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OBSERVATIONS ON THE KINETICS OF ACTION OF POLYSPERMINE-RIBONUCLEASE ON POLY(A)·POLY(U)

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Summary

Starting from the observation that double-stranded ribonucleic acids are hydrolyzed more rapidly by bovine pancreatic ribonuclease that has been cross-linked to polyspermine, we have made an initial examination of the kinetics of the process. The addition of eight residues of the polyamine serves to strengthen the binding to poly(A)·poly(U) 100-fold (K_m changes from $2.7 \cdot 10^{-4}$ to $2.7 \cdot 10^{-6}$ M in total U) and to increase V for hydrolysis of the susceptible poly(U) strand from 2.5 to 16.2 $\Delta A_{250} \cdot min^{-1}$ per mg enzyme. There is evidence for inhibition by the RNAase-resistant poly(A) tracts in the substrate; free poly(A) shows a K_i of about $8 \cdot 10^{-6}$ M in total A.

In a study of the cross-linking of bovine pancreatic RNAase A to spermine [1] a product was obtained which hydrolyzed the complementary poly(U) strand of poly(A)·poly(U) about 100-times as rapidly as did the parent enzyme at low substrate concentration ([U] = $6.1 \cdot 10^{-5}$ M). The naturally occurring dsRNA of reovirus-3 was similarly subject to rapid hydrolysis. The following experiments were designed to determine whether the increase in the activity of the derivatized enzyme toward double-stranded substrates is a result of change in K_m or V or both.

Bovine pancreatic RNAase A (type II-A) was from Sigma. Polyspermine-RNAase was prepared as previously described [1] with dimethyl suberimidate as the cross-linking agent; the product had an average of eight spermine residues per molecule; since analysis for lysine showed that nine out of ten lysine residues were unmodified, the spermines were largely in a single chain. Poly(A) and double strand poly(A)·poly(U) (prepared by extended annealing of equimolar quantities of poly(A) and poly(U)] were from P-L Biochemicals and had average chain lengths of about 400.

The enzymic degradation of the double-stranded substrate was determined spectrophotometrically at pH 7.5 in 0.015 M Tris-HCl/0.125 M in NaCl at 25°C under conditions described earlier [2,3]. The increase in absorbance at 250 nm was determined with a Zeiss M4QIII spectrophotometer equipped with a digital indicator PM-1 and a Speedomax recorder. 30-s tracings at 3-min intervals were made over a reaction period of 30 or 60 min; the rates of hydrolysis were constant over this period. The results are expressed as $\Delta A_{250} \cdot \text{min}^{-1}$. The relative resistance of poly(A) to ribonuclease action (cf. Ref. 4) was confirmed with polyspermine-RNAase by the precipitation assay with ^{14}C-labelled poly(A) [1]; the rate of hydrolysis of poly(A) was about one two-hundredth that of the action of the derivatized enzyme on poly(U).

Fig. 1 is an Eadie-Hofstee plot [5] of the hydrolysis of the complementary poly(U) strand of the double-stranded poly(A)·poly(U) by polyspermine-RNAase. The linear, least-square fit of the input data gave a V value of 16.2 $\Delta A_{250} \cdot \text{min}^{-1}$ per mg enzyme and a K_m value of $2.7 \cdot 10^{-6}$ M (mol total U per l). In contrast (Fig. 2), the parent pancreatic RNAase A gave a V of 2.5 $\Delta A_{250} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ and a K_m of $2.7 \cdot 10^{-4}$ M. At the lower concentrations of the substrate, both enzymes obey the Michaelis equation, but a lowering of the rate is observed at high substrate concentrations, as seen from both figures. Since this lowering of the rate may be a result of the binding of the complementary poly(A) strand of the poly(A)·poly(U) to the enzyme, the inhibition of the hydrolysis of the double-stranded RNA by free poly(A) was examined (Fig. 3). The graph indicates a K_i of about $8 \cdot 10^{-6}$ M, a binding

