

Crystal structure of human recombinant interleukin-4 at 2.25 Å resolution

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The crystal structure of human recombinant interleukin-4 (IL-4) has been solved by multiple isomorphous replacement, and refined to an R factor of 0.218 at 2.25 Å resolution. The molecule is a left-handed four-helix bundle with a short stretch of β sheet. The structure bears close resemblance to other cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF). Although no sequence similarity of IL-4 to GM-CSF and other related cytokines has been previously postulated, structure-based alignment of IL-4 and GM-CSF revealed that the core of the molecules, including large parts of all four helices and extending over half of the molecule, has 30% sequence identity. This may have identified regions which are not only important to maintain structure, but could also play a role in receptor binding.

Cytokine; X-ray crystallography; Protein structure; Refinement

1. INTRODUCTION

Interleukin-4 (IL-4) is a member of a family of cytokines including IL-2, IL-3, IL-6 and granulocyte-macrophage colony stimulating factor (GM-CSF), which share a similar polypeptide fold [1]. IL-4 plays an important biological role in being able to stimulate the proliferation and differentiation of a number of different cell types [2], and it has properties which may make it important pharmaceutically [3]. Molecular modeling [4] and NMR spectroscopy [5,6] have shown this molecule to be a 4-helix bundle similar in its structure to GM-CSF [7] and porcine [8] and human growth hormones [9]. Two independent atomic models in solution have been produced based on NMR data [10,11]. While crystallization of IL-4 has been previously described [12,13], no crystal structure has been reported. The present work describes solution of the crystal structure by the method of multiple isomorphous replacement (MIR), preliminary refinement of the structure, and the comparison with the structure of GM-CSF [7].

2. MATERIALS AND METHODS

Human recombinant IL-4 used in the initial investigations of the crystallization conditions was a gift of Chimtech (Moscow, Russia), while material purchased from PreproTech (Rocky Hill, NJ) was used for the bulk of the work. The polypeptide of 130 amino acids was expressed in *E. coli*, and was stored as lyophilized powder. The N-terminal methionine was present in approximately 70% of the sample, but since it was not seen in the electron density maps (see below), the

numbering used in this paper follows the native sequence, starting with His-1 and continuing to Ser-129. Commercially obtained material migrated as a single band on a polyacrylamide focusing gel, and was not further purified.

Crystals of IL-4 were grown at room temperature by the hanging drop, vapor diffusion technique [14]. Droplets containing 4 mg/ml protein in 30 mM K-phosphate buffer, pH 6.0, were equilibrated against a reservoir containing 60–63% ammonium sulfate in the same buffer. Needle-like crystals appeared in a week and reached maximum size in two to three weeks. Heavy atom derivatives were prepared by soaking crystals in solutions containing suitable compounds. A total of 25 different potential heavy atom compounds were investigated.

Diffraction data were collected for the native and derivative crystals using a Siemens multiwire area detector mounted on a Rigaku RU-200 rotating anode, operated at 50 kV and 100 mA. These data were integrated using the XGEN program system [15]. In addition, one derivative data set, and the native data used in the final refinement, were measured with an R-Axis IIc image plate detector, using an equivalent X-ray source, and were processed with the software provided by the manufacturer. Scaling of data sets and refinement of the heavy atom positions and phase calculations were accomplished with program PHASES [16], electron density maps were fitted using FRODO [17], and the models were refined with PROLSQ [18] and X-PLOR [19].

3. RESULTS

Crystals of IL-4 belong to the space group $P4_22$ (the enantiomorph established on the basis of the anomalous scattering of the heavy atoms). Unit cell parameters are $a=b=91.8$ Å, $c=46.4$ Å, with one molecule present in the asymmetric unit. Derivative data were collected to the resolution of 2.7–3.5 Å (Table I), while the native data extended to 2.23 Å and were reasonably complete (9,169 out of 10,104 possible reflections measured, with 8,223 reflections having $I > 1.5 \sigma(I)$).

