THE INCORPORATION OF 5-RIBOSYLURACIL TRIPHOSPHATE INTO RNA IN NUCLEAR EXTRACTS OF MAMMALIAN CELLS[1]

Irving H. Goldberg[2] and Murray Rabinowitz

The Departments of Medicine and Biochemistry
and the Argonne Cancer Research Hospital[3]
The University of Chicago, Chicago, Illinois

Received November 17, 1961

Previous reports have described the enzymatic formation of 5-ribosyluracil triphosphate (pseudouridine triphosphate, ψUTP) (Goldberg and Rabinowitz, 1961) and its utilization in the synthesis of 5-ribosyluracil diphosphoglycosyl compounds (Rabinowitz and Goldberg, 1961). Mention has also been made of the incorporation of P^{32} from proximally labeled ψUTP^{32}* into the interribonucleotide linkage of RNA (Goldberg and Rabinowitz, 1961) by the DNA-dependent and four nucleoside triphosphate requiring system derived from nuclear extracts of HeLa cells (Goldberg, 1961). This report compares the utilization of UTP and ψUTP in this reaction and presents evidence of partial specificity in their incorporation into RNA.

ψUTP can substitute completely for UTP in the incorporation of label from CTP^{32} into RNA (Table I). In addition, ψUTP^{32} is incorporated into RNA as efficiently as UTP^{32}. Treatment of the labeled RNA with purified

[1]Supported by grants from the National Science Foundation (G14383) and from the National Heart Institute, United States Public Health Service (H-4442 and HTS-5447).

[2]Faculty Research Associate Award from the American Cancer Society.

[3]Operated by the University of Chicago for the United States Atomic Energy Commission.

*All P^{32} labeled nucleoside triphosphates referred to in this paper are proximally labeled.

TABLE I

Comparison of Utilization of UTP and ψUTP in RNA Synthesis

Labeled Substrate	Unlabeled nucleoside triphosphate	Labeled substrate incorporated into RNA (mμmoles)
CTP^{32}	UTP, GTP, ATP	0.885
CTP^{32}	ψ UTP, GTP, ATP	0.823
CTP^{32}	GTP, ATP	0.018
UTP^{32}	CTP, GTP, ATP	0.880
ψ UTP^{32}	CTP, GTP, ATP	0.829
UTP^{32} or ψ UTP^{32}	CTP, ATP	0.012

The reaction mixture contained 3μmoles of $MnCl_2$, 100μmoles of Tris buffer (pH 8.1), 20μmoles of NaF, 0.8μmoles each of the indicated unlabeled nucleoside triphosphates, 0.1μmoles of labeled nucleoside triphosphate (12.7×10^6 counts/min./μmole), 0.1 ml. of saturated $(NH_4)_2SO_4$ (4°), pH 8.0, and HeLa aggregate enzyme containing 2.5 mg. of protein. The final volume was 1.0 ml. and incubation was for 15' at 37°. The enzyme preparation and assay procedures for incorporation of label into RNA were as described (Goldberg, 1961).

snake venom diesterase (Koerner and Sinsheimer, 1957) resulted in the liberation of 5'-ψUMP^{32} as the only labeled nucleotide, indicating that the P^{32} was carried into the polynucleotide as the 5-ribosyluracil nucleotide without prior conversion or transfer. The presence of equimolar concentrations of both unlabeled UTP and ψUTP in the reaction did not lead to any increase in incorporation of CTP^{32} over that found when only one of the isomers was included in the reaction mixture. It would appear that in the absence of the other, one of these two nucleoside triphosphates can satisfy the fourth nucleotide requirement of the system. Furthermore, Lineweaver-Burk plots demonstrate that one is able to act as a competitive inhibitor of the other. The K_M for ψUTP is approximately 5×10^{-5} and for UTP, approximately 4×10^{-5}. The K_I for ψUTP is about 3×10^{-5} and for UTP is about 1×10^{-4}.

The above data fail to demonstrate any significant qualitative differences in the utilization of ψUTP and UTP in RNA synthesis. Similarly,

the ability of 5-ribosyluracil triphosphate to form diphosphoglycosyl derivatives which can undergo 5-ribosyluridyl transfer reactions (Rabinowitz and Goldberg, 1961) as well as glucosyl transfer reactions to form glycogen[4] does not distinguish an analogue role for the 5-ribosyluracil compounds.

