Hypothesis

Analysis of three human interleukin 5 structures suggests a possible receptor binding mechanism

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Abstract We compared three crystal structures of human interleukin 5 (hIL5) expressed in either *E. coli* (hIL5$_{E.coli}$), *Sf9* cells (hIL5$_{Sf9}$) or *Drosophila* cells (hIL5$_{Drosophila}$). The dimeric hIL5 structures show subtle but significant conformational differences which are probably a consequence of the different crystallization conditions trapping this protein into one of two states. We refer to these two distinct conformations as the 'open' and 'tight' state, according to the packing around the cleft between the two subunits. We hypothesize that these two stable conformational states reflect the structure of the free or receptor bound hIL5.

Key words: Interleukin; Conformational change; Domain motion; Interleukin 5 α-receptor subunit

1. Introduction

Interleukin 5 (IL5) is a haemopoietin which induces differentiation and activation of human eosinophils and basophils. Both cell types have been implicated in the pathogenesis of chronic allergic diseases such as asthma. Given its specificity of action, antagonizing IL5 activity has attracted a considerable amount of attention for drug design (for a recent review, see [1]). Underscoring the potential clinical use of IL5 antagonists was the recent observation that no bronchial hyperreactivity, a hallmark of asthma, was observed in allergen-challenged IL5 knock-out mice [2].

IL5 belongs to a group of cytokines characterized by a typical short chain four α-helical bundle structure [3]. In this fold, the α-helices (referred to as A, B, C and D from N- to C-terminus) adopt an up-up-down-down topology. IL5 is unique within this family since it is a dimer wherein the D-helices are swapped between the monomeric domains [4]. Granulocyte-macrophage colony stimulating factor (GM-CSF) [5], and also IL3 [6], fold into a very similar tertiary structure, but both exist as monomeric proteins in solution. This fold similarity is striking since almost no homology can be detected at the primary structure level [6,7]. In contrast to IL5, the biological repertoires of IL3 (previously known as multi-CSF) and GM-CSF are much broader. However, the activities on shared target cell types, such as eosinophils, are very analogous. These observations can be explained by the fact that IL5, IL3 and GM-CSF all bind to very related receptor complexes composed of a ligand-specific α-chain and a shared β-subunit, often referred to as β-common or β_c [8,9]. Consequently, the cell type-dependent expression pattern of the α-subunits restricts their biological activity, and the shared use of the β_c receptor component provides the basis for the overlapping functionality.

Both receptor subunits belong to the class 1 cytokine receptor family, containing one extracellular and one cytoplasmic segment separated by a single membrane-spanning region. Characteristic for this receptor family is the architecture of the extracellular region consisting of so-called 'cytokine receptor modules', sometimes combined with additional immunoglobulin or fibronectin type III domains. The crystal structures of several cytokines complexed with the extracellular domains of their cognate receptors have been reported over the past few years. For class 1 cytokine receptors, these now include growth hormone/growth hormone receptor [10], interferon-γ/interferon-γ receptor α-subunit [11] and erythropoietin (EPO) peptide/erythropoietin receptor (EPOR) [12]. Typically, in these complexes, the ligands bind to the outside of the elbow formed by the two fibronectin type III domains within one 'cytokine receptor module'. In all three cases, the complex is formed between one ligand and two identical receptor molecules giving a 1:2 stoichiometry. In contrast, we have previously shown that the interaction of the IL5 dimer with the extracellular domain of its receptor α-subunit results in a 1:1 complex in solution [13]. This ratio is particularly intriguing given the homodimeric structure of the IL5 molecule. The exact stoichiometry of the IL5/IL5R complex on the cell membrane is still unclear. In this paper we describe the detailed comparison of three structures for human IL5 derived from crystallographic data [4,14]. Based on the observed differences, we propose an induced fit model for IL5 upon receptor binding, which is in agreement with the above mentioned stoichiometry.

