Molecular Weight and Structure of 7 S Nerve Growth Factor Protein*

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SUMMARY

The molecular weight of 7 S nerve growth factor has been studied in the analytical ultracentrifuge between pH values 6.8 and 7.8. At pH 6.8, where no dissociation is observed, the molecular weight was found to be 137,000 ± 7,000. Between pH values 7.4 and 7.8 there is some dissociation.

Using the data from this study and results in the literature, a model of 7 S nerve growth factor, (αβγ)₂, in reversible equilibrium with a subunit complex, (αβγ), is proposed.

The nerve growth factor (NGF), a protein which stimulates the growth of sympathetic and embryonic sensory ganglia, was originally discovered and studied by Levi-Montalcini and her colleagues (1). Their procedure for isolating NGF (2) involved chromatography on CM-cellulose at pH 4.4. They obtained a protein with an $s_{20,w}$ of 2.5 S which has been shown to have a molecular weight of 26,518 (3).

Subsequently, in another laboratory, a procedure for isolating a higher molecular weight form of NGF at neutral pH was developed (4). This form of NGF called 7 S NGF due to its sedimentation properties, was found to contain three different types of subunits called α, β, and γ each with an $s_{20,w}$ of about 2.5 S (5). The β subunit was found to have the nerve growth-stimulating property, the γ subunit was shown to be an esteropeptidase (6, 7), and the α subunit has been found to have a protective effect on embryonic sensory ganglionic cells during dissociation by trypsin (8).

The α and γ subunits themselves were found to be heterogeneous with respect to charge, each having four different electrophoretically migrating species, while the β subunit is homogeneous (5, 9). All of these species appear to combine equally well with each other to form the 7 S complex, so there are a multiplicity of forms of 7 S NGF which are structurally possible (9).

The behavior of the high molecular weight form of NGF was studied in the analytical ultracentrifuge at a protein concentration of 10 mg per ml, at 5°C from pH 3.8 to 10.3 (5). Between pH values 5 to 8, NGF has an $s_{20,w}$ of about 7 S. Outside this range, the 7 S complex dissociates into subunit complexes which at pH values 4 or 10 are dissociated into the individual subunits. This dissociation was reversible upon return to pH 6.8.

The dissociation behavior differs at high and low pH values. At higher pH values, there is a progressive decrease in the main protein component from 7 to 4.65 S at pH 9.7. A smaller protein component with an $s_{20,w}$ of 2.5 S appeared at pH 8.7 and at pH 10.3 only the 2.5 S component was present. At pH values below 5.0, two components were observed, one of about 4.9 S and the other of 2.5 S. At pH 3.8 only the 2.5 S component was observed.

Further studies of the 7 S complex between pH values 5 and 8, where the complex is stable, indicated the presence of a rapid association-dissociation equilibrium in the 7 S NGF molecule. This rapid association-dissociation equilibrium was investigated by observing the extent of incorporation of $^{125}$I-labeled subunits into unlabeled 7 S NGF at NGF concentrations of between 1 and 3 mg per ml at 4 and 25°C at pH 7.4 (9, 10). These studies showed about 40% of the activity of the free α subunit was incorporated into 7 S NGF and that the incorporation of radioactivity from free γ or free β was only about 10% of the original radioactivity. From these experiments, they concluded that the 7 S NGF complex is in rapid equilibrium with its α subunits at all pH values.

This paper describes the results of a study of 7 S NGF in the analytical ultracentrifuge between pH values 6.8 and 7.8, and its implications with respect to the stoichiometry, stability, and mode of dissociation of the 7 S NGF complex.

MATERIALS AND METHODS

Nerve Growth Factor Protein—The 7 S species was isolated by the procedure of Yaron et al. (4) and characterized by its electrophoretic properties on acrylamide gels as previously described (9, 11). Sedimentation Equilibrium—Sedimentation equilibrium experiments were performed using a Spinco model E ultracentrifuge equipped with interference and schlieren optics. Runs were made for at least 24 hours, equilibrium being assured by lack of change in the observed pattern on sequential photographs.

Samples were dissolved in the appropriate solvent and dialyzed overnight before each run. The partial specific volume of NGF was 0.73 as determined from the amino acid composition.

