INCORPORATION OF 3'-DEOXYADENOSINE-5'TRIPHOSPHATE INTO RNA BY RNA POLYMERASE FROM MICROCOCCUS LYSODEIKTICUS

Harold T. Shigeura and George E. Boxer

Merck Sharp & Dohme Research Laboratories
Division of MERCK & CO., Inc.
Rahway, New Jersey

Received September 29, 1964

3'-Deoxyadenosine isolated from the culture filtrate of Aspergillus nidulans by Kaczka et al. (1964) has been found to inhibit the biosynthesis of ribonucleic acid in intact Ehrlich ascites cells (Shigeura and Gordon, 1964). Studies with partially purified RNA polymerase from Micrococcus lysodeikticus and 3'-deoxyadenosine-5'triphosphate demonstrated that the DNA-dependent syntheses of RNA and polyadenylate by the enzyme were specifically suppressed. The formation of polyuridylate directed by either DNA or polyadenylate was not affected. The possible mechanism of this inhibition has been studied and the results as described in this paper showed that ^{14}C-labeled 3'-deoxyadenosine-5'triphosphate was incorporated to a limited extent into RNA by partially purified RNA polymerase from Micrococcus lysodeikticus.

3'-Deoxyadenosine-8-^{14}C, prepared from adenine-8-^{14}C and 3-deoxyribose (Walton et al., in press) was generously supplied by Drs. F. W. Holly and E. Walton of these laboratories. For the preparation of ^{14}C-labeled 3'-deoxyadenosine-5'triphosphate, 3'-deoxyadenosine-8-^{14}C was incubated with Ehrlich ascites cells for 3 hours in order to permit the formation of mono-, di- and triphosphates of 3'-deoxyadenosine (Klenow, 1963; Shigeura and Gordon, 1964). The phosphorylated derivatives were separated from the acid-soluble fraction of the cells by the method described by Klenow (1963). The methods used for purification and characterization of the phosphorylated

derivatives of 3'-deoxyadenosine have been described previously (Shigeura and Gordon, 1964). 3'-Deoxyadenosine-5'triphosphate will hereafter be abbreviated as 3'-dATP.

RNA polymerase was partially purified from Micrococcus lysodeikticus according to the method described by Nakamoto et al. (1964) and the ammonium sulfate fraction (Fraction V) was used as the source of enzyme.

The incubation mixture given in the legend to Table I was essentially the same as that used by Fox et al. (1964) except for the addition of ^{14}C-labeled 3'-deoxyadenosine-5'triphosphate in some experiments. After incubation for 1 hour at 30°, the reaction was terminated by the addition of cold TCA. One mg of yeast RNA was added as carrier. The acid-insoluble precipitate was washed with 10 ml of cold 5% TCA (four times), 95% ethanol and ethanol-ether (3:1). The pellet was suspended in 2 cc of 0.5 N KOH and incubated at 30° for 20 hours. 0.5 cc of cold 4 N $HClO_4$ was added and the mixture centrifuged at 4°. The pellet was washed twice with 1 ml of cold 0.2 N $HClO_4$. The supernatant fluid and washings were combined, neutralized with dilute KOH and centrifuged to remove $KClO_4$. An aliquot was plated for the determination of total radioactivity present in the hydrolyzate; the remainder of the hydrolyzate was applied on a column (0.6 x 20 cm) of Dowex-1 formate and eluted with water. This fraction containing nucleosides (Cohn, 1955) was set aside and the column was further fractionated by means of gradient elution in formic acid system, Type I (Hurlbert et al., 1954). Aliquots of the nucleoside and adenosine- and guanosine-2',3'phosphate fractions were plated and counted.

RESULTS AND DISCUSSION

The enzyme preparation used in the experiments reported here was comparable to those used by Nakamoto et al. (1964) and Fox et al. (1964).

The enzyme showed an absolute requirement for DNA, $MnCl_2$ and the four major ribonucleotide triphosphates. In the absence of spermidine phosphate, RNA synthesis was reduced by about 25%. There was no synthesis of RNA in the absence of the enzyme preparation.

The synthesis of RNA in the presence of the four common ribonucleotide triphosphates was determined with ATP-8-^{14}C as the marker. In the presence of 3.6 μmoles of each of the ribonucleotide triphosphate, 0.55 μmole (or 15.2%) of the added ATP was incorporated into acid-insoluble material (Table I, Experiment 1). These results are comparable with those obtained previously (Shigeura and Gordon, 1964) and also with those of Fox et al. (1964).

The possible incorporation of ^{14}C-labeled 3'-deoxyadenosine-5'triphosphate into RNA was then examined under similar conditions. The components of the reaction mixture were the same with the exception of the presence of 0.186 μmole of ^{14}C-labeled 3'-deoxyadenosine-5'triphosphate and unlabeled ATP. The ATP/3'-dATP ratio of 20 was used in these experiments in order to obtain measurable amounts of RNA synthesis since previous experiments (Shigeura and Gordon, 1964) had shown that RNA synthesis by the enzyme preparation from Micrococcus lysodeikticus was inhibited by more than 80% when the ratio of ATP/3'-dATP was 10. The results as shown in Table I (Experiments 2 and 3) indicated that under these conditions only 0.88 to 0.91 mμmole of 3'-dATP was incorporated. This value represented about 0.5% of the added radioactive 3'-dATP as compared to 15.2% for ATP-8-^{14}C.

