THE INCORPORATION OF AMP INTO OLIGORIBONUCLEOTIDES BY AN ENZYME FROM RAT LIVER

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Oligoribonucleotides act as primers for polynucleotide phosphorylase (Singer et al., 1960), and oligodeoxyribonucleotides are primers for DNA polymerase (Bollum, 1962) and RNA polymerase (Falaschi et al., 1963). This paper describes the synthesis of short chains of poly-A on oligoribonucleotide primers by an ATP-dependent enzyme which has been previously shown to attach AMP to the ends of RNA primers (Klemperer, 1963).

Oligonucleotides of different chain lengths were isolated from RNase digests of yeast RNA and from alkaline hydrolysates of poly-A by chromatography on DEAE cellulose columns (Tomlinson and Tener, 1963). The oligonucleotides were treated with prostatic phosphatase to remove the terminal phosphate, and incubated with a purified form of the rat liver enzyme under the conditions described previously (Klemperer, 1963).

In the presence of trinucleotides and tetranucleotides radioactivity from [^{14}C]ATP was incorporated into polynucleotide material (Table I). The dinucleotides and ribosomal RNA and poly-U were less effective primers. Other experiments showed that the incorporation was proportional to the time of incubation. Oligonucleotides which had not

TABLE I

Incorporation of AMP from ATP

Primer	[^{14}C]AMP incorporated ($\mu\mu$ moles)
-	0
A_2	940
A_3	5420
A_4	5150
R_2	80
R_3	3340
R_4	2850
RNA	1210
Poly-U	280

The reaction mixture contained (in 0.5 ml) 15 mμmoles [^{14}C]ATP, 7.5 mμmoles oligoribonucleotide (or 50 μg. ribosomal RNA or poly-U), enzyme (0.1 mg protein), 1 μmole $MnCl_2$, 2.5 μmoles mercaptoethanol, and 50 μmoles Tris-HCl (pH 8.0). After incubation at 37° for 30 minutes the tubes were heated at 100° for 45 seconds. Aliquots with added carrier ATP were applied to 2.5 x 40 cm. strips of DEAE-cellulose paper. Descending chromatography for 2.5 hours with 0.3 M ammonium formate (Falaschi *et al.*, 1963) separated [^{14}C]ATP from oligonucleotides which remained in an area extending 4.5 cm. from the origin. Radioactivity in this area was measured in a liquid scintillation counter and corrected for the value (equivalent to 50 $\mu\mu$moles ATP) obtained with unincubated controls. A_2, R_2 etc. represent the dinucleotides etc. prepared from poly A and RNA respectively.

been treated with phosphatase did not prime, and inhibited the incorporation into primer oligonucleotides. No incorporation of radioactivity was observed from labelled UTP, CTP and GTP, but [^{14}C]ADP caused 10% of the incorporation seen with [^{14}C]ATP. This is attributed to the formation of ATP from ADP by traces of myokinase which are present in the enzyme preparation.

Although the amount of primer present (7.5 mμmoles) exceeded the amount of AMP incorporated in 30 minutes (3-5 mμmoles in Table I), Table II shows that the reaction product contained terminal oligoadenylate chains attached to the primer. Alkaline digestion of the ^{14}C-labelled reaction product released radioactivity as adenosine and 2'(3')-AMP in proportions which indicate the formation of chains containing approximately 3-4 AMP residues. In longer incubations with less primer, longer chains were formed. The failure to recover ^{14}C as 3',5'-ADP shows that new oligoadenylate chains terminated by 5'-[^{14}C]AMP were not formed. After hydrolysis of the ^{32}P-labelled products formed with the RNA oligonucleotides, radioactivity was recovered almost entirely in AMP (due to internucleotide bonds in the newly-formed oligoadenylate chain) and UMP. Thus RNA oligonucleotides terminated by uridine were the preferred acceptors.

TABLE II

Location of incorporated AMP in reaction product

Primer	Precursor	% of total radioactivity recovered in					
		adenosine	AMP	UMP	CMP	GMP	ADP
A_3	^{14}C-ATP	30	70	-	-	-	< 1
R_3	^{14}C-ATP	26	74	-	-	-	< 1
	^{32}P-ATP	-	72	26	2	< 1	-

Incubation was for 30 minutes under the same conditions as in Table I except that [^{32}P]ATP (labelled in the ester phosphate) was the precursor as indicated. The areas of the chromatograms containing the reaction products were digested with 0.3 N KOH at 37° for 24 hours. After adding non-radioactive carrier compounds, the ^{14}C-labelled product was fractionated by column chromatography into adenosine, AMP and ADP, and the ^{32}P-labelled product was fractionated by paper electrophoresis into the 2'(3')-mononucleotides.

In other experiments the reaction product was fractionated by chromatography on DEAE-cellulose paper with 0.4 M ammonium formate which separates the individual oligonucleotides containing up to six AMP residues. Autoradiography showed that even after short incubation times the oligoadenylate trinucleotide primer gave rise to longer labelled chains containing two or more added AMP residues.

The enzyme studied here therefore resembles polynucleotide phosphorylase and calf-thymus DNA polymerase in attaching chains of polynucleotide to oligonucleotide acceptors. On the other hand the primer for DNA polymerase must be at least a trinucleotide, and the phosphorylase differs in that oligonucleotides terminated by 3'-phosphate will also prime.

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