LETTER TO THE EDITOR

LOW PH DOES NOT REARRANGE ANTIGENIC STRUCTURE OF PORCINE INTERFERON GAMMA BUT INHIBITS ITS RECEPTOR BINDING

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Biological acid-lability is a hallmark for all type II (gamma) interferons (IFNs). It characterizes the fact that IFN gamma looses antiviral activity when exposed to pH 2 for a few minutes (*1*). The mechanisms of this phenomenon is not fully understood. Native, bioactive IFN gamma is a homodimer composed of non-covalently bound subunits (*2*). At acidic pH the IFN gamma dissociates into monomers which can form large aggregates with reduced antiviral activity (*3,4*). It is not known, whether the pH 2 treatment induces also some structural changes in the IFN gamma monomers. For characterization of potential conformational rearrangement following the pH 2 treatment of porcine IFN gamma molecule a set of specific monoclonal antibodies (MAbs) and a polyclonal antiserum to porcine IFN gamma were employed.

Experiments were performed with recombinant *Escherichia coli*-derived porcine IFN gamma (specific antiviral activity of 2 x 10^7 U/mg) (*5*). IFN gamma was exposed to the low pH as follows: To IFN at the concentration of 50 μg/ml in phosphate-buffered saline (PBS) pH 7.2 a sufficient quantity of 5N HCl was added to attain pH 2. After 20 hrs at 4°C the pH was reversed either by dilution in an ELISA coating buffer pH 8.5 or adjusted to pH 7.2 with 5N NaOH and diluted in a cultivation medium used for antiviral bioassay.

Firstly, the effect of pH 2 on antiviral activity of porcine IFN gamma was determined. The antiviral activity was titrated by inhibition of the cytopathic effect of vesicular stomatitis virus on Madin-Darby bovine kidney (MDBK) cells and expressed in U/ml (*6*). A recent report claims that the pH 2 treatment does not affect bioactivity of natural porcine IFN gamma (*7*). We did not confirm this observation with recombinant porcine IFN gamma. Its exposition to pH 2 in PBS for 20 hrs resulted in reduction of its antiviral titer from 10^6 U/ml to 10^3 U/ml.

In the next step, the effect of the pH 2 treatment on antigenic properties of IFN gamma was evaluated using MAbs and an antiserum to recombinant porcine IFN gamma in an enzyme immunoassay (*6*). Briefly, microplates were coated with 1 μg/ml IFN gamma (pH 2-treated and untreated) and then saturated with 0.1% casein. The murine MAbs (hybridoma supernatants) or the mouse antiserum (diluted 10^{-3} in PBS) were added in triplicates (50 μl/well)

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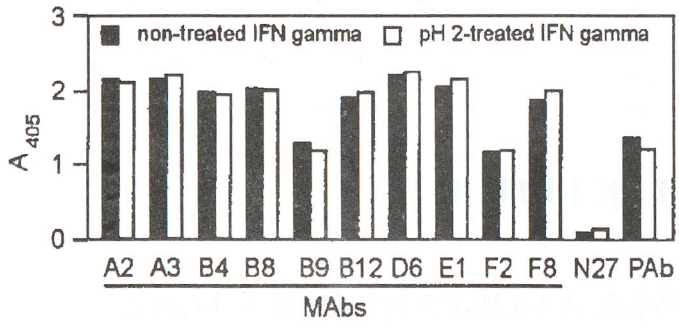
Abbreviations: IFN = interferon; MAb = monoclonal antibody; MDBK = Madin-Darby bovine kidney; PAb = polyclonal antiserum; PBS = phosphate-buffered saline



and incubated for 1 hr at 37°C. The bound antibodies were detected after incubation for 1 hr at 37°C with a peroxidase-conjugated goat antiserum to mouse Ig. Positive reactions were visualized and A_{405} was measured (see the figure). A marked reduction in antiviral activity of porcine IFN gamma following the pH 2 treatment was apparently not paralleled by its conformational rearrangement. Indeed, the MAbs were unable to detect any change in antigenic structure by the pH 2 treatment of the IFN (see the figure). These MAbs were directed to at lest three different epitopes (*6*). Theoretically, a limited number of epitopes recognized by these MAbs could not represent the entire antigenic surface of the antigen. Therefore we compared antigenic profiles of the pH 2-treated and untreated IFN gamma using the specific polyclonal antiserum (PAb) that reflected total immunogenicity of this IFN. Nevertheless, the antiserum exhibited basically similar patterns of reactivity with the acid-treated and untreated IFNs. These data provide strong support for the hypothesis that the pH 2 treatment does not introduce overall structural modifications into the IFN gamma molecule.

The reduction of antiviral potency of the pH 2-treated IFN gamma resulted most probably from unability of its aggregates to bind to a cellular receptor.The preincubation of MDBK cells with the pH 2-treated IFN gamma at the concentration of 50 ng/ml (corresponding to a titer of about 500 U/ml before the pH 2 treatment) for 1 hr at 37°C, followed by titration of the untreated IFN gamma in these cells did not decrease its antiviral titer. At the concentration used, the pH 2-treated IFN gamma alone did not exhibit significant antiviral activity on MDBK cells.

In conclusion, the loss of antiviral potency upon a pH 2 treatment is a generic feature of all the type II IFNs. The exposition of recombinant porcine IFN gamma to pH 2 resulted in an expected 1000-fold decrease in its antiviral activity. However, the acidification did not significantly alter its antigenic structure, as proved by the specific MAbs and antiserum. The preincubation of MDBK cells with the pH 2-treated IFN gamma did not affect antiviral titer of the subsequently added untreated IFN gamma. This indicates that the reduction of antiviral activity by the pH 2 treatment might be caused by inability of the acidified IFN gamma to bind to a cellular receptor.

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