References and Notes

1) Abbreviations used are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Boc=*tert*-butyloxycarbonyl, *p*NA=*p*-nitroanilide, OMe=methyl ester, Suc=succinyl.
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Enzymatic Formation of 5-Fluorouracil from 1-(Tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) in Human Tumor Tissues

1-(Tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) was phosphorolyzed to form 5-fluorouracil (5-FU) by a soluble fraction of human lung cancer. The catalysis was suppressed in the presence of excess thymidine, but not in the presence of 1-(2′-deoxy-β-D-glucopyranosyl)thymine, an inhibitor of uridine phosphorylases. The cleavage of Tegafur to 5-FU was assumed to be catalyzed by a thymidine phosphorylase activity, which is greatly enhanced in the human tumor tissues, and to represent a possible activation mechanism of Tegafur.

Keywords——1-(tetrahydro-2-furanyl)-5-fluorouracil; 5-fluorouracil; human tumor; lung cancer; thymidine phosphorylase; thymidine; 1-(2′-deoxy-β-D-glucopyranosyl)-thymine

1-(Tetrahydro-2-furanyl)-5-fluorouracil (Tegafur, FT-207) has shown an antitumor activity with a spectrum of activity similar to 5-fluorouracil (5-FU) and is considered to be a chemical depot form of 5-FU.[1] Metabolites hydroxylated at the tetrahydrofuranyl moiety were found in the urine and the plasma after the administration of Tegafur.[2–4] The hydroxylated metabolites were formed *in vitro* by rat liver microsomes.[5] One of the metabolites, 1-(4-hydroxytetrahydro-2-furanyl)-5-fluorouracil, was converted to 5-FU, while Tegafur was not, by a horse liver thymidine phosphorylase preparation.[2] It has been believed that 5-FU is generated *in vivo* by hepatic metabolism involving cytochrome P-450.[6–9] Recently, Au

and Sadée[10] reported that γ-butyrolactone was generated from Tegafur in the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) by cytosol fraction of animal liver homogenates and suggested the oxidative cleavage of Tegafur to γ-butyrolatone and 5-FU, though the formation of the latter was not proved.

In this communication, we wish to report that a soluble fraction of human tumor tissues catalyzed the phosphorolytic cleavage of Tegafur to 5-FU, which may represent another possible activation mechanism of Tegafur in human tumor tissues.

Tissues were homogenized in approximately 10 volumes of 10 mM Tris buffer (pH, 7.4) containing 15 mM NaCl and 1.5 mM $MgCl_2$. The homogenates were centrifuged at $105000 \times g$ for 90 min at 4° and the supernatant was dialyzed over night against 500 volumes of 20 mM phosphate buffer (pH, 7.4: NaH_2PO_4–Na_2HPO_4). The clear solutions thus obtained were used as enzyme preparations.

Tegafur was incubated (37°, 1 hr) in phosphate buffer (pH, 7.4; contains 0.1 M NaCl and 6 mM KCl) with the enzyme preparation and 5-FU released was determined. The enzyme activity to form thymine from thymidine was determined similarly. Protein concentrations in the enzyme preparations were determined by the method of Lowry *et al.*[11] The enzyme activity is expressed as nmol pyrimidines formed by 1 mg protein in the enzyme preparations.

For measuring 5-FU and related pyrimidine derivatives, a reversed-phase high-performance liquid chromatography assay was developed. Ethyl acetate extracts of the incubation mixtures dissolved in methanol were injected into a Waters Assoc. liquid chromatograph equipped with a UV detector operated at 254 nm. The mobile phase was water (1.0 ml/min) and the internal standard for the determination of pyrimidines was 5-chlorouracil. A μ-Bondapak C_{18}/Poracil column was used.

By the enzyme preparation from human lung tumor, Tegafur and thymidine were cleaved to form 5-FU and thymine, respectively (1.87 nmol 5-FU from Tegafur by 1 mg protein). The cleavages were only slightly observed by the use of the heat treated (100°, 15 min) enzyme preparation. 15 ng of 5-FU was formed in 1 ml of the incubation mixture (0.48 nmol/mg protein). Without the enzyme preparation, 5-FU formed in the mixture was 6.5—13 ng/ml, which is taken as the spontaneous cleavage.

The pyrimidines were not formed in significant amount by the enzyme preparation in the absence of inorganic phosphate (in Tris buffer; 0.67 nmol 5-FU/mg protein).

These results indicate that Tegafur and thymidine were enzymatically phosphorolyzed. The Tegafur phosphorolytic activity was greatly suppressed in the presence of 7-fold excess amount of thymidine (0.46 nmol/mg protein). 1-(2′-Deoxy-β-D-glucopyranosyl)thymine (GPT) reportedly inhibits uridine phosphorylase activity but does not inhibit the activity of thymidine phosphorylase.[12,13] In the present study, GPT did not inhibit the phosphorolysis of Tegafur (1.70 nmol/mg protein).

Table I. Phosphorolytic Activity of the Enzyme Preparations from Human Lung

Enzyme source	Tegafur→5-FU[a] (nmol/mg protein)	Thymidine→Thymine[b] (nmol/mg protein)
1. Normal tissue	0.045±0.005	46.5
Tumor tissue[c]	1.87 ±0.08	1066.0
2. Normal tissue	<0.04	52.5
Tumor tissue[d]	1.64 ±0.03	1105.0

a) Values are nmol 5-FU formed from Tegafur (158 nmol) in 1-hr incubation by 1 mg protein. Mean±SD in four determinations.
b) Values are nmol thymine formed from thymidine (32 nmol) in 30-min incubation by 1 mg protein. Mean of two determinations.
c) Anaplastic large cell carcinoma from 48 y male.
d) Well differenciated squamous cell carcinoma from 71 y male.

The phosphorolytic activities of Tegafur and thymidine were much higher in extracts of human tumors than in those of normal tissues of the same organ (Table I). The Tegafur phosphorolytic activity was hardly present in the enzyme preparation from mouse tumor (Sarcoma 180), which showed low thymidine phosphorylase activity.

Pyrimidine derivatives other than Tegafur and 5-FU were not detected in the incubation mixture of Tegafur and the enzyme preparations. It is unlikely to assume that a soluble fraction of tissues catalyzes the hydroxylation of Tegafur without NADPH. We assume the cleavage of Tegafur is catalyzed by a thymidine phosphorylase activity, which is greatly enhanced in tumor tissues.

Thymidine phosphorylases so far reported are highly specific for deoxyribonucleosides.[14] Purification of the enzyme in human tumor tissues is in progress in our laboratory. Preliminary experiments suggest that thymidine phosphorylase in human tumors shows broader substrate specificity than the enzymes reported.

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