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THE INTRINSIC FLUORESCENCE OF THE RECOMBINANT HUMAN LEUKOCYTE INTERFERON-αA AND FIBROBLAST INTERFERON-β1

S.I.Borukhov* and A.Ya.Strongin

Institute of Molecular Genetics, USSR Academy of Sciences, Kurchatov sq., Moscow 123182, USSR

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SUMMARY: The environment of Trp residues of the recombinant human interferons has been studied by the analysis of the emission spectra of native and denatured proteins at pH 1,5-8,5 and temperature 10-65°C in the presence and absence of the anionic, cationic and neutral to charge contact quenchers - KI, CsCl and acrylamide, respectively. The obtained data allow to suppose that in IFN-αA and IFN-β1 Trp_{141} interacts with Arg_{145} and one or several from the following residues - Phe_{124}, Ile_{127}, Tyr_{130}, Leu_{131}, whereas Trp_{77} - with Arg_{33} and Phe_{36}, Phe_{78}, Leu_{81} or Leu_{82} (Ile_{81} or Val_{82} for IFN-β1).

Recombinant human leukocyte and fibroblast interferons (IFN-αA and IFN-β1, respectively) belong to the group of proteins studied most intensively during the last decade (1, 2). Nevertheless, the tertiary structure of IFNs is still unknown that complicates understanding of the mechanism of their immunoregulatory, antiviral and antiproliferative activity and interaction with cell surface receptors. Certain amino acid residues of IFN polypeptide chain or rather short sequence regions of importance were identified within the protein molecule using site-directed mutagenasis (3, 4), protein chemical modification (5), immunochemical mapping (6, 7), constructing of hybrid IFNs (8) etc. Recently we have reported that the modification of one or two Trp residues led to the complete inactivation of antiviral activity of IFN-αA (Trp_{77}

*To whom correspondence should be addressed. Present address: Dept. of Microbiology, College of Physicians and Surgeons of Columbia University, New York, New York 10032.

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and Trp_{141}) and IFN-β1 (Trp_{77} and Trp_{141}; residues are aligned according to IFN-αA sequence) (9). It indicates, that Trp residues or the residues of their microenvironmemt might play an important role in the mechanism of IFN antiviral action.

In the present paper we describe the study of intrinsic fluorescence of Trp residues of IFNs, that allows to predict more or less precisely the residues of Trp microenvironment.

MATERIALS & METHODS Electrophoretically pure IFN-αA (antiviral specific activity $2x10^8$ IU/mg) and IFN-β1 (antiviral sp.act. $5x10^7$ IU/mg) were purified from recombinant Pseudomonas sp. and E.coli strains, respectively. Monomeric form of IFN-αA was further isolated as described in (10) and contained no reduced or oligomeric forms detectable by SDS-PAGE.

Protein concentration was determined spectrophotometrically, using the extinction coefficient at 280 nm=1,0 and 1,75 for 0,1% water solutions of IFN-αA and IFN-β1, respectively.

The fluorescence spectra of IFN-αA and IFN-β1 (0,02-0,1 mg/ml) were measured on Shimadzu RF-540 spectrofluorometer with a thermostated cell holder using the excitation wavelength 290 nm with spectral bandwidth of 2 nm for both excitation and emission monochromators. The quenching experiments were carried out at 25°C in the presence of 10-250 mM KI, CsCl and acrylamide as anionic, cationic and nonionic quenchers, respectively. The data analysis and the interpretation of the spectra were performed according to (11).

RESULTS

IFN-αA contains two Trp residues (NN77 and 141) per molecule, IFN-β1 - three Trp (an additional Trp residue is located at the position N20) (2). Fig. 1 shows the intrinsic fluorescence spectra of the electrophoretically pure IFN-αA (0,1 mg/ml; 5,2

