Crystallization and Preliminary X-ray Investigation of Recombinant Human Granulocyte-Macrophage Colony-stimulating Factor*

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Crystals of recombinant human granulocyte-macrophage colony-stimulating factor have been grown from solutions of polyethylene glycol 8000. The crystals are orthorhombic, space group P212121; the axes are a = 45.5(1), b = 58.7(1) and c = 127.3(1) Å. The crystals are stable to x-rays for at least 3 days and diffract beyond 2.8-Å resolution. Although the molecule exists as a monomer in solution, it crystallizes with two or three molecules in the asymmetric unit.

Colon-stimulating factors represent a family of glycoproteins that are required in vitro for the survival, proliferation, and differentiation of hematopoietic progenitor cells of myeloid and erythroid lineage (1-4). Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the production of colonies of granulocytes and macrophages from their precursors and also promotes the growth and differentiation of pluripotent progenitor cells. In addition, GM-CSF can enhance a variety of the functional activities of mature effector cells (reviewed in Ref. 5) and promote the differentiation of myeloid leukemic cells (6). Although human GM-CSF has been purified from natural sources (7), the low quantities produced have not permitted detailed characterization of its physicochemical and biological properties. The cloning of the cDNA encoding human GM-CSF and its expression in bacterial and mammalian cell hosts has provided a basis for the preparation of large quantities of purified recombinant protein (8-10). Based on in vitro biology and the activity of GM-CSF in animal models (11, 12), phase I/II clinical trials on recombinant human GM-CSF have been conducted in myelodysplastic syndromes (13), acquired immunodeficiency syndrome (14, 15), and cancer (16).

The mature protein sequence of human GM-CSF consists of 127 amino acids, including 4 cysteine residues that form two disulfide linkages (10, 17, 18). Physical studies have suggested that human GM-CSF is a compact globular protein from Escherichia coli and is similar to naturally occurring human GM-CSF (20) in that it does not contain N-terminal methionine, in contrast to other forms of recombinant GM-CSF (9, 18).

EXPERIMENTAL PROCEDURES

Human GM-CSF was expressed periplasmically in E. coli with a secretory vector, plNIIomPA2 (8). It was purified by conventional chromatography as described previously (21), followed by reversed-phase high performance chromatography on a Rainin Dynax-300A C-4 column utilizing a 27-72% gradient of acetonitrile in 0.1% trifluoroacetic acid.

Crystals suitable for x-ray analysis were obtained by vapor-diffusion equilibration using either hanging droplets or sandwiched droplets. For hanging droplets, 20-μl droplets containing 20 mg of protein/ml in 10% polyethylene glycol 8000 (PEG-8000), 16 mM sodium phosphate (pH 8.0) were hung from siliconized coverslips inverted on Linbro plates (Flow Laboratories, McLean, VA). These droplets were equilibrated against 1 ml of 20% PEG-8000 in 16 mM sodium phosphate (pH 8.0). After 10-15 days at 4 °C, orthorhombic crystals with dimensions up to 2.0 × 0.60 × 0.10 mm were observed. Alternatively, for sandwiched droplet experiments, 20-μl droplets containing 20 mg of protein/ml in 10% PEG-8000, 16 mM sodium phosphate (pH 8.0) were sandwiched between two siliconized coverslips in a CrystalPlate (Flow Laboratories, McLean, VA). These droplets were equilibrated against 1 ml of 20% PEG-8000 in 16 mM sodium phosphate (pH 8.0). After 15-20 days at 4 °C, crystals of the same general morphology and size appeared. In each procedure the crystals grew within the pH range of 7.0-8.0, although the best crystals were obtained at pH 8.0.

For x-ray studies, crystals were mounted in glass capillaries and were photographed on a precession camera at 22 °C using CuKα radiation from a Rigaku RU-300 rotating anode generator operating at 40 kV and 100 mA. A complete native data set was collected on a Nicolet X-100A area detector using the same radiation source.

RESULTS AND DISCUSSION

X-ray diffraction data were initially collected to 2.8-Å resolution using the area detector. Oscillation frames covered 0.25° and were measured for 10 min. A total of 6797 reflections were measured; these were merged into 3841 unique reflections. Indexing and integration of intensity data were carried out using the XENGEN processing programs (22). The Rsym value (based on I) for the data to 3.0 Å was 0.087. The data indexed in the orthorhombic system with a = 45.5(1), b = 58.7(1), and c = 127.3(1) Å. The space group P212121 was specified by the systematic absence of reflections h00 with h = 2n + 1, 0k0 with k = 2n + 1, and 00l with l = 2n + 1. Subsequent x-ray precession photographs of GM-CSF confirmed the space group and unit cell parameters (Fig. 1). The crystals are stable to x-rays at room temperature for at least 3 days and diffract beyond 2.8-Å resolution.

Based on a molecular mass of 14,477 daltons as predicted from the cDNA (10), the calculated values of Vm (23) for two or three molecules/crystallographic asymmetric unit are 3.07
and 2.05, respectively. Assuming a partial specific volume of 0.724 cm$^3$/g (19), these values correspond to solvent fractions of 59 and 39\%, respectively. Either of these values would be reasonable. The corresponding $V_m$ values for one or four molecules/crystallographic asymmetric unit are 6.14 and 1.53, respectively, which are far outside the limits normally observed for proteins (23). Therefore, it appears that the protein crystallizes with either two or three molecules in the asymmetric unit. A self-rotation function that was calculated using the native data collected on the area detector did not show a noncrystallographic 2- or 3-fold axis.

During the preparation of this manuscript, crystallization of a recombinant mutant form of human GM-CSF was reported (24). In distinction to the recombinant human GM-CSF employed here, that variant contained N-terminal methionine and an amino acid difference at one position (9, 18). Crystal growth was achieved at high salt concentrations over a period of several months.

These data have provided a basis for x-ray crystallographic analysis of the structure of GM-CSF, which is currently in progress.

REFERENCES

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