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Radioiodination of human interferon-α2 interferes with binding of C-terminal specific antibodies

Hana Schmeisser[a], Eva Kontseková[b], Iveta Vancová[a], Vojtech Mucha[a], Peter Kontsek[a,*]

[a]*Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Slovakia*
[b]*Department of Microbiology and Virology, Comenius University, 842 15 Bratislava, Slovakia*

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Abstract

Radioimmunoassays based on reactivity between a monoclonal antibody (mAb) and human ^{125}I-interferon (IFN)-α2 are frequently exploited in interferon research. In general, epitopes of antibodies specific for human IFN-α2 are located on the two immunodominant structures formed in the N- and C-terminal domains, respectively. We found that labelling of IFN-α2 with Na^{125}I by the chloramine-T method did not affect the binding of antibodies recognising the N-terminal region 30–53. In contrast, radioiodination of IFN was associated with a dramatic decrease in IFN reactivity with mAbs specific for the C-terminus (residues ~120–145~). We suggest that steric hindrance araising from the incorporation of ^{125}I into the tyrosine residues at positions 123, 130 and 136 may be responsible for the change in immunoreactivity. The adverse effect of radioiodination of IFN-α2 on the binding potency of C-terminal specific mAbs must be taken into consideration in experiments based on the interaction of such antibodies (i.e. NK2) with the labelled antigen.

Keywords: Antigenic structure; Interferon-α2; Monoclonal antibody; Radioimmunoassay; Radiolabelling

1. Introduction

Human interferon (IFN)-α2 is the most frequently studied and therapeutically exploited type I IFN. Radioiodination of IFN-α2 by the chloramine-T method is a widely used technique for both structural and functional characterisation of this cytokine, i.e. mapping of antigenic sites on the IFN-molecule, screening for antibodies induced post-therapy and the analysis of interactions between the cytokine and its receptor. In iodine labelling, the iodine atom is not part of the structure of the natural molecule and therefore there are likely to be some differences in behaviour between radioiodinated and unlabelled materials (Bolton, 1977).

There are two immunodominant structures identified on IFN-α2, which are recognised by most of the specific monoclonal antibodies (mAbs). One structure is located in the N-terminal domain centered around amino acids ~30–40~ and the other is located in the C-terminal domain spanned by residues ~112–148~ (Kontsek et al., 1991). Comparing the reactivity of mAbs with IFN-α2 in a radioim-

*Corresponding author. Fax: +421-7-5477-4284.
E-mail address: virupeko@nic.savba.sk (P. Kontsek)

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munoassay (RIA) and in an enzyme linked immunosorbent assay (ELISA) we have observed distinct binding pattern for N-terminal and C-terminal specific antibodies. We found that radioiodination of IFN-α2 was associated with a sharp drop in the binding capacity of antibodies directed to the C-terminal domain. In the present study we have addressed this topic in more detail since we believe that our results could be useful for the design and evaluation of experiments based on the interactions of C-terminal specific mAbs (the commonly used mAb NK2 also recognises this region) with ^{125}I-IFN-α2.

2. Materials and methods

2.1. Interferon

Recombinant *Escherichia coli*-derived human IFN-α2c (specific activity 10^8 U/mg) was kindly provided by Dr. G.R. Adolf (Bender, Vienna, Austria).

2.2. Monoclonal antibodies against human IFN-α2

The preparation and characterisation of murine mAbs against IFN-α2 was described previously (Kontsek et al., 1991, 1993; Kontseková et al., 1992; Karayianni-Vasconcelos et al., 1993). Four N-terminal specific antibodies: N7 (epitope 36–38), N40, 7/1 (epitope 30–41), N27 (epitope 43–53), and four C-terminal specific mAbs: N22, N39, N54 (epitope 112–148), 2–19 (epitope 93–166) were used as hybridoma culture supernatants. MAb G23 specific for human IFN-γ was used as a negative control. All antibodies were of the IgG1 isotype. The mAb concentrations were determined by ELISA using affinity purified mouse IgG1 as a standard (Kontseková et al., 1992).

2.3. RIA

Recombinant IFN-α2 was labelled with Na^{125}I by the chloramine T method without loss of antiviral activity (Kontsek et al., 1991). Immunoplates (MaxiSorp, Nunc) were coated overnight at 37°C with sheep anti-mouse F(ab′)$_2$-fragment (Sigma, 1 μg/ml, 50 μl/well in phosphate buffered saline — PBS, pH 7.2) and then saturated with 1% non-fat dried milk. Hybridoma supernatants were added (50 μl/well) and the plates were incubated for 1 h at 37°C. After washing, 50 μl of radiolabelled IFN (0.5×10^5 cpm) were added to the wells for 1 h at room temperature. Following washing the radioactivity bound to each well was measured in a gamma counter. All antibodies were assayed in triplicate. Between each step the plates were washed with PBS containing 0.05% Tween 20.

