Note

Distribution of Dihydrofolate Reductase and Activity Producing Folate Cofactors in Rat Small Intestinal Segments

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Conversion of pteroylglutamate (PteGlu) to 5-methyltetrahydroPteGlu (5-methylH₄PteGlu) involves reduction to tetrahydroPteGlu (H₄PteGlu) by dihydrofolate reductase (DFR) and subsequent methylation to 5-methylH₄PteGlu.¹⁾ Olinger *et al.*²⁾ found greater conversion by rat jejunum when a physiological concentration of PteGlu (20 nM) is used, and minimal conversion and greater transfer to the portal vein of unchanged PteGlu when a high PteGlu concentration (2 μ M) is present. Recently, we have shown similar results *in situ* in rats using the loop method.³⁾

This paper describes the distribution of DFR activity, and the activity producing folate cofactors, H₄PteGlu and 5-methylH₄PteGlu, from PteGlu in the villi and total mucosal preparations of rat small intestine.

Male Wister rats (240~260 g) were anaesthetized with sodium pentobarbital (55 mg/kg). The removed small intestine was divided into three equal parts in length. They were referred to as *upper*, *middle*, and *lower* from the proximal to distal intestine. Each part was rinsed thoroughly with 0.9% NaCl containing 1 mM dithiothreitol (DTT). The villi were prepared promptly by a modification of the procedure of Weiser⁴⁾; the longitudinally incised small intestine was dipped for 15 min at 37°C into 100 ml of buffer A, pH 7.3, containing 96 mM NaCl, 27 mM sodium citrate, 8 mM KH₂PO₄, 5.6 mM Na₂HPO₄ and 1.5 mM KCl. After decantation of the solution, the residue was stirred at 120 rpm for 30 min at 37°C in 100 ml of 0.1 M potassium phosphate buffer, pH 7.4, containing 0.9% NaCl, 1.5 mM EDTA, and 0.5 mM DTT, and centrifuged at 900 $\times g$ for 5 min. The precipitate was washed 3 times with 0.1 M potassium phosphate buffer, pH 7.4 containing 0.9% NaCl (PBS). This preparation is referred to as the villi preparation. The residual tissue was forcefully scraped to separate mucosa from the underlying smooth muscle layer and is referred to as the total mucosal preparation, which was washed

3 times with PBS. The villi and total mucosa of the *upper*, *middle*, and *lower* segments were individually suspended in 3 ml of PBS, and homogenized with equal volumes of 0.1 M mannitol–1 mM Tris HEPES buffer, pH 7.4, at 20,000 rpm for 10 sec using a Biomixer (Nihon Seiki Co., Ltd.). The homogenates were centrifuged at 32,000 $\times g$ for 20 min. The pellet and the supernatant fluid were divided into five equal samples and stored at –20°C until used for the measurement of sucrase,⁵⁾ γ -glutamyl-transferase (γ -GTase),⁶⁾ alkaline phosphatase (APase)⁷⁾ (for the pellet), and DFR activities^{8,9)} (for the supernatant), and protein content.¹⁰⁾ DFR activity was measured from the absorbance decrease at 340 nm for 10 min at pH 7.4,⁸⁾ and pH 5.4.⁹⁾ For the experiment with the activity producing folate cofactors from PteGlu, 0.5 ml of the supernatant fluid obtained from the small intestinal segments was incubated for 1 hr at room temperature with 0.2 ml of 0.5 M citrate buffer, pH 5.4, and 1 ml of hemoglobin-treated charcoal¹¹⁾ to remove endogenous folate compounds, and centrifuged for 30 min. The supernatant was concentrated with a Minicent-30 ultrafilter (Toyo Soda Co., Ltd.). The concentrate was used as the enzyme solution. The reaction mixture (0.5 ml) consisted of 0.05 M citrate buffer, pH 5.4, 0.02 M PteGlu, 0.1 mM NADPH, 20 mM 2-mercaptoethanol, and the enzyme solution (about 0.3 mg protein). After incubation for 30 min or 3 hr at 37°C, 350 μ l of methanol was added to 150 μ l of each reaction mixture and concentrated under N₂ gas blowing. A sample (25 μ l) of the concentrate was used for analysing folate compounds by high-performance liquid chromatography with electrochemical detection (HPLC-ED). The HPLC-ED conditions were described previously,¹²⁾ except that we used a short column (4.6 i.d. $\times$ 50 mm) of Cosmosil 5 Ph.

