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Partial Purification of a Thymidine Phosphorylase from Human Gastric Cancer

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A thymidine phosphorylase (TP) preparation was partially purified from human gastric cancer (poorly differentiated adenocarcinoma). The specific activity of the final preparation represented a 379-fold purification of the 7000*g* supernatant of tissue homogenate. The phosphorolytic activities toward thymidine (dThd), 5′-deoxy-5-fluorouridine (5′-DFUR), and 1-(tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) remained closely in parallel during the whole purification procedure. The results provide evidence in support of the assumption that 5′-DFUR and Tegafur are converted into 5-fluorouracil, an activated form of the antitumor agents, in human tumor tissues by a TP activity. The values of K_m of the TP preparation were 1.68×10^{-4}, 1.72×10^{-3}, 1.33×10^{-2}, and 4.76×10^{-2} M for dThd, 5′-DFUR, Tegafur, and uridine, respectively.

Keywords——thymidine phosphorylase; uridine phosphorylase; gastric cancer; human tumor; thymidine; uridine; 5-fluorouracil; 5′-deoxy-5-fluorouridine; 1-(tetrahydro-2-furanyl)-5-fluorouracil

We found[2,3] that a thymidine phosphorylase (TP) activity is greatly enhanced in human tumors as compared with normal tissues and we suggested[2–5] that the activity is responsible for the *in vivo* conversion of 5′-deoxy-5-fluorouridine (5′-DFUR) and 1-(tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) to 5-fluorouracil (5-FU). 5′-DFUR and Tegafur are antitumor agents, and their antitumor activities are manifested after cleavage to 5-FU, an activated form.[6]

The activation of 5′-DFUR by TP in human liver tumors was also suggested by Shirasaka and coworkers.[7] On the other hand, the activation of 5′-DFUR in experimental tumors of animals was proved to be catalyzed by uridine phosphorylases (UP).[6c]

These results suggest that the properties and relative activities of the two pyrimidine nucleoside phosphorylases, TP and UP, are greatly different between the tumors of humans and experimental animals. In the previous paper,[5] we reported that the substrate specificity of TP in human liver tumor tissues was broader than that of TP from other sources.

The present paper describes the partial purification of TP from human gastric cancer and the activities of the enzyme preparation for the phosphorolysis of thymidine (dThd), uridine (Urd), 5′-DFUR, and Tegafur.

Experimental

Materials——5′-DFUR and Tegafur were provided by Hoffmann-La Roche Inc., Nutley, N.J., U.S.A. and Taiho Pharmaceutical Co., Tokushima, Japan, respectively. Other chemicals were of analytical grade and were obtained from commercial sources.

Enzyme Activity——Activities of preparations for the phosphorolysis of pyrimidines were assayed according to the method reported previously.[8] Pyrimidines formed enzymatically from dThd, Urd, 5′-DFUR and Tegafur were

determined by means of high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The concentrations of the substrates were 16 μM except for Tegafur. The activity for Tegafur was measured in 5.0 mM solution. Correction for nonenzymatic degradation of Tegafur was made as described previously.[4] The specific activities of preparations were expressed in terms of the amount of the pyrimidines produced (nmol) in 1 h by 1 mg of protein.

Enzyme Preparations——Human gastric tumor tissues (91 g) were obtained at operation from a 58-year-old female and examined histologically (poorly differentiated adenocarcinoma). The following purification procedures were carried out at 4 °C. The tumor tissues were separated from normal tissues, washed in ice-cold 0.9% NaCl solution, and homogenized in 10 mM Tris buffer (pH, 7.4) containing 15 mM NaCl and 1.5 mM $MgCl_2$. The homogenates were centrifuged at 7000 × *g* for 30 min. The supernatant (preparation I) was treated with ammonium sulfate. The fractions obtained between 20 and 40% saturation were dialyzed against 20 mM sodium phosphate buffer (pH, 7.4) containing 10 mM 2-mercaptoethanol. The protein solution (preparation II) was subjected to chromatography on a Con A Sepharose column (2.1 × 8 cm) eluted with the same buffer as used in the dialysis. The eluent was collected and concentrated (preparation III). The preparation was further subjected to chromatography on a column of diethylaminoethyl (DEAE) Sephacel (1.5 × 15 cm). The sample was eluted with 20 mM potassium phosphate buffer (pH, 7.5) containing 10 mM 2-mercaptoethanol and a linear gradient of KCl (0—400 mM). The fractions with the highest dThd-cleaving activity (140—170 mM KCl) were combined and concentrated (preparation IV). The preparation was further purified by chromatography on a column of hydroxylapatite (Bio-Gel HTP) (1.5 × 13 cm) developed with 10 mM potassium phosphate buffer (pH, 7.3) containing 10 mM 2-mercaptoethanol (preparation V).

Results and Discussion

Thymidine phosphorylase was partially purified from human gastric tumor tissues by the procedure described in the experimental section. In the course of the purification procedure, preparations I—V were obtained. The specific activities of the preparations for phosphorolysis of dThd, Urd, 5′-DFUR, and Tegafur are summarized in Table I. The specific activity of preparation V for dThd represents a 379-fold purification of the initial homogenate (preparation I), and the recovery of the enzyme was 12.5%.

