Prediction of the three-dimensional structure of human interleukin-7 by homology modeling

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Introduction

Cytokines are a group of low molecular weight proteins which mediate the complex interactions among the cells of the immune system. One subgroup, the hematopoietins, act on receptors with common features, designated hematopoietin receptors (Cosman, 1993). Interleukin-7 (IL-7) is a member of the so-called short-chain hematopoietins (Sprang and Bazan, 1993), binds to a heterodimeric receptor which shares a common \( \gamma \) (\( \gamma_c \)) chain with the receptors for IL-2 and IL-4 (Kondo et al., 1993, 1994; Noguchi et al., 1993; Russell et al., 1993). Other members of this family include IL-9 (Russell et al., 1994) and IL-15 (Giri et al., 1994). Whether IL-13 also utilizes a receptor containing the \( \gamma \) chain is still a matter of discussion (He and Malek, 1995; Matthews et al., 1995).

IL-7 not only supports the growth of B-cell precursors (Namen et al., 1988a), but is also a potent growth factor of T-cells (Alderson et al., 1990; Armitage et al., 1990). More recently it has been shown to protect natural killer cells (Arment et al., 1995) and T-cells (Hernandez-Caselles et al., 1995) from death by apoptosis.

Despite having low sequence homology, the hematopoietins display a common fold, described as four-helix bundles with an up-up-down-down topology (Wlodawer et al., 1993; Davies and Wlodawer, 1995) or as left-handed type 2 four-helix bundles (Presnell and Cohen, 1989). These helices are commonly referred to as helices A through D, in sequential order of location in the sequence. The crystal structure of human growth hormone (GH) bound to its receptor (De Vos et al., 1992) gave important insight into the binding mode and suggested similar binding modes. These results were supported by mutagenesis analysis of related systems, such as IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF) and prolactin (Lopez et al., 1992; Rozakis-Adcock and Kelly, 1992; Savino et al., 1993). Extensive mutation studies on IL-7 have not been published so far.

The design of small-molecule agonists/antagonists of cytokines would be of great pharmaceutical interest. In order to achieve this goal, a detailed knowledge of the structural basis for activity of these proteins is a prerequisite. In the absence of an experimentally determined structure, we decided to perform modeling studies to predict the three-dimensional structure of human IL-7, based on known structures of other hematopoietins. An abstract reporting on a structural model of IL-7 bound to a homodimeric receptor has appeared in the literature (Srinivasan et al., 1993); however, neither coordinates nor other details have become available to the public. The model presented here is intended to suggest mutagenesis experiments, to provide further insight into the mechanism of action of this biologically important cytokine and to allow for a comparison with other hematopoietin structures.

Materials and methods

Alignment

Although the hematopoietins appear to adopt a very similar fold, the sequence homologies between the individual members are strikingly low (Arai et al., 1990). However, similar patterns of amphipathicity are present within this family (Bazan, 1990, 1991). Therefore, CAMELEON (Morris, 1988; Oxford Molecular Ltd, 1995), a graphics-based multiple sequence alignment program, was used to align the sequence of IL-7 with other members of the hematopoietins. Based on the assumption that IL-7 is a four-helix bundle with a hydrophobic core, hydrophatic residue properties were used rather than residue identities for the alignment (Figure 1). From Figure 1 it also becomes evident that structure-based alignment of other cytokines correlates very well with the alignment relying on hydrophatic properties (Figure 2). The results of this procedure were found to be in good agreement with the CAMELEON alignment.
Fig. 1. Multiple alignment of IL-7 with human GH, IL-2, IL-4 and GM-CSF. Bold font indicates helical residues. Structurally conserved residues (cf. Rozwarski et al., 1994) are boxed. Consensus buried side chains are shaded gray.

