INCORPORATION OF ADENINE NUCLEOTIDE INTO INTERNUCLEOTIDE LINKAGES OF RNA[+]

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Received October 13, 1959

In previous communications (Chung, 1958; Chung and Mahler, 1958), we reported that enzyme preparations from the cytoplasmic fraction of embryonic tissues were capable of incorporating pyrophosphate into nucleoside triphosphates, and ATP-C^{14} (but not ADP-C^{14}) into RNA. Both types of reactions were dependent on the presence of cytoplasmic RNA. Reports by others (Heidelberger, Harbers, Leibman, Takagi, and Potter, 1956; Canellakis, 1957; Edmonds and Abrams, 1957; Hecht, Zamecnik, Stephenson, and Scott, 1958; Herbert, 1958; Zamecnik, Stephenson, Scott, and Hoagland, 1957) have shown that in a similar reaction, an AMP residue derived from ATP was incorporated into RNA at a terminal position of the polynucleotide chain adjacent to CMP. The final product, adenosine -CMP-CMP-RNA, was the acceptor for the transfer of aminoacyl residues from their respective activating enzymes to the site of their ultimate incorporation into proteins (Hecht, Stephenson, and Zamecnik, 1959; Preiss, Berg, Ofengand, Bergmann and Dickermann, 1959). However, Edmonds and Abrams (1957) have reported the presence of a separate enzyme bringing about internucleotide incorporation of adenine nucleotide. Using adenine-C^{14}, Herbert (1958) found an increase in internucleotide labeling, only if his cytoplasmic preparation was supplemented by nuclei.[++] Further studies now indicate that a soluble, well-dialyzed, enzyme preparation obtained by ammonium sulfate fractionation of the soluble cytoplasmic fraction of 14-day chick embryo hearts or livers is capable of incorporating the adenylate portion of ATP-C^{14} into RNA in non-terminal positions to an extent considerable greater than reported previously. The various components of the basic incorporation system are outlined in Table I.

+ Supported by grants in aid of the American Cancer Society.

++ While this note was in preparation there appeared a communication by Weiss and Gladstone (1959) dealing with the incorporation of CMP* -P-P into RNA, catalyzed by rat liver nuclei.

Table I

Requirements for Incorporation of AMP-C^{14} into RNA

Experiment	System	Incubation time at 38°(minutes)	Counts x min^{-1} in RNA
A	Complete	10	615
	ADP-C^{14} instead of ATP-C^{14} and triphosphates replaced by diphosphates	10	2
B	Complete (0.2 mg RNA)	10	460
	Omit RNA	10	140
	Complete (0.2 mg RNA)	20	130
	Omit RNA	20	175
C	Complete	20	1000
	Omit Mg^{++}	20	620
	Omit Mn^{++}	20	700
	Omit both Mg^{++} and Mn^{++}	20	75
D	Complete (0.25 µmoles each of ribonucleoside triphosphates)	15	675
	UTP only (0.75 µmoles)	15	505
	GTP only (0.75 µmoles)	15	635
	CTP only (0.75 µmoles)	15	550
	none	15	380

The complete system contained in a total volume of 1.2 ml: 20 µmoles Tris buffer pH 9.5, 15 µmoles of $MgCl_2$, 1 µmole of $MnCl_2$, 0.4 mg of RNA isolated from the soluble fraction of a homogenate of embryonic chick livers, 2.5 µmoles each of UTP, GTP, and CTP, 0.178 µmole of ATP-C^{14} (Schwarz), containing 1.36×10^5 counts x min^{-1}, and for the different experiments different enzyme preparations in varying amounts: for experiment A - 0.42 mg. of a calcium phosphate gel treated, 40-70% $(NH_4)_2SO_4$ fraction of liver supernatant; for experiment B - 7.7 mg of a 60-100% $(NH_4)_2SO_4$ fraction of heart supernatant; for C - 0.7 mg of a 40-70% $(NH_4)_2SO_4$ fraction of liver supernatant and for D - 2.1 g. of a 40-70% $(NH_4)_2SO_4$ fraction of liver supernatant. After incubation the RNA was isolated by a modification of the method of Herbert (1958), and counted.

It will be seen that both Mn^{++} and Mg^{++} were required (the concentrations shown are saturating) and that the addition of RNA was greatly stimulatory as was the addition of GTP,CTP,UTP added singly or in combination. When ATP-C was replaced by ADP-C^{14} there was no incorporation, even when the three ribo-

nucleoside triphosphates were replaced by the corresponding diphosphates.

In aged enzyme preparations the stimulation by CYP and GTP was eliminated while that of UTP persisted. Spermidine and cadaverine, added at 5×10^{-3} M concentration, stimulated the basic system to the extent of 160, and 143%, respectively. At lower concentrations, e.g. 5×10^{-4} M, the two amines were somewhat inhibitory ($\sim$ 40%). Polyvinyl sulfate was found to be strongly inhibitory. Addition of 0.17 mg per ml of standard reaction mixture gave 80, and 0.33 mg 100 per cent inhibition. Other anionic polymers, including chitosan sulfate, chondroitin sulfate, chitosan-N sulfate, algenic acid and three different polyethylene sulfonates, all were inhibitory to a varying extent at 0.22 mg per ml, but slightly stimulatory (10-30% above the control) at 0.04 mg per ml. DNA a polyanion, closely related structurally to RNA, inhibited 40% at 0.12 mg per ml, but stimulated 80% at 0.003 mg per ml.

Table II

Requirements for Non-Terminal Incorporation of ATP-C^{14} into RNA

System	% Non-Terminal
No added metal ion	28
Omit Mn^{++}	36
Omit Mg^{++}	52
Complete	74
Complete plus spermidine or cadaverine (5×10^{-3} M)	84

RNA was isolated from reaction mixtures (5 x the amounts in Table I), hydrolyzed in 0.1 N KOH for 2 hours at 100° and neutralized; 0.25 µmoles each of adenosine, 2'- and 3'-AMP were then added and chromatographed in isobutyric acid - NH_4OH - water (66:1:33). Spots were located with a Mineralight, cut out, eluted (24 hours with 0.5 M NH_4OH), and counted.

$$\% \text{ Non-terminal incorporation} = \frac{\text{Counts in 2'- plus 3'-AMP}}{\text{Counts in adenosine plus 2'-plus 3'-AMP}} \times 100$$

Not only the total extent but also the nature of the incorporation depended on the components added, most notable the divalent ions. As shown in

Table II, maximal non-terminal incorporation was observed with both Mg^{++} and Mn^{++} added in combination and with the system supplemented by spermidine or cadaverine. The exact nature of the complex system and attempts at resolution of the various components implicated by these studies are under investigation.

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