An electron density map was computed with MIR

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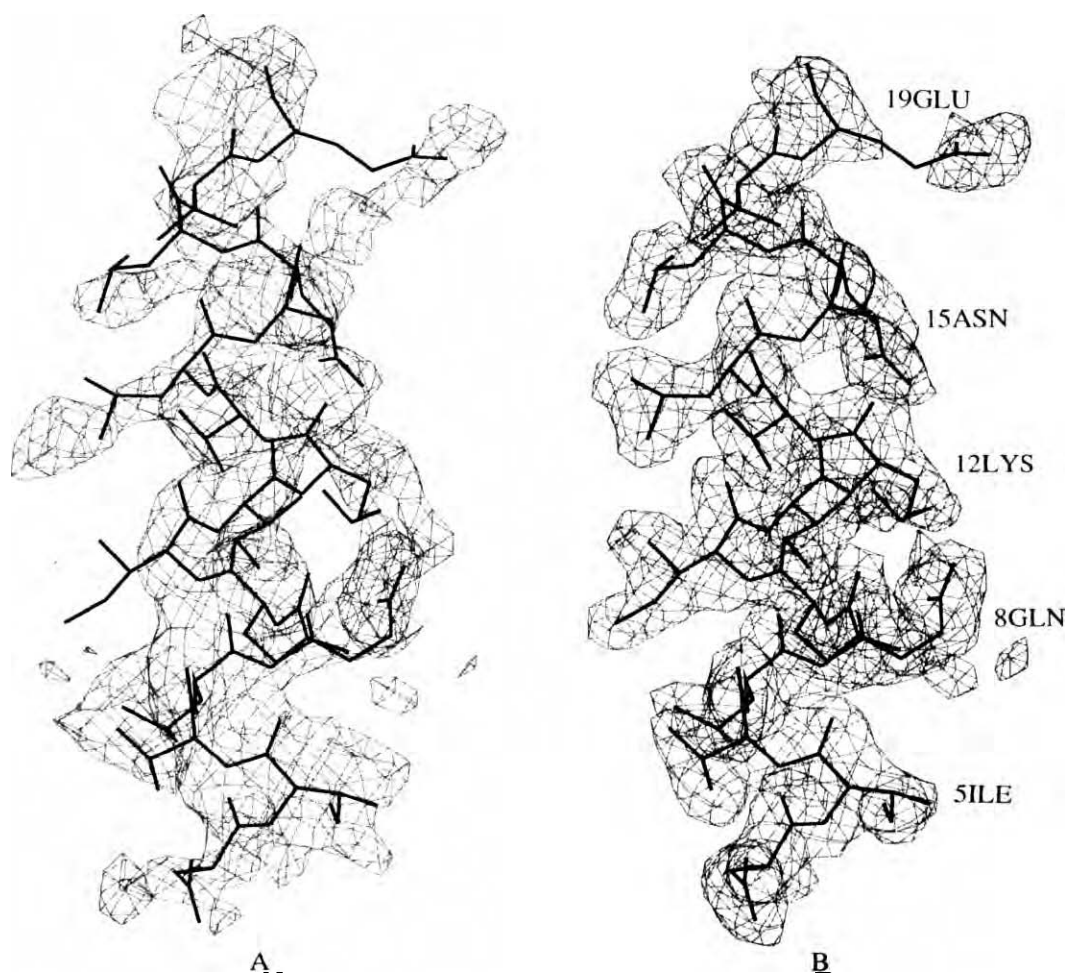


Fig. 1. Electron density corresponding to helix A (residues 4–19) contoured at 1σ level, with the coordinates of the final model superimposed. (A) MIR map computed at 2.7 Å with the phases calculated as shown in Table I. (B) $2F_o - F_c$ difference Fourier map at 2.25 Å resolution at the final stage of refinement.

phases from five derivatives, three of which also provided usable anomalous data (Table I). The map was rather noisy, even though the four helices could be iden-

tified without ambiguity (Fig. 1A). Attempts to improve the map by solvent flattening were not successful, despite the high solvent content of these crystals. The

