Novel Fold and Putative Receptor Binding Site of Granulocyte-Macrophage Colony-Stimulating Factor

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the development of and the cytotoxic activity of white blood cells. Recombinant human GM-CSF has proven useful in the treatment of blood disorders. The structure of GM-CSF, which was determined at 2.4 angstrom resolution by x-ray crystallography, has a novel fold combining a two-stranded antiparallel β sheet with an open bundle of four α helices. Residues implicated in receptor recognition, which are distant in the primary sequence, are on adjacent α helices in the folded protein. A working model for the receptor binding site is presented.

NATURAL GM-CSF IS A 127-AMINO acid residue trace glycoprotein (1, 2) that triggers the development of both granulocyte and macrophage colonies from hematopoietic progenitor cells (3). The availability of recombinant GM-CSF (2, 4, 5) has allowed a much fuller characterization of its activities, and it is now clear that GM-CSF stimulates a wide variety of hematopoietic and nonhematopoietic cell types (6). As a therapeutic agent, GM-CSF has shown promise in the treatment of aplastic anemia, myelodysplastic syndromes, acquired immune deficiency syndrome, neutropenias, and chemotherapy-induced myelosuppression (7, 8). As a step toward understanding its structure-function rela-

tions, we have crystallized nonglycosylated recombinant human GM-CSF (9). Here we report the structure at 2.4 Å resolution and define a putative receptor binding site.

The tertiary structure of GM-CSF is dominated by a bundle of four α helices and is distinctly nonspherical with dimensions of 20 Å by 30 Å by 40 Å (Fig. 1). The four helices are all antiparallel and form a twisted open barrel, with the open edge filled in by strand 1 of the antiparallel sheet. Each of the helices interacts with both of its neighbors around the barrel perimeter (Table 1) so that despite the presence of the β sheet, GM-CSF can be compared with known four-helix bundle proteins (10). The topology of the bundle places helices A and B, and helices C and D across the barrel from each other rather than next to each other. This placement gives GM-CSF a double overhand topology (10), which has previously been seen for porcine growth hormone (11). Although interesting, the simi-

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larity to growth hormone does not extend to other key features of the GM-CSF fold, including the manner in which the helices pack together and the association of the two long overhand connections to build an antiparallel β sheet. Growth hormone has long helices (20 to 30 residues) that pack with all interhelical angles close to 180° , whereas the GM-CSF helices are only between 10 and 16 residues long and cross at angles up to

43° from perfect alignment (Table 1).

For these reasons, the GM-CSF structure is clearly distinct from that of growth hormone and represents a novel fold. The topology of the GM-CSF fold as a whole is reminiscent of the β -jellyroll member of the greek-key β -barrel family (12), with four of the sheet strands replaced by helices (Fig. 1B). Interestingly, the swirl of the GM-CSF jellyroll is left-handed, whereas all known

β -jellyrolls are right-handed. The two disulfide bonds in GM-CSF are both at the same end of the molecule and connect helix B to strand 2 of the β sheet and helix C to the carboxyl terminus, respectively. Although there are no topological loop crossings, the loop from residues 43 to 54 penetrates the macrocycle formed by the carboxyl-terminal disulfide (Cys⁸⁸-Cys¹²¹) to result in a rare "threaded" topology (13). A conserved proline-rich sequence (residues 89 to 94; Fig. 2) adopts an extended conformation and makes key hydrogen bonds to stabilize the threaded interaction: the carbonyl of Pro⁹² hydrogen bonds to the amide of Cys⁵⁴ and the carboxylate of Glu⁹³ hydrogen bonds to amides of both Glu⁵⁶ and Thr⁵⁷ at the amino terminus of helix B.

Another notable feature of the fold is a stretch of 3_{10} helix from residues 68 to 73. The Pro⁷⁶ residue interrupts the helical hydrogen-bonding pattern and causes a 30° bend in the chain leading into helix C. Since Pro⁷⁶ is not conserved across species (Fig. 2), it is unclear whether the bend is also present in murine GM-CSF or whether helix C might be longer.

The solvent accessibility of each residue in GM-CSF (Fig. 2) is useful for distinguishing those residues that contribute to structural integrity from those that may be directly involved in receptor recognition. We can use the GM-CSF structure to show that many mutation studies aimed at characterizing structure-function relations for GM-CSF have been effective at locating structurally important regions rather than the receptor recognition site. A study of mutants in which various tripeptide deletions were made identified four regions in murine GM-CSF that were essential for activity (residues 18 to 22, 37 to 44, 54 to 64, and

Fig. 1. The three-dimensional structure of GM-CSF. **(A)** A ribbon diagram (23) highlighting the secondary structural elements (24): helices are shown as spirals, β strands as arrows, and disulfides as lightning bolts. **(B)** Topology diagram showing the α/β -jellyroll topology. Rectangles represent helices (A, B, C, and D), and arrows represent β strands (1 and 2). The N and C mark the termini, and the disulfides are indicated. **(C)** A stereo Co diagram with every tenth residue labeled and disulfides indicated. The view is the same as in (A). The loops at residues 31 to 38 and 45 to 52 have high mobility. Crystals of recombinant human GM-CSF ($P2_12_12_1$; $a = 47.6$, $b = 59.1$, and $c = 126.7$ Å) were grown and diffraction data were collected at room temperature on a San Diego Multiwire Systems detector (25) as described in the low-resolution structure determination (9). Two additional heavy-atom derivatives were found (methylmercury acid and HgI₂) that allowed better experimental phases to be determined. The multiple isomorphous replacement phases were refined by iterative solvent flattening (26) combined with noncrystallographic symmetry averaging (9) to yield a 3.5 Å resolution averaged electron density map that showed the characteristic spiral of the α helices and density for many side chains. One of the helices showed three large side chains pointing into the protein core and could be fit by residues 99 to 116, which include three Phe residues. No other parts of the sequence could be recognized in the electron density. After three rounds of model building and simulated annealing refinement (27) with the use of partial models, the electron density improved so that the true amino acid sequence could be assigned to the density. Five further cycles of model building and refinement were carried out and eventually used all of the data between 10 and 2.4 Å resolution to yield an R factor of 0.205. Individual atomic temperature factors were restrained, and the model has root-mean-square (rms) deviations from ideal geometry of 0.017 for bond lengths and 3.5° for bond angles. The two molecules related by noncrystallographic symmetry have an rms deviation of 1.2 Å for all atoms. The current model includes residues 5 through 123 of the protein and 64 bound water molecules. All model building was done on an Hewlett-Packard 9000/835 Workstation with the program FREIBAU (28).

Table 1. Geometry of the GM-CSF helix-packing interactions. The interhelix packing angle Ω is the angle between the vectors defining the directions of the two helices (10). A negative angle means the front helix of the pair is rotated clockwise relative to the back helix. The separation is the closest approach of the two axial vectors. Presnell and Cohen (10) also assessed the compactness of a helical bundle by the amount of surface area that became buried as each helix associated with the other three helices. For GM-CSF these numbers are 24, 21, 37, and 24% of the total accessible surface area of the four helices ($= 4400$ Å²), showing that the helices interact quite tightly with one another.

Helix pair	Separation (Å)	Ω (degrees)
A:C	8.4	-147
C:B	9.5	-171
B:D	11.4	-137
D:A	10.3	-148

ogous structures and thus apparently have evolved their receptor binding activities independently. The one thing that appears common to these structures is the high helical content and the use of residues in α helices for receptor binding.

Note added in proof: The secondary structure and topology of the cytokine interleukin-4 have been determined by nuclear magnetic resonance studies and it appears to have the same fold as GM-CSF (32).

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29. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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