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ENHANCED ACTIVITY OF DEOXYURIDINE 5'-TRIPHOSPHATASE IN REGENERATING RAT LIVER

Shigeru Hokari[+], Yoshikatsu Sakagishi[+] and Kinji Tsukada[*]

[+]Department of Biochemistry, Saitama Medical School, Moroyama, Iruma-gun, Saitama 350-04
[*]Department of Pathological Biochemistry, Medical Research Institute, Tokyo Medical and Dental University, Kandasurugadai, Chiyoda-ku, Tokyo 101, Japan

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SUMMARY: An enzyme, dUTPase, that catalyzes the conversion of dUTP to dUMP and PPi, was partially purified from regenerating rat livers. The molecular weight was estimated by gel filtration to be 60,000. The apparent Km for dUTP was 12 μM. No other deoxyribonucleoside triphosphates served as a substrate. This enzyme is active in the absence of added divalent cations or sulfhydryl reagents; the activity could be inhibited by EDTA and shows a broad pH optimum with no decrease in activity from pH 7 to 11. The specific activity of dUTPase in rat liver begins to rise 16 h after partial hepatectomy and reaches a maximum about 24 h after the operation, rising to at least 5 to 6 times the normal level.

The existence of deoxyuridine 5'-triphosphatase (EC 3.6.1.23) (dUTPase), which catalyzes the conversion of dUTP to dUMP and PPi, was demonstrated in bacteria (1, 2), mouse L-1210 cells (3) and blast cells of patients with acute lymphocytic leukemia (4). Although the function of this enzyme is still unclear, it has been thought that it removes dUTP from the cells to prevent it from serving as a substrate for DNA polymerase (5), and also provides the substrate for thymidylate synthetase to synthesize TMP.

To understand the physiological role of dUTPase, we examined the change in its activity after partial hepatectomy. As the activity was present in the cytosol in the cells with other non-specific phosphatases (3), we have partially purified it from

Abbreviations: dUTPase, deoxyuridine 5'-triphosphatase; PPase, inorganic pyrophosphatase.

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regenerating rat liver. In this communication we report some of its properties and also that its activity is increased after partial hepatectomy at the same time of increase in DNA synthesis.

MATERIALS AND METHODS

dUTP and other nucleotides of sodium salt were purchased from Sigma Chemical Co. Actinomycin D was from Boehringer/Mannheim, Cycloheximide from Wako Pure Chemical Co. Blue Sepharose CL-6B and Sephadex G-100 were from Pharmacia Fine Chemicals. Other chemicals were of analytical grade.

The rats (150-180 g) used were albino Wistar strain. Partial hepatectomy refers to the removal of about 70% of the liver (left lateral and median lobes) (6).

Mouse intestinal inorganic pyrophosphatase (EC 3.6.1.1) (PPase) was purified according to the method of Irie et al. (7). This enzyme preparation had no nucleoside triphosphate pyrophosphatase activity.

The assay of dUTPase activity was based on the release of PPi during incubation to inactivate PPase in the absence of Mg^{2+}. The dUTPase reaction mixture (0.4 ml) contained 0.1 M Tris-HCl (pH 7.8), 0.180 mM dUTP, and enzyme preparation. After incubation at 37°C for 20 min, the reaction was stopped by cooling in ice. Aliquots (0.2 ml) were transferred to another tube and 0.08 unit of mouse intestinal PPase and 10 µl of 0.4 M $MgCl_2$ were added, followed by incubation for 5 min at 37°C to convert PPi to Pi. The Pi formed was determined by the method of Itaya and Ui (8). The PPi formed in the main reaction mixture was determined and the difference calculated. One unit of activity was defined as the amount of the enzyme that produced one nmol PPi/min at 37°C.

The assay for non-specific nucleoside triphosphate pyrophosphatase activity was carried out with UTP instead of dUTP as the substrate.

To purify dUTPase from regenerating rat liver, rat livers (about 50 g) at 25 h after partial hepatectomy were homogenized with 4 vol. of 0.25 M sucrose/3.3 mM $MgCl_2$. The homogenates were centrifuged at 123,000 x g for 60 min to get the cytosol. The cytosol was brought to 50% saturation with solid ammonium sulfate, stirred 20 min, and centrifuged at 14,000 x g for 15 min. The supernatant was then brought to 75% saturation with ammonium sulfate and centrifuged at 14,000 x g for 15 min. The precipitate was dissolved in 20 ml of Buffer A (20 mM Tris-HCl, pH 8.6/2 mM $MgCl_2$) and stirred for 5 min at 57°C to inactivate PPase and other phosphatase activities. The activity remained in the supernatant after centrifugation at 15,000 x g for 10 min. The supernatant was dialyzed against Buffer A overnight. The dialyzed solution was applied on a column of blue Sepharose CL-6B (2 x 15 cm) previously equilibrated with Buffer A. The column was washed with Buffer A until the unabsorbed protein was removed. The enzyme was eluted with 0.1 M KCl. The active fractions were made 90% saturation with ammonium sulfate, and centrifuged. The precipitate was dissolved in 2 ml of Buffer A, and dialyzed against the same buffer overnight. The dialyzed solution was again applied to a blue Sepharose column (1.2 x 6 cm) equilibrated with Buffer A. The column was washed with 1 mM UTP and 1 mM dUTP successively. A large part of the activity of the enzyme was eluted by 1 mM dUTP, and these fractions were concentrated with an Amicon ultrafiltration apparatus using a UM-10 membrane.