Model generation and refinement

For generation of the three-dimensional structure of IL-7, the Composer module (Sutcliffe et al., 1987a,b) implemented in Sybyl 6.1 (Tripos, 1994) was used. A common Ca framework for the four helices was derived from root mean square (r.m.s.) superposition of the residues of GH, IL-2, IL-4 and GM-CSF listed in Table I. The fragments of the other four proteins with the highest homology to IL-7 were superimposed on the corresponding Ca framework, and appropriate side chain mutations yielded the helical core of IL-7. As the helices of the other hematopoietins extend in most cases the common core region, it was reasonable to elongate the helices of IL-7 as shown in Figure 1. In order to generate the same topology for the crossover between loops AB and CD which is observed in other short-chain cytokines, a segment of loop AB of GM-CSF (E38-E41) was inserted into the structure and mutated to the residues expected of IL-7 (N37-N40). Loop searching of a database of 347 structures (including those hematopoietins used in this study) then yielded the missing fragments of the protein. Secondary structure predictions (Chou and Fasman, 1978; Gamier and Robson, 1989) indicated that loop CD might contain a short a-helix, and residues T110-N116 were modeled accordingly.

Refinement of the structure was performed according to the following protocol. Unfavorable interactions between the residues were adjusted manually after visual inspection, followed by 100 steps of energy minimization using the Tripos force field (Clark et al., 1989). Subsequently, the protein was solvated in an 8 Å water sphere and minimized for 2000 steps with AMBER 4.1 (Pearlman et al., 1994; Cornell et al., 1995). Dynamics at 300 K was performed for 5 ps on the water molecules only, for 10 ps on the side chains, for 10 ps on loops and side chains and for 20 ps on the entire protein, applying internal constraints for the helices. Finally, after 20 ps of free dynamics, the protein was re-minimized. One should bear in mind that such a refinement procedure will not be able to correct any serious errors in the model. It will, however, help to remove unfavorable backbone/side-chain angles and interactions.

Results and discussion

General features of the model

The final model of IL-7 is displayed in Figure 3. According to this model, IL-7 contains a compact helical core and has a
3-D structure of human interleukin-7 by homology modeling

Fig. 2. Snapshots of the three-dimensional alignment procedure for helices A through D. Residues are colored according to their hydropathic properties. Green indicates hydrophobic, red acidic, blue basic and gray amphiphilic residues. Of the remaining three helices, the backbone Ca structures are shown, respectively. The picture was obtained using the program MOLSCRIPT (Kraulis, 1991).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>PDB code</th>
<th>Reference</th>
<th>Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Helix A</td>
</tr>
<tr>
<td>hGH</td>
<td>3hh</td>
<td>DeVos et al. (1992)</td>
<td>S7-L20</td>
</tr>
<tr>
<td>IL-2</td>
<td>3ink</td>
<td>McKay (1992)</td>
<td>Q11-L24</td>
</tr>
<tr>
<td>IL-4</td>
<td>1rec</td>
<td>Wlodawer et al. (1992)</td>
<td>D4-L17</td>
</tr>
</tbody>
</table>

Table I. Residues used to construct the framework of the helical core

topology identical with that of other short-chain cytokines. The total solvent-accessible surface (Connolly, 1983) amounts to 11 448 Å². The ratio of exposed hydrophobic to exposed hydrophilic surface area is 1.83, a value very similar to the corresponding ratios for hGH (1.86) and GM-CSF (1.87).

The hydrophobic core is composed of amino acids L16, M17, I20, L23, L24 (all helix A), F55, L56, A59, L63, F66 (helix B), L79, V82, T86, L89, T93 (helix C), L128, L131, L135, I138 and W142 (helix D). As also observed for other hematopoietins (Wlodawer et al., 1993), some residues not belonging to the helical regions contribute their side chains to the core. In the case of the IL-7 model, these are residues M27, F39, F41, F42 (loop AB) and F75 (loop BC). Structural alignment of IL-7 with other cytokines used in this study (Table II) reveals that amino acids F39, F41 and F42 in the AB loop have structural equivalents in IL-2, IL-4 and GM-CSF (Table III). Other hydrophobic side chains which are substantially buried are I4, V96, I145 and L146. A very short
Table II. Pairwise root mean square deviations (Å) for pairwise superposition of molecules, using residues indicated in Figure 1

