

X-ray Structure of Interleukin-1 Receptor Antagonist at 2.0-Å Resolution*

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Interleukin-1 receptor antagonist (IL-1ra) is a natural competitive antagonist of IL-1. In order to further elucidate the mechanism by which IL-1ra binds without activating the IL-1 receptor, we have solved the crystal structure of IL-1ra at 2.0-Å resolution. IL-1ra has the same overall β -trefoil fold as IL-1 α and IL-1 β and has a very similar hydrophobic core. However, there are a number of structural differences between the molecules, including significant differences at the open end of the β -barrel, which has been identified in IL-1 β as a receptor binding site.

IL-1ra¹ is a member of the interleukin-1 family (Eisenberg *et al.* 1991), which has two other characterized members, IL-1 α and IL-1 β . These three cytokines bind with high affinity to the type I IL-1 receptor (IL-1RI) found on many cells including T-cells, fibroblasts, and endothelial cells. Binding of IL-1 α and IL-1 β to IL-1RI results in multiple cellular responses including EGF receptor down-modulation, IL-1RI internalization, and increased prostaglandin E₂ production; however, IL-1ra's binding to this receptor does not elicit a detectable biological response and completely blocks the binding of IL-1 α and IL-1 β (Hannum *et al.*, 1990; Dinarello and Thompson, 1991; Dripps *et al.*, 1991a). A second IL-1 receptor, IL-1RII, is predominantly expressed on B cells, monocytes, macrophages, and neutrophils (Chizzonite *et al.*, 1989; Bomsztyk *et al.*, 1989). IL-1ra and IL-1 α bind to IL-1RII with lower affinity than IL-1 β (Dripps *et al.* 1991b). The biological function of IL-1RII is not known, although it has been shown recently that IL-1RII may inhibit the activity of IL-1 by functioning as a decoy receptor (Colotta *et al.*, 1993).

We have solved the structure of IL-1ra at 2.0-Å resolution as a part of our program to understand the mechanism by which IL-1ra binds to the IL-1RI without eliciting a biological response. IL-1ra shares 26% sequence identity with IL-1 β and 19% identity with IL-1 α (Eisenberg *et al.*, 1990) (Table I). The three-dimensional structures of IL-1 α and IL-1 β have been determined by x-ray crystallography and heteronuclear NMR spectroscopy (Finzel *et al.*, 1989; Priestle *et al.*, 1989; Graves *et al.*, 1990; Clore *et al.*, 1991; Shaanan *et al.*, 1992). These two proteins exhibit a β -trefoil topology characterized by six

β -strands forming a β -barrel, which is closed at one end by another six β -strands. The structure of IL-1ra was solved by molecular replacement using models derived from the structure of IL-1 β . IL-1ra shows the same overall fold as the other IL-1 proteins. Two regions of significant structural difference between IL-1ra and IL-1 β were observed. The first region consists of residues 83–95 of IL-1ra. This region contains the *N*-linked glycosylation site of IL-1ra and has low sequence homology to IL-1 β . The second region is located at the top of the β -barrel, which is implicated by site-directed mutagenesis studies in the interaction of IL-1RI with IL-1 β .

EXPERIMENTAL PROCEDURES

Crystallization and Data Collection—Recombinant wild type and cysteine-to-serine analogs (IL-1ra:C66S and IL-1ra:C122S) of human IL-1ra were purified by ion exchange chromatography from *Escherichia coli* lysates as previously described (Dripps *et al.*, 1991a). The cysteine-to-serine changes did not affect the affinity of the proteins for IL-1RI (data not shown). Triclinic crystals were grown by the hanging drop vapor diffusion method at 20 °C, using 25% PEG 8000, 0.8 M NaCl, 10 mM MES, pH 6.0, as the precipitant. The hanging drop contained a 1:1 mixture of precipitant and recombinant IL-1ra at 21 mg/ml in 10 mM MOPS, pH 7.0. Crystals suitable for data collection grew over a period of several weeks and required successive rounds of seeding. Large crystals (1.2 × 0.2 × 0.1 mm) were transferred to an artificial mother liquor (30% PEG 8000, 0.85 M NaCl, 50 mM MES, pH 6.0) to prevent further growth since the crystals were prone to twin. Tetragonal crystals were similarly grown using 30% PEG 8000, 0.6 M NaCl, 50 mM MES, pH 6.0, as the precipitant.

Diffraction data were collected at room temperature on a Rigaku R-Axis II at Molecular Structure Corp. and processed using the R-Axis data processing package (Sato *et al.*, 1992). The triclinic crystals belong to the space group P₁ with unit cell dimensions of $a = 58.75$ Å, $b = 43.81$ Å, $c = 35.84$ Å, $\alpha = 65.2^\circ$, $\beta = 104.8^\circ$, $\gamma = 108.3^\circ$ and have 2 molecules/asymmetric unit. The tetragonal crystals belong to the space group P₄, 2₁, 2, with unit cell dimensions of $a = b = 71.11$ Å, $c = 112.61$ Å, $\alpha = \beta = \gamma = 90^\circ$ and also contain 2 molecules/asymmetric unit.

