Crystal structure of human interleukin-10 at 1.6 Å resolution and a model of a complex with its soluble receptor

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Abstract

The crystal structure of human interleukin-10 (IL-10) was refined at 1.6 Å resolution against X-ray diffraction data collected at 100 K with the use of synchrotron radiation. Although similar to the IL-10 structure determined previously at room temperature, this low-temperature IL-10 structure contains, in addition, four N-terminal residues, three sulfate anions, and 175 extra water molecules. Whereas the main-chain conformation is preserved, about 30% of the side chains, most of them on the protein surface, assume different conformations. A computer model of a complex of IL-10 with its two soluble receptors was generated based on the topological similarity of IL-10 to interferon- γ . The contact region between the cytokine and each receptor shows excellent complementarity of polar and hydrophobic interactions, suggesting that the model is generally correct and should be useful in guiding mutagenesis experiments.

Keywords: cytokines; four-helix-bundle; interferon γ ; interleukin-10; receptor binding

Interleukin-10 (IL-10) is a cytokine secreted by B cells, T helper cells, keratinocytes, and monocytes/macrophages (Moore et al., 1993). It is mainly an immunosuppressor, inhibiting the synthesis of a number of cytokines, such as interferon- γ (IFN γ), tumor necrosis factor, IL-1, IL-2, IL-3, IL-6, IL-8, and IL-12 (de Waal Malefyt et al., 1991; D'Andrea et al., 1993). Furthermore, IL-10 shows an immunostimulatory activity toward the growth and differentiation of activated B cells (DeFrance et al., 1992; Rousset et al., 1992), cytokine-activated T-cells (MacNeil et al., 1990; Suda et al., 1990), and mast cells (Thompson-Snipes et al., 1991). It was recently shown that IL-10 potentiates cytokine production by human natural killer cells (Carson et al., 1995). BCRF1, an analogue of IL-10 found in the genome of Epstein-Barr virus (Vieira et al., 1991; Masucci & Ernberg, 1994), shares many of the biological activities of human IL-10 (hIL-10) (Vieira et al., 1991), including the capacity to stimulate B cell growth (Rousset et al., 1992).

The different activities of IL-10 appear to be mediated through formation of oligomeric complexes of IL-10 with its receptors expressed on the cell surface, in a manner somewhat similar to IFN γ (Marsters et al., 1995). Only one IL-10 receptor (IL-10R) has been characterized biochemically to date (Ho et al., 1993; Tan

et al., 1993; Liu et al., 1994). It has been shown that IL-10R belongs to the class II, or interferon-receptor like, subgroup of the cytokine receptor family (Ho et al., 1993). Recent data on the recombinant extracellular or soluble domain of human IL-10 receptor (sIL-10R) showed that it forms in solution a unique complex of two IL-10 dimers with four soluble receptors (Tan et al., 1995), is able to inhibit binding of ^{125} I-hIL-10 to the full-length receptor, and does antagonize the effect of IL-10 on cell proliferation and cytokine synthesis inhibition. Somewhat similar aggregation properties were also reported for IL-6 and ciliary neurotropic factor (CNTF). In these systems, two molecules of IL-6 or CNTF bind two IL-6 or CNTF receptors, and, additionally, two gp130 molecules (IL-6), or one gp130 and one LIFR molecule (CNTF) (Ward et al., 1994; De Serio et al., 1995). However, the only structural data for the receptor complexes of this subfamily of cytokines are derived from the recent structure of the IFN γ /IFN γ R complex, consisting of a single IFN γ dimer and of two receptor molecules (Walter et al., 1995).

In our previous work (Zdanov et al., 1995) we reported the structure of hIL-10 at 1.8 Å resolution, showing a previously unexpected similarity with the structure of IFN γ . An independent report of the structure of IL-10 in a different crystal form was published subsequently (Walter & Nagabhushan, 1995). Here we report the structure of IL-10 at 1.6 Å resolution, refined against synchrotron radiation diffraction data collected at 100 K. In addition, we present a modeled structure of an IL-10 dimer complexed with two extracellular domains of its receptor.