However, studies on the labeling pattern obtained by alkaline hydrolysis of the isolated P^{32}-labeled RNA have revealed definite qualitative differences in utilization of the two nucleoside triphosphates. Alkaline hydrolysis of the RNA results in the transfer of the P^{32} from the incorporating nucleotide to its 3'-linked neighboring nucleoside. When either ΨUTP^{32} or UTP^{32} are used for incorporation into RNA in the absence of its unlabeled isomer, the ratios of the 2'(3') nucleotides produced by alkaline hydrolysis are remarkably similar (Table II). However, the inclusion of unlabeled isomeric nucleoside triphosphate in the enzymatic reaction leads to significant changes in these ratios. The changes are of rather large magnitude, dependent upon the concentration of the unlabeled isomer, and in opposite directions for the two isomers. If no preference were expressed for special sites in the synthesized RNA for UTP or ΨUTP, one would not expect to find any alteration in the ratio of 2'(3') nucleotides from what is found with either labeled nucleoside triphosphate in the absence of unlabeled isomer. The fact that these changes are in opposite directions indicate that they are probably not due to non-specific effects. Evidence against the utilization of different templates for these reactions is provided by experiments using CTP^{32} or ATP^{32} with different concentrations of unlabeled ΨUTP and UTP. In such experiments no ratio changes could be found in the alkali released 2'(3')-labeled nucleotides.

The 2'(3') uridylic and the 2'(3') 5-ribosyluridylic acids are not separated by paper electrophoresis in 0.025 M Citrate, pH 3.5 but can be by subsequent paper chromatography in ethanol - 0.5 M ammonium acetate (5:2 v/v),

[4] unpublished experiments.

TABLE II

Ratio of Label in Alkaline Hydrolyzed Products of P^{32}-RNA

Labeled nucleotide μmoles/ml.	Unlabeled nucleotide μmoles/ml.	2'(3') Nucleotides			
		UMP ΨUMP	GMP	AMP	CMP
ΨUTP^{32} (0.1)	None	1.59	0.99	1.00	1.28
	UTP (0.1)	1.14	1.01	1.00	1.03
	UTP (1.0)	0.95	1.18	1.00	0.65
UTP^{32} (0.1)	None	1.53	0.93	1.00	1.23
	Ψ UTP (0.1)	1.99	0.68	1.00	1.65
	Ψ UTP (1.0)	2.84	0.64	1.00	2.07

The reaction conditions were the same as used in Table I except that the specific activity of the labeled nucleoside triphosphate was 40 x 10^6 counts/min./μmole; 0.8μ moles each of ATP, GTP and CTP were used, and unlabeled UTP or ΨUTP was added as indicated. The labeled RNA was isolated in the same way, redissolved in 2 ml. of 0.01 N NaOH to which 0.2 ml. of 20% potassium acetate, pH 5 was then added. The RNA was reprecipitated with two volumes of ethanol in the cold and redissolved in 1.0 ml. of 0.3 N KOH for incubation at 37° for 18 hours. The solution was acidified with 35% $HClO_4$ to pH 1 and the acid-soluble fraction neutralized and subjected to paper electrophoresis in0.025 M citrate, pH 3.5. The separated 2'(3') mononucleotides were eluted and counted in a gas-flow counter. The counts associated with each 2'(3') mononucleotide are compared with 2'(3') AMP taken as 1.00.

pH 3.8. When ΨUTP^{32} and no unlabeled UTP was included in the enzymatic reaction, 96% of the radioactivity associated with the 2'(3') "uridylic" area on paper electrophoresis was shown to be due to 2'(3') 5-ribosyluridylic acid on paper chromatography. When the concentration of UTP was increased to ten times that of ΨUTP^{32}, 90% of the label associated with the uridylic area after alkaline hydrolysis was present as 2'(3') UMP^{32}. The reverse situation pertained when UTP^{32}, with and without unlabeled ΨUTP, was used in the enzymatic reaction. The decrease in incorporation of label into RNA is less than 40% at equimolar concentrations of labeled nucleoside triphosphate and its unlabeled isomer and 70-80% at ten times concentration of isomer.

These data imply that some degree of discrimination between UTP and ψ UTP exists when both are available to the enzyme and the DNA template. When only one is available for RNA synthesis, it can satisfy the requirement for both. The nature of the RNA formed in these reactions, as well as the significance of the described labeling differences, remain to be elucidated.

REFERENCES

Goldberg, I. H., Biochim. et Biophys. Acta, 51, 201 (1961).

Goldberg, I. H. and Rabinowitz, M., Biochim. et Biophys. Acta, 54, 202 (1961).

Koerner, J. F. and Sinsheimer, R. L., J. Biol. Chem., 228, 1049 (1957).

Rabinowitz, M. and Goldberg, I. H., J. Biol. Chem., 236, PC 79 (1961).

ERRATUM

In the communication entitled "Biosynthesis of the Pyridine Ring of Ricinine from Succinate and Other Labeled Compounds," by George R. Waller and L. M. Henderson, in Biochem. Biophys. Research Communs. 5, 5 (1961), the last line on page 10 should read as follows:

"The label in the alkaloid formed from succinate-1,4-C^{14} was located 15% in the pyridone ring and 85% in the cyano group of non-flowering plants and 75% in the pyridone ring and 25% in the cyano group of flowering plants."

Author's note: These data are supported by other unpublished data which indicate that the biosynthesis of ricinine proceeds by a different metabolic pathway in flowering plants than it does in non-flowering plants.