2. The IL5 structure

Three human IL5 structures have been determined by x-ray crystallography. Crystals have been obtained of recombinant hIL5 either purified from *E. coli* inclusion bodies and subsequent renaturation [4,15,16] from the supernatant of baculovirus-infected *Sf9* cells [17] or from a *Drosophila* cell culture system [14]. The overall structures in these different IL5 crystals are very similar and consist of two domains. Each domain is built up from four helices in a prototype up-up-down-down

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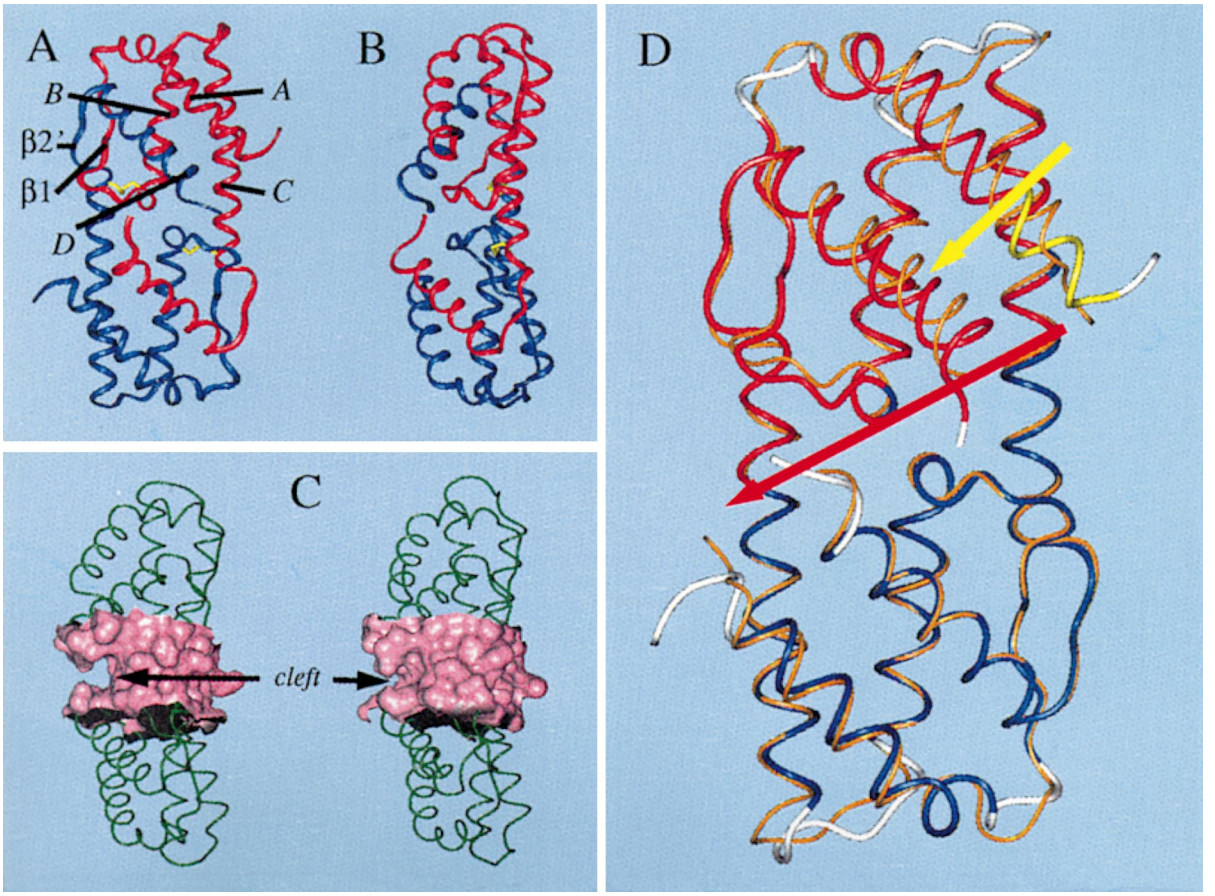

Fig. 1. Ribbon representation of the backbone of hIL5$_{Sf9}$, A: viewed along the 2-fold axis of symmetry and B: viewed by rotating figure A by 90° around an axis perpendicular to the 2-fold axis in upright position. The two monomers are shown in red (monomer a) and blue (monomer b) and are connected by two disulphide bridges shown in yellow. Each domain is built up from four helices (A, B, C from monomer a and D′ from monomer b) and two β-strands (β_1 and β_2'). C: Solvent surface representation of the cleft showing the two states: an 'open' state (hIL5$_{Eukaryotic}$, left) and a 'tight' state (hIL5$_{E.coli}$, right). Both molecules are shown in the same orientation as in B. D: Domain movements of hIL5. Shown are the backbone traces of the 'open' state (orange) and 'tight' state (other colors). The effective rotation axes are rendered as arrows in the color of the corresponding substructures. Two large segments were found: segment 1 (red, 83 residues) and segment 2 (blue, 90 residues). The 'open' and 'tight' states of segment 2 are optimally fitted so that the effect of the rotation of 11 degrees (relative error $\Delta = 1.5\%$) around the effective rotation axis (red arrow) is clearly observed in segment 1. A smaller rigid segment 3 (yellow, residues 7 to 13) was found at the N-terminal part of helix A and rotates by 27 degrees (relative error $\Delta = 0.85\%$) around its effective rotation axis (yellow arrow) in relation to segment 1.