Diffraction data from native and mercury soaked crystals were collected for both space groups (Table II). The native triclinic data were collected from an IL-1ra:C66S crystal, while the native tetragonal data were collected from a wild type crystal. Because there are no disulfide bonds in wild type human IL-1ra (Stockman *et al.*, 1992) and IL-1ra:C66S crystallizes isomorphously with recombinant wild type IL-1ra, we anticipated that the structure of IL-1ra:C66S would be virtually identical to the structure of wild type IL-1ra. Data were also collected on several derivative crystals; HG1 was a wild type IL-1ra triclinic crystal soaked for 12 h at 5% saturated concentration of 2,6-dichloromercuri-4-nitrophenol in artificial mother liquor. HG2 was an IL-1ra:C122S triclinic crystal soaked for 18 h at 10% saturated concentration of 2-chloromercuri-4-nitrophenol in mother liquor. HG3 was a wild type IL-1ra tetragonal crystal soaked for 4 days in 1 mM K₂HgI₄, 10 mM KI. HG4 was an IL-1ra:C66S tetragonal crystal soaked for 21 h at 10% saturated concentration of 2-chloromercuri-4-nitrophenol in mother liquor. However, the unit cell dimensions of these crystals differed from the native crystal by approximately 4%, and the heavy atom positions could not be unambiguously determined from difference Patterson maps. We therefore solved the structure by molecular replacement.

Determination of Molecular Replacement Positions—Two different molecular replacement methods were used to identify the molecular

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¹ The abbreviations used are: IL-1ra, interleukin-1 receptor antagonist; IL-1 α , interleukin-1 α ; IL-1 β , interleukin-1 β ; IL-1RI, type I interleukin-1 receptor; IL-1RII, type II interleukin-1 receptor; PEG, polyethylene glycol; MES, 2-(*N*-morpholino)ethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; C α , α carbon; RMSD, root mean square deviation.

TABLE I
Sequences of IL-1ra, IL-1α, and IL-1β, aligned from their x-ray structures

Residues whose Cα positions are conserved to within 2.0 Å are shown in bold. Sequences conserved between IL-1ra and IL-1β over 3 or more residues are shown in italics. The β-strands of IL-1ra are marked with double underlines. Breaks are inserted in the sequences before and after the β-strands. The residues corresponding to 84–94 in IL-1ra show particularly poor alignment between the three structures.

IL-1α	SSPFSFLSNVKNFMRIIK	Y- EFILN	DALNQ	SIIRA	NDQ	YLTAAALH	--NLDEA	51
IL-1ra	-----RPSGRKSSK	MQAFRIW	DVNQK	TFYLR	N-N	QLVAGY	LQ	43
IL-1β	-----APVR	SLNCTLR	DSQK	SLVMS	GPY	ELKALHLQ	QDMEQQ	39
β-Strands		=====	=====	=====		=====		
		S1		S2		S3		
IL-1α	VKFDMG	AYKSSKDDA --KI	TVILRI	SKTQ	LYVTAQD	--E	DQPVLLKEM	95
IL-1ra	EKIDVV	PI ----EP----H	ALFLGI	HGGK	MCLSCVK	SGD	ETRLQLEAV	83
IL-1β	VVFSMS	FVQG --EESNDKI	PVALGL	KEKN	LYLSCVL	KDD	KPTLQLESV	85
β-Strands	=====	=====	=====	=====		=====		
	S4	S5	S6	S7				
IL-1α	---PEIPKTITGS-ETNLL	FFWETH	GT	KNYFT	SVAHPN	LFIAT	K-QD	137
IL-1ra	---NITDLSENRRQD KRFA	FIRSDS	GP	TTSFE	SAACPG	WFLCT	AMEAD	128
IL-1β	DPKNYPKKKM---- EKR	FVKIEI	NN	KLEFE	SAQFPN	WYIST	SQAEN	129
β-Strands	=====	=====	=====	=====		=====		
		S8	S9	S10				
IL-1α	YVWCLA	GG---PPSIT	DFQILEN	QA	159			
IL-1ra	QPVSLT	NMPDEGVMVT	KFYFQED	E-	152			
IL-1β	MPVFLG	GT-KGGQDIT	DFTMQFV	SS	153			
β-Strands	=====	=====	=====					
	S11	S12						

TABLE II
Data collection and molecular replacement results

A. Statistics for x-ray data collection						
X-ray data	Triclinic			Tetragonal		
	Native	HG1	HG2	Native	HG3	HG4
Protein	C66S	Wild type	C122S	Wild type	Wild type	C66S
Resolution (Å)	15.00 – 2.75	15.00 – 2.50	15.00 – 2.30	8.00 – 2.00	15.00 – 2.40	15.00 – 3.00
Data coverage (%)	92.9	90.3	91.3	95.6	98.6	92.4
R _{sym} (%) ^a	6.9	6.7	8.1	6.9	5.1	5.5
B. Euler angle rotation function values for molecular replacement						
Program	Resolution	Molecule		θ ₁	θ ₂	θ ₃
	Å					
MERLOT	8.00 – 4.00	Triclinic molecule 1		294.4°	71.5°	177.7°
		Triclinic molecule 2		113.9°	87.2°	167.4°
X-PLOR	8.00 – 2.75	Triclinic molecule 1		294.7°	64.8°	177.0°
		Triclinic molecule 2		115.5°	91.4°	167.9°
	8.00 – 3.00	Tetragonal molecule 1		319.7°	13.3°	235.1°
		Tetragonal molecule 2		152.2°	28.0°	161.9°
C. Fractional translation function values for molecular replacement						
Program	Resolution	Molecule		x	y	z
	Å					
MERLOT	8.00 – 4.00	Triclinic molecule 1		0.496	0.997	0.468
		Triclinic molecule 2		0.000	0.000	0.000
X-PLOR	8.00 – 2.75	Triclinic molecule 1		0.475	0.000	0.492
		Triclinic molecule 2		0.000	0.000	0.000
	8.00 – 3.00	Tetragonal molecule 1		0.125	0.232	0.375
		Tetragonal molecule 2		0.393	0.768	0.357

