HUMAN TYPE I INTERFERONS: STRUCTURE AND FUNCTION

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Summary. – Human type I interferons (IFNs) comprise a family of 13 IFN-α subtypes and single species of IFN-β and IFN-ω. Their 20% overall sequence homology determines identical secondary and tertiary folding of polypeptides. Three-dimensional models suggest that the globular structure of type I IFNs consists of a bundle of 5 α-helices, which might form two polypeptide domains. Disulfide bond Cys 29 – Cys 139 stabilizes both domains in a bioactive configuration. The IFN molecule exerts its functional entity only as an organic polypeptide complex and therefore molecular fragments apparently lack biological activity. IFN-β, IFN-ω and some IFN-α subtypes are glycoproteins, but the sugar moiety was found to be neither structurally nor functionally relevant. Type I IFNs share a common cellular receptor, a fact that implies a high structural conservativity of their receptor-binding areas. Two conservative hydrophilic regions associated with the amino acids (aa) 30-41 and 120-145 appear to constitute the basic framework of receptor recognition site in type I IFNs. However, the individual IFN-(sub)types induce different spectra of biological effects which reflect some specificity in modelling of binding sites. Besides a subtle sequential heterogeneity in the segments aa 30-41 and 120-145, also the variable hydrophilic aa regions 23-26, 68-85 and 112-121 are responsible for structural and functional individuality among human type I IFNs. The interaction between IFN and its receptor seems to be a complex event which triggers simultaneously antiviral, antiproliferative and immunomodulating actions, although different parts of IFN molecule are not involved equally in eliciting of respective basal activities.

Key words: type I interferon; structure-activity relationship

HUMAN TYPE I INTERFERONS

The recent nomenclature of interferons (IFNs), based on gene structure, recognizes in man three distinct classes of the type I IFNs – IFN-α, β and ω. These proteins share considerable sequence homology at both the nucleotide and amino acid levels, are induced by viruses and exhibit similar biological activities after interaction with a common cellular receptor. The IFN-α family consists of at least 13 related subtypes, while IFN-β and IFN-ω are represented by a single protein. Table 1 summarizes basic characteristics of human type I IFNs (Pestka, 1983; Henco *et al.*,1985; De Maeyer and De Maeyer-Guignard, 1988; Hosoi *et al.*, 1988; Utsumi *et al.*, 1989; Adolf, 1990; Adolf *et al.*, 1990; Adolf *et al.*, 1991*a*, 1991*b*; Flores *et al.*, 1991; Kontsek *et al.*, 1991*a*; Zoon *et al.*, 1992).

PRIMARY STRUCTURE

The mature IFNs α/β consist of 166 aa with the exception of IFN-α2, which contains 165 aa (deleted Asp 44). IFN-ω is longer by 6 aa at the C-terminal end (total length 172 aa). As the only type I IFN is the basic protein IFN-ω partially pH2-labile (Kontsek *et al.*, 1991*a*). Fig. 1 shows primary structure of human IFN-α, β, and ω (adapted according Henco *et al.*, 1985; Adolf, 1987; Zoon, 1987; De Maeyer and De Meayer-Guignard, 1988; Allen and Diaz, 1994). The location of the conserved residues between different type I IFNs is depicted in Fig. 2.

Table 1. Characterization of human type I IFNs

Class	IFN- α	IFN-ß	IFN-ω
Previous nomenclature	leukocyte IFN α_I	fibroblast IFN	α_{II}
No. of functional genes	13	1	1
Chromosomal location	9	9	9
Mature protein (No. of aa)	165 – 166	166	172
M_r predicted natural	19.2 K (α2)–19.7 K (α14) 17.5 K–23.3 K	20.0 K 23.0 K	20.0 K 24.5 K
Glycosylation	some subtypes	yes	yes
Isoelectric point (pI)	4.5 – 6.9	7.8	8.5
Activity at pH 2	active	active	partially inactivated
Cellular receptor	IFN-α/ß/ωR	IFN-α/ß/ωR	IFN-α/ß/ωR

In IFN-α family is the absolute conservation of 79 (48%) of the 166 aa residues, with an average 20% difference between individual subtypes. The highest 95% homology (7 – 9 different residues) show pairs α4-α7, α4-α17, α5-α22 and α8-α10; on the opposite site are couples α1-α7 and α1-α8 with the lowest 78% sequential homology and 35-38 residues differences. The allelic/sequence variants differ on the protein level in 1 to 4 (α8a-α8b) aa positions.

IFNs α/ω contain conserved residues in 59 (36%) positions. There is about 60% homology of IFN-ω with single IFN-α subtypes, the highest (63%) with IFN-α2 and lowest (56%) with IFN-α21.

Molecules of IFNs-α/β have absolutely conserved 38 (23%) residues and sequential homology of IFN-β with the respective α-subtypes is approximately 30%. Structurally closest to IFN-β are IFNs-α2/α22 with 33% homology, the minimal (29%) homology show subtypes α1/α8/α21.

Primary structures of IFNs-β/ω have 51 identical aa positions resulting in 31% sequentional homology.

In summary, in type I IFNs the absolute conserved residues occupy 33 (20%) positions of their aa.

Of interest is the primary structure of IFNs-α2/β/ω. Both IFN-β and IFN-ω, when compared to α-subtypes, show the highest homology to IFN-α2. We infere that this homology suggests some evolutionary significance.

SECONDARY STRUCTURE

The secondary structure of polypeptides is usually represented by combination of regular motifs (α-helix and β-sheet) and irregular motifs (turn and loop). Since of the dominant secondary structure of type I IFNs is α-helix, they are classified into group of α-helical proteins (Table 2) (Zavyalov *et al.*, 1989, 1990; Boublik *et al.*, 1990). Hydrophobic helices and sheets form the core of protein, whereas hydrophilic turns and loops are situated on the surface of the protein. From functional point of view is the determination of turns and loops in a protein such as IFN of particular interest. The surface structures of IFN are likely to be involved in interaction with a specific cellular receptor, a step required to elicit a biological response in target cell.

Table 2. Structural properties of human type I IFNs

Class	Predicted content α-helix	Predicted content β-sheet	No. and location of disulfide bonds	Protein structure	Functional unit
IFN-α	66%	15%	2 1-99, 29-139	α-helical globulin	monomer
IFN-ω	68%	18%	2 1-99, 29-139	α-helical globulin	monomer (probably)
IFN-β	63%	9%	1 31-141	α-helical globulin	monomer

TERTIARY STRUCTURE

Structural models suggest that type I IFNs have globular organization, which determines a considerable physical stability of these proteins. The structural rigidity of IFN molecules is undirectly corroborated also by immunologic evidence. The existence of non-neutralizing monoclonal antibodies to IFN demonstrates that binding of a several time larger immunoglobulin molecule does not destabilize the bioactive conformation of IFN. Futhermore the binding of a monoclonal antibody (MoAb) to one epitope has not to interfere with binding of another antibody recognizing different structure on the same IFN molecule (Redlich and Grossberg, 1989; Adolf, 1990; Kontsek *et al.*, 1991*b*).

