LATENT DEOXYURIDINE MONOPHOSPHOKINASE IN CALF THYMUS*

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Deoxyuridine triphosphate (dUTP)‡ is utilized in place of dTTP for enzymic synthesis of DNA with a purified polymerase preparation (1). In spite of this fact, "natural" DNA with an exception of DNA from phage PBS 2 (2) does not contain uracil (3). Therefore, in the cell, there must be a mechanism(s) which acts to prevent the synthesis or utilization of dUTP. Actually, crude extracts from E. coli were found to be incapable of phosphorylating dUMP (1). Later, this apparent lack of the phosphorylating activity was attributed to the presence of a potent dUTPase in the extracts (4, 5).

During the course of the studies on deoxynucleotide kinases of calf thymus, a similar situation with regard to the phosphoryla-

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‡ Abbreviations: dUTP, deoxyuridine triphosphate; dUDP, deoxyuridine diphosphate; dUMP, deoxyuridine monophosphate; dTTP, deoxythymidine triphosphate; dTMP, deoxythymidine monophosphate; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid.

tion of dUMP to dUTP was observed. The crude extract of calf thymus showed no detectable dUMP kinase activity. However, upon fractionation, a fraction having the kinase activity was separated from another fraction which was capable of dephosphorylating dUTP and dUDP to dUMP.

The fractionation procedure was as follows (all procedures were done in a cold room): calf thymus was homogenized with four volumes of a medium consisting of 0.025 M potassium phosphate (pH 7.5), 0.03 M KCl, 0.005 M β-mercaptoethanol, and 5×10^{-5} M deoxythymidine and deoxyuridine. The homogenate which showed no dUMP kinase activity was centrifuged at 20,000 X g for 20 minutes. The supernatant fluid (Fraction A) was adjusted to pH 5.0 by the addition of 2 M acetic acid and centrifuged. The resulting supernatant fluid constitutes Fraction B. The precipitate was suspended in one-fourth the original volume of the medium used for the homogenization and constitutes Fraction P_5. To Fraction B at pH 5.0 was added solid ammonium sulfate slowly with stirring to 30 % saturation* and the resulting precipitate was discarded. To the supernatant fluid was added additional solid ammonium sulfate to 75 % saturation*. The precipitate so obtained was dissolved in one-tenth the original volume of homogenizing medium (Fraction C).

Activities of dUMP and dTMP kinases of these fractions were measured by two procedures which were modifications of the procedure reported by Lehman et al. (6). The details of these assay procedures will be reported elsewhere (7, 8). The results are summarized in Table I and Fig. 1. Fraction A had no dUMP kinase activity. Fraction B showed variable dUMP kinase activity according to the preparation used. Various preparations of Fraction C showed fairly constant dUMP kinase activity. When this fraction was used as the enzyme source, the amount of the products

* Saturation values are those at 25°.

Table I.

Activities of dUMP and dTMP kinases in Fractions A, B, and C

A reaction mixture contained, in a volume of 0.25 ml, 10 µmoles of Tris (pH 7.5), 1.0 µmole of ATP, 2.0 µmoles of $MgCl_2$, 0.1 µmole of dUMP or dTMP (^{32}P or ^{3}H-labeled) and 0.02 to 0.10 unit (0.05 ml) of enzyme preparation. Incubation was carried out for 20 to 60 minutes at 37°. One unit of enzyme was defined as that amount catalyzing the formation of 1 µmole product(s) per 60 minutes under the assay conditions.

Fraction	Total activity per unit weight of fresh thymus (units per gm)	
	dUMP kinase	dTMP kinase
A (crude extract)	0	1.5
B (pH 5 supernatant)+	0 - 8.0*	1.6
P_5 (pH 5 precipitate)	0	0
C (amm. sulf. 30 - 75 %)	3.1 - 3.6	1.2

+ Before assay, pH was adjusted to 7.5 by the addition of 2 M aqueous ammonia.

* Activity varied widely according to the preparation used.

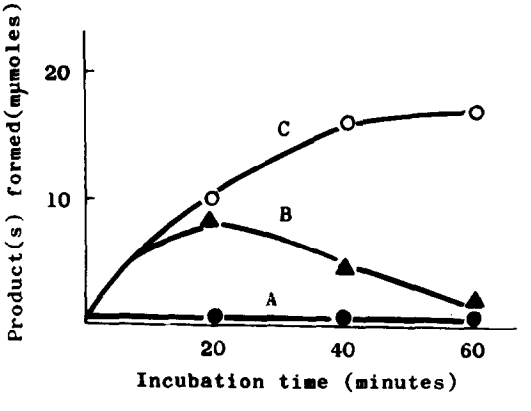

Fig. 1. Time-course of phosphorylation of dUMP under various fractions. Conditions were the same as those in Table I except that the incubation was carried out on a large scale. At each time point, an aliquot of 0.25 ml was taken from each reaction mixture and the amount of the product(s) was determined. For curves A (● —— ●) and B (▲ —— ▲), Fractions A and B were used as the enzyme source respectively. For curve C (○ —— ○), Fraction C diluted 10 times with homogenizing medium was used as the enzyme source.

increased almost linearly with time and with the amount of enzyme

added. The products from ^{3}H-dUMP were isolated from a large scale incubation mixture in which Fraction C was used as the enzyme source, and identified as ^{3}H-dUDP and ^{3}H-dUTP*.

