18. Three-dimensional Structure of Recombinant Murine Interferon- β ^{†)}

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Since we reported on the new crystal form of recombinant murine interferon- β (Mu IFN- β),¹⁾ reports on antiviral, antibacterial and anticancer activities of interferon- β (IFN- β) have been accumulated²⁾⁻⁴⁾ and the elucidation of the three-dimensional structure of this biological response modifier is urgently required. Among many such reports, a report on almost perfect antitumour activity of multiple injection of recombinant interleukin-2 in combination with recombinant Mu IFN- β against certain tumor-bearing mice indicates a strong potential for therapeutic usage of IFN- β .⁵⁾

We now report on the three-dimensional structure of recombinant Mu IFN- β as determined by X-ray crystal structure analysis. The present structure should represent the basic structural framework of the IFN- α and - β family, since there is plenty of evidence⁶⁾ showing that the IFN- α and IFN- β molecules are phylogenetically closely related (also see below). Initially the crystal structure was solved at 2.75 Å resolution through a multiple isomorphous replacement procedure using mersalyl, uranyl acetate (up to 3.5 Å) and K₃UO₂F₅ derivatives for which the "phasing power" were 1.59, 1.56 and 2.62, respectively. The structure was first refined by the simulated annealing procedure employing the program XPLOR and then refined by restrained least-squares procedure employing the program PROLSQ. Final crystallographic R-factor is 19.3% for 5153 reflections (69% of theoretical number) in the 5.0–2.5 Å range with the root-mean-square deviation from ideality being 0.013 Å for bond lengths. A total of 1437 protein atoms and 66 solvent molecules were included in the final model. Crystallographic details will be published elsewhere.

The structure shown in Figs. 1 and 2 consists of five α -helices designated as Helix A (residue 6 through 23), Helix B (50–65), Helix C (78–88), Helix D (112–129) and Helix E (137–152). The corresponding "consensus" residue numbers defined for IFN- β in Fig. 4 of reference 6) are as follows: Helix A (6–23), Helix B (54–69), Helix C (82–92), Helix D (117–134) and Helix E (142–157). Here and

†) Abbreviations: IFN- α , interferon- α ; Mu IFN- β , murine interferon- β , Hu INF- β , human interferon- β .

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Fig. 1. Schematic drawing of the α -carbon chain of recombinant murine interferon- β . Arrows indicate the polarity of five α -helices. Several amino acid residue numbers are indicated together with the N- and C-terminus. Three shaded polypeptide segments are the most likely binding region to the receptor.

hereafter the consensus residue numbers will be underlined. The interconnecting loop region between Helices A and B is named Loop AB. Likewise there are Loops BC, CD and DE. Helices A, B and D are parallel to each other while Helices C and E are running antiparallel to the former set of three helices. The average crystallographic B-factors as plotted along the polypeptide chain show the general tendency that helical regions are less flexible (without exception). Among the four interhelical loops, Loop CD exhibits highest flexibility and thus there was considerable difficulty in interpreting the weak electron density map of this region.

While overall similarity of the basic polypeptide chain folding among all the α - and β -interferons from various sources is almost obvious from the amino acid sequence homology alone (for example, see Fig. 3 of reference 7)), two additional observations arising from the present three-dimensional model further solidify the notion of overall similarity. These are as follows: (1) the disulfide linkage between residues 29(31) and 136(141) (the underlined numbers in parentheses are the consensus residue numbers), which is absent in Mu IFN- β but present in Hu IFN- β , appears geometrically feasible, the distance between the α -carbons of the corresponding residues being about 12.2 Å and (2) the disulfide linkage between residues 3(3) and 97(101), which is absent in all IFN- β molecules examined so far, but is present in Hu IFN- α (in addition to the above 29-136 linkage),

Fig. 2. Stereo pair showing the arrangement of Helices A through E. The view direction approximately corresponds to that from top to bottom in Fig. 1. N- and C-termini are marked.

appears geometrically feasible, the corresponding distance being about 12.9 Å. Another important fact related to the observation No. (1) mentioned above is the fact that introduction of the 29–136 disulfide linkage to recombinant Mu IFN- β through site-directed mutagenesis resulted in a more active and stabler product.⁸⁾ It is clear that the above two structural observations coupled with the site-directed mutagenesis experiment mentioned above give very strong support for the validity of the present model as the common chain folding of IFN- α and - β molecules in general.

Interpreting all the known biochemical, immunological and evolutionary findings obtained for various IFN- α and - β molecules in terms of the established common chain folding, we conclude that the three polypeptide segments are most directly involved in the expression of antiviral and antiproliferative activity of IFN- α and - β family. These are the segment 30 through 40 (32–42; Loop AB), the segment 70–80 (75–85; Loop BC) and the segment 120–140 (125–145; Loop DE and the flanking regions on both sides, hereafter referred to as “broader Loop DE” for simplicity). These three segments are shown as shaded regions in Fig. 1. Referring to Fig. 2 as well, it is clear that the three crucial segments, Loops AB, BC and the broader Loop DE, constitute a spatially close assembly of polypeptide chain segments. Thus it seems most likely that these regions are in fact the binding sites to the IFN- α , - β receptor(s).

The evidence, either directly or indirectly supporting the above notion of the three crucial segments, will be summarized as follows.

(1) The three segments are the regions where the amino acid sequences are relatively well conserved. In the alignment of 23 IFN- α and 5 IFN- β molecules of human, bovine, mouse and rat origins (Fig. 3 of reference 7)), 5 out of 7 “super” conservative residues are in these segments and 8 out of 14 semi-conservative residues are in these segments.

(2) Specific amino acid substitutions in Hu IFN- α 1 and α 2 in the broader Loop DE indicate that this region is critical to the determination of mouse and human cell antiviral activities.^{8),9)}

(3) In the case of Hu IFN- α 2, substitution of either Leu 30(32), Asp 32(34)

or Arg 33(35) in Loop AB by alanine resulted in disappearance of antiviral and antiproliferative activities,¹⁰⁾ while substitution of Phe 48(50), Gln 49(51) or Gln 62(64) in Helix B and the lower (to the N-terminus) flanking region resulted in no change.

(4) A monoclonal antibody raised against fibroblast Hu IFN- β neutralized the antiproliferative activity and this effect was inhibited by a fragment of Loop DE, the 132–136 (134–138) pentapeptide, of Hu IFN- α 2.¹¹⁾

(5) In the case of a modified recombinant Hu IFN- β (with a Cys 17 $\rightarrow$ Ser conversion), two species of monoclonal antibodies neutralized the antiviral activity and this effect was inhibited by certain fragments of Loop AB, the 32–56, 32–47, or 40–56 (in this case, consensus residue numbers are the same) peptides.

(6) In the case of IFN- α , amino acid substitution in the 69–79 (67–77) region (Loop BC) revealed a pronounced influence on the appropriate activity of IFN- α (as cited in p.174 of reference 7)).

Details of the present work will be published elsewhere. Site-directed mutagenesis studies of Mu IFN- β and a search for oligopeptides which are either agonistic or antagonistic to IFN are in progress.

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