2.4. Determination of mAb-affinity by RIA

The binding affinities of mAbs to IFN-α2 were determined in a competitive solid-phase RIA. For this purpose, the most simple model of antigen–antibody interaction, that based on the law of mass action, was chosen (Mucha, 1993). Briefly, increasing concentrations of the unlabelled antigen (1×10^{-12}–5×10^{-10} M) in 50 μl PBS were mixed with 50 μl PBS containing 10^5 cpm of ^{125}I-IFN-α2. The mixtures were transferred on mAb-immobilised plates for 1 h at room temperature. After washing, the bound radioactivity was counted, competition curves for each mAb were plotted and the respective affinities for IFN-α2 were calculated (Kontseková et al., 1992). The results obtained represent average values from samples at three competitor concentrations near the half inhibition point.

2.5. ELISA

Immunoplates (MaxiSorp, Nunc) were coated overnight at 37°C with IFN-α2 (1 μg/ml, 50 μl/well in PBS pH 7.2). Plates were saturated for 1 h with 1% non-fat dry milk in PBS. The hybridoma supernatants were added (50 μl/well) and the mixtures incubated for 1 h at 37°C. Bound antibodies were detected after incubation for 1 h at 37°C with peroxidase-conjugated swine antibodies specific for mouse Ig (USOL, Czechia) diluted 1/1000 in PBS. Positive reactions were visualised by the addition of orthophenylenediamine and absorbance values were measured at 492 nm (A_{492}). All mAbs were tested in triplicate. Between each step, the wells were washed with PBS containing 0.05% Tween 20.

2.6. Relative mAb-affinities determined by ELISA

Immunoplates were coated overnight with two fold dilutions of IFN-α2 in PBS (starting concentration 1 μg/ml, 50 μl/well) and saturated with 1% non-fat dry milk. The concentrations of mAbs in culture supernatants were balanced to 2 μg/ml and then were added (50 μl/well) for 1 h at 37°C. All subsequent steps were the same as described above for ELISA. Two independent measurements were performed.

The rate of change of the absorbance vs. log[IFN-α2 dilution] was plotted for each mAb. The inflection points of plotted curves were found to be localised around the absorbance value $A_{492}=0.4$. Therefore, a titre of a mAb was estimated as the reciprocal dilution of IFN-α2 giving the absorbance $A_{492}=0.4$. Since the concentrations of the mAbs were balanced, ranked titres refer to the relative affinities of respective antibodies.

3. Results and discussion

The majority of mAbs generated against human IFN-α2 can be classified into two groups, namely those specific for N-terminal epitopes and those specific for C-terminal epitopes. During experiments with these antibodies we have observed that both groups differ in their patterns of reactivity in the ELISA and RIA. To elucidate this phenomenon we performed study with eight mAbs against IFN-α2. Four antibodies recognised different epitopes located in the N-terminal domain (residues 30–53) whereas the epitopes of four others were located in the C-terminal domain (residues ~112–148~). In the enzyme immunoassay, the N-terminal and C-terminal specific mAbs exhibited a similar reactivity with the solid-phase bound antigen (Fig. 1). However, in the radioimmunoassay a different pattern of reactivity was observed. Compared to the N-terminal specific mAbs, the potency of C-terminal specific antibodies to bind radiolabelled IFN was decreased about ten fold. To characterise this effect more precisely we performed experiments comparing the affinities of these mAbs as measured by RIA and ELISA.

Firstly, mAbs to IFN-α2 were ranked according to their affinities as estimated by competitive RIA using radiolabelled IFN (Fig. 2). Only the affinities of N-terminal specific antibodies could be determined because the low binding capacity of C-terminal specific mAbs did not permitt the calculation of their affinities. Subsequently, an ELISA with immobilised IFN-α2 was used to rank the affinities of both N-terminal and C-terminal specific antibodies. In contrast to the RIA experiments, the data obtained did not indicate any difference in the affinities of the

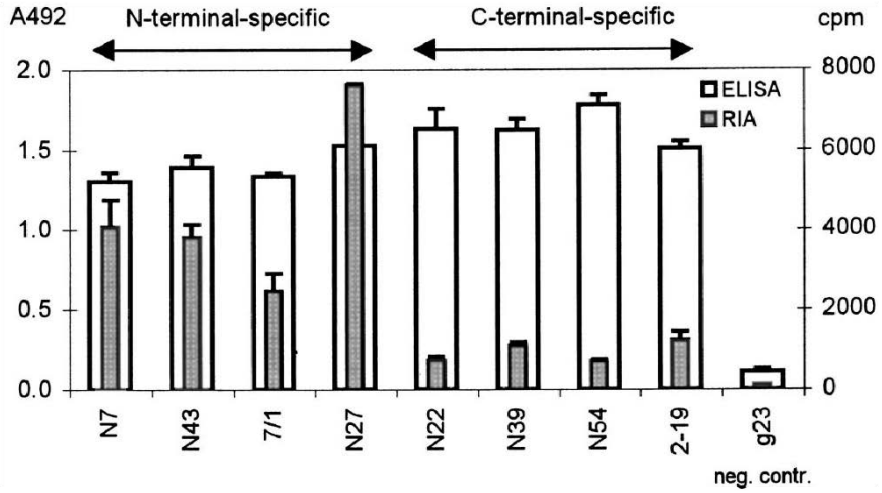

Fig. 1. Reactivity of N-terminal and C-terminal specific mAbs with human IFN-α2 in ELISA and RIA procedures. Data from one representative experiment. Error bars indicate standard deviation of the measurements.

