Thymidine triphosphate dephosphorylating activity in regenerating rat liver

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The enzyme activity of dephosphorylation of thymidine triphosphate was found in microsomal fraction of rat liver. The enzyme activity decreased at the time when [^{3}H]thymidine incorporation into DNA of regenerating liver increased. When the [^{3}H]thymidine incorporation was suppressed by 1,3-diaminopropane, the enzyme activity remained elevated. These results suggest that the enzyme activity appears to be closely linked to DNA synthesis.

Thymidine triphosphate *Thymidine triphosphate dephosphorylation* *Regenerating liver* *Cell growth* *1,3-Diaminopropane* *Polyamine*

1. INTRODUCTION

The intracellular levels of deoxyribonucleotides increase during cell proliferation [1–4] and this has been extensively studied from the view points of the enhanced syntheses of these nucleotides [5–15]. However, in addition to the increased syntheses, reductions of dephosphorylation of the nucleotides may possibly contribute to the maintenance of higher intracellular concentrations of the nucleotides. Indeed, an increase in synthesis of nucleotide and a decrease in 5′-nucleotidase activity have been reported in proliferating cells [16,17]. Reductions of ATPase [18,19] and 5′-nucleotidase activities [20,21] were also observed in growing tissues.

In regenerating rat liver, the intracellular levels of deoxyribonucleotides increase during DNA replication and among 4 deoxyribonucleotides, thymidine triphosphate level is markedly elevated [3]. Here, we have compared the activity of TTP dephosphorylation in regenerating liver with that in normal liver.

Abbreviations: TTP, deoxythymidine triphosphate; TDP, deoxythymidine diphosphate; TMP, deoxythymidine monophosphate; ATPase, ATP phosphohydrolase; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]-benzene; n.d., not determined

2. MATERIALS AND METHODS

[*methyl*-^{3}H]Thymidine triphosphate tetrasodium salt (10 Ci/mmol) and [*methyl*-^{3}H]thymidine (15 Ci/mmol) were purchased from New England Nuclear (Boston MA). Thymidine triphosphate (TTP), thymidine diphosphate (TDP), thymidine monophosphate (TMP) and thymidine were obtained from Yamasa Shoyu Co. (Chiba). 1,3-Diaminopropane was from Tokyo Kasei Co. (Tokyo).

Male Sprague-Dawley rats (180–200 g body wt) were used. These animals were starved 16 h before operation. Partial hepatectomy was done as in [22]. The rats were killed at various times after the operation and liver was perfused in situ by 0.9% NaCl to avoid contamination by blood. After perfusion, the liver was removed, minced and homogenized in 0.01 M Tris–HCl (pH 7.5) containing 0.25 M sucrose with Teflon pestle homogenizer. 1,3-Diaminopropane was administered intraperitoneally as in [23].

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2.1. *Preparation of subcellular components*

Microsomal and cytosol fractions were prepared as in [24]. The identification of mitochondrial and microsomal fractions was based on analysis of their maker enzymes, cytochrome oxidase [25] and glucose 6-phosphatase [26]. 5'-Nucleotidase activity was measured as in [17]. Nuclear fraction was separated in the presence of 3.3 mM $CaCl_2$ as in [27]. Rough and smooth microsomes were separated by sucrose gradient centrifugation [28].

2.2. *Assay of TTP dephosphorylation*

The assay mixture (0.1 ml) contained [^{3}H]thymidine triphosphate (0.01 μCi, 0.1 mM), 10 mM $CaCl_2$, 50 mM Tris–HCl (pH 9.0) and the enzyme. After incubation for 2 min at 37°C, the reaction was stopped by boiling for 2 min. Then, 10 μl reaction mixture and authentic TTP, TDP, TMP and thymidine were applied to a filter paper (Toyo roshi no. 30) and developed with a solvent composed of isobutyric acid–ammonia water–water (100:3.2:55, by vol.). After drying the paper, each compound was detected by ultraviolet absorption and extracted with 2 ml distilled water at 40°C for 1 h. The extract (1 ml) was added to 10 ml toluene scintillator containing 33% Triton X-100, 0.4% PPO and 0.01% POPOP. Radioactivity was determined by using a Packard Tri-Carb liquid scintillation counter.