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Results

The structure of IL-10

The structure of a dimer of IL-10 is shown in Figure 1A. The low temperature (LT) structure of IL-10 is almost identical to the previously reported structure of this cytokine at room temperature (RT). Although the molecule itself appears to contract slightly at LT, similar to what was found in the studies of ribonuclease A at different temperatures (Tilton et al., 1992) (the overall change in the crystal unit cell volume between RT and 100 K is 1.8% or 5336 Å³), the RMS deviation (RMSD) between the C α -atoms is 0.37 Å, between all main-chain atoms is 0.37 Å, and between all non-hydrogen atoms is 0.40 Å. To reach the best superposition, the RT model must be rotated by 0.76° and translated by -0.17 Å.

Although the functional unit of IL-10 is a dimer (Tan et al., 1993; Zdanov et al., 1995), there is a single monomer in the asymmetric part of the crystal unit cell, and 180° rotation around the crystallographic twofold axis is required to form a proper dimeric IL-10 molecule. The monomer of IL-10 consists of six helices, A–F (Fig. 1B). Helices E and F (or E' and F') of each monomer contribute mutually to the formation of compact six-helix domains of the IL-10 dimer (Fig. 1B) connected by the D–E and D'–E' linkages. A part of each domain consists of a classical four-helix bundle (Presnell & Cohen, 1989) formed by helices A, C, D, F' and A', C', D', F, respectively. An extended hydrophobic core is located inside of each domain, comprising 86% of all hydrophobic residues of the IL-10 molecule. The fact that none of the water molecules (128 in the RT structure and 303 in the LT structure) was found inside the domain core indicates how compact and hydrophobic it is.

Differences between the LT and RT structures

The main difference between the polypeptide chain conformations of the LT and RT structures of IL-10 is the observed position of the N-terminal tail Thr-6–Thr-13 (Fig. 1B). The difference between the C α atom positions increases toward the N-terminus and, de-

spite the conservation of the first disulfide bridge Cys-12–Cys-108 (Cys-12 S γ atom deviates by only 0.28 Å), the C α atom of Cys-12 shifts by 1.7 Å. At this point, the directions of the main chain diverge considerably. Although in the RT structure the Ser-11–Asn-10 tail assumes a position suggesting that the rest of the N-terminus turns away from the main body of the molecule, in the LT structure, the strand Ser-11–Thr-6 goes inside of it, toward the linkage of D and E helices (Fig. 1A,B), and ends near a potential glycosylation site, where the sulfate anion Sul-601 has been found. In fact, the nitrogen of the amide group of Thr-6 is located only 2.85 Å from the O3 of this well-defined sulfate anion molecule (Fig. 2). Because we had not seen any electron density for the N-terminal strand Ser-1–Gly-5 and we observed that the distance between this nitrogen and the negatively charged sulfate anion is very short, we had to address the question of whether the protein is truncated for some reason and Thr-6 was as its N-terminus. The N-terminal sequence analysis of the IL-10 showed that this is not the case and that the cytokine is intact. Thus, the chain Ser-1–Gly-5 exists, but is disordered and probably turns away from the interdomain interface and assumes multiple conformations in the solvent. The electron density corresponding to the strand Ser-6–Thr-11 (Fig. 2) is somewhat diffuse. Although the main chain is reasonably well defined, the side chains of Thr-6, Ser-8, Glu-9, and Asn-10 are not visible. Ser-11 and Gln-7 are the only residues in this part of the molecule for which the electron density was found for the side chains. It is notable that the side chain of Asn-10 is reasonably well defined at RT, whereas at LT it is not visible. However, this is the only example of such a phenomenon. Lys-117, Lys-119, and Asn-160, for which the side chains were not located at RT, are well defined at LT. The second large deviation of the main chain of LT IL-10 relative to RT IL-10 involves six C-terminal residues, Thr-155–Asn-160. However, taking into account the relatively large average temperature factor values of these residues in both structures (Fig. 3), we can assume that this deviation is probably due to their high flexibility.