^a R_{sym} = Σ_i Σ_j |I_{n,i} - I_{n,j}| / Σ_i Σ_j I_{n,i}, where I_n is the mean intensity of i observations of reflection n.

orientations in the triclinic crystal form. First, an initial search model was constructed from the backbone and β carbons from residues 12–33, 39–48, 56–69, 80–87, and 99–152 of the refined structure of IL-1β (Priestle *et al.*, 1989) (see Eisenberg *et al.* (1990) for sequence alignment of IL-1ra and IL-1β and Table I for structural alignment). A self-rotation function revealed that the asymmetric unit had 2 molecules related by a non-crystallographic two-fold symmetry. The orientations of the two IL-1ra molecules in the asymmetric unit were determined using the fast rotation function and translation function in the program package, MERLOT (Fitzgerald, 1967) (Table II). Second, a model structure of IL-1ra from residues 7 to 152 was generated from the refined structure of IL-1β (Priestle *et al.*, 1989) using FRODO (Jones, 1978) to replace IL-1β side chains with the corresponding IL-1ra side chains and to perform initial geometric refinement. The program X-PLOR (Brünger *et al.*, 1987) was then used with the backbone and β carbon atoms of this model to perform Patterson searches, PC refinement, and translational

searches (Brünger, 1990) (Table II, parts b and c). This procedure clearly identified orientations similar to those determined by MERLOT for both of the molecules in the asymmetric unit.

Confirmation of Molecular Replacement Positions—While none of the derivatized crystals were sufficiently isomorphous with the native crystals to provide useful phase information for either space group, the data were used to check the correctness of the orientations determined in the molecular replacement searches. Difference electron density maps were calculated using model-derived phases and intensity data from mercury derivatized crystals (data not shown). In all cases, the maps showed peaks of density (>5 σ) at positions near the cysteine residues of both IL-1ra molecules in the asymmetric unit, but the peaks were not seen in data from crystals where the cysteine residues had been mutated to serine. We were therefore confident that the molecular replacement orientations were correct for both space groups.

Refinement of Structures—The structure of IL-1ra was refined on the

TABLE III
Statistics of refinement

Refinement was executed using the program X-PLOR (Brunger *et al.*, 1987). The numbered structures are the results of the following refinement steps. Structure 1, the IL-1ra search molecule generated in FRODO (Jones, 1978), fitted to the triclinic data. Structure 2, after Powell refinement, simulated annealing to 3000° and rebuilding of regions in $2F_o - F_c$ maps. Strict non-crystallographic symmetry was enforced. Structure 3, non-crystallographic symmetry constraints removed, further manual rebuilding and refinement by simulated annealing. Group temperature factors were refined by residue. Structure 4, molecular replacement into tetragonal data set. Structure 5, tetragonal model with strict non-crystallographic symmetry enforced. Structure 6, non-crystallographic symmetry constraints removed with further refinement by model building and simulated annealing. Restrained optimization of B -factors. Structure 7, after addition of a total of 74 water molecules. R -Factors for triclinic data are for the observed data with an F_{obs} greater than $2\sigma(F_{obs})$ between 8.00- and 2.75-Å resolution (7048 reflections). R -Factors for tetragonal data are for the observed data with an F_{obs} greater than $2\sigma(F_{obs})$ between 8.00- and 2.0-Å resolution (19,076 reflections). Structures 1 and 2 were refined using X-PLOR 2.1 with the param19.pro parameter file. All other structures were refined using X-PLOR 3.1 and the parhcsdx.pro parameters of Engh and Huber (1991). In Ramachandran plots of structure 7, the only residues which fell outside the allowed regions were Asn²⁷ and Asp⁵². RMS bond length and RMS bond angle are the respective root-mean-square deviations from ideal values.

Structure no.	R -Factor (resolution)	RMS bond length deviation	RMS bond angle deviation
	Å	Å	°
1	0.525 (8.00 – 2.75)	0.07	5.1
2	0.272 (8.00 – 2.75)	0.02	4.3
3	0.229 (8.00 – 2.75)	0.02	4.3
4	0.451 (8.00 – 3.00)	0.02	4.3
5	0.344 (8.00 – 2.00)	0.02	2.1
6	0.263 (8.00 – 2.00)	0.014	2.4
7	0.236 (8.00 – 2.00)	0.011	1.9
8 ^a	0.317 ^a (8.00 – 2.00)	0.011	1.9
9 ^b	0.208 ^b (8.00 – 2.75)	0.013	2.1

^a Structure 8 was refined by simulated annealing and B -factor refinement, using a working dataset containing 90% of the reflections, as suggested by Brunger (1992). The R -factor quoted is the free R -factor derived from the 10% of reflections not used for refinement.

