structure located close to the ventricular septal defect specifically operates to permit free flow of blood from right ventricle into the aorta. However, the development of a right to left shunt during diving in the turtle is a clear example of a circulatory adaptation which ensures provision of the optimum requirements necessary for metabolism.

J. EUGENE MILLEN
H. VICTOR MURDAUGH, JR.
CATHERINE B. BAUER
EUGENE D. ROBIN

Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; Department of Medicine, University of Alabama Medical Center, Birmingham; and *Mount Desert Island Biological Laboratory, Salisbury Cove, Maine*

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7. This work was supported by research grant HE-05059 from the NIH and by a research grant from the Tuberculosis League of Pittsburgh.

9 June 1964

Specificity of Potassium-Activated Phosphodiesterase of Escherichia coli

Abstract. *A potassium-activated phosphodiesterase that hydrolyzes polyribonucleotides to 5′-mononucleotides has been purified approximately 600-fold from extracts of* Escherichia coli *B. The purified enzyme appears to be specific for single-stranded polyribonucleotides: helical forms are not hydrolyzed, nor do they inhibit the hydrolysis of single-stranded chains.*

The usefulness of nucleases that show specificity for the conformation (or secondary structure) of their DNA (*1*) substrates has been widely demonstrated (*2, 3*). In studies with RNA, pancreatic ribonuclease has been used as an indicator of helical configuration (*4*) although its specificity for attacking single-stranded polyribonucleotides is not definitive. In addition, polynucleotide phosphorylase was shown to be relatively inactive on helical polyribonucleotides (*5*). We would now like to report that the potassium-activated phosphodiesterase of *Escherichia coli*, recently described by Spahr and Schlessinger (*6*), shows a clear-cut specificity for single-stranded polyribonucleotides. The usefulness of the enzyme for studies of polyribonucleotide structure as well as the possible physiological significance of its specificity will be discussed.

The enzyme preparation described by Spahr and Schlessinger (*6*), a crude extract from which ribosomes were removed by centrifugation, hydrolyzed polyribonucleotides, including polyU and the messenger RNA of bacteriophage T_2, to 5′-mononucleotides. Thus the enzyme was distinguishable from the previously described ribonuclease of *E. coli* (*7*) which is found bound to ribosomes and produces 3′-mononucleotides. At the time of the Spahr report (*6*) we were engaged in the purification of a nuclease present in the supernatant fluid of extracts of *E. coli* B that had been centrifuged at 105,000*g*; the properties of that enzyme led us to conclude that it is the same as the potassium-activated phosphodiesterase described (*6*).

We have now purified the enzyme approximately 600-fold. The hydrolysis of C^{14}-labeled polyA was used to measure enzyme activity, one unit of enzyme being equivalent to the hydrolysis of 1 μmole of polyA to acid-soluble material per hour. Polymer concentrations are given as mononucleotide (or phosphate) equivalents. The optimum assay conditions are similar to those reported previously (*6*), namely, 1.5m*M* Mg^{++}, 0.1*M* KCl, *p*H 7.5. Approximately two-thirds of the activity of the initial crude extract (20,000*g* supernatant of an extract of sound-treated *E. coli* B) is apparently lost upon treatment with protamine sulfate or removal of the ribosomes by centrifugation at 100,000*g*. As yet, we have been unable to account for these missing units. The best preparation we obtained was purified about 600-fold compared to the supernatant fluid resulting from protamine sulfate treatment (*8*) and it had a specific activity (units per milligram of protein) of 2180. The purified enzyme showed no detectable phosphatase activity at *p*H 4.7, 7.5, or 9.0, with 5′-AMP as substrate. Under the conditions used we would easily have detected activity equivalent to one-thousandth of the phosphodiesterase activity. The maximum possible contamination with polynucleotide phosphorylase is of the same order of magnitude. With this preparation, as well as with less highly purified enzyme, the only product detected upon hydrolysis

Table 1. The effect of nonsubstrate polynucleotides on polymer hydrolysis. Reaction mixtures (0.1 ml) contained 0.1*M* tris, *p*H 7.6; 1.5m*M* $MgCl_2$; 0.1*M* KCl; 1.2 μmole of polyU or 2.2 μmole of polyA per milliliter; and 1.3 enzyme units per milliliter (hydroxylapatite fraction, specific activity 2180). Additions per milliliter were as follows: *E. coli* sRNA, 20.6 OD_{260} units; yeast sRNA, 19.4 OD_{260} units; *E coli* DNA, 0.93 μmole P in the polyU experiment, and 0.68 μmole P in the polyA experiment; and polyI, 2.8 μmole. The reaction mixtures were treated as described in the legend to Fig. 1. The results are expressed as millimicromoles of mononucleotide hydrolyzed per reaction per 20 minutes.