Sucrase as an enzyme marker was used to verify the purity of the villi preparation as described by Fortin-Magana *et al.*, who found more sucrase activity in villi of the rat small intestine than in their crypt preparation.¹³⁾ Measurement of sucrase activity in our preparations also indicated that the villi preparations from *middle* and *lower* contained mainly villi, not crypt. Greater specific activities of other membrane enzymes, γ -GTase and APase, were also present in villi than in total mucosa.

DFR activity in the supernatant fluid of the rat small intestinal segments is shown in Table I. Unlike DFR of guinea pig intestine,¹⁴⁾ DFR of the rat small intestine reduced much more H₂PteGlu and PteGlu at pH 5.4 than did at pH 7.4. The reduction rate of H₂PteGlu at pH 5.4 in the villi and total mucosal preparations of *middle* and *lower*, and the *upper* villi was greater than that in the *upper* total mucosa. DFR of villi preparations specifically reduced H₂PteGlu at pH 5.4, although as well as H₂PteGlu. Table II shows the activity producing folate cofactors from PteGlu by the cell-free extracts of the rat small intestinal segments. The extracts of the *upper* and *lower* segments produced much more H₄PteGlu than 5-methylH₄PteGlu during 3 hr of

TABLE I. DIHYDROFOLATE REDUCTASE ACTIVITY IN THE CELL-FREE EXTRACTS OF RAT SMALL INTESTINAL SEGMENTS

Small intestine		Total protein (mg)	Reductase activity ^a		
			H ₂ PteGlu (7.4) ^b	H ₂ PteGlu (5.4) ^b	PteGlu (5.4) ^b
Upper	Villi	31.9	1.85	3.26	0.89
	Total mucosa	63.9	0	0.96	0
Middle	Villi	32.7	1.72	3.64	0.92
	Total mucosa	72.9	2.67	3.89	1.68
Lower	Villi	10.3	1.82	4.41	0
	Total mucosa	73.5	2.64	3.87	3.49

^a DFR activity expressed as nmoles per min per mg protein at 37°C was measured in tissues from five rats.

^b Substrate and reaction pH in parenthesis.

TABLE II. PRODUCTION OF TETRAHYDROFOLATE AND 5-METHYLTETRAHYDROFOLATE FROM PTEROYLGLUTAMIC ACID BY RAT SMALL INTESTINAL SEGMENTS

Folate compound	Amount of folate compound ^a					
	Upper		Middle		Lower	
	Villi	Total mucosa	Villi	Total mucosa	Villi	Total mucosa
H ₄ PteGlu	16.0 (33.4)	72.2 (48.4)	0 (24.0)	5.8 (5.0)	72.0 (85.8)	8.0 (7.0)
5-Methyl H ₄ PteGlu	0 (15.8)	0 (17.2)	53.6 (31.6)	8.6 (13.6)	10.2 (0.72)	6.0 (3.0)

^a Amounts of folate compounds produced by small intestinal segments (*upper*, *middle* and *lower*) are expressed as nmoles per mg protein per 30 min and 3 hr (in parenthesis) at pH 5.4.

incubation. Since the *middle* extracts produced much 5-methylH₄PteGlu and a small amount of H₄PteGlu in spite of the high DFR activity, the *middle* was shown to have a greater methylation activity than other intestinal segments. This shows a correlation between the location of DFR activity and the appearance of 5-methylH₄PteGlu in the *middle* villi as reported by Olinger *et al.*²⁾ However, there was no correlation in the *lower*. These results suggest that various parts of rat small intestine can promptly reduce the luminal PteGlu to H₄PteGlu and 5-methylH₄PteGlu.

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