The activities toward dThd and Tegafur paralleled each other closely during the whole purification procedure. The results provide evidence in support of our proposal that Tegafur

TABLE I. Purification of Thymidine Phosphorylase

Preparation	Protein (mg)	Specific activity[a]				Recovery[b]
		dThd	Urd	5′-DFUR	Tegafur	
I	2439	197	21.3	22.5	8.08	100
II	437	991	22.8	96.1	22.6	89.9
III	115	1680	4.87	109.2	46.0	40.0
IV	11.5	11000	23.1	682	276	26.3
V	0.81	74570	164	4847	2550	12.5

a) Values are nmol of pyrimidine formed by 1 mg of protein in 1 h. The concentrations of the substrates were 16 μM for dThd, Urd, and 5′-DFUR and 5 mM for Tegafur. For details, see the text and ref. 8. *b*) Recoveries were based on the activity toward dThd.

TABLE II. Michaelis Constants of Thymidine Phosphorylases in Human Tumor Tissues

	Gastric tumor[a]		Lung tumor[b]	Liver tumor[c]
	K_m (M)	V_{max}[d]	K_m (M)	K_m (M)
dThd	1.68×10^{-4}	100	2.43×10^{-4}	2.53×10^{-4}
5′-DFUR	1.72×10^{-3}	61.6	1.69×10^{-3}	1.75×10^{-3}
Tegafur	1.33×10^{-2}	0.85		2.44×10^{-2}
Urd	4.76×10^{-2}	0.42		

a) Present study. *b*) Ref. 3b. *c*) Ref. 4. *d*) Relative values. The V_{max} value was 826 μmol (mg protein)$^{-1}$ h^{-1} with dThd as a substrate.

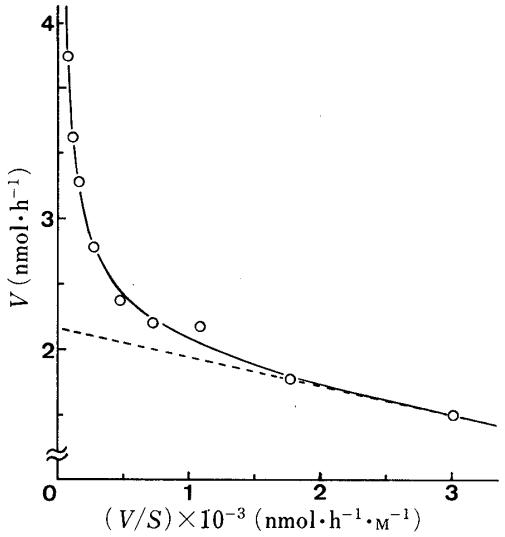

Fig. 1. Phosphorolysis of Uridine by Preparation V

Solid line, total activity; dotted line, activity catalyzed by UP.

is activated in human tumor tissues by a TP activity.[2,4]

The change of the activity toward 5′-DFUR during the purification showed that the cleavage of 5′-DFUR was mainly catalyzed by an activity which catalyzes the cleavage of dThd. The activities of preparations I and II suggest the presence of another enzyme with a limited activity, presumably UP, which catalyzes the phosphorolysis of 5′-DFUR. The cleavage of 5′-DFUR by human tumor preparations was unaffected by the presence of 1-(2′-deoxy-β-D-glucopyranosyl)thymine,[3] which inhibits UP but does not inhibit TP.[9] Thus, the major part of the activity toward 5′-DFUR can be ascribed to TP activity.

The phosphorolysis of the four substrates by preparation V was studied kinetically. The results were in agreement with Michaelis–Menten kinetics with dThd, 5′-DFUR, and Tegafur as substrates. The values of K_m and V_{max} obtained are listed in Table II along with the values reported previously.[3,4]

During the purification procedure the activities toward dThd and Urd did not remain in parallel. A considerable activity toward Urd was found in preparation I, which should indicate the presence of UP. The activity relative to that toward dThd decreased appreciably in preparations II and III.

The activity of preparation V toward Urd did not follow Michaelis–Menten kinetics. A concave curve was obtained in an Eadie plot, shown in Fig. 1, indicating that two kinds of Urd phosphorolytic activities were present in preparation V. One of them is the enzyme (UP) present in preparation I in a considerable amount, and this should account for a major part of the catalysis of preparation V, even though it was contained as a contaminant. The activity of preparation I toward Urd followed Michaelis–Menten kinetics in the concentration range of 0.1—6.0 mM and the K_m value was calculated to be 2.25×10^{-4} M. A straight line was drawn based on the K_m value and the activity of preparation V in the Urd concentration of 0.5 mM, which is shown in Fig. 1 as a dotted line. The line approximates closely to an asymptote which touches the curve of total activity at the lower concentration range and represents the UP-catalyzed cleavage of Urd. Subtraction of the UP-catalysis from the total catalytic activity should give the remaining activity. The activity thus obtained gave a linear Lineweaver–Burk plot, and we assume this activity is closely related to that of purified TP, though further work is required to establish this conclusively. From the plot, the values of K_m and V_{max} for Urd listed in Table II were calculated.

The results shown in Table II indicate that the affinity of the TP in the human tumor is in the order of dThd, 5′-DFUR, Tegafur, and Urd. The characteristics of the present TP preparation were essentially the same as those of the preparations from other kinds of human

tumors.[2-5,10]

References and Notes

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