<table>
<thead>
<tr>
<th></th>
<th>IL-7</th>
<th>hGH</th>
<th>IL-2</th>
<th>IL-4</th>
<th>GM-CSF</th>
</tr>
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<tr>
<td>IL-7</td>
<td>3.40</td>
<td>2.30</td>
<td>1.98</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>hGH</td>
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<td>2.48</td>
<td>2.37</td>
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</tr>
<tr>
<td>IL-2</td>
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<td></td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td>1.28</td>
<td></td>
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</table>

Table III. Structurally equivalent residues in loop AB contributing to the hydrophobic core

<table>
<thead>
<tr>
<th></th>
<th>IL-7</th>
<th>IL-2</th>
<th>IL-4</th>
<th>GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>F39</td>
<td>F44</td>
<td>V29</td>
<td>V40</td>
<td></td>
</tr>
<tr>
<td>F41</td>
<td>I32</td>
<td>V42</td>
<td></td>
<td></td>
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<tr>
<td>F42</td>
<td>F33</td>
<td></td>
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</table>

Fig. 3. Stereo diagram of the IL-7 model. Color coding of the helices is as follows: helix A, red; helix B, yellow; helix C, green; helix D, blue. Disulfide crosslinks and selected residues are displayed. Picture drawn with MOLSCRIPT (Kraulis, 1991).

An interesting feature of the IL-7 model is the presence of a rather long loop between helices C and D. The sequence of murine IL-7 (Namen et al., 1988b) is 23 residues shorter than human IL-7 and 22 residues shorter than bovine IL-7 (Cludts et al., 1992). Remarkably, the major deletions (20 amino acids) in murIL-7 occur at the end of helix C and the following loop (Figure 1). This indicates that this comparably long loop in the model of huIL-7 is not a consequence of a misalignment of the core sequences. The fact that human and murine IL-7 cross-react (Damia et al., 1992; Komschlies et al., 1994) also suggests that the deletion in mutIL-7 is located in a loop region. Moreover, previous alignment studies yielded
very similar results. Rozwaski et al. (1994) aligned helix C of IL-7 to other cytokines in an identical manner. Their alignment for helices A and B differed by approximately one helix turn (+4 residues for A, -4 residues for B, -3 residues for D, relative to the present alignment). Other studies (Parry et al., 1991; Manavalan et al., 1992) were performed before many hematopoietin structures became available, but gave very similar alignments/predictions.

**Disulfide bonding**

The amino acid sequence of IL-7 contains six cysteines. In the initial, unrefined model, the cysteines were located in space in such a way that three disulfide bonds could be generated unambiguously. The first one links the N-terminus of the protein chain to helix C (C2-C92), the second one being formed between loop AB and the start of helix D (C34-C129). The third disulfide bond is found between loop AB and the lower part of helix D. This disulfide pattern has been determined experimentally (Srinivasan et al., 1993). In terms of occurrence of the cysteines in the amino acid sequence, these disulfide bonds can be designated as 1-4, 2-5 and 3-6. It is interesting that in human IL-4, which also contains six cysteines, the disulfide pattern is different, and the crosslinks are formed as 1-6, 2-4 and 3-5. An attempt to model IL-7 based on disulfide bonds analogous to huIL-4 has appeared on the World Wide Web (Cosenza et al., 1995), but the resulting model does not appear to present the compact helical core and the loop topology common to other short-chain cytokines.

The only disulfide bridge connecting the same structural elements in IL-2, IL-4 and GM-CSF is formed between helix B and loop CD. In IL-4, this disulfide (3-5) appears to be crucial for function; corresponding threonine mutants of IL-4 lost 99% of activity (Kruse et al., 1991). The disulfide pattern in mouse IL-4 (1-5, 2-4 and 3-6) is different (Carr et al., 1991), but this important crosslink appears to be structurally conserved (3-6). Considering that IL-7 belongs to the same family as IL-2 and IL-4, it is an interesting feature of the IL-7 model that it does not contain an analogous disulfide crosslink. However, modifications of the alignment in order to allow the formation of such a disulfide bond did not lead to reasonable alternatives. On the one hand, rendering C129 of IL-7 available for the CD loop would result in a very short D helix and a very long loop between helices C and D. On the other hand, in order to bring C92 into an appropriate position in loop CD, the fold of the preceding sequence would be altered significantly, and the hydrophobic core would be destroyed. Above all, performing such realignments would introduce major changes in the overall structure, in order to maintain the experimental disulfide assignment. In other cytokines, such as hGH, G-CSF, M-CSF and IL-5, an equivalent disulfide bridge is not present. Recent modeling studies performed on IL-3 (Lyne et al., 1995), IL-13 (Bamborough et al., 1994) and erythropoietin (Caravella et al., 1996) also did not suggest such a crosslink.

