A NUCLEASE FROM ANIMAL SERUM WHICH HYDROLYZES DOUBLE-STRANDED RNA

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SUMMARY: Animal serum contains an enzyme which hydrolyzes synthetic and naturally occurring double-stranded RNA's. The entirely base-paired replicative form of an RNA virus (Semliki Forest Virus), the synthetic duplex poly I:poly C, as well as a highly structured RNA from uninfected animal cells were substrates for this enzyme under conditions in which these RNA's were resistant to ribonuclease A.

Among the many nucleases in normal animal sera are enzymes specific for DNA (1) and for single-stranded RNA (2,3). However an enzyme which degrades double-stranded RNA has not been detected previously in serum, although such an activity has been reported in bull semen (4,5). A number of observations suggest that a nuclease directed against double-stranded RNA may be present in animal tissue. The synthetic duplex poly I:poly C is degraded following uptake into animal cells (6). Double-stranded RNA's are found in normal (7,8) as well as in certain DNA virus infected cells (7). A catabolic mechanism for removal of this material must be invoked if this RNA is not to accumulate. This communication describes the detection and preliminary isolation of such a nuclease directed against double-stranded RNA from animal serum.

EXPERIMENTAL:

The preparation of the synthetic substrate used in the enzyme assay ^{3}H-poly I:poly C, and the assay procedure are described in the lengend of Table 1. Preincubation of the substrate with ribonuclease A was performed routinely with each assay to remove portions of the substrate susceptible to digestion by single-stranded nuclease. Since ribonuclease-A like activity was present in each of the samples to be assayed for double-stranded nuclease activity, preliminary incubation with high levels of added ribonuc-

TABLE 2: DNA-DNA hybridization of the top activity product. Using the method of Denhardt (8) the *in vitro* synthesized product was hybridized with filters to which ssDNA, RF DNA, *E. coli* DNA and no DNA were affixed. Each horizontal row represents a separate experiment with cts/min bound to blank filters subtracted in each case (this background value was between 6-12 cts/min)

	Input cts/min	Cts/min bound to:		
		ssDNA	RF DNA	*E. coli* DNA
Expt. 1	630	3	226	21
Expt. 2	1320	2	540	16
Expt. 3	463	0	168	7

The lack of hybridization with ssDNA and *E. coli* DNA along with the high level of hybridization to RF DNA (approximately 35-40% of the input counts) suggest that only the positive strand (the strand found in the phage particle) is being synthesized.

DISCUSSION: The results have answered the question concerning the feasibility of isolating the ssDNA synthesizing activity. Figures 1A, 1B, and 1C show that the appearance of this activity requires ØX-174 infection. When infected cells are pulsed with ^{3}H-thymidine late in infection and the DNA deproteinized from the region of the top activity fractions, this DNA sediments slightly faster than RF DNA with a nick in it (RF II) and is presumably the ssDNA replicating intermediate reported by Knippers *et al.* (2). This suggests that the top activity represents a DNA-enzyme complex. That the DNA polymerase and template DNA may coincidentally sediment to the same region of the sucrose gradient in Figure 1A would be excluded by Figure 1B if one assumes the polymerase would work with calf thymus DNA.

If ssDNA is synthesized according to the rolling circle model of Gilbert and Dressler (9) then one would assume the ssDNA synthesizing complex to have a double stranded RF DNA form off which a ssDNA "tail" would come as ssDNA was being synthesized. ØX-174 structural proteins would be attached to this "tail" constraining the complex to form the ØX-174 particle during ssDNA synthesis. Presumably there would be a ssDNA polymerase of phage or host origin associated

lease A made it possible to neglect the effect of endogenous single-stranded nuclease of the sample.