Table 1 lists the residues that assume different conformations at LT versus RT. Forty-five side chains of 148 for which electron

Fig. 1. Structure of IL-10. **A:** Ribbon diagram showing the structure of the IL-10 dimer. Disulfide bonds are shown in yellow, and the termini are marked. This figure was generated using RIBBONS (Carson, 1991). **B:** Superposition of the monomers of IL-10. Green, room temperature (RT) structure; red, low temperature (LT) structure; yellow, disulfide bonds.

Fig. 2. $2F_o - F_c$ electron density map in the vicinity of the potential glycosylation site (N116), where a sulfate anion is located. Side chains of the N-terminal residues Thr-6, Ser-8, Glu-9, and Asn-10 cannot be seen in this map, contoured at 0.8σ level.

density was present at RT have altered their conformations at LT. Although most of these 45 residues are polar, the rotated residues also include 4 leucines, 2 isoleucines, and 4 methionines. Thirty-nine of these residues, including all polar residues and 4 hydrophobic ones (Met-39, Leu-103, Met-156, and Ile-158) are located on the surface of the molecule. Ile-136 is partially exposed to the solvent, whereas Leu-23, Leu-26, Leu-47, Met-68, and Met-154 are found inside the molecule. Figure 4 shows as an example two conformations of Leu-103 in the RT and LT structures.

We found that two residues, Glu-54 and Arg-159, assume two alternative conformations at RT, whereas both of these residues have just one conformation at LT. Only one of these conformations is seen at LT for Glu-54, whereas the conformation of Arg-159 at LT is different from both conformations at RT. We also found that

Lys-88, Arg-106, Ser-141, and Asp-144 assume two alternative conformations at LT and one of these conformations is shared at RT.

To address the question of why there is such a large discrepancy between the RT and LT diffraction data (R_{scale} of 32.7%, Table 3),

Table 1. Residues that assume different conformations (at least one side-chain atom moved more than $1.0 \text{ \AA}$) in the RT and LT structures

Residue	Secondary structure element	Residue	Secondary structure element
Asn-10	N-terminal coil	Glu-81	Helix C
Ser-11	N-terminal coil	Gln-82	Helix C
Thr-13	N-terminal coil	Asp-84	Loop CD
His-14	N-terminal coil	His-90	Helix D
Asn-21	Helix A	Lys-99	Helix D
Leu-23	Helix A	Arg-102	Helix D
Arg-24	Helix A	Leu-103	Helix D
Leu-26	Helix A	Arg-104	Helix D
Arg-27	Helix A	Arg-107	Helix D
Ser-31	Helix A	Arg-110	Loop DE
Lys-34	Helix A	Glu-122	Helix E
Thr-35	Helix A	Asn-126	Helix E
Met-39	Helix A	Lys-130	Helix E
Lys-40	Helix A	Lys-134	Helix F
Glu-42	Strand AB	Ile-136	Helix F
Leu-47	Strand AB	Glu-151	Helix F
Glu-50	Helix B	Met-154	Helix F
Glu-54	Helix B	Thr-155	Helix F
Lys-57	Helix B	Met-156	Helix F
Met-68	Helix C	Ile-158	Helix F
Glu-70	Helix C	Arg-159	Helix F
Glu-75	Helix C	Asn-160	Coil
Glu-79	Helix C		

Fig. 3. Distribution of temperature factors (B) in both the RT (green) and LT (red) structures of IL-10.

Fig. 4. The $2F_o - F_c$ electron density maps corresponding to the different conformations of Leu-103 at RT (blue map, green coordinate) and at LT (pink map, red coordinate).

we superimposed RT structure on LT structure (including water molecules) and ran 10 cycles of scale and atomic temperature factor refinement against the LT data at 1.6 Å resolution. The *R*-factor for this structure decreased from 35.5% to 32.6%. We also performed an analogous procedure for the LT structure superimposed on the RT structure, and minimized against the RT data at 1.8 Å resolution. In this case, the *R*-factor decreased from 33.5% to 26.5%. There is a significant difference between these two structures in terms of the distribution of structure factor amplitudes, although the LT structure agrees with the RT data better than the RT structure with the LT data. The reason for this phenomenon may be primarily due to different levels of description of the solvent. Because the LT structure includes many more water molecules, it is very likely that some of them were not included in the RT structure, but they were present in the crystal at low occupancy and/or with high temperature factors.