**Putative binding interfaces**

The hematopoietins interacting with the common γ chain have two separate binding sites. The first, encompassing residues on helices A and C, binds to the individual, cytokine-specific receptor chain. The second, located mainly on helix D, interacts with γc.

Mutagenesis studies for IL-4 (Kruse et al., 1993) and murine IL-2 (Zurawski et al., 1993) revealed that acidic residues E9 (IL-4) and D34 (murine IL-2, the human equivalent being D20) on helix A are crucial for binding. An analogous residue (E21) has been identified for GM-CSF (Hercus et al., 1994). In the current IL-7 model, an acidic residue is not present at these positions, the structurally equivalent residues being S19 and L23. Alignment of D21 or D25 of IL-7 with the acidic residues of the other hematopoietins would render most of the hydrophobic residues solvent-exposed. However, helix A is considered to be involved in binding to the cytokine-specific receptor chain. Therefore, the conservation of an acidic residue depends on the nature of the amino acids at the complementary receptor site. Other amino acids in helix A of IL-7 possibly involved in receptor interaction are Y12, V15 and Q22.

The second part of the binding interface to the specific chain in IL-2 and IL-4 is composed of several residues located in helix C. In particular these include D84 and N88 for IL-2, and R81, R85 and R88 of IL-4. In analogy, this part of the binding site in IL-7 should encompass amino acids L77, K81 and E84. It would therefore be of interest to generate IL-7 mutants at one or more of these positions, in order to assess the significance of these residues for binding.

An amino acid that has been found to be sensitive to mutation on helix D of murine IL-2 is Q141 (human IL-2 Q126). The structural equivalent in the IL-7 model is K139 or T140. Additional important residues in the γc binding region of IL-4 are Y124 and S125. NMR analysis of the Y124G and Y124D variants of IL-4 indicated that this residue is directly involved in binding (Müller et al., 1994). In this context, it is of interest that the residues at the interfaces of IL-2 and IL-4 to the common receptor chain are not very well conserved. The residue in IL-7 analogous to IL-2 Y124 is K144. Other amino acids which could be part of this interface include R133, Q136 and E137. Therefore, it is possible that mutation at one of these positions in IL-7 alters the binding or receptor activation characteristics of this cytokine significantly.

**Conclusions**

A three-dimensional model of human IL-7 has been generated based on homology to other structures of hematopoietins. The model contains a compact hydrophobic core and has a topology common to other four-helix bundle proteins. The alignment procedure, which was based on the conservation of the hydrophathy profile, gave very similar results to previous alignments/predictions. 'Three-dimensional alignment', i.e. threading of the sequence through the structurally conserved helical core, agreed well with the other method. The disulfide crosslinks could be formed unambiguously without major distortions in the structure, and they are identical with the experimental assignment. The model contains a rather long loop between helices C and D, which appears to be significantly shorter in murine IL-7. Whether this loop has a particular function remains unclear.

The lack of mutation data on IL-7 makes it difficult to assess the quality of the present model in detail. However, it will be a good starting point and a valuable help in designing mutagenesis experiments. These results will allow further refinement in order to take newly discovered facts into account. In addition, the present study can serve as a starting point for generating a model of IL-7 bound to its heterodimeric receptor, and it can therefore help in gaining further insight into the cytokine–receptor interactions.

The coordinates of this model are available from the authors and will be submitted to the Protein Data Bank, Chemistry...
References

Tripos (1994), Sybyl 6.1. Tripos Assoc., St Louis, MO.
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