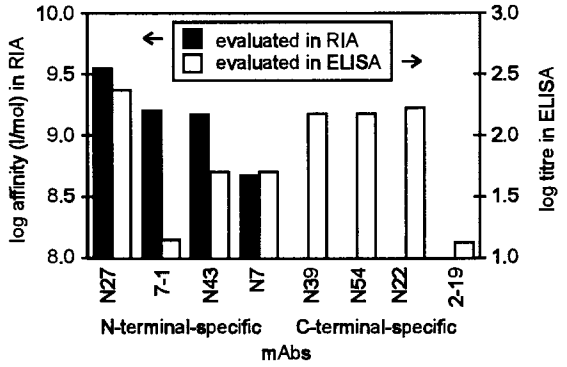

Fig. 2. Ranking of affinities for C-terminal and N-terminal specific mAbs estimated by ELISA and RIA.

two mAb-groups. Ranking comparisons showed that the affinities of N-terminal specific mAbs determined either by ELISA or RIA were comparable. On the other hand, when comparing the corresponding ELISA- and RIA-estimated affinities for C-terminal specific mAbs, a dramatic drop in their affinities for ^{125}I-IFN-α2 was observed. These data suggest some steric hindrance by the labelled antigen preventing the interaction of the C-terminal specific antibodies with their respective epitopes. What could be the nature of such interference? The chloramine-T method is the most widely used method for the radioiodination of small amounts of protein. Na^{125}I is oxidised by chloramine-T in the presence of the protein to be labelled, with the subsequent incorporation of ^{125}I into the tyrosine residues of the protein (Bolton, 1977). All five tyrosines of IFN-α2 can be localised in the three-dimensional model of this cytokine (Fig. 3). Three are located at positions 123, 130 and 136 in the D helix which represents a part of the C-terminal immunodominant region. Moreover, in the chloramine-T reaction tyrosine residues on the (immune) surface of the protein molecule iodinate most readily (Dube et al., 1964). In contrast, the N-terminal domain of IFN lacks such residues and the two remaining tyrosines at positions 86 and 90 are located in the C helix, which exhibits low immunogenicity (Kontsek, 1994). Taking into consideration both theoretical and experimental data we suggest that substitution of ^{125}I for hydrogen atoms in the tyrosine residues of IFN-α2 leads to steric hindrance for mAbs with epitopes located in the C-terminal domain. However, we do not expect significant

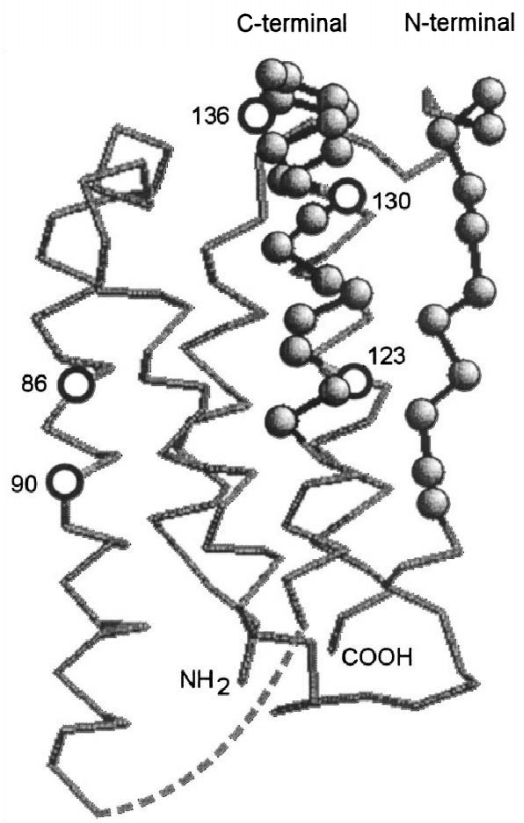

Fig. 3. Conformational model of human IFN-α2 constructed from the co-ordinates from the Brookhaven Protein Data Bank, Accession Number 1RH29 (Radhakrishnan et al., 1996). N-terminal (30–53) and C-terminal (120–145) immunodominant structures are highlighted. The positions of five tyrosines are indicated (open circles).

conformational modifications of the domain because radiolabelling is not associated with any decrease in the biologic activity of IFN-α2 (Kontsek et al., 1991).

Labelling of human IFN-α2 with Na^{125}I resulted in a sharp decrease of the reactivity of iodinated antigen with the C-terminal specific mAbs. This fact must be taken into consideration when selecting mAbs for experiments with ^{125}I-IFN-α2. In other type I IFNs (even of different species) the hydrophilic character of the D helix suggests strong immunogenicity and the potential for eliciting mAbs. Because of the number of tyrosine residues in this helix (e.g. human IFN-β has four), it is to be expected that iodination of mAbs specific for D-

helices will be deleterious. Therefore when selecting mAbs for binding to IFN (or cytokines in general) following chloramine-T radiolabelling, their epitopes should be examined for the proximity of tyrosine residues.

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