Table I
Summary of crystallographic data collection and phasing

Data set	Unique reflections $F > 2\sigma$	Resolution (Å)	Completeness (%)	R_{merge}	R_{deriv}	R_{centr}	Phasing power ⁵ iso(ano)	Heavy atom	
								Number	Location
Native ^a	8951	2.23	90.7	0.06					
Trimethyl ^a	4851	2.7	80.5	0.07	0.202	0.65	1.34 (1.48)	1	Asp-31; Glu-114
lead acetate									
$\text{K}_2\text{Pt}(\text{NO}_2)_4$ ^a	2927	3.0	97.8	0.10	0.148	0.66	1.23 (1.34)	1	His-58; His-59
Iodine ^a	3156	3.0	92.3	0.10	0.140	0.65	1.28 (1.43)	1	Tyr-56
Gold sodium ^a	2067	3.2	67.7	0.11	0.150	0.68	0.9L	1	Phs-112
thiomalate								2	Thr-18; Leu-113
K_2PtCl_6 ^a	1934	3.5	87.4	0.10	0.178	0.71	0.95	1	Thr-22; His-76
								2	Ser-107; Arg-115

The overall figure of merit is 0.524 for 5193 independent reflections with $F > 2\sigma(F)$ in resolution range 25–2.7 Å. Data collected on: ^aR-AXIS; ^bSiemens. $R_{\text{merge}} = \sum |I_{\text{obs}} - \langle I \rangle| / \sum \langle I \rangle$; $R_{\text{deriv}} = \sum |F_{\text{PH}} - F_P| / \sum F_P$; $R_{\text{centr}} = \sum |F_{\text{PH}} \pm F_P| - |F_H| / \sum |F_{\text{PH}}| - |F_P|$; ⁵Phasing power for iso = $\sum F_H / E$ and $| - | \sum 2F_H / E$

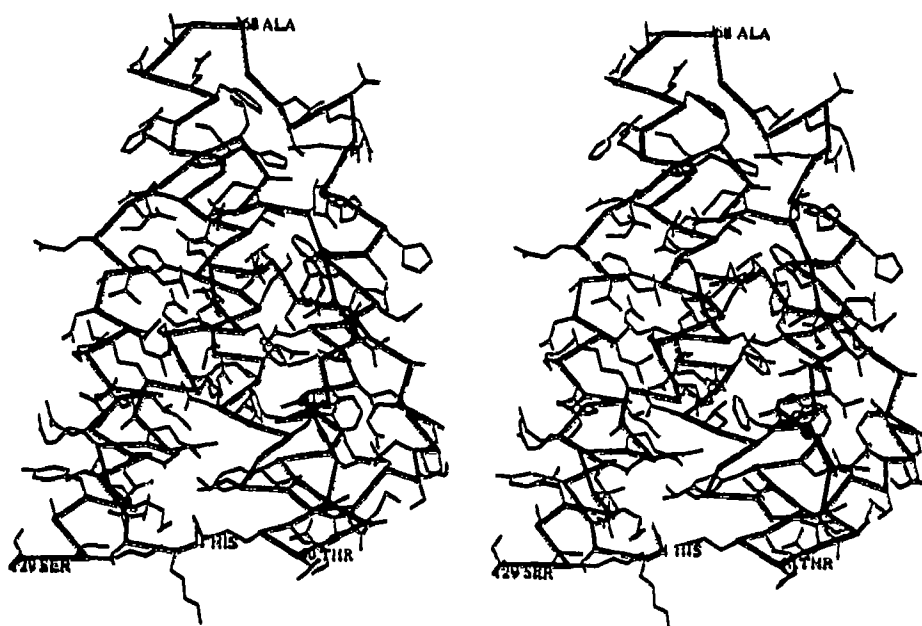


Fig. 2. Stereoview of the structure of IL-4. Main chain is presented by C_{α} trace, while all side chain atoms are shown in thin lines.

initial fit to this map was verified in a comparison with the NMR coordinates [10], which also were useful in tracing some of the broken density belonging to the regions lacking regular secondary structure.

The initial atomic coordinates were subjected to three cycles of simulated annealing [19] and to least squares refinement [18], resulting in the crystallographic *R* factor of 0.218 for a model consisting of all 129 amino acids, as well as 33 water molecules (Fig. 2). The root mean square (rms) deviation of bond lengths from the PROLSQ [18] targets is 0.020 Å, individual temperature factors are restrained, and the Ramachandran plot is acceptable except for poorly defined residues 38 and 40. The quality of the final map is good (Fig. 1B). Further studies at higher resolution, and fuller description of the solvent structure are in progress.

