RIBONUCLEASE ACTIVITY IN PREPARATIONS OF HUMAN LEUKOCYTIC INTERFERON

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A ribonuclease (RNase) with pH optimum at 7.0-7.5 was found after multistage chemical purification of preparations of human leukocytic interferon. The enzyme had an endonuclease mechanism of action. Analysis of the results of a study of the action of various substances on the RNase activity of human interferon preparations showed that many of them acted on the enzyme in the same way as on other ribonucleases. However, unlike the inactivating action on pancreatic RNase, interferon RNase was activated by dithiothreitol, a reducing agent for disulfide groups. During electrophoresis in polyacrylamide gel distribution patterns of protein and RNase were obtained.

KEY WORDS: human interferon; ribonuclease activity.

Investigations [4, 5] have shown that in the course of interferon production under the influence of virus inducers certain enzymes, especially lysosomal enzymes, leave the cells. The question of the preservation of this enzyme activity in interferon preparations and its role in the manifestation of its antiviral action consequently arose. On the other hand, both indirect [2] and direct [15, 20] evidence has been obtained of the direct action of interferons isolated from different sources on nucleic acids. The action of human interferon has not been studied from this aspect.

In the investigation described below, ribonuclease activity was found in preparations of human leukocytic interferon, some of the properties of the enzyme were studied, and views are expressed on its possible role in the antiviral action of interferon.

EXPERIMENTAL METHOD

A preparation of human leukocytic interferon with an activity of 16,000 units/ml, in accordance with International Standard B 69/19, and subjected to multistage chemical purification, was used.

Activity of the RNase was determined at 37°C by the method of Anfinsen et al. [8] in the modification [19]; the ability of the interferon to induce a hyperchromic effect was investigated by determining the rate of increase of optical density (E_{260}) of the reaction mixture [1] during incubation in a constant-temperature cuvette (37°C). Hydrolysis of cytidine-2',3'-phosphate at 37°C was investigated with continuous recording of the reaction kinetics [10].

The character of hydrolysis of the substrate by RNase of the interferon preparation was determined by methods developed previously [3, 9]. Wheat germ RNA was incubated at 37°C with the enzyme for different periods of time, at the end of which 2 ml of the reaction mixture was applied to a column (20 × 1 cm) with Sephadex G-50. Elution was carried out with 0.05 M NaCl. Fractions with a volume of 1.5 ml were collected and examined spectrophotometrically at 260 nm.

Protein in the interferon preparation was determined by Lowry's method [16].

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TABLE 1. Action of Various Substances on RNase of a Preparation of Human Interferon (M ± m)

Substance tested	Final concentration in sample	% of activity in control
Polyvinyl sulfate	0,2 mg/ml	71,5±6,1
*	2,0 mg/ml	17,4±3,0
Heparin	0,2 mg/ml	69,7±8,6
*	2,0 mg/ml	72,0±9,7
Pronase	135 units	61,6±3,0
Sodium dodecylsulfate	0,5%	2,0±1,1
$MgCl_2 \cdot 6H_2O$*	100 mM	64,7±2,0
$ZnSO_4 \cdot 7H_2O$*	2 mM	58,5±5,3
$CuSO_4 \cdot 5H_2O$*	2 mM	65,9±7,5
$CaCl_2 \cdot 6H_2O$*	100 mM	40,5±1,6
N-ethylmaleimide	1 mM	81,0±3,7
Monoiodoacetate	150 mM	44,8±5,3
Dithiothreitol	1 mM	157,9±8,1
Bovine serum albumin	0,5%	186,6±16,7

Legend. A 0.05 M K-phosphate buffer, pH 7.0, was used. Incubation continued for 2 h at 37°C. Control samples contained all components except test reagent.

*Reaction carried out in 0.05 M Tris-HCl buffer, pH 7.0.

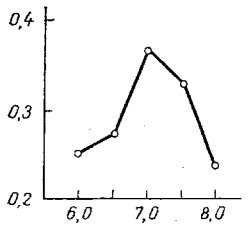

Fig. 1. Interferon RNase activity as a function of pH of medium during incubation for 24 h. Abscissa, pH values; ordinate, RNase activity (in optical density units/mg protein/min). Incubation medium contained 0.05 M K-phosphate buffer at different pH values, 1% solution of yeast RNA, and 1.58 mg interferon protein.

Electrophoresis in 7.5% polyacrylamide gel (PAG) at pH 8.3 was carried out as described by Davis [11], but without the starting and concentration gels. The interferon preparation in a dose of 100-200 μg protein was applied to each track. Electrophoresis was carried out with a current of 3-4 mA/gel for 2-3 h until all the indicator dye (Bromphenol Blue) had come out. Some of the gels were then stained with Amido Black to reveal the protein bands. In other gels the distribution of RNase activity was studied. For this purpose they were cut into pieces each 5 mm long and homogenized in 2 ml of 0.05 M K-phosphate buffer, pH 7.0, containing 0.05% of bovine serum albumin and 0.002% of Tween-80. The RNase activity [8, 19] in these homogenates was detected after incubation for 24 h at 37°C.

EXPERIMENTAL RESULTS

Incubation of human leukocytic interferon with yeast RNA led to liberation of products absorbing in the ultraviolet region and not precipitated by uranyl acetate in an acid medium. Liberation of the acid-soluble RNA degradation products continued during incubation for 48 h, but after 6 h, the reaction velocity fell appreciably. Hydrolysis of polyadenylic acid and wheat germ RNA took place much more slowly and became appreciable only after incubation for 24 and 48 h, respectively. The highest velocity of the RNase reaction was observed at pH 7.0, when it was 0.017 optical density unit/mg protein of the interferon preparation/min. The pH optimum for interferon RNase is shown in Fig. 1.