configuration. However, three helices A, B and C are from one monomer and the fourth helix D′ is contributed by the other monomer (respectively red and blue in Fig. 1A and B). Between helices A and B and C and D are two short β-strands forming a small antiparallel β-sheet (β_1 and β_2' in Fig. 1A). The two monomers are kept together by two intermolecular disulphide links and by the interaction of many hydrophobic residues at the large interface (about 7000 Å^2).

3. hIL5 crystal structures show an 'open' and 'tight' conformation

In a previous paper, Johanson et al. [14] compared the two independently solved structures of hIL5$_{E.coli}$ and hIL5$_{Drosophila}$ and noticed conformational differences but these were not further examined. We observed similar differences after superposition, in a pairwise manner, of the respective eukaryotic hIL5 and hIL5$_{E.coli}$ structures. As a measure of divergence, we calculated the root-mean-square deviations. The r.m.s. deviations for the hIL5$_{E.coli}$/hIL5$_{Sf9}$ superposition of all Cα atoms is 1.7 Å and of all non-hydrogen atoms is 2.3 Å, comparable to the 1.6 Å and the 2.1 Å for the hIL5$_{E.coli}$/hIL5$_{Drosophila}$ fitting. The superposition of the hIL5 structures expressed in eukaryotic cells resulted in a r.m.s. deviation of 0.67 Å (Cα atoms only) and are considered as identical.

Unlike hIL5$_{Sf9}$ which contains *N*-acetyl glucosamine, human interleukin 5 from *Drosophila* was enzymatically deglycosylated prior to crystallization. The only conformation difference, likely to be due to the presence of a carbohydrate group on position 28 of hIL5$_{Sf9}$, is observed in the loop between helix A and strand β_1. This suggests that glycosylation does not alter the global structure of IL5 and correlates to the observation that deglycosylation of hIL5 does not affect biological activity [18–21].

The structural differences between hIL5$_{E.coli}$ and hIL5$_{Eukaryotic}$ are reflected in the crystallographic data (Table 1). The three crystals belong to the same space group C2. hIL5 from *Sf9* and from *Drosophila* cells crystallized as an exact crystallographic dimer, in contrast to the non-crystallographic 2-fold axis found for hIL5$_{E.coli}$. The crystal of hIL5$_{E.coli}$ has expanded unit cell dimensions especially in the b and c direction (36.10 Å compared to ±24 Å, and 56.42 Å

Table 1
Crystallographic data for $hIL5_{E.coli}$, $hIL5_{Drosophila}$ and $hIL5_{Sf9}$

	$hIL5_{Drosophila}$	$hIL5_{E.coli}$	$hIL5_{Sf9}$
Space group	C2	C2	C2
a	118.30	122.10	118.80
b	24.30	36.10	24.40
c	43.80	56.42	44.50
α	90.00	90.00	90.00
ß	110.00	98.59	109.20
γ	90.00	90.00	90.00
Resolution	2.6 Å	2.4 Å	2.5 Å
	one monomer in asymmetric unit deglycosylated	dimer in asymmetric unit not glycosylated	one monomer in asymmetric unit glycosylated

The data are taken from Johanson et al. [14], Milburn et al. [4] and C. Oefner (unpublished data).

compared to ±44 Å, Table 1) and has a smaller β angle (98.59° compared to ±110°). The unit cell changes are accompanied by domain movements in hIL5, allowing to differentiate between two states: an 'open' state ($hIL5_{Eukaryotic}$) and a 'tight' state ($hIL5_{E.coli}$). In the open state the cleft between the two domains is significantly wider than in the tight state (Fig. 1C).

4. The occurrence of the two states of IL5 implies motion

Many of the conformational changes in proteins are characterized as rigid body movements of domains or substructures. These entities are often connected by flexible joints around which hinge bending movements produce different conformational states of the same protein [22].