Crystallographic model of IL-4 did not significantly modify previous descriptions of the structures solved by NMR [5,6,10,11]. The core of the molecule is formed by a left-handed helical bundle, consisting of helix A (residues 4–19), B (40–60), C (70–94), and D (109–126). The helices are connected by three loops forming the most flexible part of the molecule. Two β -strands which are part of the loops form a short fragment of antiparallel β -ribbon (residues 27–31 and 105–109) connected by four hydrogen bonds (Fig. 3).

The core of the molecule between the helices is filled by residues with mostly hydrophobic character (Thr-6, Leu-7, Ile-10, Ile-11, Thr-13, Leu-14, Leu-17 in helix A; Thr-44, Phe-45, Ala-48, Ala-49, Val-51, Leu-52, Phe-55 in helix B; Leu-79, Ile-80, Phe-82, Leu-83, Leu-86, Leu-90, Leu-93, Ala-94 in helix C; and Leu-109, Phe-112,

Leu-113, Leu-116, Ile-119, Met-120 in helix D). This core is completed by the predominantly hydrophobic residues Thr-25, Val-29, Ile-32 and Phe-33 from the irregular part of the structure. Several charged residues reside inside the hydrophobic core, forming possible ion pairs. The side chain of Glu-41 interacts with Lys-123, and His-59. Asp-62 and Arg-64 are sharing an ionic interaction (the latter two residues are located outside of the helices).

A prominent feature of the structure is the string of positively charged residues (His-74, Lys-77, Arg-81, Lys-84, Arg-85, Arg-88). All of them are exposed and their charges are uncompensated by other residues, except for Lys-84 which may form an ion pair with the side chain of Glu-60. These residues, except for Arg-88, are not involved in crystal contacts. Such formation of a positively charged 'wall' may play a role in the interaction of IL-4 with its receptor.

4. DISCUSSION

Crystal structure of IL-4 bears close resemblance to the solution structure derived by NMR [10], with 126 C_{α} atoms deviating by 1.65 Å (rms) upon superposition. Residues with significant deviations (greater than 3 Å) include the amino terminus (1–3), 36 and 37 (located in a poorly defined region), 63 and 68 (part of the strand 63–70, all of which shows large deviations), 95, 100 and 103 (in the region of 91–104 which differs significantly), 125, 126, and 129 (C terminus). All of these differences are in stretches of irregular structure outside of the helical regions, usually with high temperature factors,

