RNase Activity in Human Interferon Preparations

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The level of RNase activity in human interferon preparations was examined. Although sequential purification of interferon resulted in nearly a 300-fold increase in specific activity, RNase-specific activity remained more or less constant. The implications of this finding for the analyses of the mode of action of interferon are discussed.

Interferon (IF) is an antiviral protein produced by animal cells after viral infection or treatment with complex macromolecules such as endotoxin or polyinosinic:polycytidylic acid complexes [poly(I:C)]. Although capable of protecting cells against viral infection by inhibiting viral replication, the precise mode of action of this protein is unknown. The results of both in vivo (10) and in vitro (7) analyses suggest that IF inhibits viral replication by blocking the translation of mRNA. Furthermore, inhibitory effects of IF on both cellular (5) and viral (6) mRNA translation have been reported. It has been proposed that the site of the inhibitory activity induced by IF preparations is at the level of the ribosomes (3) and that the inhibition is mediated by a nondialyzable, heat-labile protein molecule capable of being dissociated from ribosomal fractions (8). It has been reported further that, in cell-free systems where mRNA translation is blocked by IF, not only are incomplete polypeptide chains formed but, eventually, initiation of new chains is also blocked. Such blockade may be reversed to a variable extent (3, 8) by addition of certain tRNA species. Despite the fact that IF can induce an apparent nondegradative blockade of translation, the effects of this protein on the structural integrity of mRNA must be considered as well.

In preliminary studies, therefore, we sought to examine the effects of partially purified human foreskin fibroblast IF preparations on cellular RNase activity. These early studies revealed that treatment of radiolabeled RNA substrate with IF alone resulted in large increases in soluble radioactivity, indicative of RNase activity in the IF preparation. Subsequently, examination of crude human leukocyte IF preparations (including reference samples obtained from the Research Resources Branch, National Institute of Allergy and Infectious Diseases, Rockville, Md.) revealed the presence of high levels of RNase activity. Therefore, we sought to examine the relationship between IF activity and RNase activity by testing the levels of RNase in preparations of human leukocyte IF that had been sequentially purified to yield IF of increasing specific activity (1, 2). Initially, difficulties were experienced in the interpretation of measurements of IF-associated RNase activity, which were performed using 10 mM Tris-hydrochloride buffer (pH 7.5), because we observed a sigmoidal dependence of RNase activity on the volume of IF sample utilized. Because all samples of IF contained 0.1 M sodium phosphate buffer and it appeared that activation of RNase activity was dependent upon phosphate concentration, we chose to perform all assays utilizing 5 mM sodium phosphate buffer (pH 7.0) (4). Under these conditions, enzyme activity was a linear function of enzyme sample volume over the region of sample volumes studied. Additionally, kinetics of substrate utilization were examined in the region of substrate concentrations ranging from 0 to 45 × 10⁻⁴ M and plots of (1/enzyme activity) versus (1/substrate concentration) were found to be linear and yielded an apparent $K_m$ of 1.6 × 10⁻⁴ M.

The results of testing preparations of human leukocyte IF at different stages of purification for both RNase activity and IF activity are presented in Table 1. Although it is true that, at each major stage of purification, a fraction exists having a higher IF/RNase activity ratio than fractions from the preceding stage, this fraction does not necessarily have the highest specific activity of IF attained at that stage of purification. It is evident that, at successive stages of purification, the specific activity of IF increases, whereas that of RNase remains more or less stationary. Therefore, the ratio of IF
activity to RNase activity generally increases. Thus, the fraction (PIF, pH 8.0) containing the highest specific activity of IF (3.4 × 10⁶ units/mg of protein), represented an approximate 300-fold increase in specific activity relative to crude IF. This fraction contained RNase activity (322 units/mg of protein), indicative of only a threefold decrease relative to the crude starting material. This finding indicates that levels of RNase still contaminate the IF preparation. Data has been presented by other workers which indicate that high levels of membrane-associated alkaline RNases appear in chicken embryo fibroblasts treated with IF (9). It has been proposed that IF action may result from the induction or activation of cellular RNases which would serve to degrade or reduce the translational capacity of virion mRNA. These and other data derived from the application of exogenous IF preparations must be critically analyzed, in view of the fact that even highly purified IF preparations, such as those utilized in the present study, contain significant RNase levels, and the treatment of cells with such preparations might result in the intracellular accumulation of exogenously supplied RNase. This potential difficulty might be avoided by using poly(I:C) to induce viral interference, since it is highly unlikely that under these conditions exogenous RNase will be introduced.

The most important implication of the present data is that the mode of action of IF inferred from translational studies cannot be accurately assessed in cell-free systems if IF contains levels of RNase sufficient to degrade mRNA.

### LITERATURE CITED