First three-dimensional models of human IFNs-α/β, predicted from aa sequences, were published in 1982 (Zavyalov and Denesyuk, 1982; Sternberg and Cohen, 1982). Zavyalov's upgraded structural model of type I IFN fits well in the conformation of murine IFN-β, recently determined by X-ray crystal structure analysis (Zavyalov *et al.*, 1989; Senda *et al.*, 1990, 1992). According to these studies, the

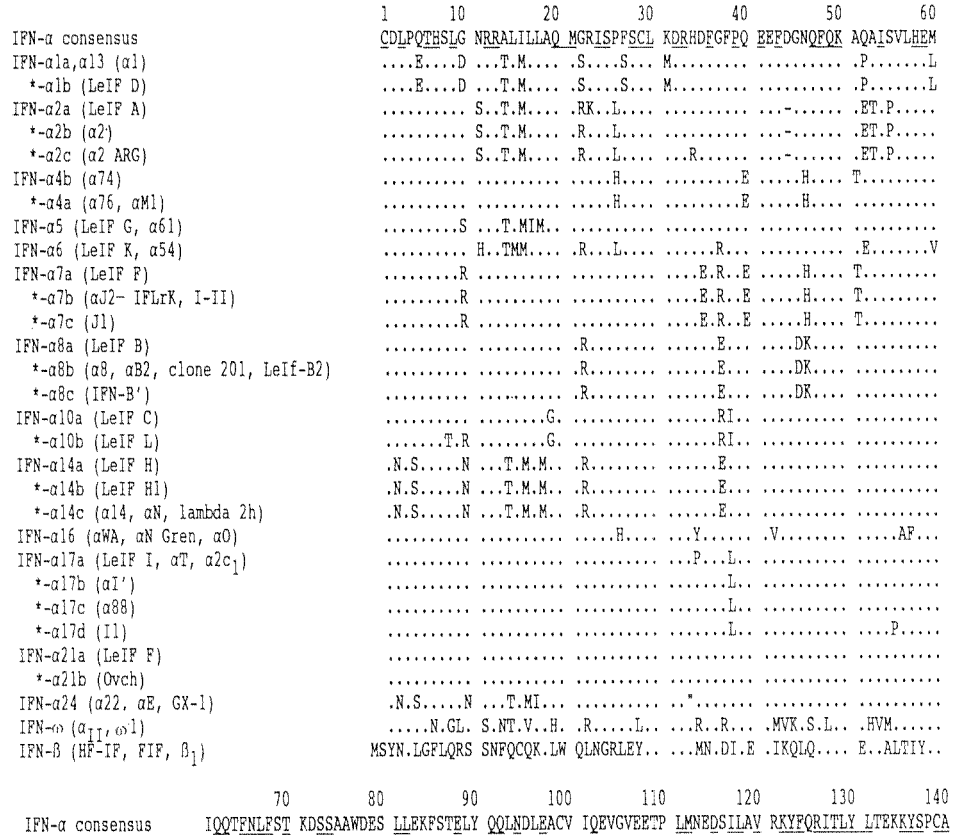

Fig. 1

Amino acid sequences of human type I IFNs

A-Ala, C-Cys, D-Asp, E-Glu, F-Phe, G-Gly, H-His, I-Ile, K-Lys, L-Leu, M-Met, N-Asp, P-Pro, Q-Gln, R-Arg, S-Ser, T-Thr, V-Val, W-Trp, Y-Tyr
Conserved aa are underlined. * allelic or sequence variants, - deleted codon.

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                          150        160    166
IFN-α consensus      WEVVRAEIMR SFSFSTNLQK RLRRKD
IFN-α1a, α13         .......... .L.L.....E .....E
  *-α1b              .......... .L.L.....E .....E
IFN-α2a              .......... ...L.....E S..S.E
  *-α2b              .......... ...L.....E S..S.E
  *-α2c              .......... ...L.....E S..S.E
IFN-α4b              .......... .L........ ......
  *-α4a              .......... .L........ ......
IFN-α5               .......... ...L.A...E .....E
IFN-α6               .......... ...S.R...E .....E
IFN-α7a              .......... ........K. G.....
  *-α7b              .......... .......... ......
  *-α7c              .......... ........K. G.....
IFN-α8a              .......... ...L.I.... ..KS.E
  *-α8b              .......... ...L.I.... ..KS.E
  *-α8c              .......... ...L.I.... N
IFN-α10a             .......... .L........ ......
  *-α10b             .......... .L........ ......
IFN-α14a             .......... .......... ......
  *-α14b             .......... ........K. G.....
  *-α14c             .......... .L........ ......
IFN-α16              .......... .......... G.....
IFN-α17a             .......... .L........ I.....
  *-α17b             .......... .L........ I.....
  *-α17c             .......... .L........ ......
  *-α17d             .......... .L........ ......
IFN-α21a             .......... ...L.KIF.E .....E
  *-α21b             .......... ...L.KIF.E .....E
IFN-α24              .......... ...V....T. ......
IFN-ω                .....M...K .LFL...M.E ...S..  RDLGSS
IFN-ß                .TI..V..L. N.Y.INR.TG Y..N
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Fig. 1 (continued)

tertiary structure of type I IFN (Fig. 3) consists of five major α-helical segments designated A (sequence 4-21), B (51-67), C (80-92), D (115-131) and E (138-158), which are interconnected by loop regions. The core of the protein is formed by the 4-α helix (A, E, B and C) bundle. Helix D is attached to the core of the molecule by a hydrophobic interaction between Tyr 120 and Tyr 121 (helix D) and Trp 138 (helix E) (Senda *et al.*, 1992).

QUASI-QUARTERNARY STRUCTURE

POLYPEPTIDE DOMAIN I AND II

Hypothesis about the two-domain organization of type I IFN proposes a model, in which the three N-terminal α-helices A, B, C form the domain I and the remaining two helices D, E constitute a smaller domain II. The borderline between the supposed domains lies at the region of aa residues 100-110 (Ptitsyn *et al.*, 1985). Observations consistent with this hypothesis have emerged from a number of studies. Experiments with recombinant hybrid IFNs indicated functional quasi-independence of larger polypeptide segments. This approach was based on quantitative differences in heterospecific effects of parental IFN-α1 and IFN-α2 or IFN-αB (Pestka, 1983; Horisberger and De Staritzky, 1987). The hybrid IFNs exerted only that heterospecificity, which followed from a combination of respective biological properties of parental proteins. Such functional independence implies also structural independence of the respective polypeptide domains – e.g. after integration of parts of different IFNs, the full hybrid protein did not assume completely new molecular architecture, but retained the original structural elements of parental proteins. This assumption was also proved by immunological analysis of hybrid proteins, prepared by combination of the 64-66 N-terminal aa

