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Studies on the ribonuclease activity of *Proteus mirabilis*

Previously we reported on the partial purification and properties of a cyclic phosphodiesterase having 3'-nucleotidase activity from *Proteus mirabilis*[1]. This enzyme catalyzes a two-step reaction in which ribonucleoside 2',3'-cyclic phosphates are hydrolyzed to the ribonucleoside 3'-phosphate which undergoes further hydrolysis to the corresponding nucleoside. The enzyme does not exhibit any ribonuclease activity. The physiological role of the cyclic phosphodiesterase is not clearly understood, although the results of the previous studies suggest that it may participate with other enzymes in the sequence of steps leading to the complete degradation of RNA. In this regard the enzyme may function together with a ribonuclease which hydrolyzes RNA to produce ribonucleoside 2',3'-cyclic phosphates. These products could in turn be acted upon by the cyclic phosphodiesterase to provide a means whereby RNA is degraded to the nucleoside level. To investigate this possibility studies were carried out on the ribonuclease activity of *P. mirabilis*. The results of these studies are described in the present report.

The ribonuclease activity of *P. mirabilis* was partially purified by fractionation of the cell-free extract by hydroxylapatite column chromatography. The hydroxylapatite had been equilibrated with 0.005 M phosphate buffer containing 0.01 M EDTA at a pH of 7.4. Elution of the enzyme activity was carried out stepwise by increasing the phosphate buffer concentration. All eluting buffers contained 0.01 M EDTA and had a final pH of 7.4. The ribonuclease activity was resolved into two components; the first eluted with 0.02 M phosphate and the second eluted with 0.15 M phosphate buffer. All studies described in the present report were carried out with the second fraction which is designated *P. mirabilis* ribonuclease-2. The *P. mirabilis* ribonuclease-2 which was purified 30-fold is free of contaminating phosphatase and cyclic phosphodiesterase activities.

Studies on the substrate specificity of this enzyme were first carried out by examining the products formed after the hydrolysis of poly (A), poly (C) and poly (U). These polynucleotides (0.4 mg in 0.1 M Tris–acetate buffer, pH 6.1) were incubated with enzyme for 22 h at 37° and the resulting mixture was chromatographed (descending) in the solvent system described by STOCKX AND VAN PARIJS[2] (Solvent 1). It was found that 60–80% of the products from each of these substrates were ribonucleoside 2',3'-cyclic phosphates. These compounds were identified from their R_F values, absorbance ratios in 0.01 M HCl ($A_{250\ m\mu}/A_{260\ m\mu}$ and $A_{280\ m\mu}/A_{260\ m\mu}$) and by their chromatographic behavior after treatment with 0.1 M HCl at room temperature[3]. Further identification of these components was carried out as follows. First, the cyclic nucleotides obtained after chromatography in Solvent 1 were eluted with water and treated with cyclic phosphodiesterase (0.6 μg) for 2.5 h at 37°, and rechromatographed in Solvent 1 or in the solvent system described by WYATT[4] (Solvent 2). Second, the components obtained after chromatography in Solvent 1 were treated with 0.1 M HCl and then treated with acid phosphatase, and rechromatographed in Solvent 1. In both cases the cyclic nucleotides were converted to their corresponding nucleoside derivative. The remaining components produced from the hydrolysis of poly (A), poly (C) and poly (U) seem to be oligonucleotides, based on their chromatographic behavior in the solvent system of MARKHAM AND SMITH[5] (Solvent 3).

The activity of *P. mirabilis* ribonuclease-2 toward various ribonucleoside 2′, 3′-cyclic phosphates had also been studied. Incubation of the enzyme with cyclic cytidylate, uridylate or guanylate for 17 h at 37° and chromatography of the digests in Solvent 1 showed that no hydrolysis of these cyclic nucleotides had taken place.

The products formed after the hydrolysis of yeast RNA by *P. mirabilis* ribonuclease-2 were also found to be predominantly ribonucleoside 2′,3′-cyclic phosphates (90% of the $A_{260\ m\mu}$ material eluted). These components were identified as cyclic adenylate, guanylate and uridylate. Cyclic cytidylate was not detected after 16–20 h of incubation, but after 44 h of incubation, a small amount of this material was observed.

The compounds produced after the hydrolysis of poly (A), poly (C) and poly (U) in the presence of both *P. mirabilis* ribonuclease-2 and cyclic phosphodiesterase have also been examined. 55% of the material produced after the hydrolysis of poly (A) was identified as adenosine. A mixture of poly (A) and poly (U) was converted almost completely to adenosine and uridine and a mixture of poly (A) and poly (C) was converted to adenosine (43%) and cytidine (24%).

In summary, the results of this study demonstrate the presence of a ribonuclease from *P. mirabilis* which hydrolyzes various synthetic polynucleotides and yeast RNA to produce predominantly ribonucleoside 2′,3′-cyclic phosphates. In contrast to the activity exhibited by certain other ribonucleases[6,7], *P. mirabilis* ribonuclease-2 does not convert the cyclic nucleotides to the 3′-mononucleotide derivative. These results therefore seem to suggest that an interrelationship exists between *P. mirabilis* ribonuclease-2 and the cyclic phosphodiesterase activity of *P. mirabilis*, in that through the combined action of these two enzymes RNA, *in vivo*, may be degraded to the nucleoside level.

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