OCCURRENCE OF POLYNUCLEOTIDE PHOSPHORYLASE IN ASCARIS LUMBRICOIDES*

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Since the discovery of polynucleotide phosphorylase (Grunberg-Manago et al.,1956), it has been found to be widespread in bacteria. Evidence for the presence of this system has been reported (Hilmoe and Heppel, 1957) for animal tissues, in which only phosphorolysis but not net synthesis of polynucleotide could be observed.

Evidence has been obtained for the presence of polynucleotide phosphorylase in a particulate fraction prepared from the reproductive tract of Ascaris lumbricoides (either male or female) The particles, which have a high content of RNA, are obtained by the use of high speed centrifugation (25,000G for 1 hour) following homogenization of the reproductive tract in dilute buffer and removal of the low speed sedimenting material.

Demonstration of the existence of this enzyme in the Ascaris particles is based on observing the phosphorolysis of polynucleotides. In investigating this reaction the esterification of P_i^{32} was measured by the use of the isobutanol extraction procedure as described by Grunberg-Manago et al. (1956). It was found

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that the particles can readily carry out phosphorolysis of synthetic polynucleotides (the authors are indebted to Miss P. Ortiz for samples of Poly A, U and C) as well as catalyze an exchange reaction between inorganic phosphate and the nucleoside diphosphates (See Table I). The products formed after phosphorolysis of polyadenylate and polycytidylate were found to be ADP and CDP exclusively.

TABLE I

PHOSPHOROLYSIS AND EXCHANGE OF P_i^{32} INTO NUCLEOSIDE DIPHOSPHATES

Phosphorolysis	mμ M	Exchange Reaction	mμ M
Endogenous	1.6		
Poly A	38	ADP	240
Poly C	51	CDP	33
Poly U	17	UDP	35
		GDP	78

The incubation was carried out in a total volume of 0.5ml., containing 0.3ml. of Ascaris particles(1.35mg. protein/ml.); 5 μM $MgCl_2$ and 10 μM P_i^{32}, pH 7.4 (which contained in the phosphorolytic reaction 4.5×10^4 cpm/ μM and in the exchange reaction 2.4×10^4 cpm/ μM); 0.5 μM of polynucleotide (as measured by optical density) or 1.0 μM nucleoside diphosphate. The reaction was stopped after 2 hours by the addition of 2.0ml. of 10% TCA. Esterified P^{32} was measured as radioactivity remaining after extraction of phosphomolybdate with isobutanol. For identification of A-R-P-P^* and C-R-P-P^* in the phosphorolytic reaction the material was first adsorbed on and eluted from charcoal and further characterized by the use of paper chromatography.

Since a small amount of AMP was present during the reaction involving ADP and Poly A, a separate experiment was carried out to show that free AMP is not responsible for the observed esterification of phosphate (Table II).

TABLE II

ESTERIFICATION OF P_i^{32} IN THE PRESENCE OF AMP, ADP AND POLY A

		mμ M
0.5 μM	AMP	3.3
0.5 μM	Poly A	15.7
1.0 μM	ADP	39.4

The incubation conditions were the same as those described in table I. The specific activity of P_i^{32} was 8.0×10^4 cpm/ μM.

In addition to the above described reactions, the forward reaction (in the direction of polynucleotide synthesis) has also been observed, as indicated by the incorporation of the nucleotide moeity of CDP^{32} (C-R-P*-P) into acid insoluble material. This reaction is currently under further investigation. Evidence has also been obtained for the presence in the Ascaris particles of a system capable of synthesizing polynucleotide from nucleoside triphosphates. The incorporation of radioactive material from CTP^{32} (C-R-P*-P-P) into an acid insoluble residue is readily catalyzed by the particles, in which there is no demonstrable CTP-ase.

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