Flexibility of the hIL5 structure is seen as a hinge motion. We used an adaptive selection procedure (HINGEFIND, [23]) for the search of rigid substructures (hereafter referred to as 'segments') of hIL5. This iterative procedure superimposes the $hIL5_{E.coli}$ and $hIL5_{Eukaryotic}$ structures using different selections of residues and the obtained best-fitted residue sets are considered as segments. For hIL5, two segments 1 and 2 (respectively red and blue in Fig. 1D), which roughly comprise the two domains of hIL5 are distinguished. In addition, we identified a small rigid segment 3 at the N-terminal part of helix A (yellow in Fig. 1D). The sequence numbers of the best-fitted residues making up the segments 1 and 2 and segment 3 are shown in Table 2. The residue sets of segments 1 and 2 are not exactly the same because of the small structural differences between both $hIL5_{E.coli}$ domains. About 80% of the hIL5 residues are part of these rigid substructures. The remaining 20% of the hIL5 residues are part of loops and N- and C-termini (white in Fig. 1D).

To localize the hinges around which the segments rotate, the HINGEFIND program superimposed the rigid sections of $hIL5_{Eukaryotic}$ ('open' state) onto the equivalent structures of $hIL5_{E.coli}$ ('tight' state). This resulted in a rigid body transformation with rotation matrices and translation vectors. Effective rotation axes were then constructed by decomposition of above rotations and the rotation angles were calculated. The relative motion of the two rigid segments 1 and 2 can be represented as a rotation of 11 degrees of both segments around the effective rotation axis (red arrow in Fig. 1D) turning the molecule from one state into the other state. This rotation axis runs through the cleft. The hinge regions on hIL5 near the rotation axis are located on helix C of both monomers and coincide with the junctions between both segments (a77/a78 and b80/b81; Table 2). Also the N-terminus of helix B is part of the hinge region (a41/a42 and a42/a43; Table 2). Probably longer stretches are needed to change conformation because only small dihedral changes are allowed in helical structures. After examining the dihedral angle variation, flexible residues were found (domain 1: residues a42, a44, a46, b87 and b88; domain 2: residues b46, a84, a86, a87 and a88; Fig. 4). We want to point out that these observed additional flexible residues may allow the relaxation required for the relative rotation of both domains. Perhaps flexibility of a residue such as a41 which has been shown by mutagenesis analysis to be involved in hIL5Rα binding may contribute to an induced fit mechanism.

Segment 3 moves around a rotation axis (yellow in Fig. 1D) shifted upwards into domain 1 compared to the main rotation axis of hIL5 and rotates about 27 degrees in relation to segment 1 (junction = a13/a14; Table 2). Due to the closer packing, in contrast to the loose packing of the cleft, shear forces are expected between segment 3 and domain 1.

Table 2
Composition of the main rigid substructures

	Segment 1	Segment 2	Segment 3
N-terminal helix A	a14-a26	b10-b26	a7-a13
Loop $Aβ_1$	a31	b31	–
Sheet $β_1$	a32-a35	b32-b35	–
Loop $β_1B$	a36-a40	b36-b40	–
Helix B	a41, a43-a56	b41-b56, a42	–
Loop BC	–	–	–
Helix C	a65-a73, a75-a77, b81-b86	b65-b80, a78-a86, a74	–
Loop $Cβ_2$	b87, b88	a87, a88	–
Sheet $β_2$	b89-b92	a89-a92	–
Helix D	b93-b111	a93-a108	–

Residues making up the junctions are underlined. Residues located in the hIL5 monomer a or b are labeled similarly (for further explanation see Fig. 1A and D).

