THE ABSENCE IN HUMAN SERUM OF A RIBONUCLEASE THAT HYDROLYSES DOUBLE STRANDED RNA

Ann M. Körner, Department of Laboratory Medicine,
Yale University, New Haven, CT. 06520

Received November 19,1975

Summary. Human serum was assayed for the presence of a nuclease that can hydrolyse double stranded RNA. The synthetic RNA duplex polyI-polyC was used as substrate. No activity was detected in unfractionated or fractionated human serum.

The presence of an enzyme in human serum that can degrade double stranded RNA has been reported (1). On this basis a survey of such an enzymatic activity was initiated in an in-patient hospital population. However, in this report evidence is presented that such a ribonuclease is absent from human serum and that hydrolysis of polyI-[^{3}H]polyC does not occur during incubation of this synthetic substrate with human serum.

Experimental. The synthetic double stranded RNA substrate polyinosate-[5-^{3}H] polycytidylate, polyI-[^{3}H]polyC, specific activity 19.5μc/μMole P, was obtained from Miles Laboratories. It was diluted 1:10 with distilled water and stored in aliquots at -20°. Ribonuclease A was purchased from Worthington Biochemical Corp., Bio-Gel P-200 from BioRad Corp. and pronase from Calbiochem. Enzymatic reactions were terminated by the addition of 5ml 5% trichloroacetic acid at zero degrees and passed through Millipore filters (HAWP 0.45μ). The filters were washed with 20ml 5% trichloroacetic acid. Radioactivity was measured in a Nuclear Chicago liquid scintillation counter using toluene-PPO-POPOP solution or PCS, a phase combining system from Amersham/Searle. Results were identical with both fluors.

Reactions to assay for the presence of ribonuclease against double stranded RNA routinely contained, in 0.25ml final volume, 50μmoles NaCl, 5μmoles Tris-HCl pH 7.0, 0.123 μg polyI-[^{3}H]polyC and from 1 to 10μl human serum either freshly

Copyright © 1976 by Academic Press, Inc.
All rights of reproduction in any form reserved.

spun from whole blood or from aliquots frozen at -20°. The protein concentration of human serum samples varies from 65 to 80 mg/ml. The serum used in most of the experiments described had a protein concentration of 71 mg/ml. Incubations were carried out at 30°. A total of 18 different serum samples was assayed for the presence of ribonuclease activity against double stranded RNA.

To ensure that the synthetic double stranded RNA substrate was resistant to digestion in 0.2M NaCl, 0.02M Tris-HCl pH 7.0 by nucleases that attack single stranded RNA, the substrate was incubated with ribonuclease A for 20 min at 30° with a ratio of RNA to ribonuclease of 400:1. Under these conditions more than 95% of the RNA was acid solubilised when the reaction was carried out in distilled water. None was solubilised when the reaction was carried out in the high salt buffer.

Incubation of polyI-[^{3}H]polyC was also carried out with 5µl of the 0.2M NH_4Cl wash of ribosomes (protein concentration 10 mg/ml) prepared by 1000,000 xg

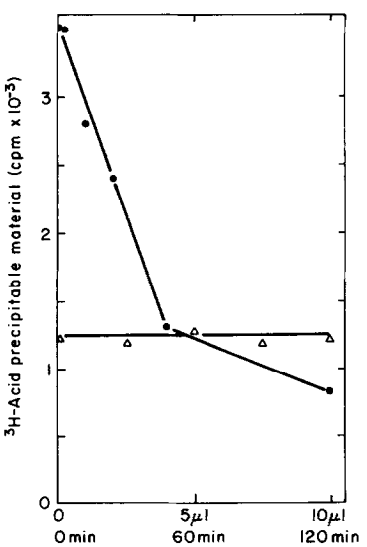

Figure 1. The effect of the addition of increasing amounts of human serum on detectable radioactivity of acid precipitable polyI-[^{3}H]polyC. The reaction is terminated after zero time at 0° by the addition of 5% trichloroacetic acid (o——o). Incubation at 30° for two hours of the standard assay mixture containing 4µl serum (△——△).

centrifugation of an extract from E. coli cells ground with alumina (gift of B. Stark). This material contains ribonuclease III, an enzyme that specifically degrades double stranded RNA (2). The reaction of the synthetic double stranded substrate with this cell fraction, carried out according to the conditions of the serum assay, resulted in the complete acid solubilisation of the substrate in 10 min at 30°.

