Crystallization and preliminary X-ray crystallographic study of interleukin-8

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Interleukin-8 (neutrophil-activating factor; NAP-1) has been crystallized by the vapour diffusion technique to give single crystals suitable for three-dimensional structural study at a resolution higher than 2.4 Å. The crystals belong to the space group $P3_121$ or $P3_221$ and have unit cell dimensions $a = b = 40.9$ Å, $c = 90.3$ Å.

Crystallization; Neutrophil-activating factor; Interleukin-8

1. INTRODUCTION

Interleukin-8 (IL-8) is a relatively newly-discovered inflammatory peptide which was isolated almost simultaneously by several groups as a secreted product of endotoxin-stimulated monocytes, and the peptide therefore has several names, including neutrophil-activating factor (NAF) [1], monocyte-derived neutrophil-activating peptide (MONAP) [2], monocyte-derived neutrophil chemotactic factor (MDNCF) [3], granulocyte-chemotactic peptide (GCP) [4] and monocyte-derived chemotaxin (MOC) [5].

It has subsequently been shown that many cell types other than monocytes can produce large quantities of Il-8 or IL-8 mRNA, in response to stimulation with endotoxin, IL-1 or tumour necrosis factor (TNF) [1,6–9].

The major form of IL-8 produced by monocytes is 72 amino acids in length and has a molecular mass of approximately 8.4 kDa (approx. 70% is this form), although both longer and shorter forms are present in stimulated monocyte supernatants [10]. However, Gimbrone et al. [11] have reported that endothelial cells produce a 77 amino acid molecule as their major form.

The activities of IL-8 are not limited to the effects on neutrophils (PMN) by which it was initially identified. It activates PMN to degranulate and exhibit respiratory burst [12,13] and is chemotactic for PMN and lymphocytes in vitro and in vivo [2,4,10,14]. Further activities include triggering of histamine and leukotriene release from IL-3-primed basophils [15], spasmogenic activity on airway smooth muscle [16], and effects on PMN adherence to endothelial cells [11,17].

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2. EXPERIMENTAL

We have produced the 72 amino acid form of IL-8 as a recombinant protein, by expression of a synthetic gene in *E. coli*[10]. The purity and homogeneity of this product were sufficient for crystallization studies.

Interleukin-8 was stored in 50 mM 2-morpholinoethanesulfonic acid (Mes) (Fluka), pH 6.5, 3 mM sodium azide (NaN_3) and 300 mM sodium chloride (NaCl) at 2.9 mg/ml. For crystallization the protein was concentrated to 20 mg/ml using an Amicon concentration cell 8010, YM2 (diameter 25 mm). Using the hanging drop, vapor diffusion method [18], concentrated protein solution (20 μl) was mixed with the precipitating solution (1–3 μl) and equilibrated against a 0.5 ml chamber of precipitating solution.

Crystallization conditions for IL-8 were initially screened at 4°C over 50 different combinations of salt, pH and precipitant. Of these, the following combinations yielded single crystals:

(1) 30%, 35%, 40% (w/w) polyethylene glycol (PEG) 20000 (Fluka), 50 mM Mes, pH 6.5, 300 mM NaCl, 3 mM NaN_3;

(2) 30%, 35%, 40% (w/w) PEG 8000, 50 mM Mes, pH 6.5, 300 mM NaCl, 3 mM NaN_3;

(3) 40% (w/w) PEG 6000, 50 mM Mes, pH 6.5, 300 mM NaCl, 3 mM NaN_3.

Crystals grew as rhomboids within 10 days to a final size of $0.5 \times 0.4 \times 0.4$ mm^3 in the first two sets of conditions and were slightly smaller with PEG 6000 (Fig. 1). For diffraction studies, crystals were mounted in 1.0 mm diameter glass capillaries and were stable in the X-ray beam for at least 24 h so that a full data set could usually be produced using one crystal.