Substrate	Addition				
	None	*E. coli* sRNA	Yeast sRNA	*E. coli* DNA	PolyI
PolyU	18			18	20
PolyA	42	44	41	38	

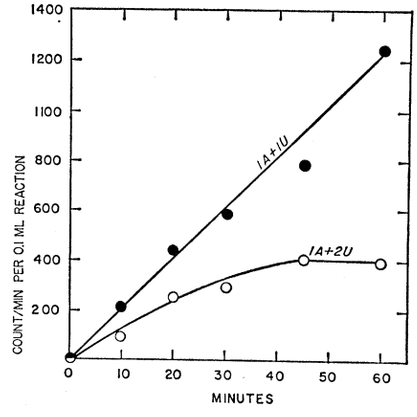

Fig. 1. Hydrolysis of complexes of polyA and polyU. Each reaction mixture contained 0.1*M* tris, *p*H 7.6; 0.1*M* KCl; 1.5m*M* $MgCl_2$; 0.1 mg of bovine serum albumin per ml; 0.76 enzyme unit (DEAE-cellulose chromatography fraction, specific activity 570) per milliliter of reaction mixture; and 1.13 μmole of polyA-C^{14} (specific radioactivity 27,300 count/min per μmole) per milliliter. They also contained polyU-C^{14} (44,300 count/min per μmole) as follows: 1) Closed circles, A + U experiment, 1.16 μmole; and 2) open circles, A + 2U, 2.32 μmole/ml. All reactions were carried out at 37°C. At the indicated times 0.1 ml portions were removed. Precipitation of undigested polymer was effected by two volumes of cold ethanol in the presence of unlabeled carrier RNA and 0.1*M* NaCl (*6*). Portions of the supernatant were counted. Results are expressed as count/min of soluble product per 0.1 ml of reaction.

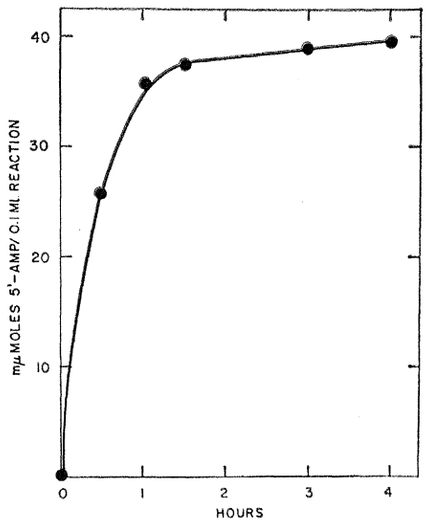

Fig. 2. Hydrolysis of mixtures of polyA + polyU. The reaction mixture and procedure were as described in the legend to Fig. 1, except that the mixture contained 1.55 μmole of polyU (unlabeled) per milliliter. The calculated amount of free polyA-C^{14} is, therefore, 0.035 μmole per 0.1 ml. Results are expressed as millimicromoles of 5′-AMP produced per 0.1 ml of reaction mixture.

of polyA or polyU was 5′-AMP or 5′-UMP, respectively. Highly purified enzyme preparations are quite unstable, even in the presence of bovine serum albumin. Approximately 95 percent of the activity is lost after 2 weeks storage at 4°C. Furthermore, in the presence of substrate, Mg^{++}, and KCl, the purified enzyme loses its activity within 15 minutes at 48°C. The relative activity of the purified fraction towards different polyribonucleotides is qualitatively similar to that reported previously (*6*), namely, polyA, 100; polyU, 55; yeast RNA of high molecular weight, 4.6; sRNA (yeast or *E. coli*), approximately 1. PolyC is hydrolyzed at a rate similar to that found with polyU.

Carbon-14–labeled polyA, polyC, and polyU and unlabeled polyA and polyUG (base ratio 0.58 uracil to 1 guanine) were prepared with polynucleotide phosphorylase purified from *Micrococcus lysodeikticus* (*9*). PolyI and polyU were obtained from Miles Laboratories. Nucleic acids were prepared in this laboratory according to the following published procedures: *E. coli* DNA (*2*), *E. coli* sRNA (*10*), and yeast sRNA (*11*). Carbon-14–labeled high molecular weight RNA from yeast was isolated (*12*) from yeast grown on C^{14}-orotic acid and was purified further by phenol extraction. All samples were assayed for radioactivity in a Packard liquid scintillation counter in 10 ml of Bray's solution (*13*) containing 0.1 ml of 1*N* NH_4OH. Paper chromatography was carried out in a solvent containing saturated $(NH_4)_2SO_4$, isopropanol, and 1*M* sodium acetate in the ratio 80:2:18 by volume, respectively (*14*).

