Inhibition of Human Plasma Deoxyribonuclease I by Ribonucleic Acid[1] (35870)

V. M. Doctor

Department of Chemistry, Prairie View A. and M. College, Prairie View, Texas 77445

In recent years, studies concerning the chemical and biological properties of different types of naturally occurring RNA and their respective roles in protein synthesis have received a great deal of attention. On the other hand, information is lacking on the possible changes brought about in the host by viral RNA during the infective phase or on the possible roles of host RNA or DNA during the defense period.

The factors influencing human plasma deoxyribonuclease (DNase) I activity have been studied (1). A method was described for assay of acid-soluble deoxyribose-purine-containing fragments from an incubation mixture consisting of serum, deoxyribonucleic acid (DNA), Mg^{2+} and buffer, pH 7.3 (1). Under these conditions, *in vitro* addition of extracts prepared from human leukocytes inhibited the enzyme activity in a competitive manner (1). The presence in leukocytes of a specific inhibitor of human serum DNase has been reported by Kurnick *et al.* (2). This inhibitor was shown to be a protein, soluble in saline and stable at 56° (2). Lehman *et al.* (3) reported the presence of DNA-specific endonuclease in *E. coli* which was inhibited by ribonucleic acid (RNA). Bovine pancreatic DNase I is reported to be inhibited by a naturally occurring protein inhibitor (4–6).

The studies reported here show that human plasma DNase I is inhibited by RNA from a variety of sources while, under the same experimental conditions, the pancreatic DNase I is unaffected by RNA.

Methods. Plasma was obtained from heparinized blood of normal human beings in the routine manner. DNase I determinations were made on the plasma samples essentially according to the procedure reported earlier (1). Two milliliters of plasma were incubated with 2 ml of 0.1 *M* Tris buffer, pH 7.3, containing 1 mg of deoxyribonucleic acid (DNA, sodium salt, calf thymus, Hammersten, highly polymerized; Mann Research Laboratories, Inc., New York) and 2 mg of $MgSO_4$. After 20 hr incubation at 45°, determinations of DNase I were made by measuring the deoxyribose-purine-containing fragments in the trichloroacetic acid (TCA)-soluble fraction. One milliliter of 55% TCA was added to the incubation mixture and the precipitate was centrifuged and discarded. The aqueous extract was assayed for deoxyribose according to the colorimetric procedure of Allfrey and Mirsky (7). Deoxyribose was used as a standard. The observed DNase I activities, as measured by the above procedure, are reported to be proportional to the amount of plasma added to the system (1). Different levels of RNA samples or of polyglutamic acid were tested by dissolving in the 0.1 *M* Tris buffer of pH 7.3 along with the 1 mg of DNA and 2 mg of $MgSO_4$.

Samples of yeast tRNA and *E. coli* tRNA were gifts from Dr. B. P. Doctor, Walter Reed Army Research Center, Washington, D. C. Polyuridylic (5^1) was purchased from Mann Research Laboratories, Inc., New York. Polyadenylic (5^1) poly-L-glutamic and yeast RNA prepared as per Crestfield *et al.* (8) were purchased from Sigma Chemical Co., St. Louis, Mo. Crystalline bovine pancreatic ribonuclease (RNase) A and yeast RNA were purchased from Worthington Biochemical Corp., Freehold, N. J.

RNase-treated preparations of RNA were made by incubating 3 mg of *E. coli* tRNA at 30° for 8 and 20 hr with 1 mg of RNase in a

[1] This work was supported by Welch Foundation and NASA.

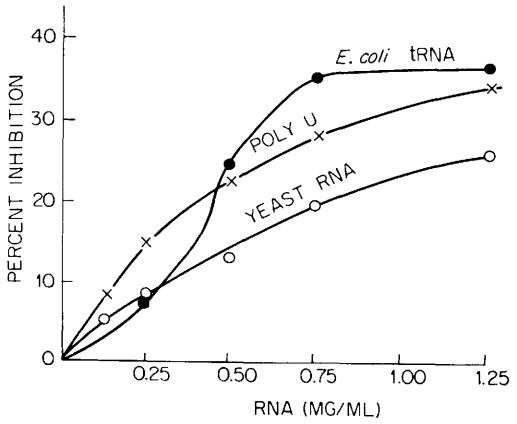

FIG. 1. Inhibition of plasma DNase I by different RNA samples. Two milliliters of plasma were incubated with 2 ml of 0.1 *M* Tris buffer, pH 7.3, containing 1 mg of DNA; 2 mg of $MgSO_4 \cdot 3H_2O$ and with, or without, various levels of RNA. After 20 hr incubation at 45°, determinations of DNase I were made by measuring the deoxyribose-purine-containing fragments in the trichloroacetic acid (TCA)-soluble fraction according to the colorimetric procedure of Allfrey and Mirsky (7). Deoxyribose was used as standard.

total volume of 2 ml of 0.1 *M* Tris buffer, pH 7.3. The incubated material was then tested for its effect on plasma DNase I activity as described above.

