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Substrate Specificity of a Thymidine Phosphorylase in Human Liver Tumor

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A thymidine phosphorylase preparation was partially purified from human liver tumor tissues (poorly differentiated adenocarcinoma). The substrate specificity of the enzyme was investigated with eleven pyrimidine nucleosides. Thymidine and 2′-deoxyuridine were good substrates, while uridine, 3′-deoxyuridine, 5′-deoxyuridine, and 2′,3′-dideoxy-3′-hydroxymethyluridine were not. Uridines substituted at the 5-position by a cyano, bromo, or chloro group were also phosphorolyzed by the enzyme, but the activity for 5-fluorouridine was much lower. 5′-Deoxy-5-fluorouridine was also cleaved. Either a 5-substituent or a 2′-deoxy structure seems to be essential for a good substrate.

Keywords——thymidine phosphorylase; uridine phosphorylase; substrate specificity; liver cancer; human tumor; thymidine; uridine; deoxyuridine; uridine 5-substituted; 5′-deoxy-5-fluorouridine

In the previous papers,[1,2] we reported that thymidine phosphorylase activity is greatly enhanced in human tumors as compared with normal tissues and is responsible for the conversion of 5′-deoxy-5-fluorouridine (5′-DFUR) and 1-(tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) to 5-fluorouracil (5-FU), an activated form. The activation of 5′-DFUR is catalyzed by uridine phosphorylases in experimental tumors of animals.[3]

Thymidine phosphorylase (TP) catalyzes the reversible conversion of thymidine (dThd) and phosphate to thymine and 2-deoxyribose 1-phosphate. Uridine phosphorylase (UP) catalyzes the reversible conversion of uridine (Urd) and phosphate to uracil and ribose 1-phosphate. UP acts primarily on Urd and also cleaves dThd and 2′-deoxyuridine. Thus, its substrate specificity seems to be broad. On the other hand, TP is reported to be highly specific for 5-substituted 2′-deoxyuridines.[4–8]

The findings that 5′-DFUR and Tegafur are phosphorolyzed by TP in human tumors suggest that the specificity of the human enzyme is somewhat different from those of the enzymes from other sources. The present paper deals with the substrate specificity of TP in human liver tumor tissues.

Results and Discussion

Thymidine phosphorylase was partially purified from human liver tumor tissues by a procedure described in the experimental section. In the course of the purification procedure, preparations I—IV were obtained. The activities of the enzyme preparations for phosphorolysis of dThd are summarized in Table I. The specific activity of preparation IV represents a 174-fold purification of the initial homogenate. The results of kinetic analyses were in

TABLE I. Thymidine Cleaving Activities of the Enzyme Preparations

Preparation	Protein (mg)	Activity[a]	Recovery
I	656.0	1.38×10^2	100
II	36.9	5.48×10^2	22.3
III	2.29	4.95×10^3	12.5
IV	0.305	2.40×10^4	8.1

a) 16 μM dThd was incubated (30 min, 37 °C) with the preparations. Values are nmol of thymine formed by 1 mg of protein in 1 h.

TABLE II. Pyrimidine Nucleoside Phosphorolytic Activities of Enzyme Preparation IV

Substrate	Protein (ng)	Incubation (min)	Activity[a]
dThd	1.23	30	14560
Urd	4.29	90	125
5-CN-Urd	2.46	30	10673
5-Br-Urd	2.46	30	4438
5-Cl-Urd	2.46	30	6612
5-FUR	2.46	30	472
2′-dUrd	2.46	30	12123
3′-dUrd	2.46	30	98
5′-dUrd	2.46	30	404
2′,3′-dUrd-3′-MeOH	2.46	30	106
5′-DFUR	2.46	30	1320

a) The nucleosides (16 μM) were incubated (37 °C) with preparation IV (*ca.* 10 d after purification). Values are nmol of pyrimidines formed by 1 mg of protein in 1 h.

agreement with Michaelis–Menten kinetics with dThd as a substrate.