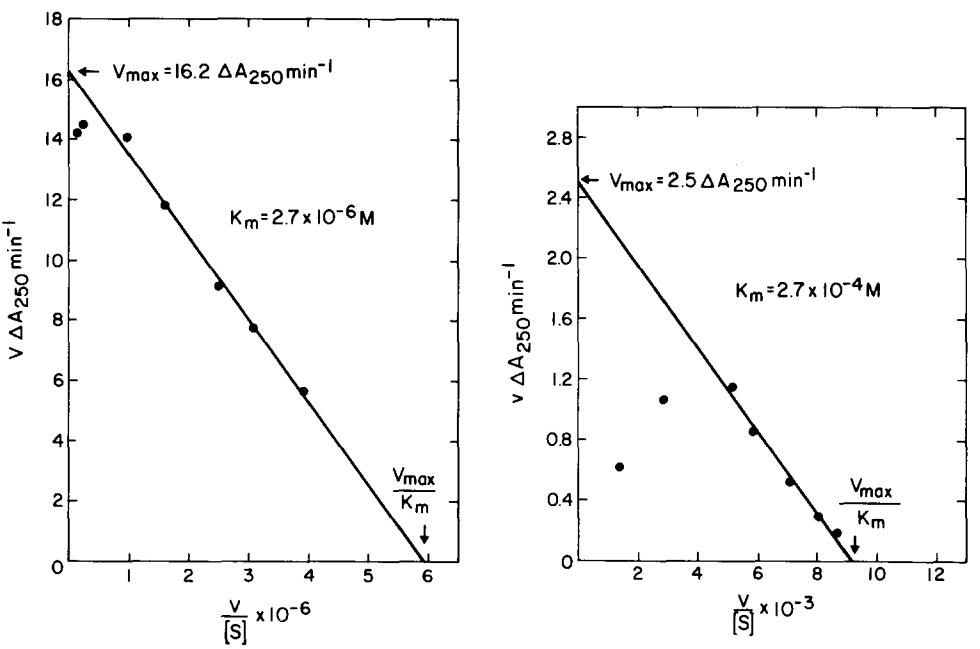

Fig. 1. Hydrolysis of poly(A)·poly(U) by polyspermine-RNAase (Eadie-Hofstee plot [5]). The initial rates of hydrolysis are expressed in terms of $\Delta A_{250} \cdot \text{min}^{-1}$ per mg of enzyme. [S] is expressed as mol of total U per l. The linear regression line has been drawn.

Fig. 2. Hydrolysis of poly(A)·poly(U) by bovine pancreatic RNAase A. (Definitions as for Fig. 1.).

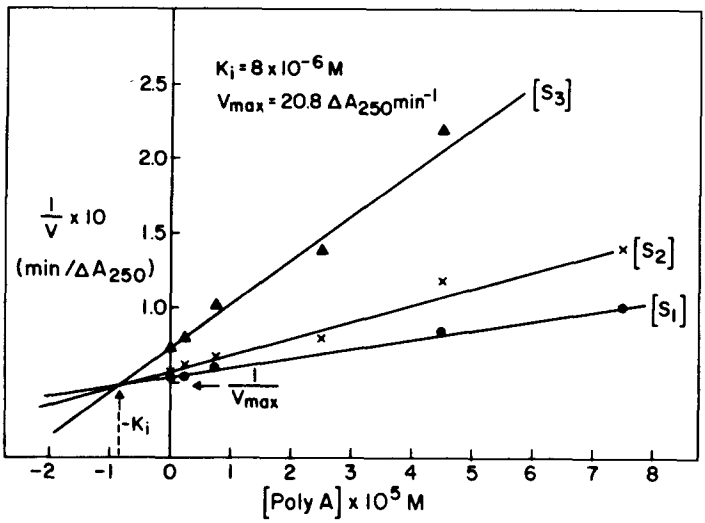

Fig. 3. Inhibition of polyspermine-RNAase hydrolysis of poly(A) ·poly(U) by poly(A) (Dixon plot [6]). The values of v are the initial rates of hydrolysis expressed as $\Delta A_{250} \cdot min^{-1}$ per mg of enzyme. The substrate concentrations (S_1, S_2, and S_3) are, respectively, $7.5 \cdot 10^{-5}$ M, $4 \cdot 10^{-5}$ M, and $7.5 \cdot 10^{-6}$ M in terms of total U. [Poly A] is expressed as mol of total A per l. The linear regression lines have been drawn.

constant close to that of the enzyme for the double-stranded substrate. The system is a complex one and the nature of the competition may be of a mixed type [7].

The observed K_m and V are thus apparent values that apply to the inherently complex system of the hydrolysis of a double-stranded RNA when one of the strands is inhibitory. The comparison of the values obtained with polyspermine-RNAase and RNAase provides a kinetic explanation of the increased rate of hydrolysis of poly(A)·poly(U) by the enzyme to which the polyspermine side-chain has been added; the derivatization increases the strength of binding 100-fold and the V is increased 6-fold. These results apply at below enzyme-saturating substrate concentrations in the range of 10^{-6} to 10^{-5} M total U.

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