HYDROLYSIS OF THE POLYMER FORMED FROM NAD WITH RAT LIVER PHOSPHODIESTERASE YIELDING NUCLEOSIDE 5'MONOPHOSPHATE

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Recently, the formation of a new polymer from NAD by isolated rat liver nuclei was reported (Sugimura et al., 1967; Fujimura et al., 1967a; Chambon et al., 1966; Nishizuka and Hayaishi, 1966). The biological significance of this polymer is still unknown. In connection with the turnover of NAD, it is important to know how this polymer is degraded in vitro and in vivo.

In studies on the degradation of RNA by rat liver we obtained a new phosphodiesterase which forms nucleoside 5'monophosphate (Futai and Mizuno, J. Biol. Chem., submitted). Moreover, we found that this phosphodiesterase hydrolyzed the new polymer described above. This is the first indication of an enzyme from animal tissue which hydrolyzes this polymer.

Abbreviations used: NAD, Nicotine amide dinucleotide; ApU, Adenyl-(3',5')-uridine.

EXPERIMENTAL

Purification of Phosphodiesterase from Rat Liver

The phosphodiesterase was purified 200 fold from a rat liver homogenate by n-butanol treatment, streptomycin sulfate precipitation, ammonium sulfate fractionation, batch-wise treatment with DEAE cellulose and finally DEAE Sephadex column chromatoraphy (Futai and Mizuno, J. Biol. Chem., submitted). The enzyme, had an optimum pH at around 10, and hydrolyzed p-nitrophenyl-uridine 5'phosphate, NAD and various oligonucleotides rapidly to form nucleoside 5'monophosphate. DNA and RNA were hydrolyzed only slowly.

Preparation of ^{14}C-labeled Polymer

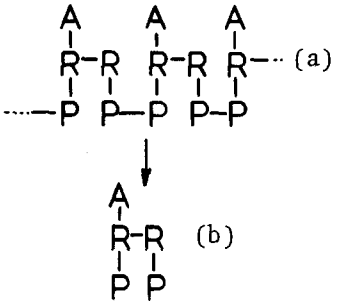
The polymer (Fig. 1(a)) was formed using the insoluble fraction of disrupted rat liver nuclei prepared according to the description of Sugimura et al., (1967; Fujimura et al., 1967a). For enzymological studies, the above preparation of polymer was treated further with phenol, RNase, DNase, spleen phosphodiesterase and finally with pronase (Sankyo Co. Japan). It was then precipitated with 5 % perchloric acid, heated at 70° for 10 minutes, dissolved in 0.3 N NH_4OH and dialyzed thoroughly against distilled water. It was essential to remove DNA, RNA and protein to obtain reproducible results. Radioactivity was measured with a low background windowless gas flow counter (Aloka Co. Tokyo).

RESULTS AND DISCUSSION

The chemical structure of the new polymer synthesized from NAD was proposed to be as shown in Fig. 1(a) (Sugimura et al., 1967).

The rat liver phosphodiesterase hydrolyzed the polymer at approximately the same initial velocity as snake venom phosphodiesterase.

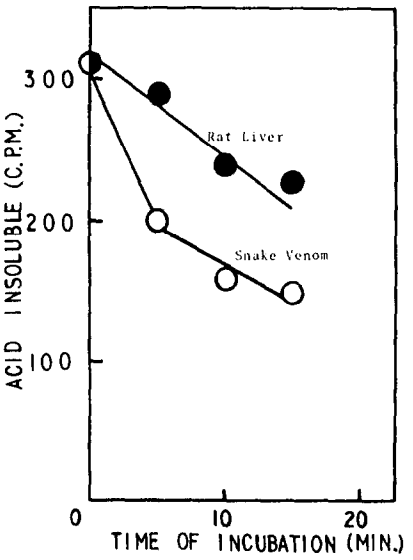
Fig. 1. Proposed Structure of the New Polymer



where:
R=Ribose
P=Phosphate
A=Adenine

As shown in Fig. 2, after 20 minutes incubation of 1m μmole of polymer containing 300 cpm, 70 cpm were solubilized with 1.7 units of rat liver phosphodiesterase, while 140 cpm were solubilized with 1.3 units of snake venom phosphodiesterase. The optimal pH for

Fig. 2. Hydrolysis of the Polymer with Rat Liver or Snake Venom Phosphodiesterase.



