INCORPORATION OF URIDYLATE INTO RNA BY AN ENZYME FROM RAT LIVER

H. G. Klemperer and H. O. Kammen

Department of Pharmacology, Yale University School of Medicine, New Haven, Conn.

Received November 13, 1961

Uridine 5'-phosphate (UMP) is known to be incorporated into RNA by enzymes in rat liver (Canellakis, 1957; Hecht et al, 1958; Herbert and Canellakis, 1961), in ascites tumor cells (Burdon and Smellie, 1961), and in pigeon liver microsomes (Strauss and Goldwasser, 1961). This paper describes a partially purified UMP-incorporating enzyme obtained from the pH 5-supernatant fraction of a rat liver homogenate subjected to centrifugation at 100,000 x g. The active fraction was prepared by precipitation with ammonium sulfate, calcium phosphate gel adsorption and elution, and chromatography on DEAE-cellulose. The final preparation still has nuclease activity, and work now in progress is designed to remove this.

RESULTS

The incorporation of radioactivity from UTP into acid-insoluble material was dependent upon the addition of both RNA and Mg^{++} (Table I). In other experiments Mn^{++}, or Mn^{++} plus Mg^{++} were less effective activators than was Mg^{++} alone. Maximum incorporation rates during a 15-minute period were found with 0.01 M $MgCl_2$ at pH 7.9. Under the conditions given in Table 1, the incorporation of radioactivity increased for 40-60 minutes and then declined, presumably because of degradation of the reaction product by nuclease. Under similar conditions UDP did not act as a precursor, CTP was only 5-10% as effective as UTP, and only traces

TABLE I

Requirements for the incorporation of UMP

Precursor	Reaction mixture	Precursor incorporated (μμmoles) 15 min.	40 min.
UTP	complete	208	314
UTP	omit RNA	1	-
UTP	omit Mg^{++}	15	20
UDP	complete	1	2
CTP	complete	19	24
ATP	complete	6	9
GTP	complete	3	2
UTP	complete + CTP + ATP + GTP	187	306

The complete system contained (in 1.0 ml): 30 mμmoles of C^{14}-labeled precursor, 70 μg. of RNA (prepared by phenol-extraction of rat liver ribosomes), enzyme (0.8 mg. of protein), 10 μmoles of $MgCl_2$, 5 μmoles of mercaptoethanol, 100 μmoles of tris-HCl, pH 7.9; where indicated, 100 mμmoles each of CTP, ATP and GTP were added. After incubation at 37° the mixture was cooled to 0° and 0.3 ml. of sodium pyrophosphate, 2M, was added to discharge "bound" nucleotides (Kammen et al, 1961). The precipitate formed on adding perchloric acid and carrier protein was washed four times with acid and plated for measurement of radioactivity.

of radioactivity were incorporated from ATP and GTP. The incorporation of radioactivity from UTP was not stimulated when the three complementary triphosphates were added, either individually (data not shown), or all together.

Soluble RNA appeared to be a poorer acceptor of radioactivity from UTP than was ribosomal RNA (Table II). DNA was neither essential for the incorporation into RNA, nor did it act as an acceptor. However, the addition of native DNA stimulated the incorporation of radioactivity from UTP into RNA by 10-20%. The reason for

TABLE II

Effects of nucleic acids on the incorporation of UMP

Additions	C^{14}-UMP incorporated (μμmoles)	
	15 min.	40 min.
RNA	133	183
Soluble RNA	83	62
RNA + RNase	3	-
RNA + DNase	127	-
DNA	2	2
DNA + CTP + ATP + GTP	2	3
RNA + DNA	150	220
RNA + DNA + CTP + ATP + GTP	132	223

Incubation conditions as in Table I, except that C^{14}-UTP was the precursor in all cases. RNA refers to ribosomal RNA (70 μg.), which was added only as stated. Other additions were 70 μg. of soluble RNA (prepared by phenol-extraction of a 100,000 x g. supernatant of rat liver homogenate), 100 μg. of calf thymus DNA, or 10 μg. of pancreatic RNase or DNase.

this stimulation is not clear, but unlike the typical DNA-dependent synthesis of RNA, it did not require the additional presence of the complementary triphosphates. Furthermore, in other experiments, denatured DNA (heated 25 minutes at 100° and cooled rapidly) no longer stimulated the incorporation of UMP into RNA; rather, it acted as an inhibitor.

Table III shows that after alkaline digestion of the reaction product formed from RNA and C^{14}-UTP, all the radioactivity was recovered in uridine and 2'- and 3'-UMP. Thus, the product contained radioactive UMP both in internucleotide positions (62%)

TABLE III

Distribution of Radioactivity in Alkaline Hydrolysate of Product

	% of total radioactivity recovered in					
Precursor	uridine	UMP	CMP	AMP	GMP	UDP
C^{14}-UTP	38	62	-	-	-	<1
P^{32}-UTP	-	77	7	6	10	<1

Incubation (40 min.) as in Table I, except that P^{32}-UTP (labeled in the ester phosphate) was the precursor as indicated. The acid-washed precipitate was hydrolyzed with potassium hydroxide, 0.3 N, mixed with non-radioactive carrier compounds, and fractionated by column chromatography. The nucleotides isolated were the nucleoside 2'- and 3'-phosphates and 3',5'-UDP. The recovery of radioactivity applied to the columns was essentially complete.

and as a "nucleoside" end-group (38%), a finding which suggests that UMP was incorporated as terminal polyuridylate chains with an average length, in this experiment, of 2-3 nucleotide residues. The failure to recover C^{14} as UDP shows that there was no significant formation of acid-precipitable polyuridylate chains terminating in 5'-UMP. After hydrolysis of the P^{32}-labeled product, all the radioactivity was recovered in the four mononucleotides; accordingly, UMP was attached in phosphodiester linkage to all four ribonucleotides. The greater percentage of P^{32} recovered in UMP, as compared with the other nucleotides, reflects the fact that a high proportion (62%) of the phosphodiester linkages formed were between newly incorporated UMP residues in polyuridylate chains.

In conclusion, this rat liver enzyme attaches UMP preferentially to UMP end groups, as do the enzymes from Ehrlich ascites carcinoma cells (Burdon and Smellie, 1961) and from pigeon liver microsomes (Strauss and Goldwasser, 1961). This behavior is

analogous to that exhibited by certain other enzymes which incorporate CMP (Hurwitz and Bresler, 1961) and AMP (Edmonds and Abrams, 1960) into terminal positions of RNA.

We wish to thank Dr. E. S. Canellakis for his interest in this problem and Miss E. Bruning for technical assistance. H. G. K. is indebted to the Anna Fuller Fund for a Research Fellowship and to the Wellcome Trust for a Travel Grant.

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