serum albumin. Light of wavelength 412 nm was used with the scanner to monitor the position of the cytochrome c boundary, and the schlieren optical system was used to record photographically the position of the partition (x_p) and solution meniscus. Although some of the potential problems associated with fixed partition separation cells have been discussed by Schachman (27), we were unable to detect any convective disturbances with the scanner during the run. The plateau region remained flat and equal on both sides of the partition. At the conclusion of the run, vacuum was released at 1000 rpm, and the chamber was opened to minimize the possibility of cell leakage with a stationary rotor under vacuum. The cell was removed, inverted gently to mix the contents above the partition, and an aliquot was withdrawn for measurement of absorbance (c_t) at 412 nm. When taken together with the initial absorbance (c_0), this procedure yielded a value of s_{z, w} = 1.61 S, whereas measurement of the scanner traces gave s_{z, w} = 1.56 ± 0.05 S.

To measure the sedimentation coefficient of NGF, the protein was dissolved in a solution of 0.1 M potassium phosphate, pH 7.0, containing 1 mg/ml of bovine serum albumin and 1 mg/ml of cytochrome c. These proteins were incorporated to minimize nonspecific adsorption of NGF to cell components. In addition, the filter paper was soaked thoroughly in the initial solution. The run was made as described above at 60,000 rpm and it was terminated when the scanner indicated that the cytochrome c boundary had moved about one-third of the distance between the meniscus and separation partition. Values of c_0 (1.11 µg/ml) and c_t (0.680 µg/ml) were measured by radioimmunoassay in quadruplicate, and a mean value of s_{z, w} = 1.90 S ± 0.05 (standard error of the mean) was obtained. As shown in Table I, the value of K calculated from this experiment is in good agreement with values determined in the conventional manner.

**Estimation of K by Gel Filtration**—Fig. 4 presents Sephadex G-75 elution profiles of NGF at two initial loading concentrations, 61.5 ng/ml and 3.62 µg/ml. Ten milliliters of each solution were applied to the column to establish a plateau region. Cytochrome c (1 mg/ml) was incorporated into both NGF solutions to ensure reproducibility of column operation, although only one cytochrome c profile is shown in Fig. 4. (From run to run, the elution volume of cytochrome c was constant to within 0.05 ml.)

As can be seen, the elution volume of NGF decreases with increasing NGF concentration. Using the equations given by Henn and Ackers (18), together with the assumption that the Stokes radii of monomer and dimer are closely similar, values for the association constant K were calculated from the data of Figs. 1 and 4, and

\[
\sigma_w = \frac{V_e - V_a}{V_e} = \frac{V_v}{V_i}
\]

where V_v, V_e, and V_i are the elution volumes of NGF, the void volume, and the internal column volume, respectively, and \(\sigma_w\) is the weight-average partition coefficient. V_v was taken to be the midpoint of the symmetric NGF profile as constructed.
chrome c was included in both solutions applied to the column, and its solvent was 0.1 M potassium phosphate, pH 7.0, containing 1 mg/ml of bovine serum albumin. O—O, NGF with c. = 3.62 pg/ml; O—O, cytochrome c.

from radioimmunoassays of the fractions, and K was obtained from

\[ \alpha = \frac{\sigma_1 - \sigma_2}{\sigma_1 + \sigma_2} \]

\[ K = \frac{1 - \alpha}{\sigma_1' \sigma_2'} \]

where \( \sigma_1 \) and \( \sigma_2 \) are the partition coefficients for pure monomer and dimer, respectively. To estimate these numbers, a plot of \( \ln V_c \) versus \( \ln \sigma \) (molecular weight) was constructed from values of \( V_c \) and molecular weight for chymotrypsinogen and cytochrome c (Fig. 1). Then by interpolation, \( \sigma_1 \) and \( \sigma_2 \) were evaluated from the monomer and dimer molecular weights of NGF. Table I summarizes the results.

The mean value of K, taking together all the data of Table I, is 9.4 ± 1.9 (S.E.M.) \( \times 10^{-5} \) M\(^{-1}\). Using this number, we have calculated a theoretical curve for the dissociation reaction. Fig. 5 illustrates a plot of percentage of monomer in the equilibrium mixture as a function of total NGF concentration. Fig. 5 also shows that an NGF solution of concentration 1.4 \( \mu \)g/ml consists of an equal mixture of monomer and dimer. At \( c_0 = 1 \mu g/ml \), the mixture contains greater than 99% monomer, and this is 20 to 30 times the NGF concentration which has been shown to be biologically active (28).

The results presented above indicate that solutions of NGF at neutral pH comprise a monomer \( \rightleftharpoons \) dimer equilibrium system and further, that at concentrations of the protein usually employed in bioassay (1 to 10 ng/ml), the equilibrium mixture consists almost entirely of monomer. In an earlier study Frazier et al. (29) coupled NGF to BrCN-activated Sepharose in the presence of a high concentration of guanidine hydrochloride, and the resulting NGF-Sepharose conjugate was shown to be biologically active. Because this concentration of guanidine hydrochloride is known to dissociate the NGF dimer (20), and because reassociation of monomers coupled to Sepharose would be statistically improbable after removal of guanidine, Frazier et al. (29) concluded that monomeric NGF possesses biological activity. The results of the present study indicate that the biological activity of native NGF at concentrations in the nanogram/ml range is mediated by its monomeric form.