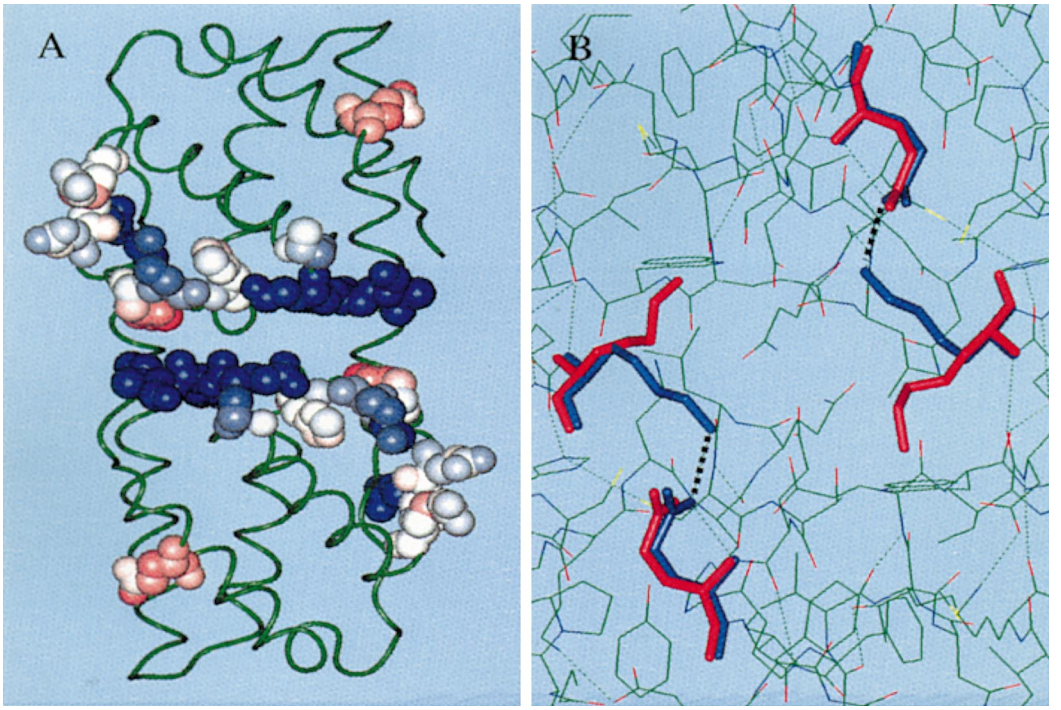

Fig. 2. A: Localization of hIL5 mutations affecting hIL5Rα binding, represented by van der Waals radii of the side chains and colored by their temperature factors (blue = high temperature). The mutants located on loop β_1B and helix B (H38, K39 and H41) and on helix D (T109, E110, W111 and I112) that are lying in or near the cleft have higher temperature factors. Residue 13 on α-helix A has a lower temperature factor (red = low temperature). B: The residue pair Glu-46-Lys-83 forms salt bridges in $hIL5_{Sf9}$ (blue) which are not present in $hIL5_{E.coli}$ (red). In both structures and also in $hIL5_{Drosophila}$, they have high temperature factors.

The root-mean-square deviations of a least squares fit of both states of hIL5, with the joints assumed flexible, and these of the proposed rotational fit are similar (respectively 0.53 Å and 0.50 Å for segment 1 and 2; respectively 0.38 Å and 0.4 Å for segment 1 and 3) and thus proves that hinge bending is the ultimate mode of motion for hIL5.

5. Many important residues for α and β_c subunit binding are located on the moveable structural segments

To localize the binding domains on the hIL5 structure, an almost complete alanine scan of the charged residues has been performed [24,25]. All mutants were tested in two different assay systems. First, a hIL5Rα-specific solid-phase binding assay was performed to pinpoint the mutants affecting the hIL5Rα interaction. Second, the hIL5 mutants were tested for their biological activity in a proliferation assay. Such signalling only occurs when hIL5 engages an $\alpha\beta_c$ complex.

Most residues shown to be involved in binding to the α-receptor are clustered in a loop connecting β-strand 1 and helix B and the first residue of this helix B (residues H38, K39 and H41), in β-strand 2 (residues E89, R91, weaker effect for E90) and close to the C-terminal helix D (residues T109, E110, W111 and I112) (Fig. 3). Clearly, several of these residues are lying near or in the cleft between both IL5 monomeric domains and the position of these residues is changed by the observed domain rotation.

Residue E13 in segment 3 does not contribute to hIL5Rα binding but is critical for interaction with the β_c receptor chain (Fig. 2A). Mutations at a homologous position in GM-CSF (E21) abolished interaction with the β_c chain and charge reversal mutations (E21R, E21K) result in a mutant with antagonistic activity [26–28]. Mutations at the corresponding position in IL-3 (E22) seemed also to affect the interaction with the β_c subunit, although to a lesser extent [28,29]. We previously showed that the hIL5 E13Q mutant still binds to the receptor complex but cannot induce proliferation of IL5-responsive TF1 cells and furthermore has antagonistic properties [24]. This residue shows normal interaction with the hIL5Rα subunit but a reduced interaction with hIL5Rβ_c subunit (Van Ostade et al., manuscript in preparation). Since such a single amino acid change can profoundly affect the biological activity, the motion of helix A (as described above) may have important consequences on the biological activity of hIL5.