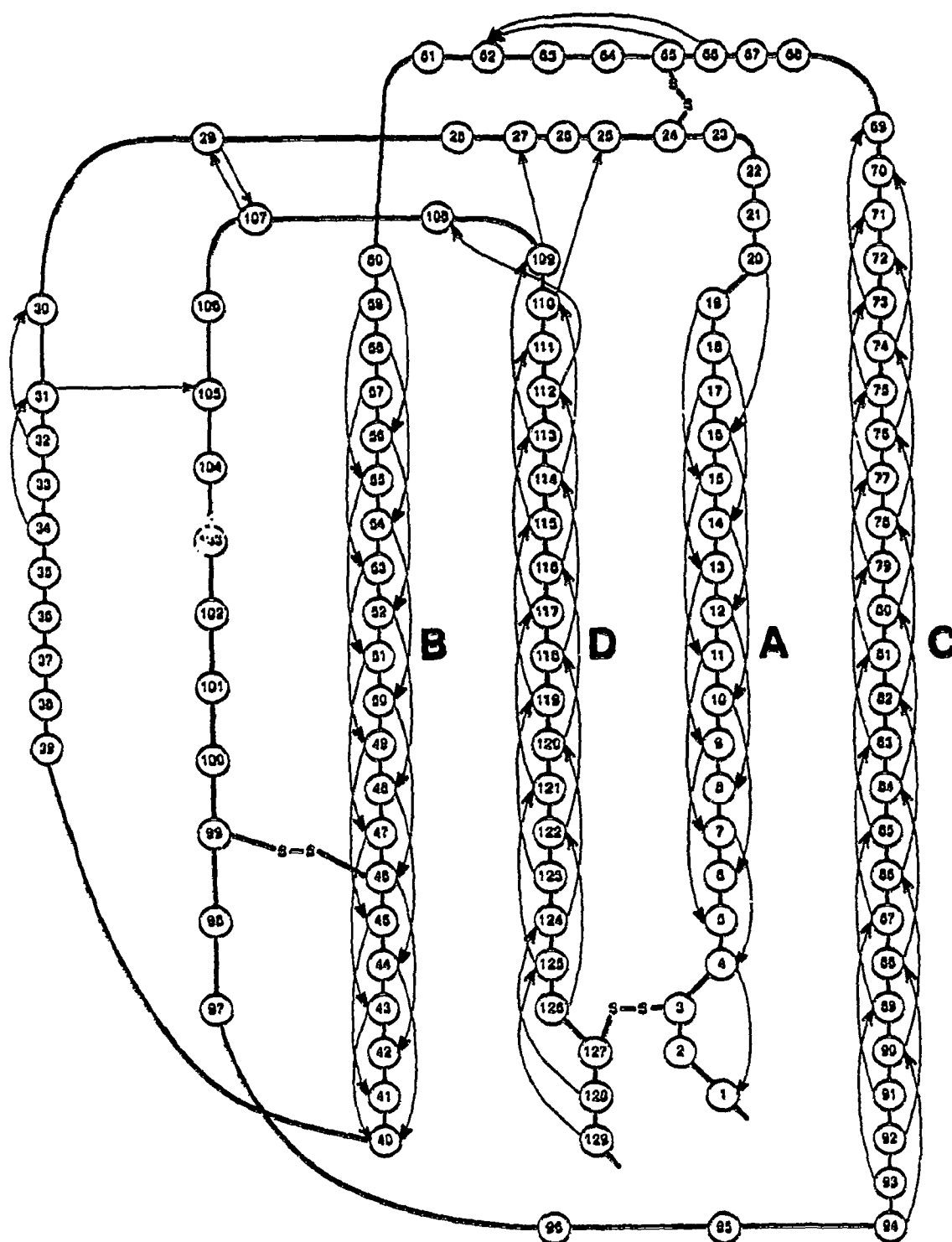
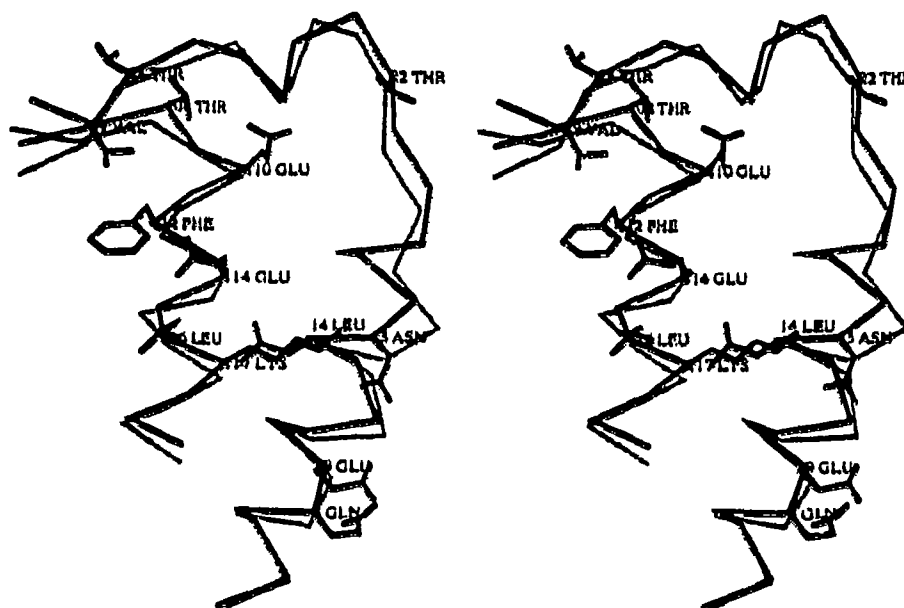


Fig. 3. Secondary structure diagram of IL-4, showing all main chain hydrogen bonds (indicated by arrows), as well as the three disulfide bonds.

and may have resulted from the influence of crystal packing, as well as from artifacts of structure determination by either method. In addition, large deviations are seen for many side chains, including bulky ones such as Trp-91. These differences are considerably larger than those reported in a similar comparison of solution

and NMR structures of interleukin-8 [20], but it is clear that the quality of both the X-ray and NMR structures of IL-4 is significantly lower.

GM-CSF is the other related cytokine with publicly available atomic coordinates, and therefore its comparison with IL-4 is possible. Both of these structures are



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