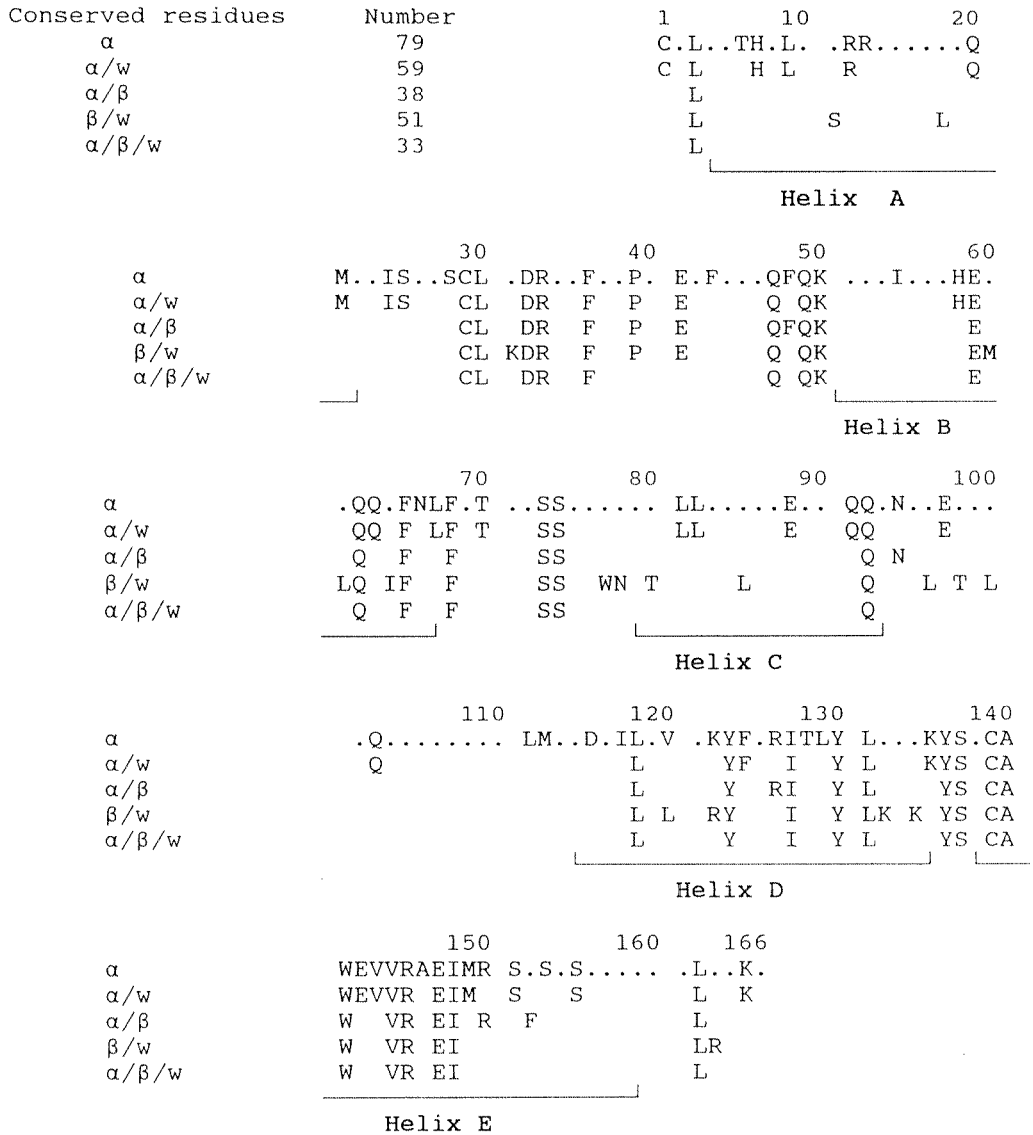

Fig. 2
Conserved position in human type I IFNs

and 101-108 C-terminal aa of IFN-α2 and IFN-ω (Adolf, 1990; Karayianni-Vasconcelos *et al.*, 1993). Neutralizing and immuno-enzymatic assays unambigously demonstrated that combined segments in hybrid molecules had an antigenic structure identical with the corresponding structure in the parental protein. Physico-chemical analysis of synthetic peptides corresponding to aa positions 24-81 and 111-166 of IFN-α1 found their secondary structures, particularly of the latter peptide, similar to their conformation in the native protein (Leist and Thomas, 1984). Many local distorsions in N- or C-terminal segment of IFN molecule did not cause major changes in the overall conformation of the protein. Large N-terminal deletions (21-29 aa) that inactivated IFN-α2 did not interfere with binding of NK2-antibody, recognizing C-terminal epitope at positions 112-130 (Edge *et al.*, 1986; Alexenko *et al.*, 1991). Analogically, replacements of aa in the region 31-48 of IFN-α2 greatly influenced the antiviral effect, but except the position 38 only minimally affected the NK2-reactivity (Shechter *et al.*, 1990). An analog of IFN-α2 with changed aa 30-33 and without receptor-binding ability, still specifically bound NK2-antibody. Replacement of aa 5, 27, 31 and 59 in IFN-α2 by corresponding aa of IFN-α1 led to N-terminal structural changes of the analog, resembling by activity IFN-α1, but its C-terminus still maintaining the specific structure of IFN-α2 (Edge *et al.*, 1986). Variation in the antigenic structure of

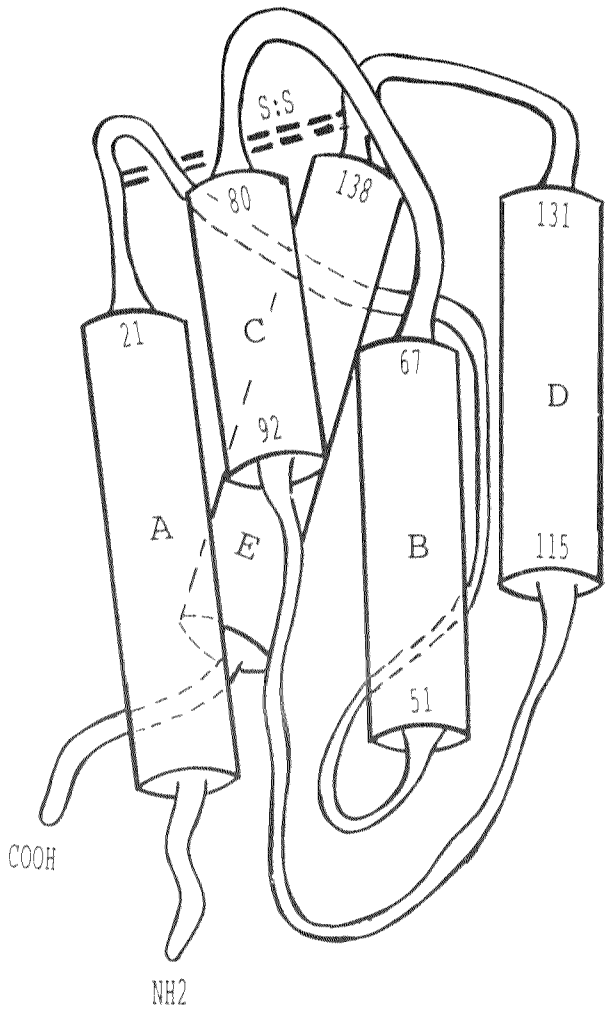

Fig. 3
3D - structure of type I IFN
Five α-helices, C- and N-termini of molecule are designed by letters (adapted according to Kontsek *et al.*, 1992).

IFN-α2 -subvariants resulting from replacement of aa 34 did not influence antigenic properties of the rest of molecule (Karayianni-Vasconcelos *et al.*, 1993). On the other side, there are indications that the aa segment 100-110 connecting both domains is structurally and functionally "silent". X-ray structural analysis of IFN-β found this region (loop CD) to be the most flexibile part of the molecule (Senda *et al.*, 1992). The aa segment 101-110 in IFN-α2 can be deleted, or the region 93-107 can be replaced by unrelated sequence from IFN-gamma with only little effect on specific activity or NK-2 reactivity (Edge *et al.*, 1986). Analysis of a set of IFN-α2 mutants revealed that the longest segment carrying biologically tolerated point mutations was bound by residues 80-110 (Petrenko *et al.*, 1987). The possible existence of two domains was experimentally supported also by the thermodynamic analysis of IFN-α2 (Zavyalov *et al.*, 1989).

DISULFIDE BONDS

The higher structural organization of IFN molecule is in accordance with the role of disulfide bonds in stabilizing the active conformation of IFN (Wetzel, 1981; Wetzel *et al.*, 1982*a*). The four Cys residues in positions 1, 29, 99 and 139 are conserved almost among all human type I IFNs. Two exceptions are IFN-α8a with Leu in position 99 and IFN-β with three Cys, but two of them are found in positions homologous to Cys 29 and Cys 139 of IFNs-α/ω. Disulfide bonds are formed between Cys residues 1-99 and 29-139 in IFN-α/ω, or 31-141 in IFN-β.

Structure-functional significance of these disulfide bridges in IFN is not equivalent. The homology between sequences of human type I IFNs indicated that the conserved disulfide bridge 29-139 could be more relevant. As it was experimentally demonstrated the Cys 1-Cys 29 bond lacks functional or structural role (Streuli *et al.*, 1980; Nisbet *et al.*, 1985; Edge *et al.*, 1986). The conformational integrity of the N-terminal domain is consistent with a fact that the "intradomain" disulfide bond 1-99 is not essential. In contrast, the integrity of Cys 29-Cys 139 bridge is critical for biological activity (Shepard *et al.*, 1981; Morehead *et al.*, 1984). Variants of IFN-α with one or both Cys 29 and Cys 139 substituted by another residue exerted a great reduction in specific antiviral activity. Substitution of Cys 29 by Ser reduced the activity of IFN-α1 to 6% of its original value (Nisbet *et al.*, 1985), IFN-α4 with Arg 29 showed only 0.2% of the original activity (Waine *et al.*, 1992) and IFN-α2 analog with both Cys in positions 29 and 129 replaced by Ser retained some 4% of the activity of the parental protein (Edge *et al.*, 1986). Available data suggest that the disulfide bond 29-139 is not essential for achieving a suitable tertiary structure of single N- or C-terminal domain, but is necessary for stabilization of the native molecule in bioactive (quarternary ?) configuration.

