55. Crystallization of Human Recombinant Interleukin-2^{†)}

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Interleukin-2 (IL-2), also referred to as T-cell growth factor, is a lymphokine produced by activated T-cells.¹⁾ Recently mRNA's for human IL-2 of leukaemic T-cell line, Jurkat 111, were isolated and cDNA's were cloned, sequenced and expressed in *E. coli*.²⁾ The expressed protein, which had the characteristic biological properties of IL-2, was purified to homogeneity from a cell extract by successive chromatographies including reversed phase high performance liquid chromatographies (RP-HPLC).³⁾ The procedure of extraction and purification of IL-2 from *E. coli* cells will be described elsewhere (manuscript in preparation). The primary structure of a single polypeptide chain of human IL-2 consisting of 133 amino acids (molecular weight 15,500) has been known.²⁾ However, crystallization conditions and three-dimensional structure of IL-2 have not been reported. Here we report on the crystallization of human recombinant IL-2.

The optimum conditions of crystallization of IL-2 were established by vapor diffusion method in the hanging drop mode. General settings were as follows: hanging drops of 10 μ l each were equilibrated with reservoir solutions of 1.0 ml each containing one of such precipitants as ammonium sulfate, sodium chloride and polyethylene glycol. Each hanging drop contained 15 to 20 μ g of IL-2 dissolved in an appropriately buffered solution containing the same (but at a lower concentration) precipitant as used for the reservoir solution. The reservoir solution was buffered at the same pH as, or at the slightly lower pH than, the droplet.

After about a week, several different types of crystals appeared in the droplets depending on the conditions employed. Each type of these crystals was identified as IL-2 by means of RP-HPLC and biological assay (CTLL assay).^{4), 5)} The large hexagonal bipyramidal crystals as shown in Fig. 1 were obtained at 25°C. The precipitant used was ammonium sulfate, the concentrations of which were 0.8 to 1.3 M and 1.5 to 2.5 M for the droplet and reservoir well solution, respectively. Such hexagonal crystals were obtained only when the pH was in the range 6.3 to 7.3. The maximum dimensions of thus obtained hexagonal crystals were 0.8 $\times$ 0.5 $\times$ 0.5 mm and extinction along the long axis (*c*-axis) was observed under a polarizing microscope. The crystal belongs to a hexagonal space group P6₂22 or P6₄22 with $a=b=105.8$ Å, $c=122.2$ Å, giving a cell volume of $V=1.18\times 10^6$ Å³. One of the precession photographs is shown in Fig. 2. The X-ray diffraction spots were seen up to the spacing of 3.4 Å. The crystals showed no

^{†)} Abbreviations: IL-2, interleukin-2; RP-HPLC, reversed phase high performance liquid chromatography.

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Fig. 1. A crystal of human recombinant interleukin-2.
The long axis (*c*-axis) is about 0.8 mm.

Fig. 2. The 10° (*h k 0*) precession photograph of a hexagonal crystal of IL-2. The exposure time was 6 hrs using nickel-filtered Cu- $K\alpha$ radiation from a Rigaku RU-200 rotating anode generator operating at 50 kV and 90 mA. The focus size was 3.0×0.3 mm.

significant radiation damage even after 7 days of exposure to X-rays. Assuming 2 molecules of IL-2 per asymmetric unit, the ratio of cell volume to weight (V_M) is $3.2 \text{ \AA}^3/\text{dalton}$ and solvent content (V_{solv}) is 61%. Assuming 3 molecules per asymmetric unit, on the other hand, V_M is $2.1 \text{ \AA}^3/\text{dalton}$ and V_{solv} is 42%. Both values of V_M are within the range reported by Matthews.⁶⁾

The fact that the present preparation of human recombinant IL-2 has now been crystallized, strongly indicates that the preparation does not contain any significant amount of heterologous molecular species such as irregularly aggregated, misfolded or partially processed molecules. It is noteworthy that this holds despite the fact that all of the three cysteine residues in a human IL-2 molecule were found to be in free state in the original product stored as inclusion bodies

appeared in *E. coli* cells and these were oxidized to a correct pattern of linkage (a disulfide bridging between Cys-58 and Cys-105 and one free Cys-125)^{7),8)} through a specific procedure devised in the present laboratory (manuscript in preparation).

Characterization of other crystal forms, which have already been obtained, and search for heavy-atom derivatives are under way.

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