6. Implications for the mechanism of IL5 receptor interaction

The first requirement for receptor triggering is the binding of hIL5 to hIL5Rα. One can hypothesize that the observed conformational transition in hIL5 may be induced upon hIL5 α-receptor interaction. An induced fit mechanism can be proposed that transforms the cleft from one state into another, whereby the interacting residues lying around the cleft would make closer interactions with the residues of the α-receptor. Human IL5 has α-helices interweaved from both monomers. The hIL5 monomers interpenetrate extensively, burying ± 7000 $Å^2$ of subunit interface area. As a result, conformational shifts are easily transmitted from one domain to the other. These findings may offer an explanation for the 1:1 stoichiometry found for the IL5 dimer/IL5Rα complex in solution. If hIL5 is considered as a rigid homodimeric structure

then a 1:2 ratio could be expected. This study clearly shows that hIL5 can undergo structural changes which may affect the stoichiometry of the complex. In such a model, a conformational change may be induced in the hIL5 structure upon binding of one hIL5Rα, disrupting the second hIL5Rα binding site (Fig. 3).

An alternative explanation follows the suggestion of Chaiken and coworkers [30] who proposed a physical occlusion model to explain the 1:1 stoichiometry. They used a single-chain interleukin 5 to obtain heterodimeric mutants. Their results lead to a model of receptor recruitment by IL5 whereby residues from both domains interact with the α-receptor. It was suggested that the α-receptor could bind to IL5 in two alternative but occlusive modes, favoring contact with either one or the other four α-helical bundles.

It should be noted that other α-helical cytokines were crystallized in a 1:2 binding ratio to their receptor hence excluding sterical hindrance. These include the growth hormone/growth hormone receptor and interferon-γ (IFN-γ)/interferon-γ receptor complexes. In the latter case the IFN-γ homodimer is complexed with two IFN-γ receptor α-subunits.

Dickason et al. [31] engineered an IL5 monomer with a biological activity close to that of native IL5. This mono-5 variant supports maximal proliferation of an IL5-dependent cell line albeit at an approximately 10-fold reduced specific activity. These results imply that most structural parameters necessary for IL5 function are present within one domain. However, the stoichiometry of the IL5Rα complex on a cell surface is still unclear at present. Hence, dimerization of mono-5 on the cell surface receptor complex cannot be excluded. If this situation occurs in IL5/IL5Rα complex formation, an analogous packing of two IL5 monomers in such a complex could occur with less structural constraints. An example for such dimerization of α-helical cytokines is the IL6 binding mode that leads to the formation of an IL6 hexameric receptor ligand complex which contains two IL6 molecules (reviewed in [32]).

We also want to point out that the proposed induced fit not only may affect the hIL5-hIL5Rα interaction, but also the binding of hIL5 with its β_c receptor. The movement of segment 3 alters the position of residue E13, a residue which is

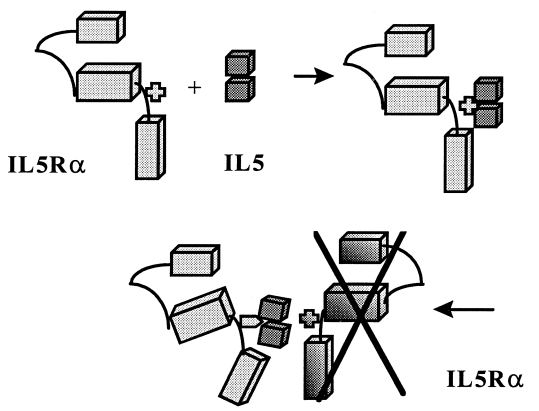

Fig. 3. A model of a 1:1 complex of IL5 with IL5Rα and the conformational isomerization of both interacting molecules based on the observations in this study. The receptor α-subunit binds IL5, inducing a conformational change in IL5 which masks the other binding site. A second IL5Rα molecule does not recognize the IL5 isomer and fails to bind IL5.