Considering the available experimental data we suppose that the quasi-quarternary structure of biologically active type I IFN consists of two structurally independent polypeptide domains involving residues 1-92 and 111-166 (172), sterically stabilized in an optimal configuration by the 29-129 disulfide bridge. The linking chain 93-110 seems to play a minimal structural and functional role.

CONFORMATIONAL VARIABILITY

The human IFN-α family is the main source to gain information on structural determination of its functional

variability. Despite the primary structures of single subtypes of IFN-α differ from each other only up to by 20%, they exhibit a considerable variability in many biological functions. The individual profile of a biological activity results from the interaction between the active surface of a particular IFN molecule and the receptor of target cells. Therefore just a subtle variability in primary structure among α-subtypes must be of much greater influence on the surface modelling of molecules. Immune system provides an optimal tool for defining of surface differences between proteins. An extent of changes in surface configuration between individual IFN-α subtypes is most complexly measured with specific polyclonal antisera. An antiserum characterizes the total immune response to the whole surface of the molecule and reflects the immunodominance of single antigenic sites. Antiserum to IFN-α1 neutralized IFN-α2 (with 83% aa homology) only with 3% of its efficiency to IFN-α1 and vice versa (Exley *et al.*, 1984). Using analogical bioassays Adolf (1987) reported that antiserum to IFN-α1 neutralized subtypes α2, α8, α10 and α21 (all with an about 80% sequential homology to IFN-α1) with an activity ranging only in 0.2 – 1.4% of its specific inhibiting capacity, and, antiserum to IFN-α2 showed only 0.4 – 3% of its specific neutralizing efficiency to subtypes α1, α8, α10 and α21.

In contrast to significant differences in total configuration of active surface, the overall conformation of individual polypeptides belonging to IFN-α family is not absolutely subtype-specific . The conformational originality of a respective subtype appears to be a result of specific combination of (a few) unique discrete structural elements. Therefore the same structural elements can be found, in another composition, also in molecules of other subtypes. For demonstration of this idea MoAbs are particularly suitable with their ability to recognize only one specific structure. It was observed that MoAbs raised to one IFN-α subtype always exert cross-reactivity when tested against a sufficiently large panel of other subtypes. It indicates that a particular epitope can be shared by several IFN-α subtypes, whereas the global antigenic structures of these IFNs are different. Common structural (antigenic) motifs were found in IFN-α1/α2 (Arnheiter *et al.*, 1981; Exley *et al.*, 1984; Meager and Burg, 1986), IFN-α1/α2/α4b/α6 (Tsukui *et al.*, 1986; Cheetham *et al.*, 1991), IFN-α2/α7, IFN-α2/αcon., IFN-α2/α7/αcon (Shearer *et al.*, 1984), IFN-α2/α88 (Andersson *et al.*, 1991). Antibody NK2 recognizes IFN-α2/α4a/α6/α8/α10/α14/α17 (Alexenko *et al.*, 1991; Sattayasai *et al.*, 1991). Alkan *et al.* (1985) analyzed the reactivity of 12 MoAbs with five IFN subtypes (IFN-α1, -α2, -α7, -α8 and -α21). They detected common antigenic structures on IFN-α1/α2/α7, IFN-α1/α8/α21, IFN-α1/α7/α8, and IFN-α2/α7/α8/α21. These experiments showed that despite individual common epitopes can be concurrently located on several IFNs, each IFN-α subtype possess its own epitope combination. Moreover, the same reactivity of MoAb with several subtypes in binding experiments does not mean that the common structures are also functionally identical, because such MoAb may differ in neutralizing activity against the different subtypes (Alkan *et al.*, 1985; Tsukui *et al.*, 1986).

CARBOHYDRATE CONTENT

Except the majority of IFN-α subtypes all other human type I IFNs are glycosylated. Sugar chains are bound to proteins by N- or O-glycosidic linkage. A tripeptid Asn-aa-Ser/Thr is required for the glycosylation of Asn residues. In the IFN-α family IFN-α14 has two such sites. IFN-ω, although with the highest structural similarity to IFN-α, contains glycosylation site in the aa position 78 correponding to localization of glycosylated Asp 80 in IFN-β. The analyzed preparations of natural IFN-β and IFN-w did not contain O-linked carbohydrates. The only demonstrable O-glycolysated type I IFN is IFN-α2 (Adolf *et al.*, 1991*b*). The carbohydrate moiety is not essential for expression of functions of IFN *in vitro* (see, e.g., Zoon and Wetzel, 1984; Adolf, 1987), but may be of some physiological importance for its activity *in vivo* (Bocci, 1983). The glycosylation increases the calculated molecular mass of type I IFNs by about 15 – 25%. Comparison of structures of natural and bacterial IFNs-β using physical methods (circular dichroism, nuclear magnetic resonance) did not reveal substantial conformational differences (Utsumi *et al.*, 1986). Immunologic analyses of the surface structures with polyclonal antisera found no dramatic differences in overall antigenic structure between glycosylated and unglycosylated IFN-β (Mark *et al.*, 1984; Colby *et al.*, 1984; Pestka, 1985). However, a more detailed mapping of surfaces with MoAbs allowed detection of local antigenic variability between glycosylated and unglycosylated forms of IFN-β (Sugi *et al.*, 1987; Redlich and Grossberg, 1989), but neither for IFN-ω (Adolf, 1990; Kontsek *et al.*, 1991*a*) or IFN-α2 (Kontseková *et al.*, 1992; Karayianni-Vasconcelos *et al.*, 1993). Linked carbohydrates probably only sterically interfere with binding of some antibodies to respective epitopes without causing conformational distorsions of the surface . It is in accordance with the same specific activities of natural and bacterial IFN-β, because they can hardly differ in active surfaces (Colby *et al.*, 1984).

LENGTH OF POLYPEPTIDE AND ACTIVITY

To assume a biologically functional conformation, the IFN polypeptide requires besides a proper aa sequence also appropriate length parameters. It is generally accepted that deletions of up to 10 aa residues from either end of IFN

molecule are tolerated without a substantial loss of its bioactivity. However, the specific activity of such truncated IFNs is mostly substantially reduced.

N-TERMINAL VARIATIONS

Deletion of 1 to 4 aa residues from N-terminus do not change antiviral, antiproliferative or immunomodulatory potency of IFNs. It was shown by recombinant truncated analogs of IFN-β (2-166), IFN-α2 (4-165), IFN-α2 (5-165) and by hybrid IFN-α2 (5-62)/α1 (64-166) (O'Rourke *et al.*, 1985; Edge *et al.*, 1986; Fish *et al.*, 1989). The correlation between length and function was studied in detail by Edge *et al.* (1986) . The replacement of segment 2-7 of IFN-α2 by completely unrelated residues 2-7 of human IFN-τ did not affect the activity of IFN-α2. Truncated IFN-α2 (10-165) and IFN-α2 (12-165) had hardly detectable antiviral effect, but owing to their low production in bacteria a definitive conclusion could not be drawn. All shorter IFN-α2 variants containing aa 14-165, 16-165 and 21-165 were biologically inactive. Devoid of antiviral effect was also analog IFN-α4 (29-165) (Cheetham *et al.*, 1991).

These findings suggest that destabilization of the biologically potent conformation of IFN is initiated by deletion of 8-9 N-terminal aa residues and after removal of further 4-5 residues the inactivation of IFN is completed.

On the other side, the extended N-terminus does not change the activity or antigenic properties of IFN, as demonstrated by IFN-α1 with 9 or 22 additional aa residues (Nisbet *et al.*, 1985; Masucci *et al.*, 1980), IFN-α2 longer by 4 or 18 residues (King *et al.*, 1983; Karayianni-Vasconcelos *et al.*, 1993) and IFN-ω extended by 2 residues (Adolf *et al.*, 1990).