The polymer (300 cpm/1m μmole) was incubated with enzyme for the indicated times. The incubation mixture contained 1m μmole polymer, 0.05 M Tris-Cl (pH 9.0) and 1.7 units of rat liver (●—●) or 1.3 units of snake venom phosphodiesterase (○—○), in a total volume of 0.4 ml. After incubation at 37°, 0.4 ml of 10 % and 1.0 ml of 5 % perchloric acid were added and then after 30 minutes storage in the cold, the tube was centrifuged at 3,000 × g for 3 minutes. The precipitate thus obtained was dissolved in aqueous 3N ammonia and radioactivity was measured as described in the text.

hydrolysis of the polymer was around pH 10 (Fig. 2). This alkaline optimal pH, which is different from that of nucleotide pyrophosphatase from rat liver (Schiselfeld, Eys and Touster, 1965), was quite similar to that for the hydrolysis of p-nitrophenyl uridine 5'monophosphate, NAD or ApU by rat liver phosphodiesterase, as reported separately (Futai and Mizuno, J. Biol. Chem., submitted). Hydrolysis of the polymer was almost completely inhibited by 10^{-3}M EDTA or $HgCl_2$, and slightly inhibited by 10^{-3} M p-chloromercuric benzoate.

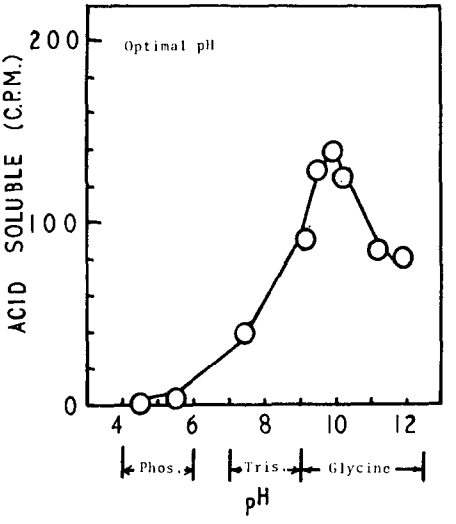


Fig. 3. Effect of pH on Hydrolysis of the Polymer by Rat Liver Phosphodiesterase.

The polymer was incubated in the presence of 0.1 M NaCl with rat liver phosphodiesterase as described in the legend of Fig. 2 except that various buffers were used.

A slight activation was observed with 0.1 M NaCl or 10^{-3} M $MgCl_2$. These effects were also shown using p-nitrophenyl uridine 5'monophosphate, NAD or ApU as substrate. The above results suggest that the polymer was hydrolyzed by phosphodiesterase.

The hydrolyzed product was found to be as shown in Fig. 1(b) by paper chromatography using two solvent systems (Fujimura et al., 1967a). The polymer synthesized from NAD with the precipitable fraction of the nuclei was degraded in vitro on prolonged incubation, as shown previously by Fujimura et al., (in press). The present enzyme may be partly responsible for this degradation, since the

enzyme and the polymer synthesizing systems are both present in the nuclear fraction precipitated by centrifugation.

This polymer was not hydrolyzed by pancreatic DNase, RNase, spleen phosphodiesterase or potato NAD pyrophosphatase as shown by Fujimura et al., 1967a and Hasegawa et al., (1967). Thus this phosphodiesterase is the first enzyme found in animal tissue which hydrolyzes the polymer formed from NAD.

Details of this work will be published elsewhere.

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