Acta Cryst. (1997). D**53**, 329–330

Crystallization and preliminary X-ray studies of mouse tumor necrosis factor

K. J. BAEYENS,[a] H. L. DE BONDT,[a] A. RAEYMAEKERS,[b] C. J. DE RANTER[a]* AND W. FIERS[b] *at* [a]*Laboratorium Analytische Chemie en Medicinale Fysicochemie, E. Van Evenstraat 4, B-3000 Leuven, Belgium, and* [b]*Laboratorium voor Moleculaire Biologie, VIB en U. Gent, Ledeganckstraat 35, B-9000 Gent, Belgium. E-mail: camiel.deranter@farm.kuleuven.ac.be*

(*Received 2 October 1996; accepted 13 December 1996*)

Abstract

Well diffracting crystals of recombinant mouse tumor necrosis factor (mTNF) have been obtained. The sitting-drop vapor-diffusion method was used to grow crystals suitable for X-ray studies from solutions containing methoxypolyethylene glycol 2000 and isopropanol as precipitants. The crystals belong to the space group $P1$ with unit-cell dimensions $a = 49.40$, $b = 48.24$, $c = 51.13$ Å, $\alpha = 115.06$, $\beta = 103.32$, $\gamma = 91.27°$ and one trimer in the asymmetric unit. Crystals are stable to X-rays and diffract beyond 2.0 Å. Cooling the crystal during data collection is necessary since crystals dissolve at room temperature.

1. Introduction

Tumor necrosis factor-α (TNF-α) belongs to the cytokine family of polypeptide mediators, a group which includes the interferons and the interleukins. TNF plays a central role in inflammation and the cellular immune response (Vassalli, 1992; Fiers, 1995). A broad spectrum of physiological effects such as fever, septic shock, rheumatoid arthritis, inflammatory tissue destruction, antiviral and antimalarial activity are associated with TNF (Vassalli, 1992). The potentialities for TNF in pharmacology and pathology are increasing as TNF has been proven to work as an anticancer agent (Lejeune *et al.*, 1995) and antibodies against TNF have been used in case of rheumatoid arthritis (Feldmann, Brennan, Elliott, Williams & Maini, 1995) and Crohn's disease (Murata, Ishiguro, Itoh, Munakata & Yoshida, 1995). TNF, a trimer of 17 350 Da subunits, exerts its diverse biological properties by binding to and clustering distinct cell-surface receptors of 55 kDa [TNF receptor-1 (TNFR1)] and 75 kDa [TNF receptor-2 (TNFR2)] (Vandenabeele, Declercq, Beyaert & Fiers, 1995), both members of the large TNF receptor superfamily. Cellular signaling occurs by triggering one or the other, or both receptors, depending on the cell type and conditions. Human TNF (hTNF) in the mouse only interacts with TNFR1, and hence is a specific ligand for the latter. Remarkably, in the normal mouse, hTNF is 50-fold less toxic as compared to mTNF, indicating that the interaction of mTNF with TNFR2 contributes in a specific way to the systemic toxicity. In order to investigate in more detail these receptor-specific interactions, the three-dimensional structure of mTNF is being determined.

2. Purification and crystallization

2.1. *Preparation of mTNF*

The cDNA coding for mTNF (Fransen *et al.*, 1985) was expressed in *E. coli* under control of the Trp promoter. The cell paste was disintegrated twice in a French press, and nucleic acids were removed from the cleared supernatant by polyethylenimine (final concentration 0.4%). The protein fraction precipitating between 40 and 60% $(NH_4)_2SO_4$ saturation was loaded on a Phenyl Sepharose CL-4B column (Pharmacia), which was eluted with a gradient from 25% $(NH_4)_2SO_4$ 10 m*M* Tris–HCl, pH 6.8, to 20 m*M* ethanolamine, pH 9. Active fractions were dialyzed and loaded on a Q-Sepharose Fast Flow column (Pharmacia), which was eluted with a gradient from 0 to 1 *M* NaCl and 20 m*M* ethanolamine, pH 9. Active fractions were then passed over a heparine Sepharose Cl-6B column (only contaminants bound to the column, not mTNF). The flow-through was brought to 25% $(NH_4)_2SO_4$ and loaded on a Butyl Sepharose 4B column, which was eluted with a decreasing gradient of $(NH_4)_2SO_4$ to 10 m*M* phosphate buffer pH 7. The active fractions were further purified by FPLC on a Mono Q HR16/10 column (Pharmacia) with a gradient from 0 to 1 *M* NaCl in 10 m*M* phosphate buffer, pH 7. The active fractions were diluted fivefold and re-run on the same column, which was now eluted with a gradient from 0 to 1 *M* NaCl in 10 m*M* phosphate buffer, pH 6. Finally, the active fractions were purified by gel filtration on a Fractogel HW50 column, equilibrated with 0.9% NaCl, 10 m*M* Hepes buffer, pH 6.8.