The initial experiments to assay for ribonuclease activity against the double stranded substrate appeared to indicate that such an activity was indeed present. Incubations with 4µl of serum for 90 min at 30° showed a loss of 40% of the acid precipitable counts that had been added to the reaction as polyI-[^{3}H]polyC. However, as can be seen in Figure 1 this same decrease can be observed if the reaction is terminated immediately following the mixing of substrate and serum at 0°. As shown also in Figure 1, the decrease at 0° in zero time of acid precipitable radioactivity is dependent on the amount of serum added.

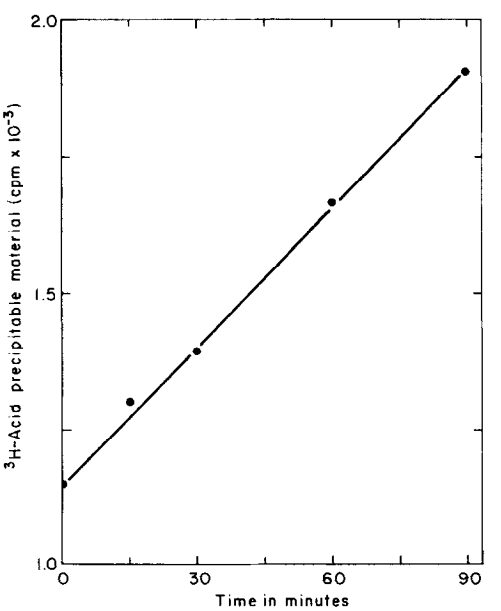

Figure 2. Incubation of the standard assay mixture containing 5 µl serum and 200 µg/ml pronase.

To eliminate the possibility of an almost instantaneous reaction it can be shown that the same disappearance of radioactivity can be obtained by passing the trichloroacetic acid precipitate of an equivalent amount of serum over a Millipore filter on which the RNA substrate has previously been dried.

The apparent disappearance of radioactivity from the acid precipitable substrate is due to the high concentration of protein in serum (approximately 70 mg/ml) and can be reproduced exactly using bovine serum albumin at the same concentration. No radioactivity can be detected in the eluate following the washing of the Millipore filters. If the protein held on the filters is, in fact, reducing the efficiency of detection of the radioactivity in the RNA, it should be possible to eliminate the quenching by solubilisation of the protein. As shown in Figure 2, incubation of the reaction mixture with pronase at a concentration of 200 μg/ml is accompanied by a gradual return of detectable radioactivity. The degree of quenching is independent of the amount of RNA added. A given volume of serum reproducibly reduced detectable radioactivity in RNA samples by the same fraction, dependent only on the amount of protein added.

Since no activity against double stranded RNA was detected in unfractionated human serum, an attempt was made to repeat the previously reported partial purification of the activity on Bio-Gel P-200 (1). 0.2ml of human serum was placed on a Bio-Gel P-200 column (1.5 cm i.d. x 100 cm) and eluted with a buffer containing 0.2M NaCl and 0.02M Tris-HCl pH 7.0. Fractions of 1.7ml were collected and 0.2ml of each fraction assayed for activity against double stranded RNA by incubation for 60 min at 30° with 0.123μg of the synthetic substrate. The optical density at 280 nm was determined for each fraction. The "activity" against double stranded RNA paralleled the protein concentration (absorbance at 280 nm) exactly. Furthermore incubation of increasingly large aliquots of the peak fraction with substrate for zero time at 0° showed that the "activity" is solely a reflection of quenching by increasingly large amounts of protein.

Discussion. The experiments reported here demonstrate that there is no

activity in human serum that degrades synthetic double stranded RNA to acid soluble material. This is a direct contradiction of the result reported in earlier work by Stern (1). It appears that this result is due to an artifact of quenching arising from the very high protein concentration in human serum. With respect to the sera of other animals tested by Stern, it must be emphasized that the results presented here apply to human serum and bovine serum albumin alone. No experiments were carried out using the sera of other animals.

Acknowledgements. This work was carried out in the laboratory of Dr. D. Seligson under a postdoctoral fellowship (1 F22 CA00090-01) from the National Institutes of Health. The help given by Dr. S. Altman and Dr. H. D. Robertson in the preparation of this manuscript is gratefully acknowledged.

References.

1. Stern, R., Biochem. Biophys. Res. Comm., 41, 608-613 (1970).
2. Robertson, H. D., Webster, R. E., Zinder, N. D., J. Biol. Chem., 243, 82-91 (1968).