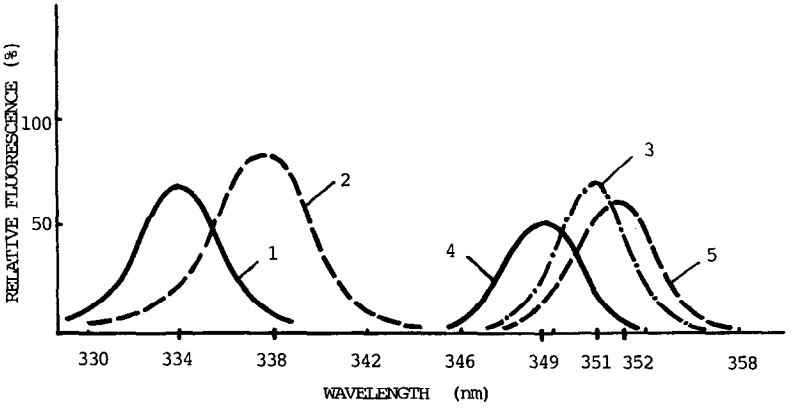

Fig. 1. Emission spectra of IFN-αA (1, 4), IFN-β1 (2, 5) and free Trp (3). The concentrations are 5,2, 5,2 and 10,5 μM, respectively. 1, 2, 3 - 0,1M Na-phosphate buffer, pH 7,2, 4, 5 - 0,1 M Na-phosphate buffer, pH 7,2, containing 7 M guanidium hydrochloride.

μM) and IFN-β1 (0,1 mg/ml: 5,2 μM) in 0,1 M Na-phosphate buffer, pH 7,2, the emission maximum (λ_{max}) values of these proteins being equalled to 334 and 338 nm, respectively. Under the same experimental conditions 10,5 mM solution of free Trp possesses the same emission intensity, the λ_{max} value being equalled to 351 nm. It shows that Trp residues within the IFN molecules are in highly hydrophobic environment. Moreover, when the temperature of protein solution is increased from 10° to 65°C the intensity of IFN-αA fluorescence monotonously decreases without any change of λ_{max}, equalled to 334 nm. These results suppose the presence of polar or charged micro-environment of Trp residues in IFN-αA. The treatment of IFN-αA and IFN-β1 with 7 M guanidium hydrochloride, causing the complete denaturation of proteins leads to shifting of the λ_{max} value to 349 and 352 nm, respectively, indicating that Trp residues in IFNs become accessible to solvent and more resemble free Trp (Fig. 1).

The decrease of the pH of protein solution from 8,5 to 3,2 does not practically effect the emission spectra of IFNs, since within the pH 3,2-2,7 IFN-αA in contrast to IFN-β1 is characterized with 40-45% increase of the fluorescence intensity (Fig. 2), λ_{max} being shifted from 334 to 336 nm. Evidently, carboxy group with the pK value about 3 (presumably, from Asp residue) is located nearby one or both Trp residues in IFN-αA.

Further the microenvironment of Trp residues was studied in water solutions at pH 2,0, 7,2 and 8,5 with KI, CsCl and acrylamide as anionic, cationic and neutral to charge contact quenchers, respectively, in the presence and absence of organic

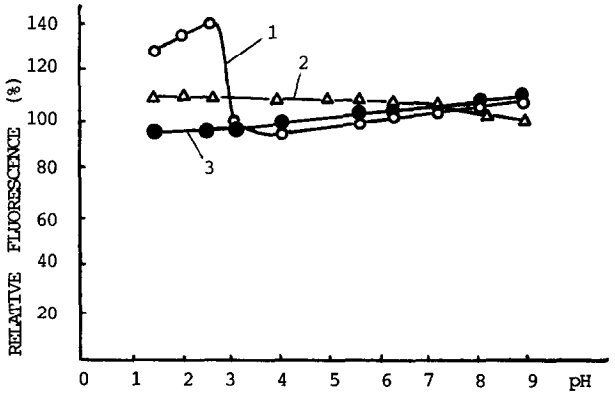

Fig. 2. pH dependence of intrinsic fluorescence of IFN-αA (1), IFN-β1 (2) and free Trp (3).