^b Structure 9 is the result of transforming the tetragonal solution (without water molecules) to the orientations of the molecules in the triclinic crystals, followed by simulated annealing and B -factor refinement. Thus, the tetragonal structure also provides a more accurate solution of the triclinic structure.

triclinic crystal data to 2.75-Å resolution by conjugate gradient minimization and simulated annealing using X-PLOR, as described in Table III. The progress of the refinement was monitored by examination of $2F_o - F_c$ maps, omit maps, and interactive model building using FRODO (Jones, 1978) (Table III). The refined IL-1ra structure from the triclinic crystal form was then used to search the tetragonal data set, using X-PLOR as described above. Unambiguous orientations for the two molecules in the unit cell were determined, whereas searches using homology-built models of IL-1ra were unsuccessful in identifying the molecules' positions. The IL-1ra structure was then refined to 2.0 Å using X-PLOR, as described in Table III.

RESULTS AND DISCUSSION

Structure Determination—The structure of IL-1ra was solved by molecular replacement methods, using 2.75-Å resolution data from triclinic crystals and 2.0-Å resolution data from tetragonal crystals. We were forced to use the lower resolution triclinic data first, because we were unable to find a molecular replacement solution for the tetragonal data set using any starting model derived from IL-1β. This was probably due to the very high number of false minima in this high symmetry space group. We therefore first solved the structure in the triclinic crystal and then used this to solve the tetragonal case. The orientations of the two IL-1ra molecules in the triclinic unit cell were determined from polyalanine search molecules, which were constructed using the structure of IL-1β (Priestle *et al.*, 1989) as a template. Similar searches were used to determine the orien-