C-TERMINAL VARIATIONS

Data on functional significance of the C-terminus of IFN are more controversial. Leukocyte IFN-α lacking last 10 aa residues (Levy *et al.*, 1981) and recombinant IFN-α2 (1-152) with 13 residues removed by proteolytic cleavage (Wetzel *et al.*, 1982*b*) had specific activity close to that of full-length protein. In contrast, C-terminaly truncated recombinant IFN-α2 variants shorter by 5, 10 and 11 residues exhibited 3-4 times lower antiviral activity than the complete molecule (Franke *et al.*, 1982; Edge *et al.*, 1986). IFN-α4 (1-158) with deletion of 8 C-terminal residues had reduced specific antiviral activity to 3% of unmodified IFN-α4 and after removal of further 7 residues the activity of IFN-α4 (1-151) was less than 0.2 % (Cheetham *et al.*, 1991). Also truncated IFN-α2 (1-151) exerted minimal effect on bovine cells (De Chiara *et al.*, 1986). IFN-α4 (1-141) with removed 25 C-terminal aa residues and IFN-α2 variants truncated in positions 138, 143, 147 and 149 lacked completely antiviral activity (De Chiara *et al.*, 1986; Edge *et al.*, 1986; Cheetham *et al.*, 1991). Recombinant IFN-ω (1-163) with deleted 9 residues from the C-terminus was biologically active (Adolf *et al.*, 1991*a*).

These studies indicate that reduction of biologic potency of type I IFNs is initiated by removing of 6-7 C-terminal aa residues , it progressively increases by deletion of further residues and a deletion of more than 15-16 residues leads a to total loss of biological activity.

INTERNAL DELETIONS

Considering the biological activity of IFN-α2 with deleted Asp 44 it is obvious that this deletion would occur other IFNs also without pronounced structural or functional changes. The additional insertion of Asp in position 44 in IFN-α2 was without biological effect (Edge *et al.*, 1986). Of interest is observation that a decapeptid 102-111 in IFN-α2 could be deleted without abolishing the bioactivity (De Maeyer and De Maeyer-Guignard, 1988).

INTERFERON FRAGMENTS

There is a general opinion, based on studies with proteolytic or synthetic peptides, that fragments of human IFNs are biologically inactive. Even in a high concentration, they were unable to elicit antiviral effect in human or bovine cells, as demonstrated with synthetic IFN-α1 peptides corresponding to residues 1-81, 24-81, 71-166 and 111-166 (Leist and Thomas, 1984), single proteolytic IFN-α2 fragments 1-15, 60-110, 22-58, 112-148 and 149-165 or their mixtures (Wetzel *et al.*, 1982*a*; Lydon *et al.*, 1985), synthetic IFN-α2 peptides from regions 30-36, 132-137, 124-144, 124-138 and 129-144 (Borecký *et al.*, 1985; Shevalier *et al.*, 1990) and synthetic IFN-β fragments 32-47, 40-56, 32-56 and 118-126 (Redlich *et al.*, 1991).

More than the bioactivity we could expect from some fragments the ability of binding to cellular IFN-receptors. Synthetic peptides, in particular, are frequently so designed, that they span the sequences contributing to the formation of receptor recognition site of IFN. Neither this assumption was experimentally confirmed. Pretreatment of cells with proteolytic or synthetic IFN-α2 peptides 1-15, 22-58, 60-110, 105-125, 112-148 and 124-144 (Wetzel *et al.*, 1982*a*; Lydon *et al.*, 1985; Trown *et al.*, 1985; Shevalier *et al.*, 1990), or IFN-β peptides 32-47 and 40-56 (Redlich *et al.*, 1991) did not reduce antiviral effect of the parental intact IFNs.

However, IFN-fragments can possess antigenic and immunogenic properties, which are in some extent similar to those of full-length proteins. This was demonstrated by the reactivity of fragments with antibodies raised against com-

plete IFNs or by the possibility to exploit peptides for preparation of antibodies which react with an intact IFN molecule. Polyclonal antibodies elicited against IFN fragments provide another evidence that 3D structure of peptides usually differs from the conformation of the corresponding region in intact polypeptide. Such antisera might react specifically with partially denaturated IFNs (in immunochemical assays) as well, but they did not bind (neutralize) IFN in its native conformation, even several peptides were derived from the epitopes recognized by IFN-neutralizing antibodies. Such reactivity showed antisera to the synthetic IFN-α1 fragments 99-111 and 111-166 (Arnheiter *et al.*, 1981; Sattayasai *et al.*, 1988), IFN-α4 peptides 37-50 and 142-151 (Sattayasai *et al.*, 1988, 1991), or sera against fragments 1-21, 18-45, 32-47 and 40-56 of IFN-β (Chow *et al.*, 1984; Redlich *et al.*, 1991). However, the probability should not be ruled out that the small population of peptides can assume conformation which is identical with a "native" one resulting thus in raising of an adequatelly frequent population of neutralizing antibodies. It is our explanation for isolation of a MoAb against synthetic IFN-α2 peptide 133-147, which neutralized antiviral activity of IFN-α2 (Barasoain *et al.*, 1989). Noteworthy is also the neutralizing ability of antisera against synthetic IFN-α2 peptides derived almost from identical region 124-138 and 129-144 (Shevalier *et al.*, 1990).

In contrast with data indicating lack of biological effects of IFN fragments are results of few studies. Two reports from the same laboratory (Ackerman *et al.*, 1984; Attalah *et al.*, 1987) describe the residual antiviral, antiproliferative and immunomodulatory activities of the 110 aa long N-terminal fragment, obtained by proteolytic digestion of IFN-α2, on human cells. This discrepancy might be explained by contamination of the N-terminal fragment preparation with undigested IFN. The synthetic IFN-α2 peptides 69-80 displayed weak antiproliferative activity, but significantly increased antiviral activity of IFN-α1 and IFN-α2 (Eichmann *et al.*, 1990). The same group found that the synthetic octapeptide corresponding to aa residues 131-138 of human IFN-α2 competed with human IFN-α2 for receptor on mouse thymocytes. Moreover, the octapeptide having no antiviral activity showed some immunomodulatory function and in presence of concanavalin A,it induced blastic transformation of mouse thymocytes (Zavyalov *et al.*, 1991). Finally, synthetic C-terminal fragment 124-138 of human IFN-α2 inhibited mitogen-induced proliferation of human blood cells (Danilkovich *et al.*, 1991).

RECEPTOR-BINDING SITE

The fact that all type I IFNs recognize identical cellular receptor implies a common structural framework of corresponding IFN-receptor-binding domains. It is postulated that aa that are conserved in sequences of type I IFNs signalize their potentional importance for structure and/or function of these proteins. Among human type I IFNs invariant aa residues occupy 33 positions, most of them clustered in regions 29-50 (10 residues), 59-68 (4 residues) and 118-148(14 residues) (Fig. 2). Generally, sites for molecular recognition of proteins are formed by hydrophilic loops and turns located on the surface of polypeptide. Therefore also the receptor-binding domains of IFN should comprise such hydrophylic blocks. The highly hydrophilic segments of human type I IFNs are spanned by residues 31-51, 69-79 and 121-145 (Zavyalov and Denesyuk, 1982). The localization of conserved and hydrophylic segments coincides in two regions – in the N-terminal part of 29-50 molecule, or around residues 120-145 at the C-terminus. In 3D model of type I IFN sequences 29-50 and 120-145 are in close spatial proximity. The N-terminal segment 29-50 is comprised in the loop AB which has a relatively rigid conformation and surface location. The adjacent antiparallel C-terminal sequence 120-145 forms partially the helices D and E, which stabilize the conformation of the loop DE (Fig. 4). Both these domains define a fairly large spatially continuous area, which most likely represents the contact site for interaction with receptor (Senda *et al.*, 1992).

