THE HYDROLYSIS OF "SOLUBLE" RIBONUCLEIC ACID BY SNAKE VENOM PHOSPHODIESTERASE*

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Venom phosphodiesterase (VPD) is primarily an exonuclease releasing 5'-nucleotides from the 3'-hydroxyl end of a nucleic acid (Razzell and Khorana, 1959). It is in addition a 5'-phosphate forming endonuclease. The endonuclease action is much slower than the exonuclease. Endonuclease action is demonstrated by the splitting of cyclic oligonucleotides (Razzell and Khorana, 1959). It is also shown in the splitting of oligonucleotides terminating in a 3'-phosphate since a nucleoside-3',5'-diphosphate is released last rather than first (Felix et al, 1960).

This paper presents a titrimetric analysis of the course of the complete digestion of sRNA by a VPD preparation, the method employed for purifying the VPD, and analyses for contaminating enzymes which would interfere with the use of the VPD for structure studies on sRNA.

The starting material for the enzyme purification is the commercial VPD (Worthington Biochemical Corp.) which has been purified from Crotalus adamanteus venom by Step 1 of Williams, Sung, and Laskowski (1961). The procedure adopted for the further purification of this VPD is patterned after the cation-exchange step used by Björk (1961) for the purification of VPD from Ringhals cobra venom. This step was

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adopted in the present study when it was found that VPD purified in this way digests sRNA to completion in a nearly linear fashion producing mononucleotides and only a trace of nucleosides. Digestion of bulk yeast sRNA by the commercial VPD under the conditions used here stops short at from 60 to 80% of completion.

For the purification, commercial VPD is applied to a Dowex 50 column at pH 5.8 and is recovered in the effluent immediately following the dead volume of the column. Dowex 50 W-X8 (200-400 mesh, 1.9 meq/ml wet volume, Baker Analyzed Reagent) is washed with 5 N NaOH, 1 N NaOH, water, and 0.3 M sodium acetate buffer, pH 5.8. A 7 ml column of the washed resin, 0.6 x 20 cm, is equilibrated with 0.005 M acetate pH 5.8. The column is operated at room temperature and by hand as the total effluent to be analyzed is only about 4 ml. The drops as they emerge from the column are analyzed following the dead volume of about 2.2 ml. A convenient test for protein can be run on each drop by withdrawing a few microliters into a fine capillary and then drawing up 0.6 N $HClO_4$ so that the solutions mix in the capillary. A visible turbidity can be seen with as little as 0.2 mg protein per ml.

The contents of a 5-6 mg bottle of commercial VPD is dissolved in 1.0 ml water. This solution on analysis shows from 8 to 12 absorbancy units determined at 280 mμ (AU_{280}). This solution of VPD is applied to the equilibrated Dowex 50 column followed by more of the 0.005 M acetate pH 5.8. The purified VPD emerges between about 2.2 and 4.0 ml of effluent, the exact volume being determined by the capillary protein test. Before the purified VPD is frozen for storage, the pH of the solution is adjusted to about 7.5. Recovery of activity is about 60%, and of AU_{280} about 45%. The loss of activity is due to retention of VPD on the Dowex 50 and occurs only the first time that a column is used. If a column which has been used and loaded in the above fashion is washed with 0.3 M acetate pH 5.8, reequilibrated with 0.005 M acetate pH 5.8,

and used again, recovery of activity will be practically quantitative and recovery of AU_{280} will be about 80%. A loaded column can be used repeatedly in this way. It must be kept at 4° when it is not in use.

All the assays for contaminating enzymes in this purified VPD preparation were run under the conditions used for the digestion of sRNA as given in the heading of Fig. 1. The 5'-nucleotidase was found to be reduced by the purification step to 16-25% of that in the commercial VPD. With an enzyme concentration giving an A_{280} of 0.25, 0.001 M A-5'-P is hydrolyzed under the above conditions at the rate of approximately 0.01 μmoles/ml/hr. The purified VPD showed no significant hydrolysis of A-2'(3')-P. This shows the absence of phosphomonoesterase activity.

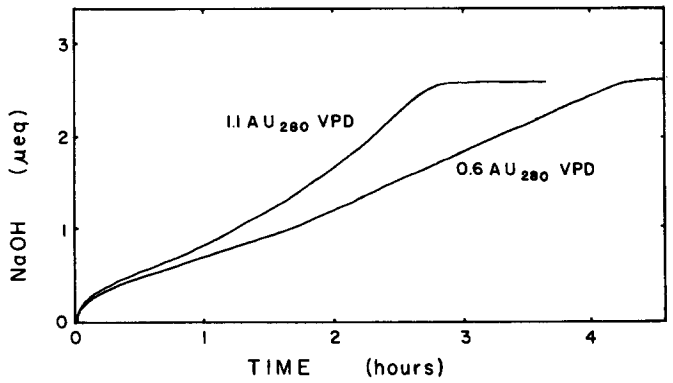

Fig. 1. The digestion of sRNA by purified VPD at pH 7.4 and 37° followed by titration of acid released in a pH stat. Atmospheric CO_2 was excluded from the enclosed titration vessel by a stream of nitrogen. The vessel contained in the final volume of 2.0 ml, 0.6 or 1.1 AU_{280} purified VPD as indicated, 0.06 mmoles $MgCl_2$, and 0.85 mg yeast sRNA (2.5 μmoles nucleotides). The digestion was initiated by the addition of the sRNA. The curves are corrected for the initial titration which occurs when sRNA is introduced into 0.03 M $MgCl_2$.

To test for 3'-P forming endonuclease activity, a complete digestion of sRNA by the purified VPD was carried out, and the digest was analyzed for nucleoside-3',5'-diphosphates by paper electrophoresis. Only mononucleotides and a trace of nucleosides could be detected. Since phosphomonoesterase is absent, each split in the sRNA brought about by a contaminating 3'-P forming endonuclease (Georgatsos and Laskowski, 1962) would give rise to a nucleoside-3',5'-diphosphate in the complete digest.

The titrimetric analysis of the complete digestion of sRNA by the purified VPD is shown in Fig. 1. Digestions were run at pH 7.4 rather than at the pH optimum of the enzyme which is about 9, because yeast sRNA alone at pH 8.8 is hydrolyzed at an appreciable rate as shown by a pH-stat titration. No specific stimulation by Ca^{++} of the type reported by Nihei and Cantoni (1963) is seen under the conditions described here.

The VPD digestion of sRNA shows a rapid initial phase during which about 10% of the phosphodiester linkages are split. This is followed by a much slower rate of digestion perhaps due to the stability of the secondary structure of the sRNA in the presence of Mg^{++}. If, after digestion of one portion of sRNA, a second portion is added, the initial rapid phase is again observed, which shows that the slow second phase is not due to product inhibition. The rate accelerates during the second half of the digestion.

The extent of 5'-P forming endonuclease action of the VPD during such a digestion is difficult to determine. At the high enzyme level required for the complete digestion of sRNA, pancreatic RNase "core" oligonucleotides are hydrolyzed at a very appreciable rate, and digestion of such oligonucleotides has been shown to be endonucleolytic (Felix et al, 1960). Any small fragments released from the sRNA by the 5'-P forming endonuclease action would escape detection because the enzyme acts more rapidly on such small fragments than on the structured sRNA.

The purified VPD preparation described here has proved to be satisfactory for the sequence analysis of large oligonucleotides by a new method developed by Holley, Madison, and Zamir (1964).

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