There are several reasons for thinking that the monomer \( \rightleftharpoons \) dimer reaction of NGF is a chemically specific one. For example, polymerization appears to stop at the dimer stage—a conclusion which is supported by the sedimentation equilibrium studies of Angeletti et al. (20) and the sedimentation velocity results of Bocchini and Angeletti (5). Moreover, recent crystallographic studies of NGF (30) reveal that each unit cell contains 12 asymmetric units, and that the asymmetric unit is the monomer. Although the NGF structure has not been solved, the crystals were grown at a sufficiently high protein concentration that NGF would be expected to crystallize as the dimer. (It should be noted that x-ray determination of the size of the asymmetric unit does not establish whether the unit cell contains monomer or a multiple of monomer.) Consequently, the packing arrangement between monomer chains must be highly stereospecific and related by a 2-fold rotation axis (30), since a random, nonspecific collection of dimer aggregates would not be expected to crystallize.

One possible explanation for the existence of a specific monomer \( \rightleftharpoons \) dimer equilibrium should not be overlooked—that is that the monomer is biologically active and the dimer, inactive. Several authors have reported that when biological assays are carried out as a function of NGF concentration, a maximum occurs in the dose-response curve. Larrabee (31), working with 13- to 14-day-old chick embryonic sympathetic ganglia, observed maximal activity between 100 ng/ml and 1 \( \mu g/ml \) of NGF, and the biological response decreased sharply between 1 and 10 \( \mu g/ml \). Pearce (32) found a peak of activity at about 100 ng/ml with a sharp decrease in activity at 1 \( \mu g/ml \). Greene (38), using a very sensitive bioassay with dissociated sympathetic neurons, noted a maximum in the dose-response curve close to 1 ng/ml of NGF, with activity falling at higher concentrations. In earlier studies, Levi-Montalcini and Angeletti (33) demonstrated (8-day-old chick embryonic sensory ganglia) that 10 ng/ml of NGF produced a halo of neurites, whereas 1 \( \mu g/ml \) of NGF did not. However, when ganglia which had been treated with 1 \( \mu g/ml \) of NGF were sectioned and stained, they observed a closely packed fibrillar capsule of neurites enclosing the ganglia, and concluded that although the morphological response to NGF was atypical, a biological effect could be demonstrated with 1 \( \mu g/ml \) of NGF. Their paper
does not indicate whether concentrations of NGF higher than 1 μg/ml were tested. Finally, Burnham and Varon (34) have measured the effects of 7 S NGF (19) upon uridine incorporation by both intact and dissociated sensory ganglia. They also find a maximum in the dose-response curve, with decreasing uridine incorporation at the higher concentrations of NGF examined. Thus by both morphological and biochemical criteria, a maximum in the NGF dose-response curve has been demonstrated.

The question of whether the NGF dimer is biologically active has been examined by Stach and Shooter (35), who prepared NGF cross-linked with dimethylsuberimidate. This preparation was found to be indistinguishable from native NGF in the sensory ganglion assay. While this result argues against the notion that the dimer may be inactive, there is another possible explanation, since it is not known how extensively (in a three-dimensional sense) the two chains were cross-linked. That is, it remains possible that at high dilution, the inter-chain noncovalent bonds are broken with concomitant substancial unfolding of the cross-linked dimer and exposure of biologically active segments of individual monomer chains. We note in this connection that Stach and Shooter (35) also observed a maximum in the dose-response curve for cross-linked dimer at 5 ng/ml, followed by a marked decrease in activity between 100 ng/ml and 1 μg/ml.

If the monomer is biologically active and the dimer inactive, this could serve to explain why an optimum in dose responsivity to NGF is consistently observed, provided that the monomer and dimer compete for NGF binding sites. In this regard, Frazier et al. (36, 37) have studied the binding of 125I-NGF to sympathetic and sensory ganglia as well as other tissues. In the case of ganglia, multiple binding affinities were observed, and the binding was nonsaturable at concentrations of NGF as high as 10−4 M (about 0.26 μg/ml). From Scatchard plots, Frazier et al. (37) calculated an association constant of 9.0 × 10⁵ M⁻¹ for the high affinity binding sites and 1.4 × 10⁶ M⁻¹ for the low affinity sites. Since an NGF solution of concentration 0.26 μg/ml would be expected to contain considerable dimer (Fig. 5), the data are compatible with the hypothesis that dimers can bind. It should be noted that a monomer ≡ polymer equilibrium system (with monomer active, polymer inactive) could be a rather simple control mechanism for regulating the biologic activity of a molecule as a function of its local concentration in vivo.

Last, in calling attention to some of the pitfalls associated with measurements of hormone-receptor interactions, Cuatrecasas and Hollenberg (38) have suggested that the nonsaturable binding of NGF could be due to its continued self-association. In light of the present studies which demonstrate appreciable dissociation of the NGF dimer over the concentration range studied by Frazier et al. (36, 37), such an interpretation would seem to be unlikely.

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Molecular size of nerve growth factor in dilute solution.
M Young, J D Saide, R A Murphy and B G Arnason


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