N-TERMINAL BINDING SEGMENT

Loop AB (aa 22-50)

The analysis of bioactivity of the analogs of human IFN-α2, α4 and β made by site-directed mutagenesis showed that critical substitutions of the conserved aa over the region 26-49 involved (except of a previously analyzed Cys 29) Leu 30, Arg 33 and Phe 36 (Edge *et al.*, 1986; Petrenko *et al.*, 1987; Tymms *et al.*, 1989; Waine *et al.*, 1992). The single replacements in indicated positions reduced the initial antiviral or antiproliferative activity of IFNs on human cells by more than 100-fold. Substitutions of conserved Asp 32 and Glu 41 only slightly decreased the activity of IFN-α4 on human cells, however the position 41(consensus 39) was critical for the bioactivity of IFN-β (Stewart *et al.*, 1987). The conserved Glu 49 was without effect on the antiviral or antiproliferative activity of IFN-α2 (Valenzuela *et al.*, 1985; Shechter *et al.*, 1990). Among residues in variable positions , as critical for antiviral activity was found the substitution of Phe 38 in IFN-α2 (Shechter *et al.*, 1990) and Glu 42 (consensus 40) in IFN-β (Stewart *et al.*, 1987). A considerable reduction in antiviral activity was found after substitution of non-conserved residues in position 31 (IFN-α4), 40 and 42 (IFN-α2 and α4) , whereas single replacements in positions 26, 27, 34, 35 and 37 in

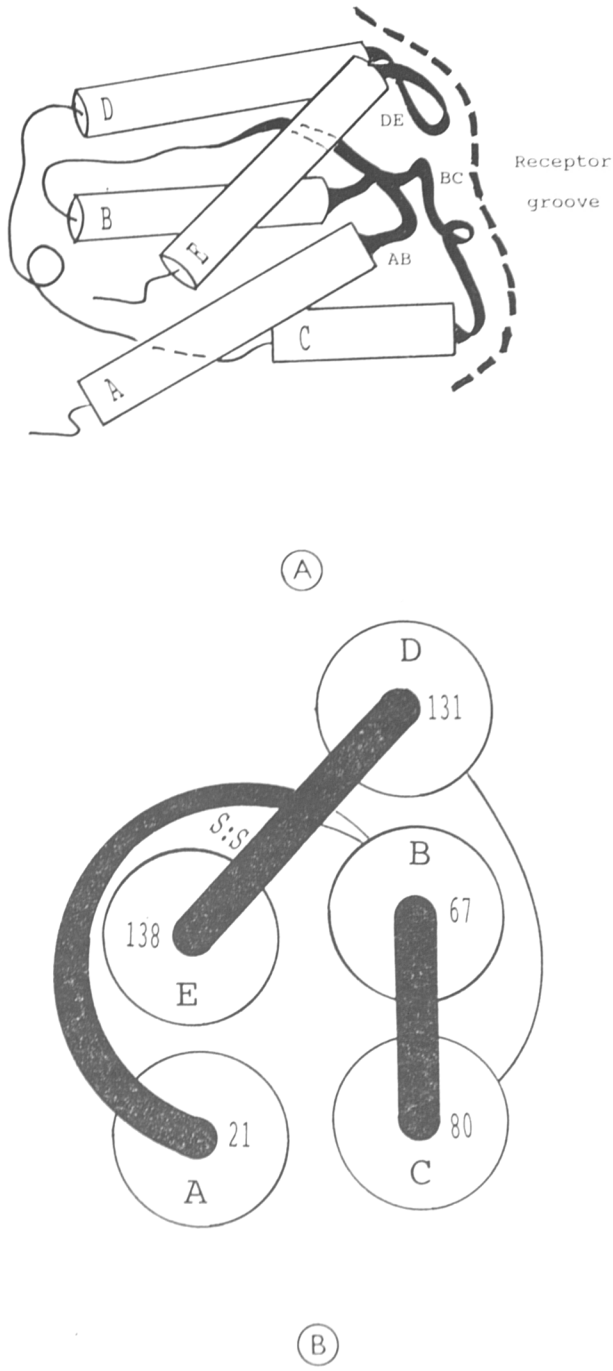

Fig. 4
Interaction of type I IFN with receptor groove
A – frontal view, B – vertical view. The segments of molecule that contribute to formation and stabilisation of the receptor binding site are marked (adapted according to Kontsek *et al.*, 1991*c*, 1992).

IFN-α4, or in positions 45 and 48 in IFN-α2, had only minor or insignificant effects on antiviral acitivity in human cells (Shechter *et al.*, 1990; Waine *et al.*, 1992). Residues Ser 27 and Met 31 in particular, significantly contributed to low specific activity of IFN-α1 on human cells (Edge *et al.*, 1986). The integrity of the chain encompassing the conserved residues 30, 32 , 33 and 36 appears to be extremely critical for the binding and bioactivity of IFN-α on human cells, but just critical for its biological activity on bovine cells (Marcucci and De Maeyer, 1986). This region seems to be differently involved in the interaction of IFN-α with human or bovine IFN-receptor. Also single residue replacements in positions 30, 31, 32, 33 and 36 of IFN-α2 and IFN-α4 produced an apparent drop in antiviral activity on human cells, in comparison with relative stability of antiviral effects on bovine cells. A substitution in the variable position 34 in IFN-α2c (Arg 34) resulted not only in the local modification of antigenic structure (Karayianni-Vasconcelos *et al.*, 1993), but also decreased specific activity in human cells (von Gabain *et al.*, 1990). In the N-terminal segment 10-44 of human IFN-αJI there were identified residues 42-44 as significant for binding with receptor on bovine and human cells, among them Phe 43 conserved in IFN-α (Shafferman *et al.*, 1987).

Immunological study of type I IFNs, as well, allowed to identify in the N-terminal binding domain biologically relevant antigenic sites. The immunodominant epitopes of neutralizing MoAbs were mapped into region 30-38 in IFN-α1 and 30-35 or 36-41 in IFN-α2 (Kontsek *et al.*, 1991*c*; 1991*d*; 1992). Antibodies recognizing structure (residues 30-41) inhibited binding of IFN-α2 on human and bovine cells, while antibodies to the corresponding region of IFN-α1 lacked this ability. The distal part of the loop AB probably contributes also to the direct interaction of IFN-α2 with receptor. Conservative alteration of Arg with Lys in position 23 in IFN-α2a/b did not affect its biologic potency, but modified antigenic structure in the N-terminal domain (Meager and Burg, 1986; Siemers *et al.*, 1988). The neutralizing antibodies whose epitopes contained Arg 23 inhibited binding of IFN-α2b to human cells (Siemers *et al.*, 1988; Kandefer-Szerszen and Lundgren, 1992), and the same effect had also MoAb to the N-terminal half (1-91) of IFN-α2a (Lim and Langer, 1993). For binding of neutralizing antibodies to an antigenic structure located in the N-terminal segment 32-56 of IFN-β there were important residues 41-43 with conserved Pro 41(consensus 39) and Glu 43(consensus 41) (Redlich *et al.*, 1991).

In the loop AB the sequence 30-41 in particular, seems to be critical for structural integrity and function of the N-terminal binding domain. The region 23-28 appears to be involved in the formation of functional binding site too, while the region 42-50 could be structurally and functionally less relevant. Helix A might play probably a stabilizing role in the interaction between loop AB and receptor. The N-terminal truncations of IFN-α2 molecule comprising more than 10 aa residues, or impairment of hydrophilic

cluster in the region 10-15 caused dramatic loss in activity (Edge *et al.*, 1986). Consistent with this is also lack of antiviral activity of IFN-α4 with deleted segment 1-28 on human and bovine cells (Cheetham *et al.*, 1991).

Loop BC (aa 68-78)

More controversial is contribution of the loop BC (68-78) to the interaction with receptor. This structure in type I IFNs, although strongly hydrophilic, is not highly conservative. Senda *et al.* (1990) have implicated this loop for receptor binding because of its spatial proximity with loops AB and DE, but later, after an extensive literature survey they disclaimed this assumption (Senda *et al.*, 1992). However, recent experimental knowledge on architecture of the N-terminal binding domain follows mainly from analysis of IFN-α2 and IFN-α4. Also our study with IFN-α2 has found limited contribution of the region 43-85 for eliciting of bioactivity (Kontsek *et al.*, 1993). In contrast, the structure-function study of IFN-α1 revealed that MoAbs recognizing sequence 63-67 inhibited binding of IFN-α1 to bovine and human cells. It is plausible that although the conserved segment 30-41 maintains the proper conformation of N-terminal domain, various IFNs might interact with receptor also troughout different parts of this domain, as it has been demonstrated e.g. for IFN-αJI. Localization of the two immunodominant structures of IFN-α1 and IFN-α2 showed that both molecules have one large antigenic structure in the region 30-40, but they differ in location of the other. The second immunodominant site contains in IFN-α1 residues 63-85, while in IFN-α2 residues 112-148 (Kontsek *et al.*, 1991*c*, 1992).