When polyA and polyU are mixed under the aforementioned standard assay conditions, a triple helix containing one strand of polyA and two strands of polyU is formed (*15*). In Fig. 1, the lower curve shows the release of soluble radioactive material when a mixture of 1 mole of polyA-C^{14} and 2 moles of polyU-C^{14} is treated with enzyme. The reaction is quite slow compared to the hydrolysis of either polymer alone (see legend) and stops after only a small percentage of the possible radioactivity has been released. Paper chromatography of a portion of the reaction mixture after 1 hour of incubation showed both 5′-AMP and 5′-UMP as products. Elution from the paper strips by quantitative technique indicated that twice as much 5′-UMP as 5′-AMP had been formed. It is possible that the formation of the small amount of mononucleotide reflects the time required for completion of triple helix formation (*15*).

The upper curve in Fig. 1 shows the release of radioactivity from an equimolar mixture of polyA-C^{14} and polyU-C^{14}. In this case, formation of the expected triple helix leaves one half of the total polyA free in solution. If, as is suggested by the experiment with a mixture of polyA and polyU (1:2), the triple helix is resistant, only the polyA should be hydrolyzed. As thereby predicted, paper chromatography of portions of the reaction mixture after 1 hour of incubation showed 5′-AMP as the only detectable product.

These results are confirmed by the experiment shown in Fig. 2. In this case a small excess of unlabeled polyU over the amount of polyA-C^{14} was present, and the reaction was allowed to go to completion. The reaction stopped when the calculated amount of free polyA-C^{14} had been hydrolyzed. Again, 5′-AMP was the only detectable product.

Under our standard assay conditions mixtures of polyA and polyI also form a triple helix containing one polyA strand and two polyI strands (*16*). Figure 3 shows the rate of release (at 23°C and 37°C) of 5′-AMP from polyA-C^{14}, a mixture of 1 mole of polyA-C^{14} and 1 mole of polyI, and a mixture of 1 mole of polyA-C^{14} and 2 moles of polyI. The results are similar to those obtained with the mixtures of polyA and polyU. No hydrolysis of polyI itself could be detected, and this finding may reflect an ordered structure for polyI under our conditions (*17*). However, the polyI was not simply a nonspecific enzyme inhibitor since, as will be shown, it did not inhibit polyU hydrolysis.

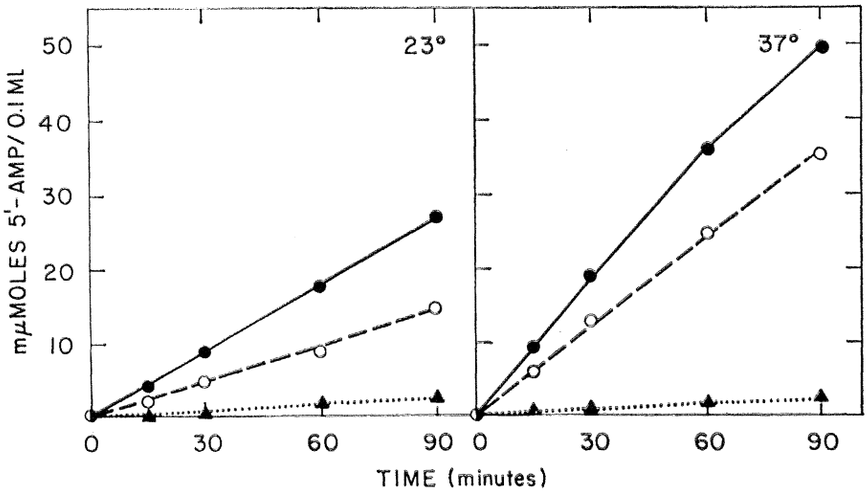

Fig. 3. Hydrolysis of mixtures of polyA and polyI. Reaction mixtures and procedure were as described in the legend to Fig. 1, except that they contained 0.36 enzyme unit (hydroxylapatite fraction, specific activity 2180) per milliliter of reaction. In addition, appropriate tubes contained polyI as indicated. Closed circles, no polyI; open circles, 1.1 μmole of polyI per milliliter; closed triangles, 2.2 μmole of polyI per milliliter.

