COMPETITIVE INHIBITION OF ACID DEOXYRIBONUCLEASE BY POLYRIBONUCLEOTIDES

by

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Received September 11, 1964

Acid deoxyribonuclease (DNase) has been recently prepared as a homogeneous protein from hog spleen (Bernardi, Griffé and Appella, 1963 ; Bernardi and Griffé, 1964). A study of the mechanism of degradation of deoxyribonucleic acid (DNA) by acid DNase has revealed that native DNA is degraded according to both a "single hit" and a "double hit" kinetics (Bernardi and Sadron, 1961 ; 1963 ; 1964 ; Mac Hattie, Bernardi and Thomas, 1963). The "double hit" degradation takes place through the random splitting of one or another of the two strands, as in the case of the degradation by pancreatic DNase (Thomas, 1956 ; Schumaker, Richards and Schachman, 1956), whereas the "single hit" degradation apparently occurs through the simultaneous splitting of both strands at the same level. Besides its activity on DNA, acid DNase shows a phosphodiesterase activity on Ca bis (p-nitrophenyl) phosphate $_2$ and the p-nitrophenyl esters of deoxyribonucleoside-3'-phosphates (Bernardi and Griffé, 1964).

The results of Lehman, Roussos and Pratt (1962) and of Lehman (1963) on the competitive inhibition by polyribonucleotides of E. coli endonuclease, an enzyme which resembles acid DNase in several respects, prompted a similar investigation on the spleen enzyme. It was thought that, if present, the inhibition phenomenon

might be a useful tool for understanding the enzyme-polynucleotide interaction and possibly some features of the catalytic action of acid DNase.

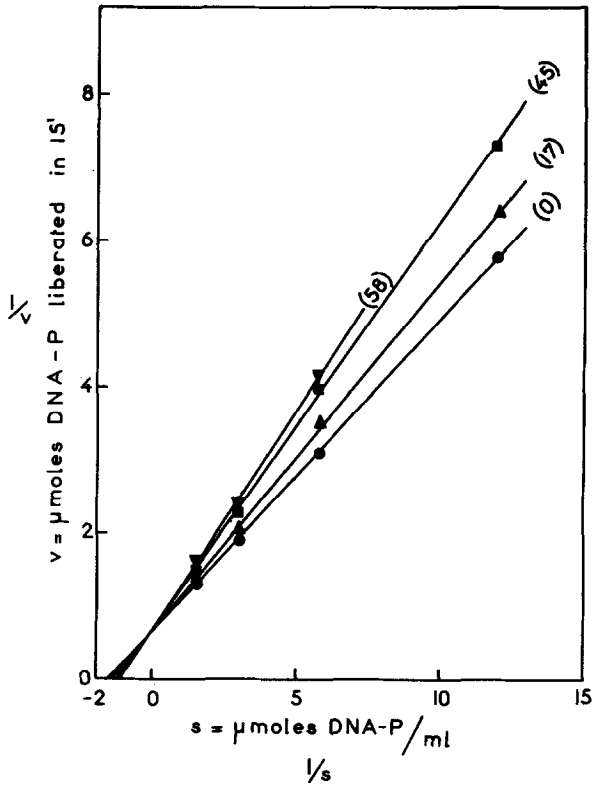
The results obtained with polyribonucleotides will be briefly reported here. A detailed account of this work, also dealing with the more recently discovered inhibition of acid DNase by enzyme-resistant polydeoxyribonucleotides, will appear elsewhere in due time.

The following natural or synthetic polyribonucleotides were assayed for their inhibitory activity : yeast soluble ribonucleic acid (s-RNA ; a preparation from Prof. Ebel's laboratory), ribosomal RNA from Ehrlich ascites tumour cells (a preparation already used in previous work by Bernardi and Timasheff, 1961), polyadenylic acid (poly A ; Miles, Clifton, N. J. ; or Calbiochem, Los Angeles, Calif.), polyuridylic acid (poly U ; Miles, or Calbiochem), polycytidylic acid (poly C ; Miles), polyinosinic acid (poly I ; Miles) and the poly A - poly U complex.

All the above substances were generally used at a 10-100 ug level ; the DNA present in the incubation mixture varied between 200 and 800 ug. The DNase assay was carried out as described by Bernardi and Griffé (1964), the only difference being that cytochrome c replaced bovine serum albumin in the enzyme diluting solution.

With the remarkable exceptions of poly A and poly C, which did not show any effect on the DNase activity, all polyribonucleotides tested exhibited an inhibitory activity, this being highest for the poly A - poly U complex. In every case inhibition was of the competitive type, as indicated by both 1/v vs. 1/s, and 1/v vs. I

plots (v being the hydrolysis rate, s the substrate concentration, and I the inhibitor concentration). Figure 1 shows a Lineweaver-Burk plot obtained using three different s-RNA concentrations. Very interestingly, the phosphodiesterase activity of acid DNase was also inhibited by the polyribonucleotides.



Competitive inhibition of hog spleen acid DNase by yeast s-RNA. The numbers in parentheses indicate the mumoles of s-RNA - P / ml.

These results are to a large extent similar to those reported for E. Coli endonuclease, the similarity of the effects of s-RNA and poly A - poly U on the two enzymes being particularly striking. Here, too, inhibition is specific and does not simply represent the binding of a polyanion by a basic protein ; in fact some

polyribonucleotides are ineffective as inhibitors, and excess cytochrome c, a strongly basic protein, does not interfere with inhibition. Some differences are also evident, however. For instance, poly U has a definite inhibitory effect on acid DNase, yet it does not affect the E. coli enzyme ; poly A does not inhibit either enzyme, but this similar behaviour is rather misleading, since the secondary structure of poly A is quite different at the two pH values used in the enzymatic digestions (5.0 for acid DNase, 7.5 for the bacterial enzyme).

These data show that the inhibitory activity cannot be correlated in an elementary way with the natural or synthetic origin of the polyribonucleotides nor with their secondary structure. It is evident that the formation of the acid DNase - polynucleotide complex is extremely specific as far as the structure and composition of the polynucleotide is concerned. Since acid DNase may be prepared essentially free of ribonuclease activity and in sizable amounts (Bernardi and Griffé, 1964), physical chemical investigations on the acid DNase - polyribonucleotide complex formation appear to be feasible. Preliminary results obtained in this laboratory using sedimentation techniques indicate the existence of well defined complexes in the case of the acid DNase - s-RNA interaction. Our understanding of this specific type of interaction seems to be of obvious importance for other enzyme - polynucleotide systems as well, for instance those involving DNA and RNA-polymerase, or s-RNA and the activating enzymes.

Acknowledgements

This work has been aided by grant UR-E9-(10-60)-80 from the U. S. Department of Agriculture.

The author thanks Prof. C. Sadron for his interest and encouragement. The excellent technical help of Miss Betty Nubel is gratefully acknowledged.

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