Differences in spatial configuration between regions directly involved in the interaction of IFN-α1 and IFN-α2 with human receptor are supported also by other experimental evidence. The binding and specific activity of IFN-α1 in human cells are about 100-fold lower than with IFN-α2, while the binding affinity and activity of both IFNs on bovine cells are equal. However, human cells expressing the gene for the bovine IFN-receptor recognized IFN-α1 as a subtype with high specific activity (Mouchel-Vielh *et al.*, 1992). IFN-α2 is supposed to bind bovine receptor through the region 62-122 (Raj *et al.*, 1986). Moreover, synergism in antiviral effect between IFN-α1 and IFN-α2 was observed on human cells but not on bovine cells (Revel, 1983). All these data support a hypothesis that IFN-α1 binds to human and bovine cells via the loop BC, whereas receptor interaction of IFN-α2 with human cells is mediated by the loop AB and with bovine cells by the loop BC. However, the segment 63-86 present in IFN-α2 molecule is not "silent" from the view of activity on human cells. The hybrid IFN-α2/α1/α2 with only three residues specific for IFN-α1 in positions 69, 80 and 86, had 7-times decreased specific activity on human cells as compared to IFN-α2 (Wetzel, 1981). The drop in activity could be induced only by Thr 69 and Asp 80 since substitution of Ser 86 by Cys in IFN-α4 (with an about the same activity on human cells as for IFN-α2) did not influence its antiviral effect on human cells (Cheetham *et al.*, 1991). The mutation in position 74 resulted in a loss of antiviral activity of IFN-α2 on human cells (Petrenko *et al.*, 1987). Also we have mapped epitopes of two antibodies neutralizing antiviral and antiproliferative effects of IFN-α2 into regions 63-76 and 77-85 (Kontsek *et al.*, 1993).

The data presented above indicate, that the spatial arrangement of the hydrophilic sequence 68-78 might form a part of an active site in the type I IFN , which determines the high-affinity binding to the human cellular receptor (Fig. 4B).

CARBOXY-TERMINAL BINDING SEGMENT

The segment 120-145 is comprised of two clusters of conserved hydrophilic aa. The one forms hydrophilic surface of the helix D from Tyr 123 to Leu 131, and the other determines hydrophilic surface of the helix E in region 139-145. It is hypothesized that both convergent rigid helices stabilize the conformation of the hydrophilic loop DE (Zavyalov *et al.*, 1990; Fish, 1992).

Helix D

Specific aa substitutions in helix D were criticial for activity of human IFN-α1, α2 and α4 on human and mouse cells, but they affected much less their biological potency on bovine cells. Among substitutions in the sequence 121-124 of IFN-α1 and IFN-α4 the most pronounced reduction (100-fold) in antiviral and antiproliferative action resulted from substitutions of Tyr 123, less effective were replacements of adjacent residues 121 or 122.

The variability in antiviral and antiproliferative potency of IFN-α1 with substitued Tyr 123 was proportional to a change in binding to human but not to bovine cells (Mc Innes *et al.*, 1989). Replacements in positions 121,122 and 123 in IFN-α4 had additive negative effect, and an analog with triple substitution exerted an antiviral activity reduced by more than 1000-times, as well on human as on bovine cells (Cheetham *et al.*, 1991). Conservative replacement of Arg 121 by Lys increased twice the potency of IFN-α4 on human cells (Cheetham *et al.*, 1991). Such potentiation of the activity was surprising, since IFN-α1 with Lys 121 exerts a relatively low specific activity on human cells. Double aa substitution involving position 126 and conserved Ile 127 reduced only slightly the activity of IFN-α2 on human cells (Edge *et al.*, 1986). In the region 130-135 as critical for biologic activity of IFN-α were found conserved residues Tyr 130 and Leu 131. IFN-α2 with double

replacement in these positions was inactive. Radical substitutions in single positions 130 and 131 abrogated antiviral activity of IFN-α4 on both human and bovine cells (Cheetham *et al.*, 1991) and the substitution of Leu 131 diminished also its antiproliferative effect (Tymms *et al.*, 1990). Replacements of variable residues in positions 132 and 133 of IFN-α4, and in positions 134 and 135 of IFN-α1 and IFN-α4 had relatively small or none effect on antiviral potency on human or bovine cells (Tymms *et al.*, 1989; Cheetham *et al.*, 1991).

MoAb NK2 allowed identification of a large immunodominant epitope in IFN-α2 (residues 112-130) (Edge *et al.*, 1986; Alexeenko *et al.*, 1991), structurally corresponding to helix D and adjacent part of the loop CD. For NK2-epitope there are critical positions 114, 116, 119 and 121. NK2 bounds IFN subtypes α2, α4a, α6, α8, α10, α14 and α17 but did not react with subtypes α1, α4b, α5, α7 and α21 (Alexenko *et al.*, 1991; Cheetham *et al.*, 1991; Sattayasai *et al.*, 1991). It is of interest that two subvariants of IFN-α4 are antigenically distinct in this epitope because of the substitution Glu(4a) 114 Val(4b).

Antibodies recognizing NK2-epitope or close antigenic sites neutralize the antiviral, antiproliferative and immunomodulatory activities of IFN-α2 (see Cebrian *et al.*, 1987; Kontsek *et al.*, 1991*c*). The ability of these antibodies to inhibit the receptor-binding was not determined unambigously, that might be caused by the used antibody concentration. Neither Cebrian *et al.* (1987) nor Kontsek *et al.* (1991*c*) observed significant effect of such antibodies in concentration 1 μg/ml on cellular binding of IFN (antibodies to the N-terminal binding domain at the same concentration inhibited the IFN- receptor interaction). Whittall *et al.* (1984) inhibited binding of IFN-α2 to human cells using NK2 in concentration 20 μg/ml. Also Kandefer-Szerszen and Lundgren (1992) reported a decrease in IFN-α2 binding to human cells with one antibody to the N-terminus and two others (NK2 and NK2-like) to the C-terminus of IFN. However, to achieve the same inhibition, the C-terminus-specific antibodies had to be used in 10-times higher concentration than the N-terminus-specific antibody. These data are consistent with a hypothesis that the C-terminal part of IFN molecule is involved in the interaction with the low-affinity receptor (Streuli *et al.*, 1981). Therefore also a low amount of antibody specific for N-terminus (responsible for the high-affinity interaction of IFN with receptor) significantly inhibited cellular binding of IFN, whereas detection of inhibition of low-affinity binding required an additive effect of a substantially higher number of C-terminus-specific antibody molecules.

Helix E

The sequence 136-148 with 9 invariant positions is superconserved in type I IFN. Similarly to N-terminal helix A, also helix E apparently influences the receptor binding. Helix E is implicated to maintain orientation of segments that are linked by the disulfide bond 29-139 (Cheetham *et al.*, 1991). This helix is suggested to be distant from the receptor-binding surface, since non-neutralizing MoAb with the epitope located in region 151-166 recognized IFN-α2 bound to the bovine receptor (Arnheiter *et al.*, 1983). However it should be pointed out that the contact epitopes of IFN-α2 for human and bovine receptors are different. We assume that beside some stabilizing role, at least the proximal part of helix E could interact directly with the receptor. Hydrophilic region 138-145 has surface location because the sequence 139-149 in IFN-α2 is highly susceptible to proteolytic cleavage (Kostrov *et al.*, 1985). Furthermore changes in positions 146 and 151 in IFN-α2 (Petrenko *et al.*, 1987) and in conserved positions 139, 140, 147 and 150 in IFN-β (Stewart *et al.*, 1987) diminished or greatly reduced their biological potency. Also the impairment of integrity of helix E by the C-terminal shortening of IFN-α molecules was associated with the progressive loss of biological effectivness (see paragraph *C-terminal variations*).