The nuclease activity directed against double-stranded RNA was examined in bovine serum. The rate of hydrolysis of ^{3}H-poly I:poly C was proportional to the amount of serum added to the reaction mixture in the range from 1 to 10 ul. The rate was also linear throughout the 60 min incubation period. A unit of activity was not defined for the enzyme because the total number of acid-precipitable radioactive counts varied among different preparations of ^{3}H-poly I:poly C. It was observed that 3 ul of bovine serum solubilized half the amount of substrate in the standard reaction mixture or 10 mumoles of duplex polynucleotide phosphate in 60 min. The nuclease directed against double-stranded RNA was examined in a number of animal sera. Table 1 demonstrates that the highest levels of activity were found in rat and rabbit sera. Guinea pig, chick, bovine, and human sera contained intermediate levels while activity was barely detectable in fetal bovine, burro, and horse sera.

Nuclease activity directed against double-stranded RNA was also observed in several tissue homogenates and in extracts of cells in tissue culture. However activity was irregular and proportionality to enzyme could not be demonstrated suggesting the presence of an intracellular inhibitor.

Other base-paired RNA's were tested as substrates in the standard reaction mixture. The double-stranded RNA or replicative form of the Semliki Forest Virus, a group A arbovirus, was extracted from infected ^{32}P-labeled chick embryo fibroblasts (9). This RNA was also a suitable substrate for the bovine serum nuclease. Another ribonuclease A resistant RNA possessing many of the properties of a base-paired structure has been described in normal animal cells (7,8). RNA of this type was prepared (10) from Burkitt lymphoma cells (11) and from chick embryo fibroblasts (9) following 30 min preincubation with actinomycin D and 40 min of labeling with ^{3}H-uridine and with ^{32}P-phosphoric acid respectively. Following preliminary incubation with

ribonucleases A and T_1 (5.0 and 0.5 ug/ml for 1 hr at 37°) both of these RNA's were further hydrolyzed to acid soluble material by bovine serum in the standard reaction mixture.

The nuclease activity directed against double-stranded RNA was also found in preparations of bovine serum albumin (Armour, fraction V). 0.5 mg of albumin contained an amount of activity equivalent to that found in 2.8 ul of bovine serum containing 72 mg protein/ml. No activity was detected in solutions of methylated albumin, crystallized trypsin (Worthington Biochemical Corp.) or in an acetone extract of porcine pancreas (Pancreatin , Lilly Labs.).