IL-10/sIL-10R complex

The topology and architecture of the IL-10 dimer are very similar to those reported for IFN γ (Zdanov *et al.*, 1995). Both IL-10 and IFN γ receptors belong to the same (class II) family of cytokine receptors (Ho *et al.*, 1993). Taken together, these data suggest that, with a very high probability, a complex formed by IL-10 and IL-10 receptor should bear considerable similarity to the IFN γ /IFN γ R complex. For this reason, we generated a computer model of the IL-10/sIL-10R complex based on both the high-resolution structure of IL-10 presented above, and a model structure of its receptor, derived from the structure of soluble IFN γ R (sIFN γ R). The structure of the complex of human IFN γ with the extracellular domain of its receptor has been published recently (Walter *et al.*, 1995). Although the resulting coordinates have not been released, another group has been independently conducting structural work on the IFN γ /sIFN γ R complex, and they generously agreed to provide us with their very preliminary coordinates (S. Ealick, *pers. comm.*).

Because the complex of IFN γ /sIFN γ R is formed by a single IFN γ homodimer bound to two receptor molecules, where each soluble receptor binds to one domain of the cytokine and the receptors do not interact with each other (Walter *et al.*, 1995), we may suppose that the domain is the minimal functional unit of the complex. In other words, it should be possible first to build a

model of the complex between one domain of IL-10 and sIL-10R and later to generate the entire complex (IL-10 dimer with two sIL-10R) by applying twofold symmetry. In general, however, this approach may be not entirely correct, because IFN γ from different species was found in the crystals both as a complete dimer in the asymmetric unit (Ealick *et al.*, 1991) and as a monomer similar to IL-10 (Samudzi *et al.*, 1991).

The domains of IL-10 and of IFN γ were superimposed (RMSD 2.6 Å) and the resulting orientation of the receptor molecule of IFN γ provided a starting point for the modeling. The amino acid sequences of human IL-10R (Swissprot data base, accession U00672) and IFN γ R were aligned by using the Wisconsin program package (Genetics Computer Group, 1994) followed by manual correction (Fig. 5). Based on the assumption of its similarity to sIFN γ R, we built the model of sIL-10R in accordance with this alignment. The resulting molecule of sIL-10R consists of two domains, and each domain has an immunoglobulin fold-like architecture (Bazan, 1990), constrained by two conserved disulfide bridges, one in each domain. It appears that human IL-10R is a unique member of the family of class II (Bazan, 1990) cytokine receptors, because it lacks Cys-68 (IL-10R numbering) which is a complement of Cys-59 in the formation of the conserved disulfide bridge in the N-terminal domain. An equivalent cysteine is, however, found in murine IL-10R (Tan *et al.*, 1993). However, the human IL-10R contains Cys-40, which corresponds to valine in IFN γ R (Fig. 5) with the distance between Val-40 and Cys-59 in the IFN γ R structure being about ~ 7 – 8 Å. For this reason, we decided to build a new disulfide bridge between Cys-40 and Cys-59. We modified the conformation of corresponding β -strands as well as loops, which were missing from the original sIFN γ R structure (S. Ealick, *pers. comm.*) or corresponding to deletions/insertions (Fig. 5). Surprisingly, the IL-10/sIL-10R interface did not require any major rebuilding of the main chains. The rotations of χ_i permitted the formation of reasonably good interactions between the polar side chains on both sides of the interface. Subsequently, we subjected the model of the complex comprising one IL-10 domain and one receptor molecule energy minimization by X-PLOR and revised the model again using computer graphics. Several cycles of this procedure allowed us to obtain the final model of an IL-10 domain (residues 6–115 and 116'–160') complexed with a sIL-10R molecule (residues 10–209). The entire complex of a dimer of IL-10 with two receptor molecules was generated by applying twofold symmetry, as found in the crystal structure of IL-10.

Fig. 5. Alignment of amino acid sequences of human IFN γ R and IL-10R. Identical residues are boxed in red; structurally similar residues are boxed in blue.