Loop DE

The loop DE is from the structural and evolutional point of view an extremely remarkable region of human type I IFN. The loop represents the shortest interhelical segment in the molecule spanned approximately by residues 132-138. In spite that it corresponds to the region with maximal hydrophilicity in IFN-α2 and IFN-ω, this is not reflected in immunodominant properties of this structure. Fish (1992) supposed this loop serving as recognition epitope for interaction with (low-affinity) cell receptor. Zavyalov *et al.* (1991), following binding activities of IFN-α2 peptides to mouse thymocytes, deduced as critical for receptor interaction the sequence 136-138 (Tyr-Ser-Pro) with Tyr 136 and Ser 137 conserved among human type I IFNs. The region 132-137 is a constituent of the unique evolutionary conserved epitope which, until now, represents the only evidence of an antigenic homology between human IFN-α and IFN-β (Kontsek *et al.*, 1990*a*). This common epitope, recognized by antibody B6, is conformational. The antibody B6 did not neutralize IFN-α1 even when its sequential homology with IFN-α2 in the part 132-137 is higher than homology of corresponding parts between IFN-α2 and IFN-β. It follows from Table 3, that Lys 132 can be critical for spatial arrangement of the antigenic structure recognized by antibody B6. We propose the other Lys in position 31 as an important component of the conformational B6-epitope, because in this position the subtypes α1 and α2 are also different, but IFN-α2 and IFN-β are identical. In 3D model of type I IFN are three lysine residues in positions 31, 132 and 134 spatially close each other. Moreover, the confor-

Table 3. Comparison of amino acid sequences of selected IFNs in parts supposed as critical for B6-epitope

Consensus position	31	.....	132	133	134	135	136	137
IFN-α1	Met	.....	Thr	Glu	Lys	Lys	Tyr	Ser
IFN-α2	Lys	.....	Lys	Glu	Lys	Lys	Tyr	Ser
IFN-ω	Lys	.....	Lys	Glu	Lys	Lys	Tyr	Ser
IFN-β	Lys	.....	Lys	Ala	Lys	Glu	Tyr	Ser
Conserved aa	Lys	.....	Lys	.	Lys		Tyr	Ser

mation of site B6 in IFN-α2 is acidolabile, and pH 2, though unaffecting the biological activity of IFN-α2, it abrogated reactivity of IFN-α2 with antibody B6 (Kontsek *et al.*, 1990*b*). The physico-chemical causality of the pH 2-induced conformational changes of IFN-α2 molecule might reside in above mentioned cluster of 3 basic residues 31, 132 and 134. Such basic cluster can be destabilized at low pH, because at abnormally low pH Lys or Tyr residues in IFN-α2 were found to ionize (Shire, 1982). Thus, induced changes in the contact area between N- and C-terminal domains might alter the configuration of molecular surface. It is noteworthy that IFN-ω, in positions 31 and 132-137 identical with IFN-α2, was partially inactivated at pH 2 (Kontsek *et al.*, 1991*a*).

Considering the presented theoretical and experimental data we draw the following conclusion. The binding area in human type I IFN represents a multi-domain complex which consists at least of 4 segments: loop AB (23-41), loop BC (68-78), helix D(120-131) and loop DE (132-138/145). The N-terminal part 23-78 determines the high-affinity binding with receptor and the C-terminal region 120-145 is involved in the low-affinity receptor binding. In spite that these 4 segments constitute a spatially continuous area, there exists a possibility that this area could be a combination of more distinct binding sites (Senda *et al.*, 1992).

MUTUALITY OF BIOLOGICAL EFFECTS

The manifestation of antiviral, antiproliferative and immunomodulatory activities reflects an intrinsic biological potency of IFN. Mutual correlation between these 3 fundamental effects was demonstrated with recombinant IFNs and their analogs, as well as by use of neutralizing MoAbs. Analysis of biological potency of 60 analogs of IFN-α2 showed no significant separation of antiviral from antiproliferative or NK-stimulating activities. The extent of antiviral activity reflected also activities in the remaining two assays (Edge *et al.*, 1986). The only exception was truncated IFN-α2 (4-155), which as compared to the full-lenght protein required 10-times more antiviral units to produce the same antiproliferative effect. Waine *et al.* (1992), analysing the region 26-37 in IFN-α4, observed that changes in antiviral activity on human cells were accompanied by similar changes in antiproliferative activity. Similarly, aa replacements in the segment 121-135 in IFN-α1 and IFN-α4 produced shifts in antiviral activity which were proportional to changes in antiproliferative effect (Tymms *et al.*, 1989, 1990). Furthermore, also studies with neutralizing antibodies are mostly consistent with the idea that inhibition of one activity of IFN simultaneously neutralizes its other effects (Thompson *et al.*, 1986; Cebrián *et al.*, 1987; Siemers *et al.*, 1988; Kontsek *et al.*, 1989*a*, 1989*b*, 1991*c*, 1992*a*; Redlich and Grossberg, 1989).

The extent of the high-affinity binding to receptor on human cells correlated with antiviral and antiproliferative activities of IFN-α subtypes (Aguet *et al.*, 1984; Fish *et al.*, 1989). The most potent in this respect were IFN-αcon1, IFN-α2 and IFN-α7, the lowest activity exerted IFN-α1, IFN-α21 and IFN-α10. However, the relative ratio between antiviral and antiproliferative potency of individual IFN-α subtypes is different, thus indicating that both activities are not generated within the identical molecular segments (Pestka, 1983). Detailed quantitative analysis of antiviral and antiproliferative actions of the hybrid IFN- α2 (5-62)/α1 (64-166) indicated that the antiproliferative activity could be determined by the low-affinity binding of C-terminal domain (Le *et al.*, 1988). This is in accord with the finding that IFN-α2 (4-155), truncated at the N-terminus by only three and at the C-terminus even by ten residues, exerted in comparison to full-length protein almost unreduced antiviral but significantly reduced antiproliferative potency (Edge *et al.*, 1986). The contribution of single polypeptide segments of IFN to generation of a particular effect is different, as supported also by the immunomodulatory study on IFN-α. The aa replacements at positions 69, 80, 96 in IFN-α2 by residues from IFN-α1 resulted in a relatively greater decrease in NK-boosting potency than in antiviral or antiproliferative activity (Pestka, 1983). The binding to human cells correlated concurrently with antiviral, antiproliferative and immunomodulatory effects of IFN-α2, α8 and α21. However, IFN-α1 and α10, with low antiviral and antiproliferative potency, according to the strong NK-activity boosting potency surprisingly resembled subtypes IFN-α2 and α8. In contrast, IFN-α7 with high binding, antiviral and antiproliferative activity on human cells showed only minimal (but not abrogated) potency of boosting of NK activity (Ortaldo *et al.*, 1984).

In general, appart from evidence about the different contribution of various molecular segments to a particular biological effect, the absolute separation of a single activity of type I IFNs has not been observed. Of course, there are

also few exceptions. Barasoain et al. (1989) raised MoAb against synthetic peptide corresponding to the conserved region of IFN-α2 133-142. The antibody neutralized antiviral effect of leukocyte IFN-α (immunoaffinity-purified on NK2-antibody) without affecting its antiproliferative activity. In contrast, Zavyalov *et al.* (1991) demonstrated the immunomodulatory activity of a nearly identical IFN-α2 fragment 131-138 without inducing antiviral protection.

Viewed together, these data allow us to infere that the binding area of biologically active IFN, when docks in the receptor groove on the target cell, triggers simultaneously all basic biological effects of IFN. Specifically modelled surface of binding site of particular IFN governs only the expression level of individual activities in target cell. It seems to be impossible to eliminate selectively and totally only some of basal effects of IFN, however, there is possibility to modulate (suppress or potentiate) manifestation of a particular activity in relation to remaining effects.

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