The Enzymic Incorporation of Ribonucleotides into Polyribonucleotides and the Effect of DNA

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Received July 12, 1960

Cell free extracts of Escherichia coli utilize the nucleoside triphosphates for the incorporation of UMP, AMP, CMP and GMP into an acid-insoluble product. Enzyme fractions which catalyze the incorporation of AMP and CMP (Preiss and Berg, 1960, Alexander et al, 1960) require the addition of RNA, ATP or CTP, and Mg^{++}. This fixation is unaffected by the addition of UTP, GTP, or DNase but is sensitive to RNase. In contrast to these requirements, it has been observed that the incorporation of radioactivity from UTP is dependent on the addition of the nucleoside triphosphates ATP, GTP and CTP. Similar observations have been reported by Weiss and Gladstone (1959) for CMP incorporation with liver nucleic preparations. This incorporation of UMP by E. coli preparations can be prevented if RNase or DNase is included in the reaction mixture. The latter observation suggested that the incorporation of the UMP might require the presence of DNA. A DNA-dependent incorporation of the ribonucleotide CMP has previously been reported with enzyme fractions from E. coli (Hurwitz, 1959) as well as thymus gland (Krakow and Kammen, 1960).

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The enzyme fraction responsible for the incorporation of UMP was prepared from either glass bead extracts or from sonically disrupted cells. The enzyme activity has been purified approximately 30-fold by precipitation with protamine sulfate and elution with 0.005M phosphate buffer, pH 7.4, followed by ammonium sulfate fractionation (30-45% saturation). Such preparations exhibit the requirements summarized in Table I. Omission of any one of the nucleoside triphosphates results in a marked decrease in UMP incorporation. In the presence of RNase or DNase no detectable acid-insoluble product is formed. The nucleic acid preparation used in these experiments (Table I) was obtained from ribosome-free fractions of E. coli by lanthanum precipitation followed by phenol treatment (Kirby, 1956). Other DNA preparations from thymus gland (Kay et al, 1952), from the bacteriophage T2, or from rat liver

Table I

REQUIREMENTS FOR UMP INCORPORATION

	mμmoles incorporated
Complete System	0.81
-ATP	0.05
-GTP	0.07
-CTP	0.05
-ATP, GTP or CTP	<0.02
- Nucleic acid	0.17
Complete + DNase (1 μg)	<0.02
Complete + RNase (1 μg)	<0.02

The complete system (0.5 ml) contained uridine P^*-P-P (30 mμmoles, 1 x 10^6 cpm/μmole), GTP, ATP and CTP (50 mμmoles) E. coli nucleic acid (2 optical density units at 260 mμ), $MgCl_2$ (2 μmoles), $MnCl_2$ (2 μmoles), mercaptoethanol (1 μmole), acetyl phosphate (4 μmoles), 0.04 units of acetokinase (Rose et al.), and 40 μg of an ammonium sulfate fraction obtained from E. coli W. After 20 minutes at 38°, the reaction was stopped with 0.2 ml of 7 per cent $HClO_4$ and 0.05 ml of 10 per cent albumin was added as carrier. The acid-insoluble material was washed 3 times with 1 per cent $HClO_4$ and dissolved with NH_4OH, plated and the radioactivity measured.

nuclei[1], similarly stimulate UMP incorporation (Table II). These nucleic

Table II

NUCLEIC ACID REQUIREMENT

Exp.	Nucleic Acid Fraction	mμmoles incorporated
1	E. coli nucleic acid	0.32
2	T2-DNA	0.52
3	Thymus DNA	0.40
4	E. coli RNA	0.03
5	No addition	0.04
6	1 or 3 + DNase	<0.02
7	1 or 3 + RNase	<0.02

The additions were as in Table I with the exception that T2 DNA (1 optical density unit at 260 mμ) and thymus DNA (2 optical density units at 260 mμ) replaced the E. coli nucleic acid fraction where indicated and Mg^{++} (8 μmoles) was added instead of the Mg^{++} - Mn^{++} mixture. The enzyme preparation was pretreated with DNase prior to the assay (0.005 μg of DNase per ml of enzyme solution); this treatment reduces the amount of incorporation in the absence of added DNA.

acid fractions are inactivated by pretreatment with DNase. RNA isolated from E. coli does not replace DNA in this system (Table II).

The acid-insoluble radioactive material produced in the reaction has the properties of RNA. The reaction mixture was dialyzed against large volumes of a salt solution to remove the starting material and small oligonucleotides. The non-dialyzable radioactive product was sensitive to treatment with NaOH or RNase, which rendered it completely acid soluble, but was not affected by treatment with DNase. Following hydrolysis with NaOH, the mononucleotides were separated by paper electrophoresis; all four nucleotides were labelled. The distribution of P^{32} in cpm was as follows: cytidylate 220, adenylate 420, guanylate 390 and uridylate 230. This was not exclusively end-group addition since degradation of the product with snake venom phosphodiesterase (Razell

[1] The authors are indebted to Dr. R. Rendi of the Department of Biochemistry for this preparation.

and Khorana, 1959, Singer et al, 1958) resulted in liberation of both radioactivity and ultraviolet absorbing material at parallel rates.

With respect to the requirement for all four nucleoside triphosphates (Table I) it has been established that these are also incorporated into an acid-insoluble product. The possibility that RNA is also involved has not been completely excluded since the enzyme preparations contain small amounts of RNA.

This enzyme system may be related to that previously described (Hurwitz, 1959) for the incorporation of CMP into DNA. In each case inactivation of the enzyme preparations during storage can be reversed by the addition of Mn^{++} to the incubation mixture. This effect of Mn^{++} can be related to inhibition of nucleases in the enzyme preparations. In the previous work a requirement for all four ribonucleotides for the incorporation of CMP was detected and the system was also dependent upon the presence of DNA.

While the present results with UMP incorporation are in accord with the formation of chains of ribonucleotides, the nature of the product, and the precise role of DNA must await further purification of the system.

This program was supported by grants from the National Institutes of Health.

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