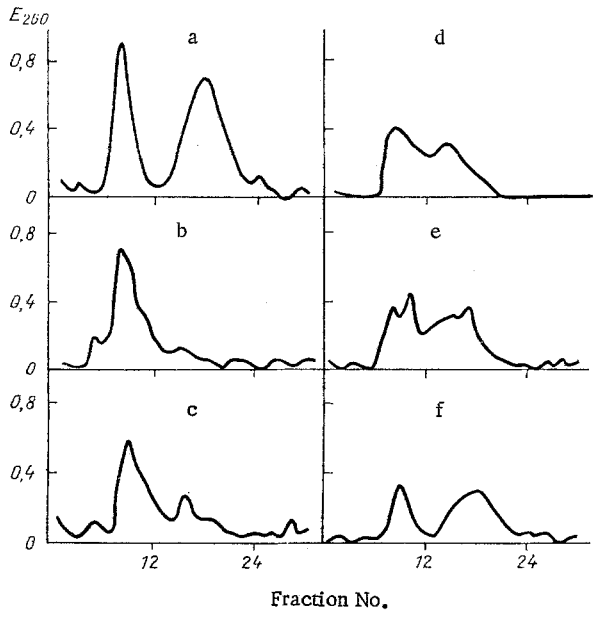

Fig. 2

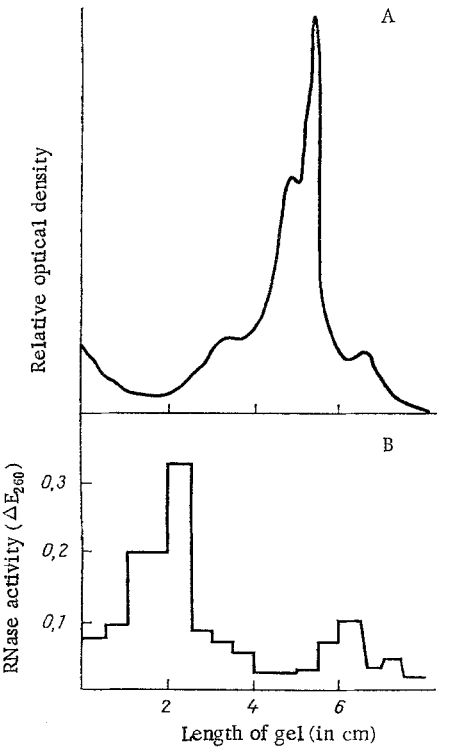

Fig. 3

Fig. 2. Gel-filtration of hydrolysis products of wheat germ RNA on Sephadex G-50 after various periods of incubation with interferon. Incubation mixture consisted of 0.025% wheat germ RNA, 0.6-0.8 mg protein of human leukocytic interferon, and 0.05 M K-phosphate buffer, pH 7.0 (total volume 2.5 ml). a) Elution profile of mixture of RNA and mononucleotides (control); b, c, d, e, f) chromatography of reaction mixture of RNA and interferon after incubation for 5, 15, 60, and 120 min and 24 h, respectively.

Fig. 3. Comparison of protein and RNase spectra during electrophoresis of human leukocytic interferon preparation in polyacrylamide gel: A) electrophoresis of protein; B) of RNase activity.

Data on the effect of various substances – RNase inhibitors, bivalent metallic ions, sulfhydryl reagents for RNase of the human interferon preparation – are given in Table 1. In most cases the action of these substances agreed with data in the literature for their effect on other ribonucleases, especially pancreatic RNase [3, 6, 7, 17]. However, by contrast with the depression of activity of pancreatic RNase by various reducing agents for disulfide groups, dithioerythrite activated RNA hydrolysis by the human interferon preparations.

Addition of interferon to the incubation mixture containing various types of RNA led to a continuous increase in the E_{260} value of the solution. The hyperchromic effect depended on the type of RNA and its value for yeast RNA, polyadenylic acid, wheat germ RNA, and poly-(inosinic-cytidylic) acid was 8.8, 8.5, 12.8, and 2.5 units ($\Delta E_{260} \cdot$ mg protein$^{-1} \cdot$ min$^{-1} \cdot 10^3$) respectively.

As the results of continuous spectrophotometry showed, the interferon hydrolyzed cytidine-2',3'-phosphate. When the latter was present in a concentration of 33 mM, the rate of increase of E_{284} of the solution (0.05 M Tris-HCl buffer, pH 7.0) was 9.2 ± 0.7 units ($\Delta E_{284} \cdot$ mg protein$^{-1} \cdot$ min$^{-1} \cdot 10^3$).

It can be concluded from the results in Fig. 2 that the enzyme had an endonuclease mechanism of action, for the peak of the low-molecular-weight fractions coincided with the mononucleotide peak only after incubation for 24 h. These results are in agreement with those obtained for preparations of chick [20] and mouse [15] interferons, which also contain endonucleases.

Electrophoresis of the human leukocytic interferon preparations in 7.5% PAG showed the heterogeneity of their protein composition (Fig. 3); the maxima of RNase activity did not coincide with the main protein peak. According to some workers' observations [13, 18], the maxima of antiviral activity of interferons from different

sources likewise did not coincide with the principal protein peaks of electrophoresis in PAG. On the other hand, nuclease activity cannot be separated from antiviral activity by chromatography on CM-Sephadex [20]. Nevertheless, the question of whether the RNase belongs to interferon itself or to its impurities cannot yet be answered because preparations of interferon completely freed from impurities are unstable [12, 14]. It has been suggested that nucleases may play an important role in the mechanism of the antiviral action of interferon [20] and, in particular, that they may selectively destroy viral messenger RNAs [15].

Preparations of human leukocytic interferon thus have endonuclease activity. The question of the role of this activity in the antiviral action of interferon is not yet settled and requires further study.

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