The model structure of sIL-10R consists of the N-terminal and C-terminal domains. Each domain has a fibronectin type III-like topology (Bork et al., 1994), formed by seven β -strands, α_1 - α_2 and α_3 - α_4 for the N- and C-terminal domains, respectively (Fig. 5). The linkage between the domains, consisting of 10 amino acids, contains a short α -helix. The overall sIL-10R structure is similar to that of sHGHR, sPRLR, and sIFN γ R (de Vos et al., 1992; Somers et al., 1994; Walter et al., 1995). The differences between sIL-10R and sIFN γ R are mainly in the different pattern of disulfide bridges. sIFN γ R has four disulfide bridges, one in the N-terminal domain, two in the C-terminal domain, and one in between. These disulfide bridges appear to make the overall sIFN γ R structure more rigid (Walter et al., 1995). sIL-10R has only two such bridges, one in the N-terminal domain, and the other, common to both structures, in the C-terminal domain.

Because we generated the model of the IL-10/sIL-10R complex on the assumption that IL-10 binds its receptors in a manner similar to IFN γ binding sIFN γ R, the overall architecture of both complexes is necessarily very similar (Fig. 6). Each IL-10 domain is bound to one receptor molecule and these functional units are related to each other by a 180° rotation around the twofold axis, which is most likely perpendicular to the cell membrane. Unlike the HGHR/sHGHR complex, the sIL-10R moieties are separated by a distance of about 40 Å and do not interact with each other. The interface between IL-10 and sIL-10R covers the C-terminal part of helix A, the A-B loop, the C-terminal part of helix B, and helix F' on IL-10. The residues of sIL-10R that interact with IL-10 originate on loops c_1 - d_1 , e_1 - f_1 , b_2 - c_2 , d_2 - e_2 , f_2 - g_2 , linkage f_1 - a_2 , and strands f_1 and g_1 . The hydrophobic patch formed by Leu-46, Leu-53, Ile-145, Tyr-149, and Ala-152 on the IL-10 surface is extended in

Fig. 6. Model structure of the IL-10/sIL-10R complex. N and C termini are marked as such; disulfide bonds are colored yellow. Loops and coil regions of the sIL-10R are shown in white with a short interdomain helix in red.

the complex by the aromatic rings of Tyr-48, Tyr-75, Tyr-141, and Phe-145 of the receptor. This hydrophobic core of the IL-10/sIL-10R interface is surrounded by polar residues, forming a number of possible hydrogen bonds and ionic interactions listed in Table 2.

Discussion

In this report, we describe the crystal structure of recombinant human IL-10 at 1.6 Å resolution at LT. This LT structure represents the highest resolution and the best-refined structure of a helical cytokine available at this time (Davies & Wlodawer, 1995). The structure of IL-10 is basically the same both at RT and 100 K, although the crystal unit cell contracts a little at LT. Although the conformation of the main body of the protein is conserved, the position and conformation of the N-terminal strand (Thr-6–Cys-12 at LT and Asn-10–Cys-12 at RT) are different. This difference appears to reflect the high flexibility of the N-terminus and agrees very well with the results obtained for IL-10 crystallized in a different crystal form (tetragonal, $P4_32_12$), where none of the residues 1–17 are visible (Walter & Nagabhushan, 1995). This disordered region includes the disulfide bond Cys-12–Cys-108. Although the RMSD between the LT and RT structures is only 0.4 Å for all nonhydrogen atoms, about 30% of the side-chains, mostly on the protein surface, assume different conformations (Table 1). Whereas the overall distribution of the temperature factors of individual residues is similar (Fig. 3), the LT structure is clearly less flexible in three regions: 13–37, 81–106, and 135–154. So far we could not find any reasonable explanation for this phenomenon. In contrast, residues 38–44, 108–110, and 160, which are not present in the structure of IL-10 in the tetragonal crystal form (Walter & Nagabhushan, 1995), have about the same temperature factors in both the RT and LT structures (Fig. 3). This result suggests that the regions with relatively high flexibility conserve this property regardless of the temperature, whereas the less

flexible parts become more rigid. The average temperature factors for all nonhydrogen atoms are 27.2 Å² and 23.2 Å² at RT and LT, respectively.