Protein was determined by the method of Lowry et al. (9) with bovine serum albumin taken as the standard.

Table I

Substrate specificity of dUTPase from regenerating rat liver

Substrate	Addition	Activity
		PPi (nmol)
dUTP		18.5
dCTP		<0.3
dTTP		<0.3
dATP		<0.3
dGTP		<0.3
BrdUTP		<0.3
UTP		0.6
dUTP	dCTP*	18.1
dUTP	dTTP*	17.8
dUTP	dATP*	17.0
		Pi (nmol)
dUTP		<0.3
dUDP		1.9
dUMP		<0.3

The purified enzyme was used with the same amount of each substrate in the assay procedure described under "MATERIALS AND METHODS"
* The concentration was 4-fold greater than that of dUTP.

RESULTS AND DISCUSSION

The dUTPase was purified from regenerating rat livers about 300-fold with a yield of 13%. The purified enzyme was free from non-specific nucleoside triphosphate pyrophosphatase and PPase activities. The molecular weight of dUTPase as determined by gel filtration of Sephadex G-100 was approximately 60,000 (data not shown). Enzyme activity was measured in the pH range of 4 to 11. A broad plateau of activity was found above pH 7.0, with no decrease in activity up to pH 11. The apparent Km value for dUTP was about 12 μM (data not shown). No hydrolysis of dCTP, dTTP, dATP, dGTP and BrdUTP was detected after 30 min of incubation with 10 units/ml. The addition of dCTP, dTTP or dATP at concentration 4-fold greater than that of dUTP did not inhibit the hydrolysis of dUTP (Table I). UTP was slightly hydrolyzed by the preparation (Table I). The

Table II

Effect of various divalent cations and some chemicals on dUTPase activity

Addition	Concentration	Activity
	mM	%
None		100
Mg^{2+}	5	133
Mn^{2+}	5	74
Ca^{2+}	5	31
Zn^{2+}	5	77
EDTA	1	9
NaF	5	102
NaCN	5	113
Dithiothreitol	0.5	109
N-Ethylmaleimide	0.5	118

The purified enzyme was used with the same assay procedure described under "MATERIALS AND METHODS".

addition of Mg^{2+} enhanced the activity of the enzyme about 30% over that of control (Table II). Several divalent cations tested exert an inhibitory effect on this enzyme. The activity was reduced by the addition of EDTA, and the addition of NaF or NaCN was not inhibitory. The activity was not influenced by the addition of sulfhydryl reagents (Table II). This purified dUTPase converted dUTP to dUMP and PPi stoichiometrically (data not shown).

The activity of dUTPase has been shown to reside in cytosol (3). When the cytosol was heated at 57°C for 5 min, almost all of PPase and some of non-specific nucleoside triphosphate pyrophosphatase activities present in the cytosol were inactivated, without loss of the dUTPase activity. Fractionation of heated cytosol on a single blue Sepharose column indicated an increase in dUTPase activity in regenerating rat liver. Fig. 1 shows the blue Sepharose column chromatography profile of dUTPase and non-specific nucleoside triphosphate pyrophosphatase from the heated cytosol. The column was washed with Buffer A until the unabsorbed protein was removed.

