Molecular Weight of Recombinant Human Tumor Necrosis Factor-α*

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Recombinant DNA-derived human tumor necrosis factor-α from Escherichia coli was examined by equilibrium ultracentrifugation under conditions similar to those where gel filtration experiments suggested an oligomeric structure. Short-column equilibrium experiments at concentrations in the range 0.015–0.12% at pH 8.5 in 0.04 M Tris/Tris-HCl gave molecular weights corresponding to 3 times the sequence molecular weight both in the presence and absence of 0.1 M NaCl. Long (2.6 mm)-column experiments under the same solvent conditions indicated molecular weights of 51,900 ± 900 in the absence of added NaCl and 52,600 ± 700 in the presence of added 0.1 M NaCl. No evidence of any species other than the trimer was found.

Tumor necrosis factor-α (TNF-α) is a protein of $M_r = 17,000$ as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Aggarwal et al., 1985) and exhibits a variety of biological activities such as cytolysis on transformed cells (Sugarman et al., 1985; Wang et al., 1985), protection of cells from viral infection (Mestan et al., 1986; Wang and Goeddel, 1986; Arakawa et al., 1987), cachexia (Beutler and Cerami, 1986), and macrophage activation. Di- verse molecular weights of human TNF-α ranging from 34,000 to 140,000 have been obtained with relatively crude preparations of the natural protein (Mathew, 1981; Nissen-Meyer and Hammerstrom, 1982). However, these values suggest that TNF-α is oligomeric in solution.

Recently, the amino acid sequence of human TNF-α has been deduced from cDNA clones (Pennica et al., 1985; Mar- menout et al., 1985; Wang et al., 1985; Shirai et al., 1985) and also directly determined from the protein purified from serum-free cell culture supernatant of the HL-60 promyelocytic leukemia cell line induced by 4β-phorbol 12-myristate 13-acetate (Aggarwal et al., 1985). We have synthesized and cloned the gene coding for TNF-α, expressed the correspond- ing protein in E. coli, and purified the protein from E. coli cells to near homogeneity by sequential chromatog- raphies (Davis et al., 1987). In a previous paper (Davis et al., 1987), we reported the results of gel filtration, CD, and SH titration of the protein. The gel filtration showed the molecular weight of TNF-α to be 46,000, suggesting its dimeric or trimeric structure, in agreement with other published values (Aggarwal et al., 1985; Shirai et al., 1985). We report here results of the sedimentation equilibrium experiments of recombinant E. coli-derived human TNF-α at pH 8.5 where $M_r = 46,000$ was observed.

MATERIALS AND METHODS

TNF-α was purified as described previously (Davis et al., 1987) and stored in 0.04 M Tris/Tris-HCl, 0.1 M NaCl, pH 8.5 (determined at room temperature) at -20 or 4 °C. The purified protein showed a cytotytic activity of 1–2 × 10^6 units/mg when assayed on mouse L929 cells in the absence of actinomycin D, and an antiviral activity of 5 × 10^6 units/mg when assayed in encephalomyocarditis virus-infected HeLa cells (Arakawa et al., 1987). The protein was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and activity prior to use.

Protein concentrations were determined using the extinction coefficient of 1.62 at 280 nm for 0.1% protein solution (Davis et al., 1987).

Sedimentation equilibrium experiments were performed using a Model E ultracentrifuge (Beckman) equipped with a pulsed argon ion laser (Paul and Yphantis, 1972), a digital laser controller (Yphantis et al., 1984), and an automated control system for photography (Laue et al., 1984). Rayleigh interferograms were taken on “Technical Pan” 35-mm film (Kodak 2415) and were measured using a Digital Equipment Co. PDP-8/L mini-computer controlled system (Laue and Yphantis, 1979; Laue, 1981) that provides estimates of fringe displacement as a function of position. Typically, the images were measured every 8 μm (corresponding to about every 4 μm in the ultracentrifuge cell).

Data analyses were performed using nonlinear least squares approaches (Johnston et al., 1981) with various assumed models. The programs used were derived from a version of the NOMLIN programming system written in FORTRAN for a PDP 11/32 minicomputer (Digital Equipment Co.). The indicated ranges of the fitting parameters returned by these programs correspond to a confidence range of about 65% probability. (For Gaussian error distributions these 65% confidence regions are essentially equivalent to ranges of the mean plus or minus 1 standard deviation.)

Short-column equilibrium experiments were carried out in multi-channel centerpieces (Yphantis, 1966) loaded with 15–20 μl of each solution; they were run at a temperature near 20 °C for a minimum of 2 h. Values of $M_r$, the apparent z-average molecular weights, were determined from nonlinear least squares fits of the fringe displacements in a solution channel to a model of a single ideal component. This procedure is similar to an orthogonal linear least squares procedure (Correia, 1980, 1983) and can be shown to be equivalent to determination of the $M_r$ of the ratio of the second derivative to the first derivative of the fringe displacement (Yphantis, 1964).