The position of the analogue in the newly-synthesized polynucleotide chain was next studied by subjecting the polymer to alkaline hydrolysis and subsequent fractionation by means of Dowex-1 chromatography. The analogue attached to the growing terminal position of the polynucleotide should be present in the nucleoside fraction of the alkaline hydrolyzate while those present originally in diphosphate linkages (internal positions)

TABLE I

The reaction mixture in a volume of 5.0 ml contained 0.72 mM ATP, GTP, CTP and UTP, 0.1 M Tris buffer (pH 7.6), 1.5 mM $MnCl_2$, 200 µg calf thymus DNA, 0.5 mM spermidine phosphate, 0.037 mM ^{14}C-3'-deoxyadenosine-5'triphosphate (32,600 cpm) and enzyme preparation (1 mg protein). The mixture was incubated at 30°. After 1 hour, 0.5 cc of cold 50% TCA was added. The nucleoside and purine nucleotide fractions were obtained from the acid-insoluble precipitate as described under experimental procedure.

Fraction 1 represents the total activity in the original acid-insoluble residue. Fractions 2, 3 and 4 represent the activity found in the water eluate and formic acid eluates of the RNA hydrolyzate applied in Dowex-1 formate column.

Experiment 1 represents the total activity in the acid-insoluble RNA in which 0.72 mM ATP-8-^{14}C (47,000 cpm) was added without 3'-dATP.

Fraction	Total cpm		
	Expt. 1	Expt. 2	Expt. 3
1. RNA hydrolyzate	7100	160	154
2. Water eluate		123	135
3. Adenosine-2',3'PO_4		2	2
4. Guanosine-2',3'PO_4		1	0.5

should be found to co-chromatograph with adenosine-2',3'phosphate. The results (Experiment 2 and 3, Fractions 2, 3 and 4) showed that practically all of the radioactivity recovered was present in the nucleoside fraction. Due to the low level of incorporation of 3'-deoxyadenosine-5'triphosphate, no attempt was made to characterize the radioactive material in the water eluate. The results also showed that only a trace of activity was present in the nucleotide fractions.

The results obtained previously (Shigeura and Gordon, 1964) suggested that 3'-dATP was a metabolic analogue of ATP and competed with ATP for certain sites during the synthesis of RNA. The results reported here indicated that 3'-dATP once incorporated in lieu of ATP into a growing polynucleotide chain, prevented the further elongation of that nascent RNA.

The absence of a hydroxyl group on carbon-3 of the deoxyribose moiety of the analogue would prevent attachment by the next incoming nucleotide, thus inhibiting further synthesis of RNA.

The incorporation of various analogues of purine and pyrimidine analogues into internucleotide positions of RNA has been shown by Kahan and Hurwitz (1962). The presence of ribose in these analogues, however, permitted the continued synthesis of polynucleotides. The effects of the incorporation of 3'-deoxyadenosine-5'triphosphate into RNA is unique in that synthesis of RNA appears to be abruptly terminated upon incorporation of the analogue into the growing polynucleotide.

The possible presence of 3'-deoxyadenosine-containing polynucleotides in the acid-soluble fractions of growing cells is presently being investigated.

SUMMARY

^{14}C labeled 3'-deoxyadenosine-5'triphosphate was found to be incorporated to a limited extent into RNA by partially purified RNA polymerase from Micrococcus lysodeikticus. Upon alkaline hydrolysis and subsequent fractionation of the RNA hydrolyzate, practically all of the radioactivity was found to be present in the nucleoside fraction. The results indicated that incorporation of 3'-deoxyadenosine into nascent polynucleotide prevented the further elongation of the polymer.

This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, under the National Institutes of Health, Contract Number SA-43-ph-1886.

REFERENCES

Cohn, W. E., in "The Nucleic Acids" (E. Chargaff and J. N. Davidson, eds.) Academic Press, Inc., New York, Vol. 1, page 211 (1955).
Fox, C. F. and Weiss, S. B., J. Biol. Chem., 239, 175 (1964).
Hurlbert, R. B., Schmitz, H., Brumm, A. I., and Potter, V. R., J. Biol. Chem., 209, 23 (1954).

Kaczka, E. A., Dulaney, E. L., Gitterman, C. O., Woodruff, H. B., and Folkers, K., Biochem. Biophys. Res. Comm., 14, 452 (1964).
Kahan, F. M. and Hurwitz, J., J. Biol. Chem., 237, 3778 (1962).
Klenow, H., Biochim. Biophys. Acta, 76, 347 (1963).
Nakamoto, T., Fox, C. F., and Weiss, S. R., J. Biol. Chem., 239, 167 (1964).
Shigeura, H. T. and Gordon, C. N., J. Biol. Chem. In press.
Walton, E., Jenkins, S. R., Nutt, R. F., and Holly, F. W., J. Am. Chem. Soc., 86, 2952 (1964).