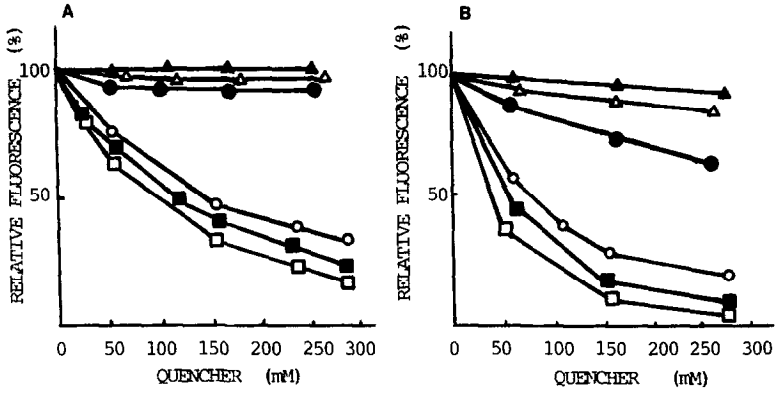

Fig. 3. The quenching of intrinsic fluorescence of IFN-αA (A) and IFN-β1 (B) in 0,1 M Na-phosphate buffer, pH 7,2 (-▲-, -●-, -■-) and 0,1 M Na-citrate buffer, pH 2,0 (-△-, -○-, -□-) in the presence of CsCl (-▲-, -△-), KI (-●-, -○-) and acrylamide (-■-, -□-).

solvent, 10% dioxane (12, 13). Fig. 3A demonstrates that acrylamide at pH 7,2 significantly suppresses the fluorescence intensity of IFN-αA, whereas KI and CsCl have no effect. It seems, that both Trp are located at or near to the surface of the protein, contacting with positively and negatively charged residues. On the other hand, in the presence of 10% dioxane the effect of KI is similar to that of acrylamide - 200 mM KI suppresses the fluorescence intensity about 4-fold (data not shown), CsCl also having no effect. This might be explained by the conformational changes induced by dioxane and leading to the removal of charged Asp or/and Glu residues out of the Trp environment. The same effects of quenchers are revealed for IFN-αA at pH 1,5-2,5. The obtained data allow to suppose that positively charged groups form more tight contacts with Trp residues in IFN-αA in comparison with negatively charged ones.

The analogous data were obtained for IFN-β1. At pH 7,2-8,5 acrylamide significantly suppresses the fluorescence intensity of IFN-β1. It shows that at least two from three Trp residues of IFN-β1 are located at or near to the surface of the protein and accessible to solvent (presumably, the residues at the same positions NN77 and 141 as in IFN-αA). These results correlate well with the data of Raman spectroscopy of IFN-β1 (14). The quenching characteristic for KI is more pronounced at pH 2,0, then at pH 7,2-8,5 (Fig. 3B). Presumably, negatively charged Asp or Glu residues are lacking within the envirinment of one or two Trp residues in IFN-β1. As in case of IFN-αA 250 mM CsCl does not practically effect the emission spectra of IFN-β1.

DISCUSSION

The obtained results might be summerized as followed, the residues of interest being shown on Fig. 4. At pH 8,5, that is comparatively close to the pK value of ε-amino group of Lys, CsCl does not influence on the emission spectra of both IFNs. It shows that Trp residues (presumably, sequence conserved Trp_{77} and Trp_{141}) contact with Arg (presumably, sequence conserved Arg residues), which are more basic then Lys ones. The lack of CsCl-induced fluorescence quenching at pH 2,0, that was common for both IFNs, as well as at alkaline pH in the presence of 10% dioxane allowed to predict that Trp-Arg interaction in both IFNs was rather tight. Conserved Arg positions in IFNs are NN 33, 126, 145, 150 and 163. According to the predicted model of IFN structure (15) it is most probably that Arg_{33} contacts with Trp_{77} and Arg_{145} - with Trp_{141} in IFN-αA and IFN-β1 molecules. Maybe the third Trp_{20} of IFN-β1 interacts with Arg_{150} and/or Arg_{163} (Fig. 5). The negligible shift in the λ_{max} value of both native IFNs at pH from 1,5 to 8,5 support also that Trp-Arg contact is rather tight, as well as indicates homology of hydrophobic residues within the spatial environment of Trp_{77} and Trp_{141}. According to the amino acid sequence and predicted structure for Trp_{77} these residues might be Phe_{36} and Phe_{78}, which are conserved in IFNs. The other hydrophobic residues, which might be also located in tertiary structure adjacent to Trp_{77} are Leu_{81} or Leu_{82} for IFN-αA and Ile_{81} or Val_{82} for IFN-β1.