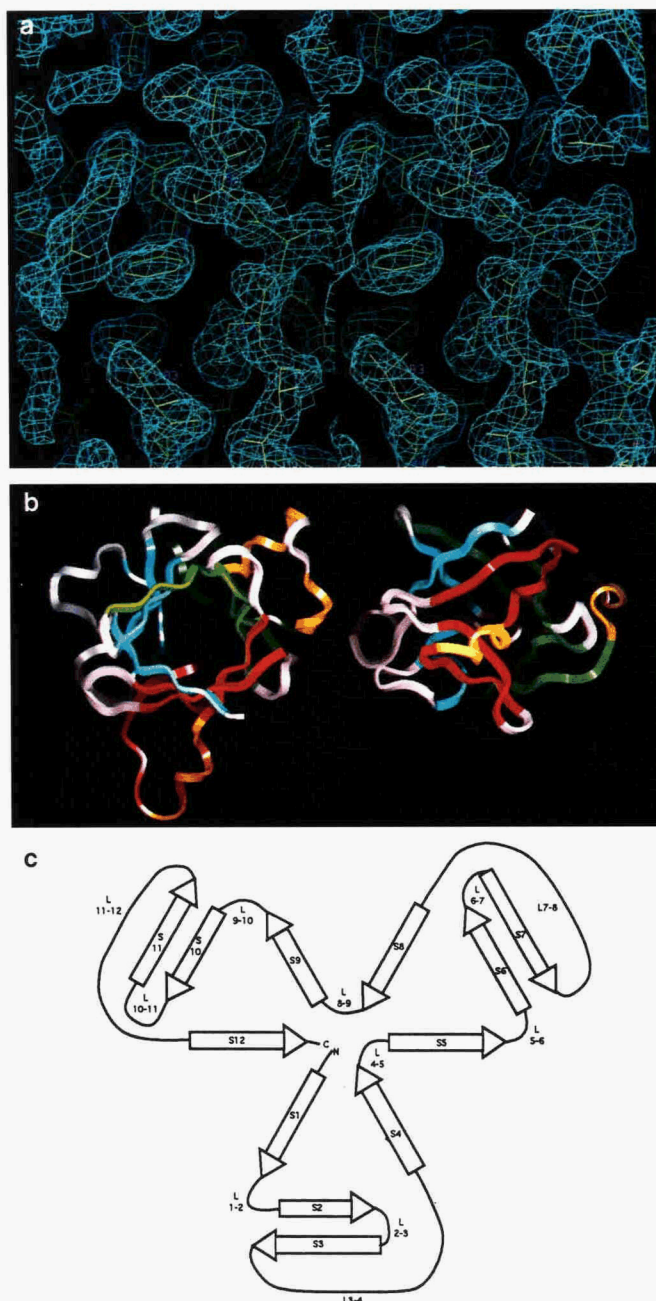


FIG. 1. *Panel a*, $2F_o - F_c$ map of the present structure of IL-1ra (8.0–2.0-Å resolution), contoured at 1σ . The map is centered on L67 of molecule 1, which lies in strand 7. *Panel b*, ribbon diagram of IL-1ra viewed down the pseudo-3-fold axis of the β barrel (*left*) and rotated 90° (*right*). The β strands are shown in red, green, and blue for the first, second, and third trefoil, respectively. The three short 3_{10} helices are shown in yellow and are located at residues 37–43, 84–88, and 92–99. The first two of these helices were also found in the NMR structure, but the third helix was not seen, presumably because no assignments were made for residues 94 and 95 in the NMR structure. *Panel c*, schematic diagram of IL-1ra showing the overall topology of the β -trefoil fold. The β -sheet assignments of our x-ray structure (using deviation of the (ϕ, ψ) angles from the ideal angles (-140° , 135°) (Richardson, 1981)) are as follows: S1, Met¹⁰–Trp¹⁶; S2, Thr²²–Arg²⁶; S3, Gln²⁹–Gln³⁶; S4, Glu⁴⁴–Val⁴⁹; S5, Ala⁵⁵–Ile⁶⁰; S6, Met⁶⁵–Lys⁷¹; S7, Glu⁷⁵–Val⁸³; S8, Phe¹⁰⁰–Ser¹⁰⁵; S9, Thr¹⁰⁸–Glu¹¹²; S10, Trp¹¹⁹–Thr¹²³; S11, Gln¹²⁹–Thr¹³⁴; S12, Lys¹⁴⁵–Asp¹⁵¹. The loop regions between the strands are as follows: L1–2, Asp¹⁷–Lys²¹; L2–3, Asn²⁷–Asn²⁸; L3–4, Gly³⁷–Glu⁴³; L4–5, Pro⁵⁰–His⁵⁴; L5–6, His⁶¹–Lys⁶⁴; L6–7, Ser⁷²–Asp⁷⁴; L7–8, Asn⁸⁴–Ala⁸⁹; L8–9, Gly¹⁰⁶–Pro¹⁰⁷; L9–10, Ser¹¹³–Gly¹¹⁸; L10–11, Ala¹²⁴–Asp¹²⁸; L11–12, Asn¹³⁵–Thr¹⁴⁴.