2.3. *Assay of incorporation of [^{3}H]thymidine into DNA*

[^{3}H]Thymidine (10 μCi) was administered to rats intraperitoneally 23 h after partial hepatectomy. After 1 h labelling, the rats were killed and livers were removed. The livers were homogenized in 5% trichloroacetic acid and the radioactivity of the acid-insoluble fraction was measured.

2.4. *Measurements of protein and nucleic acids*

Protein was determined as in [29] using bovine serum albumin as standard. Insoluble protein was solubilized and measured as in [30]. DNA and RNA were measured by using diphenylamine [31] and orcinol reagent [32], respectively.

Table 1

Intracellular distribution of TTP dephosphorylating activity in rat liver

Fraction	TTP dephosphorylating activity (μmol.mg protein^{-1}.min^{-1})	Cytochrome oxidase (ΔE.mg protein^{-1}.min^{-1})	Glucose-6-phosphatase (nmol.mg protein^{-1}.min^{-1})	5'-Nucleotidase (nmol.mg protein^{-1}.min^{-1})	DNA (μg/mg protein)	RNA (μg/mg protein)
Expt 1						
Homogenate	0.38	1.02	33.7	2.97	n.d.	n.d.
Mitochondria	0.64	6.79	28.9	11.95	n.d.	n.d.
Microsomes	1.52	0.27	194.4	12.20	n.d.	n.d.
Cytosol	0.06	0.00	0.3	1.20	n.d.	n.d.
Expt 2						
Homogenate	0.62	1.00	38.3	2.30	13.95	2.09
Nuclear	0.75	0.72	61.1	10.70	143.00	22.90
Expt 3						
Microsomes	0.58	n.d.	n.d.	n.d.	n.d.	n.d.
Smooth microsomes	0.96	n.d.	n.d.	n.d.	n.d.	14.70
Rough microsomes	0.29	n.d.	n.d.	n.d.	n.d.	45.80

Subcellular fractions were obtained from 10 g rat liver as in section 2. Conditions for enzyme assay were the same as described in text. Each value represents the average of 2 independent expt; n.d. = not determined

3. RESULTS

3.1. *Intracellular distribution of TTP dephosphorylating activity*

Intracellular distribution of TTP dephosphorylating activity in rat liver is shown in table 1. The activity in cytosol was low and most of the activity was found in the microsomal fraction. The mitochondrial and nuclear fractions had some activity, but the specific activity of those fractions was lower than that of microsomal fraction. The activity of the smooth microsomal fraction was higher than that of the rough microsomal fraction. Therefore, we used the microsomal fraction as an enzyme source in the following experiments.

3.2. *TTP dephosphorylating activity in sham-operated and regenerating liver*

Fig. 1 shows that TTP dephosphorylating activity in liver of sham-operated rat remained high until 48 h, but the activity of regenerating liver decreased to 50% of sham-operated liver at 36 h after operation.

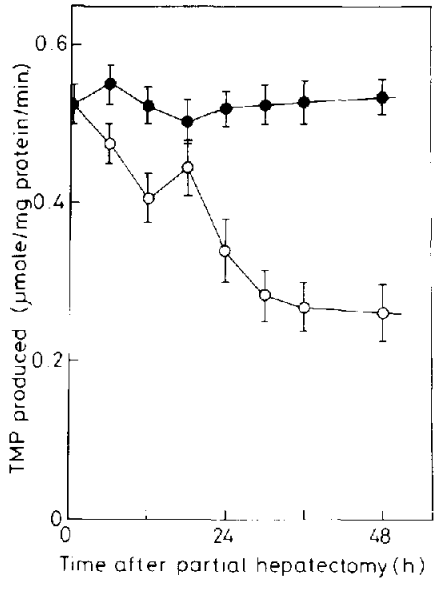

Fig. 1. Thymidine triphosphate dephosphorylating activity during liver regeneration. Each value and vertical bar shows the mean of 3–5 rats and standard error, respectively: (○) regenerating liver; (●) sham-operated liver.