The experiments done using schlieren optics were treated ac-
according to the procedure of Lamm (12, 13)

\[ M_s = \left( \frac{2RT}{(1 - \bar{v}\omega^2)} \right) \left( \frac{d \ln \frac{1}{r} \frac{dr}{d(r^2)}}{d(r^2)} \right) \]  

(1)

where \( M_s \) is the \( z \) average molecular weight, \( \bar{v} \) is the partial specific volume, \( \rho \) is the density of the solution, \( c \) is the concentration, \( r \) is the distance from the axis of rotation, \( \omega \) is the rotor speed, \( T \) is the absolute temperature, and \( R \) is the gas constant.

The experiments with interference optics used the procedures of Yphantis (14) and Chervenka (15). The solution column length was 7 mm in all experiments. The data were analyzed using the well known equation

\[ M_w = \left( \frac{2RT}{(1 - \bar{v}\omega^2)} \right) \left( \frac{d \ln (c)}{d r^2} \right) \]  

(2)

where \( M_w \) is the weight average molecular weight.

In addition, the number average molecular weight, \( M_n \), was calculated as described by Yphantis (14) using the equations

\[ M_n(r_k) = \left( \frac{RT}{(1 - \bar{v}\omega^2)} \right) \left( \int_{r_k}^{r_{\infty}} \frac{c(r) \, dr}{r} \right) \]  

(3)

\[ I = \left( \frac{RT}{(1 - \bar{v}\omega^2)} \right) \left( \frac{c(r_{\infty})}{M_n} \right) \]  

(4)

where \( m \) is the horizontal magnification factor and

\[ I = \left[ \frac{RT}{(1 - \bar{v}\omega^2)} \right] \left[ \frac{c(r_{\infty})}{M_n} \right] \]  

(5)

\( M_n \) is calculated by trapezoidal integration from \( r_1 \) to \( r_2 \) where \( c(r_1) \) is at least 100 pm. The integral centripetal to \( r_1 \) is approximated by \( I \) using for \( M \) the value of \( M_w \) obtained in (2) for fringe displacements between 100 and 150 pm.

The mathematical analyses were done on an IBM 360-60 computer using a least squares procedure for pointwise slopes of Equations 1 and 2 and for the extrapolations of molecular weight versus concentration plots to zero concentration.

**RESULTS**

**Molecular Weight of NGF Complex**—The pH, ionic strength, and rotor speed were varied to obtain conditions where the 7 S complex is stable as well as to examine the dissociation of the 7 S complex. The experiments at pH 6.8 enabled us to unambiguously determine the molecular weight of 7 S NGF. The \( \ln c \) versus \( r^2 \) plot (Fig. 1A) and the molecular weight versus concentration plot (Fig. 1B) are linear over a concentration range of 0.200 to 1.8 mg per ml. Moreover, the Lamm plot (Fig. 2) is also a straight line. The values obtained from two different Rayleigh interference patterns (three fringes averaged per pattern) were 137,396 ± 344 and 136,253 ± 377 for \( M_w \). The value of \( M_s \) obtained from the Lamm plot is 136,724 ± 340. When the errors attributable to \( \nu, \rho, \omega, \) and \( T \) are considered, a value of about 137,000 ± 7000 is obtained for the 7 S NGF complex.

**Effect of pH on Molecular Weight of NGF Complex**—Experiments done at pH values 7.4, 7.5, and 7.8 indicate that 7 S NGF undergoes some dissociation at protein concentrations be...
A, sodium potassium phosphate buffer; I = 0.05, pH 7.4, at 25° ± 0.1 M NaCl; temperature, 25.8°; rotor speed, 14,000. B, Tris-HCl buffer; I = 0.05, pH 7.4, at 25°; temperature, 20.8°; rotor speed, 18,000. C, sodium potassium phosphate buffer; I = 0.05, pH 7.8, at 25° ± 0.1 M NaCl; temperature, 21.9°; rotor speed, 14,000.