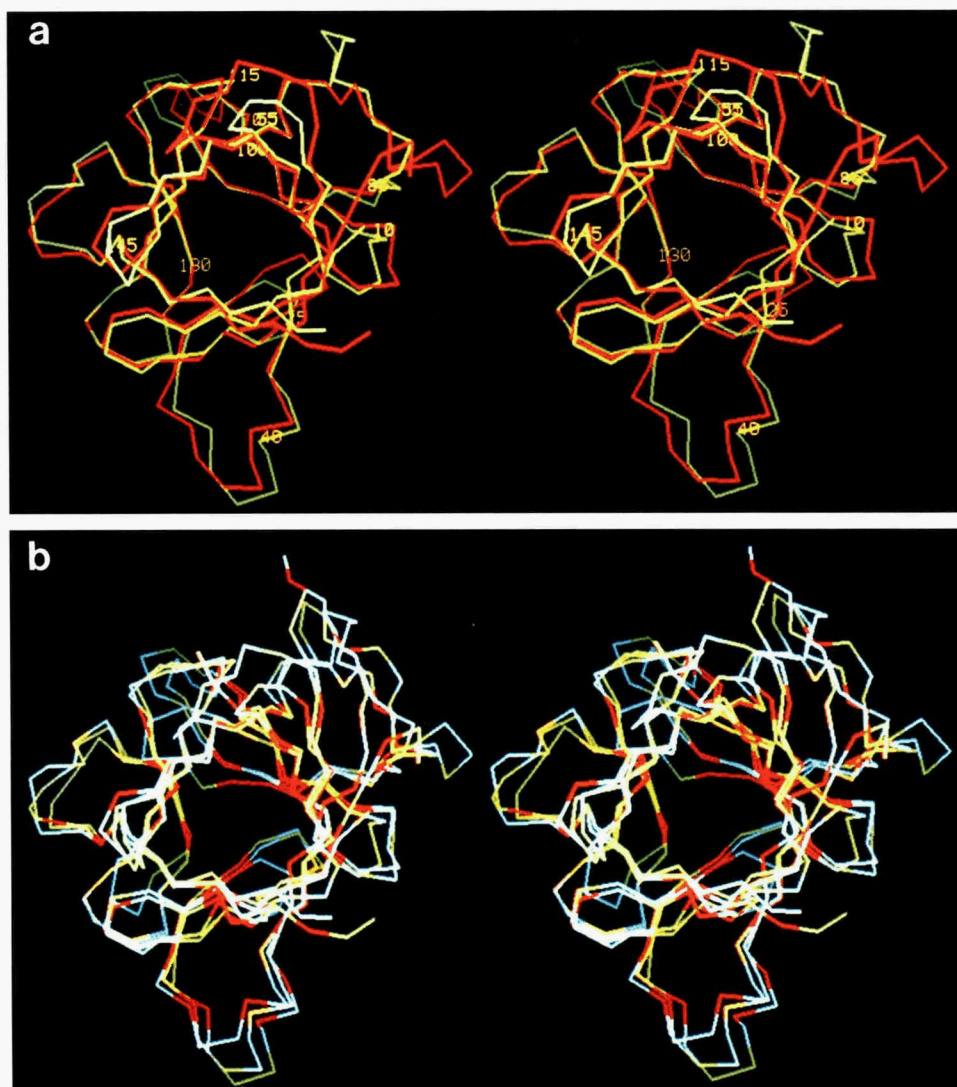


FIG. 2. *Panel a*, stereo view of the α carbon backbones of IL-1ra (green) and IL-1 β (red), viewed down the pseudo-3-fold axis of the β barrel. Every 15th residue of IL-1ra is labeled. *Panel b*, stereo view of the α carbon backbones of IL-1 α , IL-1 β , and IL-1ra, color-coded by hydrophobicity (red-yellow-green-blue for most hydrophobic to most hydrophilic). There are 6 structurally conserved hydrophobic residues in the strands that form the walls of the β -barrel, and another 5 in the strands that close off the bottom of the barrel. The hydrophobic residues conserved between IL-1 α , IL-1 β , and IL-1ra are as follows. Sides of barrel: for IL-1 α , the residues are Leu²⁰-Val⁶⁶-Leu⁶⁸-Ile⁷⁰-Val⁷¹-Val⁸⁶; for IL-1 β , the residues are Leu¹⁰-Val⁵⁸-Leu⁶⁰-Leu⁶²-Leu⁶⁹-Leu⁸⁰; for IL-1ra, the residues are Ile¹⁵-Leu⁵⁶-Leu⁵⁸-Ile⁶⁰-Leu⁶⁷-Leu⁷⁸. Bottom of barrel: for IL-1 α , the residues are Leu³⁶-Val⁸⁶-Leu⁸⁸-Val¹³⁶-Leu¹³⁸; for IL-1 β , the residues are Leu²⁶-Leu⁸⁰-Leu⁸²-Val¹³²-Leu¹³⁴; for IL-1ra, the residues are Leu³⁰-Leu⁷⁸-Leu⁸⁰-Val¹³¹-Leu¹³³.

tations of the partially refined IL-1ra structure in the tetragonal unit cell. In both cases the positions of the molecular replacement solutions were confirmed from heavy atom derivative data, as described under "Experimental Procedures."

The triclinic IL-1ra structure was refined using X-PLOR. Initially, strict non-crystallographic symmetry was enforced between the two subunits (Structures 1 and 2 in Table III), and the structure was refined by conjugate gradient minimization, simulated annealing, and manual model building into $2F_o - F_c$ maps. This was followed by refinement in which the two molecules were allowed to diverge. Finally, group temperature factors were refined (Structure 3 in Table III). No water molecules were added to the structure. The final triclinic model had an R -factor of 22.9% (Table III).

The tetragonal structure was refined in the same way (Structures 4–6 in Table III). The last step in the refinement was to add a total of 74 water molecules (Structure 7 in Table III). The only region of IL-1ra that required major modification from the triclinic model was residues 84–94, which were poorly defined in the triclinic structure. The final tetragonal model consists of 2338 non-hydrogen atoms plus 74 water molecules. The first 10

residues (Met⁰ to Lys⁹) were not well defined in the electron density maps. The agreement of the atomic model with crystallographic data at the present stage of refinement is characterized by an R -factor of 23.6% (Fig. 1a and Table III). The accuracy of the IL-1ra structure is supported by the free R -factor (Brünger, 1992), which is 31.7% after simulated annealing to 3000° and recalculation of the B -factors. A Ramachandran plot of the ϕ - ψ torsion angles of the polypeptide chain indicate that only 2 non-glycine residues (Asn²⁷ and Asp⁶²) have conformations outside the allowed regions. Both of these residues are in loops that make close intermolecular contacts in the crystal.

Structure of IL-1ra: Overall Topology—IL-1ra exhibits an overall folding topology, which has been termed the β -trefoil fold (Murzin *et al.*, 1992). Other proteins known to have this folding topology are IL-1 α , IL-1 β , acidic and basic fibroblast growth factor, soybean trypsin inhibitor, *Erythrina caffra* trypsin inhibitor, the wheat proteinase K/ α -amylase inhibitor, and the pH-sensitive actin-binding protein hisactophilin (Graves *et al.*, 1990; Finzel *et al.*, 1989; Clore *et al.*, 1991; Zhu *et al.*, 1991; Eriksson *et al.*, 1991; Zhang *et al.*, 1991; Onesti *et al.*, 1991; Zemke *et al.*, 1991; Habazettl *et al.*, 1992). As shown in Fig. 1 (b

and c), IL-1ra has 12 β -strands (S1–S12), which form three β -trefoils. Each trefoil consists of four β -strands. The first and last strands of each trefoil (S1, S4, S5, S8, S9, and S12) pack in an up-and-down manner to form a six-stranded β -barrel. The barrel is closed at one end by the other six β -strands. The approximate dimensions of the molecule are 25 Å across the top (open end of the β -barrel), 35 Å across the bottom, and 25 Å from the top to the bottom of the barrel. The loops connecting the β -strands vary considerably in length and in conformation. Eight of the loops are compact (L1–2, L2–3, L4–5, L5–6, L6–7, L8–9, L9–10, and L10–11), consisting of 2–6 residues, while the other three loops connecting the third and fourth strands in each trefoil (L3–4, L7–8, and L11–12) have between 7 and 16 residues. Three short 3_{10} helices are present in L3–4 and L7–8 between residues 37–43, 84–88, and 92–99.

The secondary structure of IL-1ra has been determined by three-dimensional NMR (Stockman *et al.*, 1992). A comparison of the β -strand assignments from the x-ray structure with the assignments determined by NMR showed very good agreement, averaging ± 2 -residue difference between the independent assignments of each strand (Fig. 1b). The hydrogen-bonding pattern deduced from NMR studies is also consistent with all the relative positions of the residues on adjacent β -strands in the x-ray structure. A homology-built model of IL-1ra (Veerapandian, 1992) was also compared to the crystal structure. The RMSD between these structures is 1.10 Å for the $C\alpha$ atoms in the β -strands and 2.88 Å for all the $C\alpha$ atoms. As expected, the β -strands that form the structural core of the molecule were modeled reasonably well, whereas most of the loops were less precisely modeled.