Because the topological similarity of IL-10 and IFN γ is obvious (Zdanov et al., 1995), both their receptors belong to the same type II class of cytokine receptors (Bazan, 1990; Ho et al., 1993), and the coordinates of the crystal structure of the 1:2 IFN γ /sIFN γ R complex became available to us (S. Ealick, pers. comm.), we have felt encouraged to generate a computer model of the IL-10/sIL-10R complex. The extracellular portion of the IL-10 receptor consists of two domains having fibronectin type III-like topology (Bork et al., 1994), connected by a 10-residue linkage with a short helix in the middle. Similar features were reported for all known crystal structures of cytokine receptors (de Vos et al., 1992; Somers et al., 1994; Walter et al., 1995). The unique property of human sIL-10R is that it lacks a conserved cysteine residue at position 68, and thus there is no disulfide bond Cys-59–Cys-68 between β -strands d_1 and e_1 , observed in the structure of sIFN γ R. Instead, sIL-10R has a nonconserved Cys-40, which appears to form a new disulfide bond, Cys-40–Cys-59 between strands c_1 and d_1 . Each domain of the IL-10 dimer binds one receptor molecule and these units are related in our model by 180° rotation around a twofold axis, which is presumably perpendicular to the cell surface.

Studies of the HGH/sHGHR complex, in which all contact residues on the receptor side of the protein–protein interface were replaced one by one with alanine, showed the importance of the central hydrophobic contact region (Clackson & Wells, 1995). This region, dominated by two tryptophans (104 and 169), accounts for more than three-quarters of the estimated binding energy. A similar region, comprising Tyr-52, Trp-85, and Trp-210, was also found in sIFN γ R structure (Walter et al., 1995). Interestingly, only one of these three key hydrophobic residues is conserved in sIL-10R. In the latter structure, both tryptophans became arginines, which are involved in ionic interactions through the interface surface. Actually, none of the tryptophans in sIL-10R is involved in a direct contact with the ligand, although the overall structure of the protein–protein interface with a hydrophobic core, including Tyr-48, Tyr-75, Tyr-141, and Phe-145 of the receptor and Leu-46, Leu-53, Ile-145, Tyr-149, and Ala-152 of IL-10, surrounded by salt bridges and hydrogen bonds, is preserved.

Unlike the HGH/sHGHR complex, which represents a biologically functional aggregate (de Vos et al., 1992), the complexes of IFN γ /sIFN γ R (Walter et al., 1995) and IL-10/sIL-10R are not functional. The former complex requires for signaling at least one more receptor subunit–IFN γ R β (Marsters et al., 1995). Although no other receptors of IL-10 are known, the mode of its aggregation may be quite different. It was shown recently (Tan et al., 1995) that an extracellular domain of the IL-10 receptor binds to IL-10 in the proportion two IL-10 dimers:four molecules of sIL-10R. In light of these data, our model may represent molecular arrangement in an intermediate step during signal transduction. We speculate that two such complexes have to find each other in order to initiate an intracellular event. A crystal structure of such a complex is needed to answer any of these questions.

Materials and methods

The purification and crystallization protocols utilized in this study were the same as reported in our previous work on IL-10 at RT (Zdanov et al., 1995). The X-ray diffraction data were collected from a single crystal at 100 K by using synchrotron radiation, on

Table 2. Possible hydrogen bonds and ionic interactions in the interface between IL-10 and sIL-10R

IL-10		Distance (Å)	sIL-10R	
Arg-27	NH1	3.3	Asp-105	OD1
Gln-38	O	3.4	Arg-81	NH1
	NE2	2.9	Asn-99	OD1
	OE1	2.8	Thr-100	OG1
Asp-41	OD2	3.1	Arg-81	NH1
	OD2	3.4	NH2	
Asp-44	OD2	3.0	Arg-81	NH2
	OD1	3.4	NH2	
Leu-46	N	3.1	Tyr-48	O
Lys-49	NZ	3.4	Glu-51	OE2
Glu-50	OE1	3.2	Tyr-48	OH
	OE2	3.2	OH	
Lys-57	NZ	3.0	Glu-142	OE2
Asp-144'	OD2	3.0	Arg-196	NH2
	OD1	3.4	NH2	
Asn-148'	OD1	2.9	Arg-196	NH1
Glu-151'	OE1	3.0	Arg-149	NH2
	OE2	3.1	NH1	
Arg-159'	NH1	3.4	Phe-148	O
Asn-160'	O	3.1	Lys-167	NZ