3.3. *Effect of 1,3-diaminopropane on [^{3}H]thymidine incorporation into DNA and TTP dephosphorylating activity*

Since ornithine decarboxylase activity increased markedly prior to DNA synthesis [33] and the inhibition of the accumulation of intracellular polyamines resulted in suppression of DNA synthesis [34], we tested the effect of administration of 1,3-diaminopropane on TTP dephosphorylating activity and on [^{3}H]thymidine incorporation into DNA (fig. 2). The increase in [^{3}H]thymidine incorporation into DNA and the decrease in TTP dephosphorylating activity induced by partial hepatectomy were inhibited in dose-dependent manner by administration of 1,3-diaminopropane and the incorporation of [^{3}H]thymidine into DNA was inversely proportional to the activity of TTP dephosphorylation.

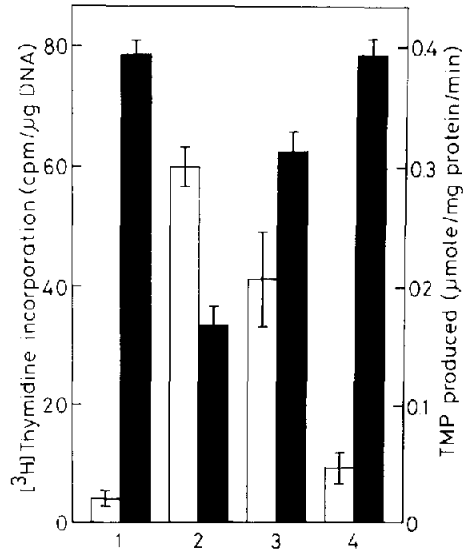

Fig. 2. Effect of 1,3-diaminopropane treatment on activity of thymidine triphosphate dephosphorylation and incorporation of [^{3}H]thymidine into DNA in regenerating liver. Each value represents the mean of 3–5 rats and bar shows standard error: (▭) incorporation of [^{3}H]thymidine into DNA; (▬) activity of thymidine triphosphate dephosphorylation; (1) sham operation; (2) partial hepatectomy; (3) partial hepatectomy + 1,3-diaminopropane (50 μmol/100 g body wt); (4) partial hepatectomy + 1,3-diaminopropane (100 μmol/100 g body wt).

3.4. *Effect of polyamines on TTP dephosphorylating activity*

Since the administration of 1,3-diaminopropane caused inhibition of the decrease in TTP dephosphorylating activity, we examined whether polyamines had inhibitory effects on the enzyme activity. When putrescine, spermidine or spermine at the concentration of 1 mM was added to reaction mixture, the enzyme activities were 98.8, 90.2 and 88.3% of control, respectively. From these results, it is unlikely that polyamines accumulated in cells inhibited the enzyme activity, leading to lower activity in regenerating liver than in sham-operated liver.

4. DISCUSSION

The increases in intracellular concentrations of deoxyribonucleotide triphosphates occurred prior to DNA synthesis in growing cells [1–3] and these increases might be due to stimulation of synthesis of the nucleotides [4–14]. However, it is possible that the decrease in deoxyribonucleotide dephosphorylation also contributes to the increase in nucleotide levels. Eker showed that dephosphorylation of TDP and TMP but not of TTP decreased in growing phase of Chang human liver cells [35]. Fig. 1 shows that dephosphorylation of TTP decreased markedly at the time when DNA synthesis increased. Furthermore, when DNA synthesis was suppressed by 1,3-diaminopropane, TTP dephosphorylating activity remained elevated, suggesting that activity of dephosphorylation appears to be closely linked to DNA synthesis. The mechanism of the decrease in the activity of TTP dephosphorylation in regenerating liver is not yet clear, but it is unlikely that low- or high-M_r inhibitor of the enzyme causes the decrease in the enzyme activity in regenerating liver as:

(i) Dialysis of enzyme solution obtained from regenerating liver did not result in the increase in the enzyme activity;

(ii) Mixture of enzyme extracts obtained from normal liver and regenerating liver showed an additive enzyme activity;

(iii) The addition of polyamines to the reaction mixture did not inhibit TTP dephosphorylating activity, eliminating a possibility that polyamines accumulated in regenerating liver inhibit the enzyme activity through direct interaction to the enzyme protein.

However, it is possible that accumulation of polyamines, the decrease in TTP dephosphorylating activity and the increase in TTP concentration are the sequential obligatory steps for the increase in DNA synthesis and that the inhibition of the accumulation of polyamines results in the inhibition of the decrease in TTP dephosphorylating activity, although mechanism of the decrease is not clear.

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