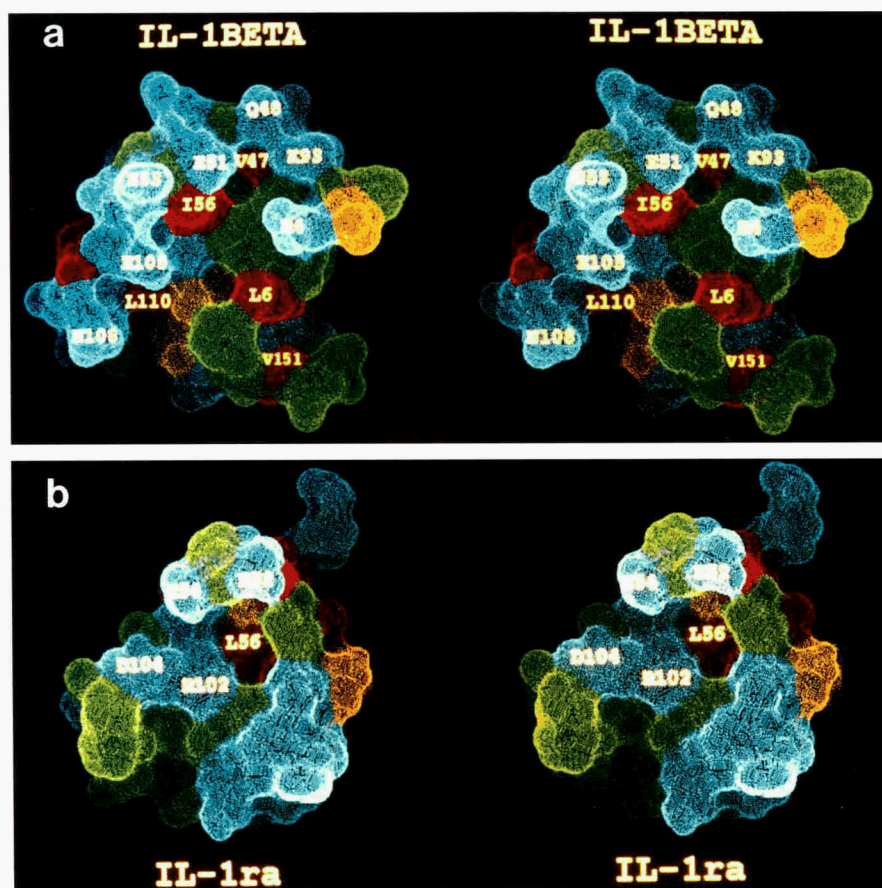
Comparison of IL-1ra with IL-1 α and IL-1 β —As shown in Fig. 2a, the refined structure of IL-1ra is similar to the structures of IL-1 α and IL-1 β . The RMSD between $C\alpha$ atoms in IL-1ra and IL-1 β , ignoring insertions and deletions (see Table

I), is 2.7 Å, while the RMSD between the α carbons in the β strands is 1.0 Å. The corresponding RMSDs between IL-1ra and IL-1 α are 2.7 and 1.2 Å, while the RMSDs between IL-1 α and IL-1 β are 3.6 and 1.0 Å. Over 70% of the residues that are identical in IL-1ra and IL-1 β are in the β -strands. The cores of the β -barrels are strongly hydrophobic in all three proteins, with many hydrophobic residues conserved between them (Fig. 2b). Of the five regions of IL-1ra that show 100% sequence identity with IL-1 β over 3 or more residues, four of these regions (Leu⁶⁷–Val⁷⁰, Leu⁷⁸–Glu⁸¹, Lys⁹⁶–Phe⁹⁸, and Phe¹¹¹–Ala¹¹⁴) are adjacent to each other on one side of the molecule (Table I and Fig. 2b). In IL-1 β , residues in these four highly conserved regions have been shown to be involved in forming a very early folding intermediate (Varley *et al.*, 1993), suggesting that these regions may be conserved for structural purposes.

There are also several notable structural differences between IL-1ra and IL-1 β . The largest difference is shown in Fig. 2a and consists of residues 84–94 in L7–8 of IL-1ra. This region lies on the side of the β barrel, along the edge of a large receptor binding site of IL-1 β (see below), and shows very low sequence homology to IL-1 α or IL-1 β . Only 1 residue in this region, Lys⁹³, is homologous to IL-1 β by sequence alignment (Eisenberg *et al.*, 1990); however, in the structures the $C\alpha$ atoms of these residues are 9.1 Å apart. This region is also different in that native human IL-1ra has at least two forms glycosylated at Asn⁸⁴, in addition to the non-glycosylated form (Hannum *et al.*, 1990). Glycosylation alone is not responsible for the different structure observed in this region in IL-1ra, however, since the IL-1ra used for structural studies was produced in *E. coli* and therefore was not glycosylated.