beamline X4A at the Brookhaven National Laboratory. The crystal was mounted in a fiber loop (Mitchell & Garman, 1994) with Sigma M-3516 mineral oil used as a cryoprotectant. A total of 91 frames of 1.5° oscillation each, with an exposure time of 55 s per frame, was collected at the wavelength of 0.958 Å. The data were processed with the DENZO program package (Otwinowski, 1992) to the resolution limit of 1.5 Å. Table 3 summarizes the X-ray data statistics both for the previous RT experiment (Zdanov et al., 1995) and the current LT experiment. In the latter case, the unit cell parameters have changed a little, the difference between unit cell axes *a* and *c* became 1.0 Å, whereas at RT it was 0.04 Å. The R_{scale} between these two data sets is as high as 32.7%.

The refinement of the IL-10 structure against LT data was conducted with the program packages X-PLOR (Brünger, 1992) and PROLSQ (Hendrickson, 1985). The rigid-body refinement of the RT model of IL-10 (no water molecules), followed by the slow-cool protocol, brought the *R*-factor value to 26.2% at 1.8 Å resolution. The model was subsequently revised against $2F_o - F_c$ and $F_o - F_c$ electron density maps, a number of water molecules were added, and the refinement proceeded further. Several cycles of minimization with the program PROLSQ, followed by revision of the current model using computer graphics against difference electron density maps (program FRODO; Jones, 1985), allowed the location of four additional N-terminal amino residues, a total of 303 water molecules, and three sulfate anions (Table 3). The final *R*-factor is 16.3% in the 10.0–1.6 Å resolution shell. The RMSDs for the bonds and bond angles are 0.016 Å and 0.048 Å, respectively, and the deviation from planarity is 0.016 Å. Average temperature factors for the main-chain atoms and all non-hydrogen atoms are 20.3 Å² and 23.2 Å², respectively.

The IL-10/sIL-10R model was obtained by superimposing atomic coordinates of the high-resolution structure of the domain of IL-10 described above on the structure of the domain of IFNγ complexed with its soluble receptor molecule. The latter coordinates used for this purpose were the result of an unpublished study (S. Ealick, pers. comm.). The original sequence of sIFNγR was replaced by the sequence of sIL-10R based on the alignment shown in Figure 5, and the missing parts were inserted as necessary. Interaction interface between IL-10 and sIL-10R was optimized manually, with minimal changes to the main chains of the protein. Program FRODO (Jones, 1985) was used in all of these steps. The energy minimization procedure (X-PLOR; Brünger, 1992) was applied to

obtain the final model of IL-10 domain (residues 6–115 and 116'–160') complexed with sIL-10R molecule (residues 10–209). The entire complex of a dimer of IL-10 with two molecules of soluble IL-10 receptor was generated by applying twofold symmetry, as found in the crystal structure of IL-10.

The atomic coordinates and structure factors have been deposited in the Brookhaven Protein Data Bank (accession numbers 2ILK and R2ILKSF, respectively).

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Table 3. Statistics of data collection at RT and LT

Parameter	RT	LT
<i>a</i>	70.27 Å	69.53 Å
<i>b</i>	70.27 Å	69.53 Å
<i>c</i>	70.31 Å	70.54 Å
Space group	P3 ₂ 21	P3 ₂ 21
Diffractionmeter	RAXIS-IIC	X4A beamline
No. of reflections	15,324	21,495
<i>R</i> _{merge}	6.1%	7.2% (1.5 Å)
Resolution	1.8 Å	1.6 Å
<i>R</i> -factor	0.156	0.163
No. of residues	10–160	6–160
No. of H ₂ O	128	303
No. of SO ₄	0	3
<i>R</i> _{scale}		32.7%

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