As mentioned, yeast RNA of high molecular weight is hydrolyzed at a rate about 5 percent of that of polyA. Soluble RNA, known to contain a high degree of secondary structure (*18*), is hydrolyzed at 1 percent of the rate of polyA hydrolysis. Indeed, the experiments on sRNA were carried out at the limit of detectability, and it may be that sRNA is not hydrolyzed at all by the purified enzyme. We have already mentioned that polyI is resistant to hydrolysis. In addition, polyUG containing 0.58 mole of uracil to 1 mole of guanine is resistant to hydrolysis. The properties of this polymer have been described (*19*), and it is known to contain a high degree of secondary structure.

The data presented in Table 1 indicate that the addition of helical polynucleotides to reaction mixtures does not inhibit the breakdown of single-stranded polyribonucleotides. Thus, polyI which is itself resistant to enzymic attack does not affect polyU hydrolysis, and sRNA does not affect polyA hydrolysis. In addition, native DNA is not inhibitory. Actually, this finding can also be inferred from the data in Fig. 1. In the experiment shown in the upper curve, the concentration of free polyA should be 0.55 μmole per milliliter of reaction and the rate of hydrolysis is 24 μmole per hour per milliliter of enzyme. A separate experiment, in which we studied the rate of polyA hydrolysis as a function of polyA concentration, gave just such a rate at 0.55 μmole of polyA per milliliter of reaction mixture.

The evidence indicates that the potassium-activated phosphodiesterase of *E. coli* is unable to degrade helical polyribonucleotides. In this sense it differs from the ribosome-bound ribonuclease of *E. coli* (*7, 20*) but is similar to polynucleotide phosphorylase (*5*). As suggested by Spahr and Schlessinger (*6*) therefore, this enzyme, as well as polynucleotide phosphorylase, may be responsible for the rapid breakdown in vitro of messenger RNA that has been noted by others (*21*). The mechanism by which the enzyme functions in vivo, particularly with respect to messenger RNA bound to ribosomes, remains unclear. The binding of the enzyme to ribosomes is of interest from this point of view.

This diesterase should be a useful tool for studying both the primary and secondary structure of polyribonucleotides. The absence of detectable phosphomonoesterase and polynucleotide phosphorylase from the purified fractions enhances this utility. The apparent lability of the purified enzyme will be a disadvantage unless stabilizing conditions can be found. It should perhaps be pointed out that this enzyme appears to be distinct from the *E. coli* phosphodiesterase described by Lehman (*2*). That enzyme, although it also produces 5′-mononucleotides and prefers single-stranded polynucleotides, is specific for polydeoxyribonucleotides, and has a *p*H optimum of about 9.

MAXINE F. SINGER
GEORGE TOLBERT
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

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1. Abbreviations: 5′-AMP and 5′-UMP refer to 5′-adenylic and 5′-uridylic acids, respectively; DNA is deoxyribosenucleic acid and RNA is ribosenucleic acid. In addition, sRNA indicates transfer (soluble) RNA; and the capital letters A, U, C, G, and I are used for the nucleotides adenylic, uridylic, cytidylic, guanylic, and inosinic acids, respectively, when they are residues in a polyribonucleotide chain (for example, polyA is polyriboadenylic acid); also OD, optical density; DEAE, diethylaminoethyl.
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20 April 1964

Human Erythrocyte Lactate Dehydrogenase: Four Genetically Determined Variants

Abstract. *Four variants of human erythrocyte lactate dehydrogenase (LDH) are described. One of these is similar to the one previously described by Boyer and is interpreted as a variant of LDH-B. The other three are thought to represent variants of LDH-A. Studies of families indicate that LDH-A and LDH-B are under separate genetic control and are inherited as autosomal co-dominants. Evidence is presented that the LDH-A locus is not closely linked to the loci for the ABH and Duffy blood group systems.*

Lactate dehydrogenase (LDH) is an enzyme which has a molecular weight of approximately 135,000 and which can be dissociated into four subunits of equal size (*1*). Electrophoresis on starch granules, starch gels, or acrylamide gels separates LDH into five distinct isozymes (*2*). It has been suggested that LDH exists in two electrophoretically distinguishable forms, LDH-A and LDH-B, and that the five isozymes are tetramers formed by random association of the LDH-A and LDH-B subunits—that is, they have the following composition: LDH-1, A_0B_4; LDH-2, A_1B_3; LDH-3, A_2B_2; LDH-4, A_3B_1; and LDH-5, A_4B_0 (*1*).

The relative amounts of the isozymes vary with the tissue and species studied. In the lactate dehydrogenase of erythrocytes the LDH-B subunits predominate so that on electrophoresis the LDH-4 zone is faint and the LDH-5 band is absent (*3*). Boyer *et al.* (*4*) have observed a variant pattern of erythrocyte LDH in an individual from Nigeria; Nance *et al.* (*5*) have presented evidence for another erythrocyte LDH variant. We have studied the LDH isozyme patterns of