Results. Inhibition of DNase I by different RNA. The results presented in Fig. 1 show an inhibition of human plasma DNase I by various levels of yeast RNA (Worthington or Sigma). The inhibition increases with increasing levels of yeast RNA and then levels off at 1.5 mg/ml, or higher, concentrations. Since this RNA is probably a mixture of the lower molecular weight amino acid acceptor RNA (tRNA) and a larger RNA component found in ribosomes, studies were conducted on the effect of different levels of *E. coli* tRNA on the human plasma DNase I activity. Figure 1 shows an increase in inhibition of DNase I by increasing levels of *E. coli* tRNA; however, at concentrations higher than 0.75 mg/ml the inhibition levels off. Yeast tRNA also inhibited the plasma DNase I by 20% at 1 mg/ml level. The ribonucleic-like polynucleotides, *viz.*, polyuridylic acid (5^1) and polyadenylic acid (5^1) or polyglutamic acid, another high molecular weight polyanion, were tested for possible inhibitory effect of plasma DNase I. The results presented in Fig. 1 show that polyuridylic acid (5^1) also inhibited the enzyme in a manner similar to the other two RNA samples (Fig. 1). Polyadenylic acid (5) inhibited the DNase I by 20% at 1 mg/ml level while polyglutamic acid was found to be inert as an inhibitor even at 2 mg/ml.

Inhibition of plasma DNase by RNase-treated E. coli *tRNA.* The results of this study show a destruction of inhibitor activity of *E. coli* tRNA by pancreatic RNase. The 0 hr RNA sample inhibited DNase I by 22% while 8 and 20 hr RNase incubated RNA samples inhibited the enzyme by 14 and 7% respectively. Thus the RNase treated (8 or 20 hr) *E. coli* tRNA was less inhibitory to plasma DNase I compared with the 0 hr sample.

Specificity of RNA for Plasma DNase I. Another enzyme which attacks DNA, *viz.*, crystalline pancreatic DNase I (Worthington Biochemical Corporation, Freehold, N.J.) was tested at 10.0 μg/ml under the above experimental conditions and the DNase I activity was found to be unaffected by 1 mg/ml of RNA samples. Similar results have been reported for bovine pancreatic DNase I by Lehman *et al.* (3)

Discussion. An *E. coli* endonuclease (3) is reported to be inhibited by many naturally occurring RNA while the ribonucleic acid-like polynucleotides or polyphosphates were reported to be essentially inactive. The plasma DNase I, on the other hand is inhibited by both, the naturally occurring RNA and the enzymatically synthesized ribonucleic acid-like polynucleotides. Human plasma is of complex composition and the possibility cannot be excluded that the inhibitory effect on plasma DNase I by RNA is mediated by one or more of these constituents and therefore indirect.

Summary. Human plasma deoxyribonuclease I is inhibited by many naturally occurring RNA samples and also by ribonucleic acid-like polynucleotides, *viz.*, polyuridylic acid (5^1) or polyadenylic acid (5^1), while

polyglutamic acid, another polyanion, was found inactive. The significance of this partial immobilization of DNase I by RNA is not clear.

1. Doctor, V. M., Arch. Biochem. Biophys. **96**, 475 (1962).

2. Kurnick, N. B., Schwartz, L. I., Pariser, S., and Lee, S. L., J. Clin. Invest. **32**, 193 (1953).

3. Lehman, I. R., Roussos, G. G., and Pratt, E. A., J. Biol. Chem. **237**, 829 (1962).

4. Dabrowska, W., Cooper, E. J., and Laskowski, M., J. Biol. Chem. **177**, 991 (1949).

5. Lindberg, M. V., J. Biol. Chem. **241**, 1246 (1966).

6. Lindberg, M. V., Biochemistry **6**, 323 and 343 (1966).

7. Allfrey, V., and Mirsky, A. E., J. Gen. Physiol. **36**, 227 (1952).

8. Crestfield, A. M., J. Biol. Chem. **216**, 185 (1955).

Received April 16, 1971. P.S.E.B.M., 1971, Vol. 138.