The activities of the partially purified enzyme preparation for phosphorolysis of eleven pyrimidine nucleosides were measured. The nucleosides used were dThd, Urd, 5-cyanouridine (5-CN-Urd), 5-bromouridine (5-Br-Urd), 5-chlorouridine (5-Cl-Urd), 5-fluorouridine (5-FUR), 2′-deoxyuridine (2′-dUrd), 3′-deoxyuridine (3′-dUrd), 5′-deoxyuridine (5′-dUrd), 2′,3′-dideoxy-3′-hydroxymethyluridine (2′,3′-dUrd-3′-MeOH), and 5′-DFUR.

The results are summarized in Table II. Preparation IV was virtually inactive for phosphorolysis of Urd. Phosphorolysis of dThd and 5′-DFUR by the preparation was unaffected in the presence of 1-(2-deoxy-β-D-glucopyranosyl)thymine (GPT), which was reported to inhibit UP activity but not TP activity.[5,9] These results indicate that the TP preparation was not contaminated by UP.

TP in human tumor tissues was practically inactive towards Urd, 3′-dUrd, 2′,3′-dUrd-3′-MeOH, and 5′-dUrd. On the other hand, 2′-dUrd was a good substrate. These findings indicate that TP acts on 2′-deoxypyrimidine nucleosides. That the 3′-hydroxyl group is essential for the substrate was shown by the present results, together with others which will be reported shortly.

5-CN-Urd, 5-Br-Urd, and 5-Cl-Urd were phosphorolyzed by the preparation. 5-FUR cleaving activity was very low. Thus, pyrimidine ribosides with a group or a bulky atom at the 5-position are good substrates of TP.

Phosphorolytic activity of the preparation towards 5′-DFUR was about three times as much as that towards 5-FUR. Almost the same relationship is seen in the activities towards 5′-dUrd and Urd. These results indicate that the absence of the 5′-hydroxy group in

substrates increased the phosphorolytic activity.

The present results suggest that the substrate specificity of TP in human tumor tissues is broader than that of TP from other sources. Either a 5-substituent or a 2′-deoxy structure seems to be essential for a substrate of the enzyme. Purification of TP from various human tumors and further studies on the enzyme kinetics are in progress. The preliminary results indicate that the characteristics of TP from other human tumors are essentially the same as those of the present preparation.

Experimental

Materials——5-Chlorouridine,[10] 5-bromouridine[11] 5-cyanouridine,[12] 5′-deoxyuridine,[13] and 2′,3′-dideoxy-3′-hydroxymethyluridine[14] were prepared by the cited procedures. 5′-DFUR and 3′-deoxyuridine were provided by Hoffmann-La Roche Inc., Nutley, N.J., U.S.A. 1-(2-Dexoy-β-D-glucopyranosyl)thymine (GPT) (NSC 402666) was obtained from the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, U.S.A. Other chemicals were of analytical grade and were obtained from commercial sources.

Enzyme Activity——Pyrimidines formed enzymatically from the nucleosides were determined by means of high-performance liquid chromatography with UV detection, details of which were reported previously.[15] The activity of preparations was expressed in terms of the amount of the pyrimidines produced (nmol) in 1 h by 1 mg protein.

Enzyme Preparations——Human liver tumor tissues metastasized from gall bladder (poorly differentiated adenocarcinoma) were obtained at operation from a 49-year-old female and examined histologically. The tumor tissues (31 g) 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 $7000 \times g$ for 30 min at 4 °C. 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 purified by chromatography on a ConA Sepharose column (2.1 × 8 cm) eluted with the same buffer as used in the dialysis. The effluent, preparation III, was further subjected to chromatography on a column of diethylaminoethyl (DEAE) Sephacel (1.5 × 15 cm). The column was developed with 20 mM potassium phosphate buffer (pH, 7.5) containing 2-mercaptoethanol (10 mM) and a linear gradient of KCl (0—400 mM). The fractions with the highest dThd cleaving activity (115—225 mM KCl) were collected (preparation IV).

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