**DISCUSSION**

**Molecular Weight and Stoichiometry of 7 S NGF—**At pH 6.8 the molecular weight of 7 S NGF is about 137,000 which is that expected from the sedimentation coefficient. This value combined with the subunit molecular weights can be used to determine the formula of 7 S NGF. The molecular weight of the α and γ subunits have been determined by sedimentation equilibrium in the analytical ultracentrifuge. The values for α and γ are 25,600 ± 500 and 18,000 ± 2,000, respectively. The value for β is 26,518 as determined from the sequence (3). These values are most simply fitted to a formula for 7 S NGF of (αβγ)2 which has a molecular weight of 140,636 ± 5,000, in good agreement with our value for 7 S NGF. With this formula, the NGF complex is a symmetric molecule containing an equal number of each subunit. Since the α and β subunits have similar molecular weights, other formulas are consistent with the data. In our discussion of the dissociation behavior of 7 S NGF we will advance further arguments for the existence of symmetry in the 7 S NGF complex.

**Stability of 7 S NGF Complex—**Previous work (5) showed no dissociation of 7 S NGF between pH values 5 and 8 at a protein concentration of 10 mg per ml. Our results extend the stability of 7 S NGF to about 0.2 mg per ml at pH 6.8. Between pH values 7.4 and 7.8 there is some dissociation at protein concentrations below 1 mg per ml. This would be expected since this is approaching the pH value where 7 S NGF dissociates at concentrations of 10 mg per ml.

**Nature of Dissociation of 7 S NGF Complex—**The data of Fig. 3 and the subunit exchange experiments (10) indicate that 7 S NGF is undergoing a reversible association-dissociation equilibrium at neutral pH values. The work reported here combined with previous work in other laboratories enables us to propose a model for dissociation of the 7 S NGF complex. One implication of our model is to suggest a structure and stoichiometry for 7 S NGF.

There are two modalities by which the dissociation can proceed; a symmetric dissociation into two species of similar molecular weight or an asymmetric dissociation into at least two species of differing molecular weight. In the symmetric model, there are only two differing molecular weight species present, one being a multiple of the other. For the asymmetric case there are at least three species with different molecular weights in equilibrium with each other.

The evidence is that 7 S NGF is a single peak or band using gel filtration, sedimentation velocity experiments (4, 5) and sucrose gradients (10). In addition, polyacrylamide gel electrophoresis, which would be especially sensitive to the presence of at least three species due to its ability to separate on the basis of charge as well as molecular weight, shows a single band for 7 S NGF between pH values 7.4 and 8.2 (5). These observations suggest that 7 S NGF is in rapid equilibrium with subunit complexes which are of similar molecular weight and charge. This equilibrium can be represented as (αβγ)2 ↔ (αβγ).

We are proposing that 7 S NGF with a molecular weight of 137,000 dissociates into a molecule of molecular weight 68,500 with an s20,w of about 4.7 s. There is evidence for the existence of this molecule from two sources. The first is the previously mentioned sedimentation velocity experiments (4) where the existence of species with an s20,w between 4.85 and 4.9 s was reported. The second, the results at neutral pH values in this paper, are more direct evidence for this model. It was found that at pH values 7.5 and 7.8 both Mm and Mw extrapolate to molecular weights between 65,000 and 70,000 (Table I). The results at pH 7.4 where there is less dissociation may be interpreted using the function 2Mm - Mw which for limited association approximates the monomer molecular weight (14). In this case the value of 2Mm - Mw is 68,900.

In summary the evidence of this paper as well as previous studies are all consistent with 7 S NGF being in equilibrium with one species with a molecular weight of about 68,000, which

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**Table I**

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<tr>
<th>pH</th>
<th>Mm</th>
<th>Mw</th>
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<tbody>
<tr>
<td>6.8</td>
<td>137,400 ± 350</td>
<td>137,400 ± 350</td>
</tr>
<tr>
<td>7.4</td>
<td>74,800 ± 800</td>
<td>80,700 ± 1500</td>
</tr>
<tr>
<td>7.5</td>
<td>70,000 ± 700</td>
<td>67,800 ± 1500</td>
</tr>
<tr>
<td>7.8</td>
<td>67,100 ± 900</td>
<td>64,300 ± 3000</td>
</tr>
</tbody>
</table>

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would have the formula $\alpha \beta \gamma$. Further dissociation of this species into the individual subunits could occur by changing buffer pH, ionic strength, or protein concentration.

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REFERENCES

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