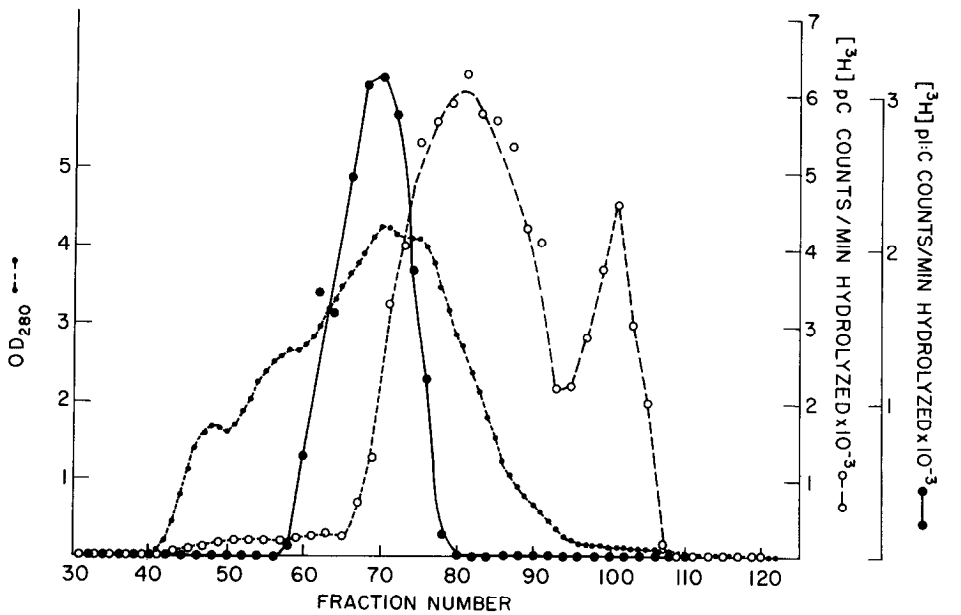

FIGURE 1: Ribonuclease activites in bovine serum directed against single and double-stranded RNA's following molecular sieve chromatography. 0.2 ml of bovine serum was placed on a column (1.6 cm i.d x 110 cm) of Bio-Gel P-200 (BioRad Corp,) and eluted with a buffer containing 0.02 M Tris-HCl (pH 7.6) and 0.2 M NaCl. Fractions of 2.2 ml were collected and stored at 4° until assayed. 0.2 ml of each fraction was added to the standard reaction mixture. For the assay of the ribonuclease activity directed against single-stranded RNA, 0.1 ml of each fraction was added to 0.4 ml of buffer containing 0.02 M Tris-HCl (pH 7.6) and 10 ul of ^{3}H-poly C containing 1 mumole of polynucleotide phosphate and 10 muCi. This mixture was incubated for 15 min at 24° and then processed exactly as described in the legend of Table 1. Protein concentrations were estimated by spectrophotometry at 280 mu. (●—●) ^{3}H-poly I:poly C hydrolyzed; (o—o) ^{3}H-poly C hydrolyzed; (●—●) absorption at 280 mu.

In an attempt to resolve the various serum ribonuclease activities from each other, 3 ml of bovine serum were placed on a Bio-Gel P-200 column for molecular sieve chromatography (Fig. 1). Single-stranded ribonuclease activity was assayed in each fraction using ^{3}H-poly C as a substrate. The peak of nuclease activity directed against double-stranded RNA preceded the two peaks of ribonuclease A-like activity from the column and coincided with the major protein peak.

The argument can be invoked that the activity directed against double-stranded RNA in animal serum reflects the presence of a serum protein which binds to the ribonuclease A in the standard reaction mixture and alters its substrate specificity. However no difference in the profile of nuclease activity directed against double-stranded RNA on the Bio-Gel P-200 column was obtained when the ribonuclease A was omitted from the standard reaction mixture and the column fractions were reassayed.

The difference in molecular size between the double-stranded and the single-stranded ribonucleases suggested from the results of molecular sieve chromatography was confirmed by density gradient centrifugation in sucrose. Using bovine hemoglobin (MW 68,000) and ribonuclease A (MW 13,700) as markers, a relative molecular size of 45,000 to 55,000 was estimated for the nuclease directed against double-stranded RNA.

DISCUSSION:

An enzyme which degrades double-stranded RNA has been detected in uninfected *E. coli* (12). This enzyme is also able to degrade the RNA moiety of DNA-RNA hybrids (13) but not single-stranded RNA. The enzyme isolated from bull semen (4,5) is non-specific and is able to hydrolyze single-stranded as well as double-stranded RNA's. The profile of ribonuclease activities in Fig. 1 indicated that the serum nuclease directed against double-stranded RNA had little or no activity against the single-stranded substrate. However further purification of the enzyme would be required to establish this point unequivocally. Recently, an enzyme activity has been found in animal cells

which hydrolyzes the RNA moiety of DNA-RNA hybrids (14). This enzyme has no activity directed against double-stranded RNA's.

Double-stranded RNA's have evoked considerable interest. These RNA's had previously been thought to occur in nature only in the replicative cycle of RNA viruses. Now double-stranded RNA's have been discovered in normal presumably uninfected (7,8,15,16) and in certain DNA virus infected (7,17) animal cells and in DNA phage infected E. coli (18). Double-stranded RNA's are potent inducers of interferon (9,19). Antibodies directed against these double-stranded RNA's occur in the serum of patients with lupus erythematosus (20,21,22) and in the serum of New Zealand black mice (20), animals which have a propensity for developing autoimmune disease. These observations suggest that double-stranded RNA's play a variety of important roles in nature. That an enzymatic activity directed against these base-paired RNA's circulates in normal animal serum supports this contention. In addition, the existence of this serum nuclease has major clinical importance in the evaluation of antiviral agents such as poly I:poly C and naturally occurring duplex molecules (23) which act as inducers of interferon. These molecules would have a finite half-life in the presence of the serum enzyme described in this communication.

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