It has been recently reported that in IFN-αD Lys_{121}, Arg_{125} and Thr_{132} located within the α-helix NN115-132 /according to

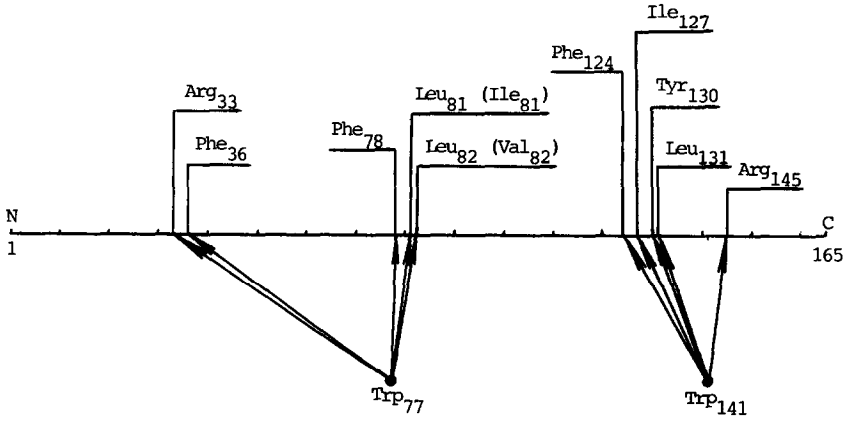

Fig. 4. The positions of amino acid residues forming probable microenvironment of Trp residues in IFN-αA and IFN-β1. The residues shown in brackets are characteristic for IFN-β1.

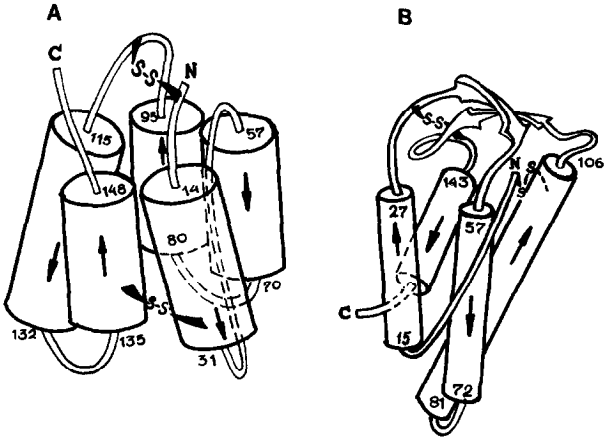

Fig. 5. The predicted models of IFN tertiary structure according to Zavyalov and Denesiuk (15) /A/ and Sternberg and Cohen (16) /B/.

the model shown on Fig. 5A (15), the α-helix includes also conserved hydrophobic residues of Phe_{124}, Ile_{127}, Tyr_{130} and Leu_{131}/ on the protein surface are able to bind specific cell receptors (3). According to the same model, this α-helical region coinsides with the α-helix NN135-148, carrying Trp_{141}. Presumably certain residues from the following ones - Phe_{124}, Ile_{127}, Tyr_{130} and Leu_{131} might contact with Trp_{141}. According to the alternative model (Fig. 5B) (16), it is most probably that Trp_{141} contacts with Phe_{124} or Ile_{127}. The study of Trp fluorescence quenching of IFNs in the presence and absence of dioxane, as well as distinct effects of KI at pH 2,0 and pH 7,2 (Fig. 3) indicates that the residues with the pK around 3 (most probably Asp) contact with Trp_{77} and Trp_{141} in IFN-αA and maybe IFN-β1, but this type of interaction is not tight enough to predict the positions of negatively charged residues.

Thus, the obtained data allow to suppose that in IFN-αA and IFN-β1 Trp_{141} interacts with Arg_{145} and one or several from the following residues - Phe_{124}, Ile_{127}, Tyr_{130}, Leu_{131} and Trp_{77} - with Arg_{33} and Phe_{36}, Phe_{78}, Leu_{81} or Leu_{82} (Ile_{81} or Val_{82} for IFN-β1). These data allow to speculate that the model of IFN structure designed by Zavyalov and Denesiuk (15) more resembles the real tertiary structure of these proteins, than the model of Sternberg and Cohen (16).

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