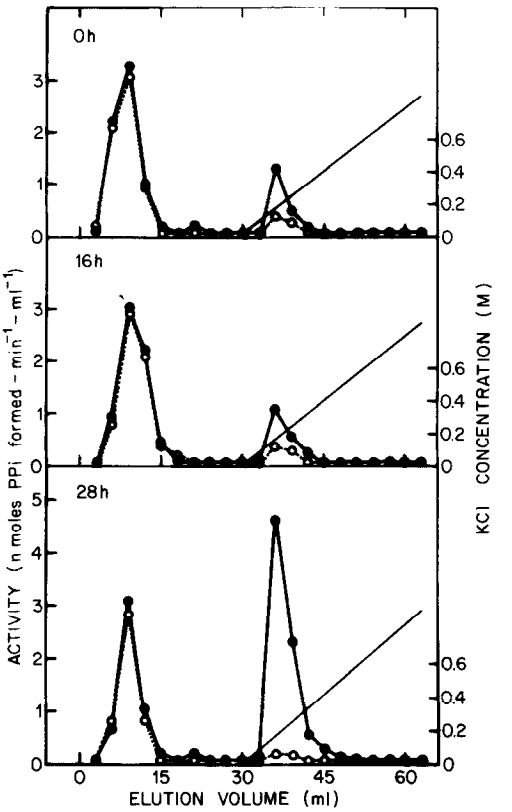

Fig. 1. Blue Sepharose column chromatography of dUTPase from the heated cytosol. The cytosol from rat livers (3 g of wet weight) was heated at 57°C for 5 min. The supernatant after centrifugation was brought to 80% saturation with ammonium sulfate. The precipitate after centrifugation was dissolved in a small amount of Buffer A, and dialyzed against the same buffer overnight. The dialyzed solution was loaded onto a column of blue Sepharose (1.2 x 7 cm) equilibrated with Buffer A. The column was washed with Buffer A until the unabsorbed protein was removed. The absorbed protein was then eluted with a 100 ml linear gradient of Buffer A containing 0 to 1.0 M KCl, and fractions of 3 ml were collected. Aliquots (0.1 ml) of each fraction were assayed for dUTPase activity using dUTP or UTP as a substrate as described under "MATERIALS AND METHODS". The salt concentration was determined by conductance measurements. The numbers represent the hour after partial hepatectomy. ● , dUTP; ○ , UTP.

A first peak of non-specific pyrophosphatase activity, that liberated PPi from dUTP and UTP, appeared in wash-through fraction, while a second peak, that hydrolyzed mainly dUTP, was eluted at approximately 0.1 M KCl. As shown in Fig. 1, the total activity of dUTPase (second peak) was almost the same in liver of normal rat and the liver at 16 h after partial hepatectomy and then showed a large increase. The non-specific pyrophosphatase activities (first peak)

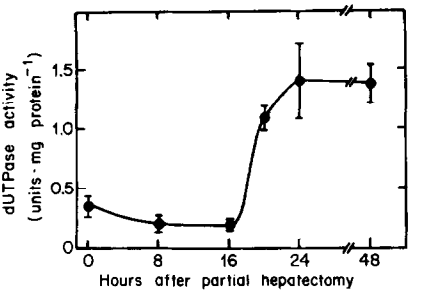

Fig. 2. Increase in dUTPase activity of the cytosol from liver as a function of time after partial hepatectomy. Analysis of dUTPase activity (second peak in Fig. 1) was carried out as in Fig. 1. Each point represents the mean ± S.D. of the results obtained from 3 to 4 independent analyses.

were not significantly altered. The rise in the activity of dUTPase began after a lag of about 16 h and was maximal at about 24 h after the operation (Fig. 2). The time course of the response of this enzyme activity was similar to that of DNA ligase activity as well as DNA synthesis measured with [^{3}H]thymidine incorporation after partial hepatectomy (10). Specific activity of the enzyme maintained the same level up to 48 h. These results suggested that dUTPase activity is correlated with increased DNA synthesis. Administration of cycloheximide (100 μg) or actinomycin D (20 μg) into a rat immediately after partial hepatectomy caused a decrease in the induction of dUTPase activity. However, when the same amount of each antibiotic was administered at 24 h after partial hepatectomy, no decrease of the enzyme activity was observed (data not shown). These data suggested that the increase of dUTPase activity was due to newly-synthesized enzyme protein.

This enzyme appears to resemble the enzyme in bacteria (1, 2) and the human enzyme (4) with respect to the stoichiometric conversion of dUTP to dUMP and PPi, the lack of inhibition by NaF, and low activity with UTP and other deoxyribonucleoside triphosphates. These enzymes are active in the absence of added Mg^{2+} and are inhibited by EDTA. The enzyme from rat liver differs significantly from Yoshida sarcoma (11) in that the latter hydrolyzes dUDP well.

Labow and Maley (12) have reported that breakdown of dUTP by the tissue extracts increased in proliferating rat tissues, such as thymus, embryo and regenerating livers, although they did not characterize the enzyme. While the exact role of dUTPase activity in mammalian is unknown, it is of interest to note that the dUTPase activity of regenerating rat liver paralleled the incorporation of [^{3}H]thymidine into DNA. It has been postulated that dUTPase serves to keep the concentration of dUTP low enough to avoid incorporation into DNA by DNA polymerase. When uracil incorporation into DNA occurs, an enzyme, uracil-DNA glycosidase, which is present in both mammalian (13) and bacterial cells (14), has been reported to release uracil from the DNA.

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