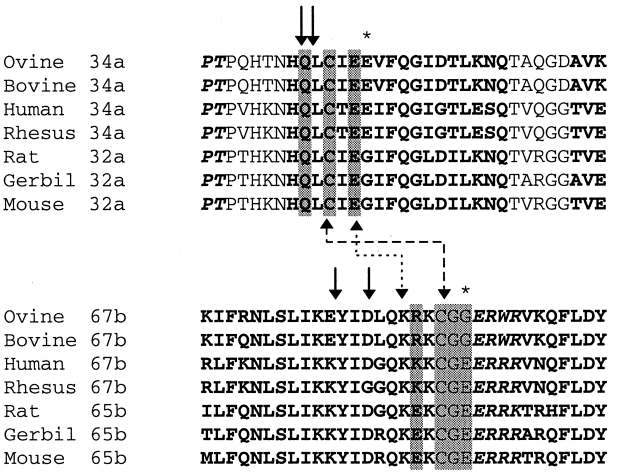

Fig. 4. Alignment of known IL5 sequences of which only residues lying in or near helix C are shown. The location of α-helices are in bold, these of the β-sheets are in italic bold. The flexible residues are highlighted. Two residue positions (Glu-47 and Glu-88) near or in the flexible position that are substituted for glycines in other species are indicated (*). Also shown are the joints (arrow) between the two rigid fragments, the salt bridge between Glu-46a and Lys-83b (dotted arrow) and the disulfide bridge between Cys-44a and Cys-86b (dashed arrow).

critical for hIL5-hIL5Rβ_c interaction. This may result in a conformation of hIL5 optimal for β_c receptor binding. The temperature factors of the E13 residues in both crystal structures are low, reflecting its rigidity (Fig. 2A). The role of E13 as possible anchor residue in the hIL5-hIL5Rβ_c is thereby suggested.

7. Further support for an induced fit model

(1) Comparison of IL5 sequences of different species supports the notion that the residues near the joints are important for the global motion. The residue Glu-47 next to the flexible residue Glu-46 is substituted by glycine for rat, gerbil and mouse IL5. The same substitution happens at position 88 for ovine and bovine IL5 (Fig. 4). These substitutions may underscore the necessity for flexibility in this region.

(2) The residue pair Glu-46-Lys-83 at the back of the cleft forms salt bridges in hIL5$_{Sf9}$ which are not present in hIL5$_{E.coli}$ structure. These residues have high temperature factors which means they are mobile and can redirect their side chains more easily (Fig. 2B). Internal or external forces exerted on the domains could destabilize the salt bridges and allow the hIL5 dimer to adopt another conformation. The hIL5$_{Sf9}$ structure has these salt bridges formed at the back of the cleft holding the cleft in the open state. The pH used for the crystallization is about 6.5 (hIL5$_{Sf9}$) which is lower than the 8.5–9 used for the hIL5 structure determination of *E. coli*. Protonation of the lysine residues could be involved in the transition between the open and the tight state. These two residues are conserved in the IL5 primary structures of different species.

(3) The possibility that conformational changes are coupled to the hIL5/soluble hIL5Rα binding reaction is provided from binding thermodynamic and kinetic experiments [14]. The data suggest that binding does not fit to a simple A plus B to AB binding model despite a 1:1 stoichiometry but rather fit a conformational isomerization model. The total amount of

surface area buried in the binding reaction of hIL5 and its soluble α-receptor that has been estimated is very large and cannot be attributed to the interface between the two proteins alone. These authors suggested that significant conformational changes occur in the receptor although hIL5 isomerization can also be included. In a recent paper [33], the formation of both 1:1 and 2:1 complexes of the soluble extracellular domain of EPOR and a recombinant EPO is examined by circular dichroism. Occupation of the first binding site on EPO resulted in a conformational change which further increased upon occupation of the second binding site. This change could occur in EPO, in the EPOR or in all molecules of the dimer or trimer. Inactive mutants of EPO did not show any conformational change, in agreement with a connection between binding and conformational change.

In summary, we suggest that the observed transition of the hIL5 molecule might be important in hIL5Rα recognition and binding. The fact that two hIL5 conformations are possible is reminiscent of an induced fit mechanism, in which the hIL5 monomeric domains wrap around a binding site resulting in a more tight receptor fit. Such an induced fit would then result in a loss of the second α-receptor interaction explaining the observed 1:1 stoichiometry and would also enable β_c receptor binding. Confirmation of this model will await the elucidation of the three-dimensional structure of the complex. Finally, we want to point out that our observation suggests that the cleft between the domains may represent an alternative target for drug design.

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