From the results mentioned above, it was inferred that a component(s) which inhibited the dUMP kinase was removed during the fractionation. Actually, as seen in Fig. 2 a, addition of P_5 to Fraction C at 0 minute caused a remarkable inhibition of dUMP kinase. Addition of P_5 at 60 minutes resulted in a rapid degradation of the product(s) which had been formed during the previous 60 minutes incubation (Fig. 2 b). This result and the fact that the assay of the kinase measures the amount of nucleoside polyphosphates resistant to phosphomonoesterase, suggest that the latency of dUMP kinase in the homogenate and in Fraction A is attributed, at least in part, to the presence of a component(s) in Fraction P_5 (pH 5 precipitate) which dephosphorylates dUDP and dUTP to dUMP.

Kinase activities toward dUMP and dTMP of Fraction A, B and C were compared. The results summarized in Table I show that no increase in total activity of dTMP kinase was observed through the steps from Fraction A to Fraction C, whereas dUMP kinase, which was never detected in Fraction A, became relevant in Fractions B and C, suggesting that Fraction P_5 dephosphorylates dUTP and dUDP preferentially to dTTP and dTDP. Purification and characterization of the dephosphorylating enzyme are in progress.

Further fractionation of Fraction C revealed that dUMP kinase and UMP kinase are different entities. The former, dUMP kinase, was obtained in the same fraction in which dTMP kinase was recovered. Whether or not the phosphorylation of dUMP and dTMP is catalyzed by a single enzyme merits further investigation.

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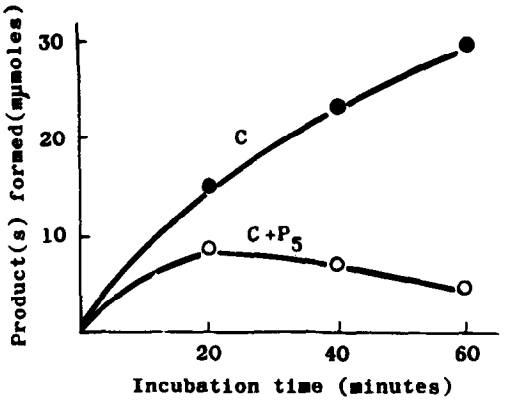
* Calf thymus extracts contains a very active nucleoside diphosphokinase which is recovered in Fraction C (9).



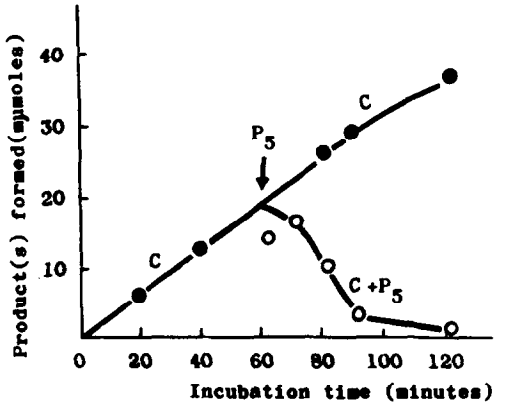


Fig. 2. Effect on dUMP kinase of the addition of P_5.

a) For curve C (● —— ●) Fraction C diluted 10 times with homogenizing medium was used as the enzyme source. Other conditions were the same as those described in Fig. 1. For curve C + P_5 (○ —— ○), a mixture consisting of 0.1 ml of Fraction C, 0.4 ml of Fraction P_5 and 0.6 ml of homogenizing medium was used as the enzyme source. Other conditions were the same as those for curve C.

b) Conditions were the same as those described in Fig. 1. Incubation was carried out as usual until 60 minutes (● —— ●). At 60 minutes, two 2.0 ml aliquots were taken from the reaction mixture. To the one was added 0.4 ml of Fraction P_5, and the incubation was continued (curve C + P_5, ○ —— ○). To another 2.0 ml aliquot was added 0.4 ml of homogenizing medium, and the incubation was continued (curve C, ● —— ●). At each time point after 60 minutes, an aliquot of 0.3 ml was taken from each reaction mixture and the amount of the product(s) formed was determined.

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