Other differences between IL-1 β and IL-1ra include residues 86–89 and 96–99, which in IL-1 β form type-1 β -turns (Finzel *et al.*, 1989), whereas in IL-1ra the corresponding residues (84–88 and 92–99) have the same hydrogen-bonding pattern (Richard-

FIG. 3. Stereo views of the solvent accessible surfaces (calculated by the method of Connolly (1983)), viewed down the pseudo-3-fold axis, are shown for IL-1 β (top) and IL-1ra (bottom). Residues are colored by hydrophobicity as described in legend to Fig. 2. Key residues are labeled. In IL-1 β , residues Arg⁴, Lys⁹³, Gln⁴⁸, Glu⁵¹, Asn⁵³, Glu¹⁰⁵, and Asn¹⁰⁸ form a hydrophilic ridge around the 4 hydrophobic residues Val⁴⁷, Ile⁵⁶, Leu¹¹⁰, and Leu⁶. In IL-1ra, only 1 of the 4 hydrophobic residues is conserved (Leu⁵⁶), and the ridge of hydrophilic residues is greatly reduced in extent. Thus the region that forms a large receptor binding epitope of IL-1 β is greatly altered in IL-1ra.



son, 1981) but form more extensive 3_{10} helices. Another difference between the structures is that L3–4 in IL-1ra is shifted in position by as much as 6 Å from the analogous loop in IL-1 β , but this difference could be due to crystal packing.

Differences in the Receptor Binding Sites of IL-1 β and IL-1ra—Mutagenesis experiments on IL-1 β indicate that a large number of residues are involved in type I IL-1R binding (Labriola-Tompkins *et al.*, 1991). The largest identified binding epitope in IL-1 β is located at the open end of the β -barrel and consists of residues at the amino and carboxyl termini, L4–5, L7–8, and L9–10. In this region, 7 hydrophilic residues (Arg⁴, Lys⁹³, Gln⁴⁸, Glu⁵¹, Asn⁵³, Glu¹⁰⁵, and Asn¹⁰⁸) form a horseshoe-shaped ridge around 5 hydrophobic but solvent-accessible residues (Leu⁶, Val⁴⁷, Ile⁵⁶, Leu¹¹⁰, and Val¹⁵¹) (see Fig. 3). There are no other areas on either molecule that are as hydrophobic as this area of IL-1 β , supporting a role in receptor binding. In IL-1ra, however, L4–5 is considerably more compact, with 6 fewer residues, than the corresponding loop in IL-1 β (Fig. 2a). This, together with alterations at the positions structurally equivalent to Gln⁴⁸, Glu¹⁰⁵, and Asn¹⁰⁸ in IL-1 β , makes the solvent accessible surface of IL-1ra much flatter than the equivalent surface of IL-1 β (Fig. 3). Furthermore, there is only 1 hydrophobic solvent-accessible residue in this region of IL-1ra (Leu⁵⁶). Thus the large binding epitope of IL-1 β appears not to be structurally conserved in IL-1ra. This is consistent with mutagenesis experiments showing that none of the residues in this area of IL-1ra are important for receptor binding (Eisenberg *et al.*, 1993).²

There are 3 additional residues of IL-1 β implicated in Type I IL-1R interactions: Arg¹¹, His³⁰, and Lys¹⁴⁵, which lie at separate locations on the side of the β -barrel. Alignment of the tertiary structures of IL-1 β and IL-1ra shows that the areas surrounding these 3 residues are structurally conserved in IL-1ra, with Trp¹⁶ replacing Arg¹¹ and Tyr³⁴ replacing His³⁰. Mutagenesis of Trp¹⁶ and Tyr³⁴ in IL-1ra results in analogs of IL-1ra with reduced receptor binding activity (Evans *et al.*, 1991). Lys¹⁴⁵ in IL-1ra is structurally homologous to Asp¹⁴⁵ in IL-1 β , and mutation of this residue in IL-1ra to Asp results in a protein with limited agonist activity (Ju *et al.* 1991). These results suggest that these sites of receptor interaction are conserved in IL-1ra and IL-1 β .

CONCLUSIONS

We have solved the structure of IL-1ra³ at 2.0-Å resolution, by molecular replacement from the published structure of IL-1 β (Finzel *et al.*, 1989; Priestle *et al.*, 1989; Graves *et al.*, 1990; Clore *et al.*, 1991; Shaanan *et al.*, 1992). The sequence identity between IL-1ra and IL-1 β is low (26%), and for this reason we had to solve the structure in two stages, using first 2.7-Å resolution data in the P₁ space group and then 2.0-Å resolution data in the P₄₃2₁2 space group. This approach extends the usefulness of molecular replacement to more distantly related proteins, where initial rotational and translational solutions are more difficult to determine. We also used diffraction data from heavy atom derivatives to confirm the correctness of the molecular replacement solutions, even though the data were not sufficiently isomorphous to generate useful phases. This methodology provides a worthwhile check on molecular replacement solutions.

Not unexpectedly, a comparison of the structures of IL-1ra and IL-1 β show that their structural similarities are much greater than their differences. Both of these proteins have the same overall folding topology, a conserved hydrophobic core,

and a small number of structurally conserved receptor binding residues. These similarities are consistent with the fact that IL-1ra and IL-1 β interact with the same cellular receptors, IL-1RI and IL-1RII. It is interesting, however, that the greatest structural differences between IL-1ra and IL-1 β are located in the region that has been identified as the major receptor binding epitope in IL-1 β . Whether these differences are responsible for the respective antagonist and agonist activities of these molecules is yet to be elucidated. Additional studies, including site-directed mutagenesis and crystallographic studies of IL-1ra and IL-1 β complexed with IL-1RI, will be required to further our understanding of these molecules' interactions.

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³ The α carbon coordinates have been submitted to the Protein Data Bank and will be released 1 year after publication.