Long-column experiments were carried out in 12-mm optical path length “external loading cells” (Ansevin et al., 1970) with three pairs of channels. Blanks were run both before and after the set of runs to correct for optical distortion. The ultracentrifuge was run at a temperature of 22.9 °C and at 24,000 rpm for 22.5 h and then at 30,000 rpm for 28.5 h. These ultracentrifuge times significantly exceed estimates of the time required to attain equilibrium that were made using the relation of van Holde and Baldwin (1958) with their ω parameter taken as 0.001 and with the assumption of a diffusion coefficient of $6 \times 10^{-7}$ cm^2 s^-1.

The partial specific volume, $\bar{v}$, of isionic recombinant TNF-α protein was estimated as 0.7377 ml g^-1 from the known sequence using the method of Traube as detailed by Cohn and Edsall (1943). Solvent densities were estimated from data in the International Critical Tables (1929) and in the Handbook of Chemistry and Physics (1980).

* The abbreviation used is: TNF-α, tumor necrosis factor-α.

3 T. M. Laue and D. A. Yphantis, manuscript in preparation.

4 M. L. Johnson, personal communication.

5 J. J. Correia and D. A. Yphantis, manuscript in preparation.

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Molecular Weight of TNF-α

TABLE I
Apparent z-average molecular weights for TNF-α at pH 8.5 from short-column equilibrium experiments

| Concentration | $M_z \times 10^{-3}$ in 0.04 M Tris/Tris-HCl at 30,000 rpm |
|---------------|---------------------------------------------------------|
| g/liter       |                                                       |
| 0.45          | 55.4 ± 1.5                                             |
| 0.34          | 54.2 ± 1.1                                             |
| 0.23          | 52.8 ± 2.0                                             |
| 0.15          | 54.5 ± 2.3                                             |
| Average       | 54.2 ± 1.1                                             |

TABLE II
Molecular weights for TNF-α at pH 8.5

These molecular weights were obtained by least squares fitting of measurements from long-column (2.6 mm) sedimentation equilibrium experiments to the model of a single ideal component.

| Loading concentration | Solvent | $M_z \times 10^{-3}$ |
|-----------------------|---------|----------------------|
| g/liter               |         | At 24,000 rpm       | At 30,000 rpm       |
| 0.06                  | 0.0     | 51.9 ± 0.6           | 52.6 ± 0.3          |
| 0.44                  | 0.0     | 52.5 ± 0.5           | 53.1 ± 0.4          |
| 0.44                  | 0.0     | 51.8 ± 0.4           | 51.0 ± 0.4          |
| Average               | 0.0     | 51.9 ± 0.9           |                   |
| 0.15                  | 0.1     | 53.5 ± 0.5           | 52.5 ± 0.4          |
| 0.46                  | 0.1     | 52.8 ± 0.8           | 51.9 ± 0.8          |
| Average               | 0.1     | 52.6 ± 0.7           |                   |

RESULTS

Sedimentation equilibrium experiments were carried out on TNF-α in 0.04 M Tris/Tris-HCl at pH 8.5 in short (0.75 mm) columns both with and without the presence of 0.1 M NaCl. The averages of the apparent z-average molecular weights presented in Table I correspond to 3.12 ± 0.06 and 3.05 ± 0.03 times the molecular weight calculated for the sequence of the monomer, indicating that this protein is a trimer under these conditions.

Experiments were also performed using longer (2.6 mm) solution columns at both 24,000 and 30,000 rpm. Fits of the observed fringe displacements to the model of a single ideal component gave residuals with no obvious systematic trends and with a root mean square of 0.015-0.03 fringe. The molecular weights returned by the least squares procedures when fitting the data to the model of a single ideal component are presented in Table II. In the absence of NaCl the average of these values corresponds to 2.99 ± 0.05 times the sequence molecular weight and to 3.03 ± 0.04 times the monomer molecular weight in the presence of 0.1 M salt. The magnitudes of the fitting deviations were not lowered significantly by assumptions of more complex fitting models that included simple nonideality, association to higher polymers, heterogeneity, and/or dissociation to monomers. Thus, we conclude that this recombinant protein behaves as an ideal trimer under these conditions.

DISCUSSION

The recombinant TNF-α used in this study was purified from the insoluble pellet from E. coli after solubilization in urea followed by folding into 0.04 M Tris/Tris-HCl, 0.1 M NaCl, pH 8.5. We have shown in a previous paper (Davis et al., 1987) that this material has a conformation and size identical to that purified from the soluble fraction of TNF-α in disrupted E. coli cells. The molecular weight of the protein was 46,000 as determined by gel filtration, a value identical to that for protein purified from the nature source (Aggarwal et al., 1985). These results suggest that the protein preparation used in this study, obtained by denaturation and renaturation, is comparable in structure to protein that never was exposed to these treatments. This is supported by the observed biological activities of the protein as described under “Materials and Methods” and by the observed lipogenic activity of the preparation used, which had been observed for the natural TNF-α (Beutler and Cerami, 1986).

Gel filtration experiments showed the molecular weight of TNF-α to be 46,000 under similar solvent conditions (Aggarwal et al., 1985). Since the molecular weight determination by gel filtration is based on the elution position of globular, monomeric proteins, comparison of this value with the actual molecular weight suggests that TNF-α assumes a compact structure in solution. Therefore, it will be of interest to examine the orientation of each subunit in the trimer leading to such compact structure.

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