2.2. *Crystallization of mTNF*

Crystals were initially grown by the hanging-drop vapor-diffusion technique at 278 K using Linbro multiwell tissue-culture plates. Microcrystals were obtained out of the sparse-matrix screening (Jancarik & Kim, 1991). Replacing polyethylene glycol 4000 by methoxypolyethylene glycol 2000 lead to clustered crystals after several weeks. These spontaneous crystals were used for microseeding. Suitable crystals were grown in hanging drops consisting of 2 µl protein solution (10 m*M* Hepes pH 6.8, 0.9% NaCl), concentrated to

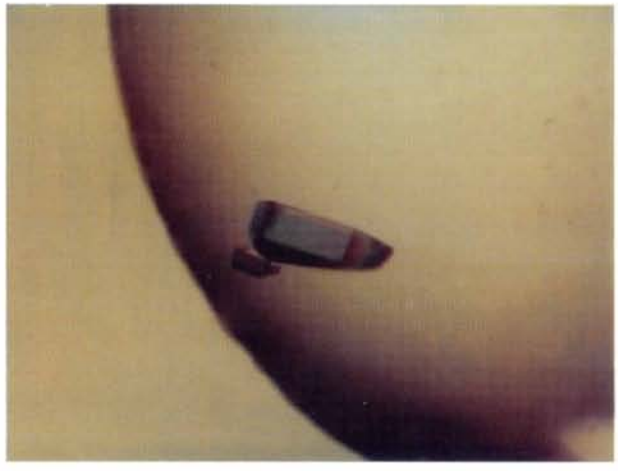

Fig. 1. A typical crystal of mTNF. The crystal was obtained by sitting-drop vapour diffusion after microseeding as described in the text. The dimensions of this crystal are 0.5 × 0.2 × 0.2 mm.

13 mg ml^{-1} in an Amicon microcon, and 2 µl reservoir solution (30% methoxypolyethylene glycol 2K, 7% isopropanol, 0.1 *M* Tris pH 8.5). After one week of equilibration between the drop and the reservoir solution, streak seeding with a hair was performed. Single well shaped crystals appeared after 2 d and were fully grown after two weeks. Switching to ACA crystallization plates and sitting drops resulted in bigger crystals reaching a typical size of 0.4 × 0.2 × 0.2 mm (Fig. 1). The crystals dissolve at room temperature.

3. Data collection and analysis

X-ray diffraction data were collected at 130 K after flash freezing the crystal in a cold stream cooled by liquid nitrogen. The mother liquor turned out to be a good cryoprotectant. Data collection was performed on a Siemens X-1000 multiwire area detector mounted on a Siemens P3 three-axis goniometer. The Cu $K\alpha$ X-rays were produced by a Siemens sealed-tube generator. The data were collected with a 7 cm crystal-to-detector distance, a 2θ setting of 0°, an oscillation range of 0.1° and an exposure time of 40 s per frame. The Siemens software was used for data collection and data reduction. Crystals belong to the space group $P1$ with unit-cell dimensions of $a = 49.40$, $b = 48.24$, $c = 51.13$ Å, $\alpha = 115.06$, $\beta = 103.32$, $\gamma = 91.27°$ and diffract beyond 2.0 Å. The volume of the unit cell suggests one trimer of mTNF in the asymmetric unit and the value of the Matthews constant (Matthews, 1968) gave $V_m = 2.173$ Å^3 Da^{-1} corresponding to a solvent content of 43.3%, which are normal values for proteins.

A total of 28 897 diffraction intensities were collected to 2.05 Å, from which 19 245 are unique. Data reduction yielded a 86.9% complete ($I \geq 3.0\sigma$) data set to 2.40 Å resolution and 72.6% complete ($I \geq 3.0\sigma$) data set to 2.05 Å resolution with a R_{sym} value of 3.05%.

A self-rotation search using *X-PLOR* (Brünger, 1992) confirmed the presence of a trimer with non-crystallographic symmetry. The molecular-replacement method is being employed, with hTNF as the search model (Eck & Sprang, 1989; Jones, Stuart & Walker, 1989). The structure of mouse TNF will provide a firm basis to explain the structure–function relationship of receptor-specific mTNF muteins.

HDB is a Postdoctoral Research Fellow of the National Fund for Scientific Research (Belgium).

References

Brünger, A. T. (1992). *X-PLOR. Version* 3.1. *A System for X-ray Crystallography and NMR*. Yale University, Connecticut, USA.
Eck, M. J. & Sprang, S. R. (1989). *J. Biol. Chem.* **264**, 17595–17605.
Feldmann, M., Brennan, F. M., Elliott, M. J., Williams, R. O. & Maini, R. N. (1995). *Ann. NY Acad. Sci.* **766**, 272–278.
Fiers, W. (1995). *Biologic Therapy of Cancer*, 2nd ed., edited by V. T. DeVita Jr, S. Hellman & S. A. Rosenberg, pp. 295–327. Philadelphia: Lippincott.
Fransen, L., Müller, R., Marmenout, A., Tavernier, J., Van der Heyden, J., Kawashima, E., Chollet, A., Tizard, R., Van Heuverswyn, H., Van Vliet, A., Ruysschaert, M. R. & Fiers, W. (1985). *Nucleic Acids Res.* **13**, 4417–4429.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Jones, E. Y., Stuart, D. I. & Walker, N. P. C. (1989). *Nature (London)*, **338**, 225–228.
Lejeune, F., Lienard, D., Eppermont, A., Schraffordt Koops, H., Rosenkaimer, F., Gerain, J., Klaase, J., Kroon, B., Vanderveken, J. & Schmitz, P. (1995). *J. Infusional Chemother.* **5**(2), 73–81.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Murata, Y., Ishiguro, Y., Itoh, J., Munakata, A. & Yoshida, Y. (1995). *J. Gastroenterol.* **8**, 56–60.
Vandenabeele, P., Declercq, W., Beyaert, R. & Fiers, W. (1995). *Trends Cell Biol.* **5**, 392-399.
Vassalli, P. (1992). *Ann. Rev. Immunol.* **10**, 411-452.