3. RESULTS AND DISCUSSION

A native data set to 2.4 Å resolution was collected on a fast area detector using CuK_α-radiation (40 kV, 70 mA) produced by a rotating anode X-ray generator (FR571). Indexing by the ENDEX program available in MADNES [19] and subsequent refinement (fixing $\alpha = \beta = 90^0$) using 250 reflections gave a cell with $a = 40.86$ Å, $b = 40.87$ Å, $c = 90.26$ Å and $\gamma = 120.03^0$. The data were then processed using COLLECT and

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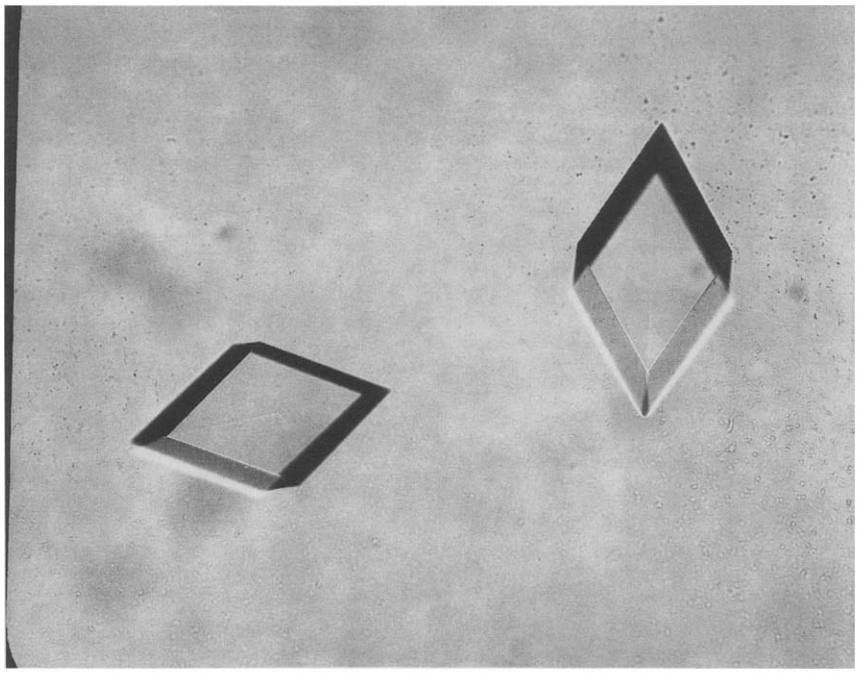

Fig. 1. Crystals of interleukin-8 expressed in *E. coli*.

PROCOR [20] (11249 reflections) and merged in AGROVATA (CCP4-package) using all possible point-groups containing one 3-fold axis. Point group 321 yielded $R_{sym} = 3.4\%$ (on intensities) for 10626 measurements (comprising samples of all symmetry equivalent reflections) of 3099 independent reflections (no rejections). X-Ray precession photographs subsequently confirmed the point group and fixed the space-group at $P3_121$ or $P3_221$. The unit cell volume is 130 800 Å^3. One chain of molecular mass = 8386 Da per asymmetric unit gives $V_M = 2.60$ Å^3/Da which is within the normal range for protein crystals [21]. Heavy atom derivative data sets have been collected and are currently under analysis.

Although the solution structure of the interleukin-8 dimer has been solved recently by nuclear magnetic resonance spectroscopy and hybrid distance geometry-dynamical simulated annealing calculations [22] and was shown to consist of two antiparallel α-helices lying on top of a six-stranded antiparallel β-sheet platform, there are 3 main reasons for a determination of the crystal structure of IL-8.

Firstly, it will be important to see whether there are major differences between the crystal structure of the protein and its conformation in solution. Secondly, the 72 amino acid form of the protein used for our crystallographic study is the basis of an extended mutagenesis programme at Sandoz intended to define the minimum structure required for full activity. Nine different mutants are now available [23] and results from biological test systems show marked variation in activity. Crystallization experiments with the most interesting derivatives are in progress. Lastly, the relationship between the biological function of these mutants and their precise structure, especially of the two symmetry-related helices could yield important information for the design of potential IL-8 inhibitors.

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