Thiamine Diphosphatase in Rat Small Intestine

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Summary. TDPase is located mostly in the proximal portion of the small intestine and its activity, like that of ALPase, decreased markedly in thiamine deficiency. The decreased enzyme activities were restored after thiamine or vitamin D_3. Kinetic and other studies of the purified enzyme indicated the identity of the two enzymes.

It is widely accepted that intestinal transport of thiamine is linked with a carrier-mediated mechanism that is dependent either on thiamine phosphorylation-dephosphorylation coupling, or on some metabolic energetic mechanism, possibly activated by Na^+[2]. However, little information is available concerning the enzymes involved in phosphorylation and dephosphorylation of thiamine esters in the intestine. We have begun a series of experiments designed to elucidate a possible physiological role of thiamine metabolism in the intestine at enzymic level.

In this paper, in view of a report[3] that human intestinal alkaline phosphatase (ALPase) is able to hydrolyze thiamine diphosphate (TDP), comparative studies on TDPase and ALPase in the intestine were undertaken. We report here an evidence suggesting that the two enzyme activities may be part of a single enzyme.

Materials and methods. In order to exclude inorganic phosphate, TDP was purified by chromatography on a column of Amberlite IRC-50 (H^+)[4]. Male Sprague-Dawley rats, 200–250 g, were used. Thiamine-deficient and pair-fed rats were prepared by the method of IWATA et al.[5]. The weight of the intestinal mucosa reduced to the same degree in thiamine-deficient and pair-fed groups comparing to control animals. Duodenal mucosa, scraped off with a glass slide, was homogenized with 20 vol. of 5 m*M* EDTA (pH 7.4) and the homogenate was diluted with 40 m*M* *Tris*-buffer (pH 7.4) (Table II, experiment 1) or distilled water (Table II, experiment 2).

TDPase activity was determined as previously reported[6], except that an incubation time of 10 min was used for the experiment shown in Table II, III and Figure. ALPase activity was determined by the method of RUSSELL et al.[7]. Protein content was measured by the procedure of LOWRY et al.[8].

Results and discussion. As shown in Table I, TDPase activity was high in the intestine, kidney, liver and pituitary, and in endocrine organs. We found a similar distribution for TDPase as KRAWITT et al.[9] found for ALPase. Both activities were much higher in the mucosa than in the muscle coat (data not shown).

Table I. Distribution of TDPase activity in various tissues of normal rats

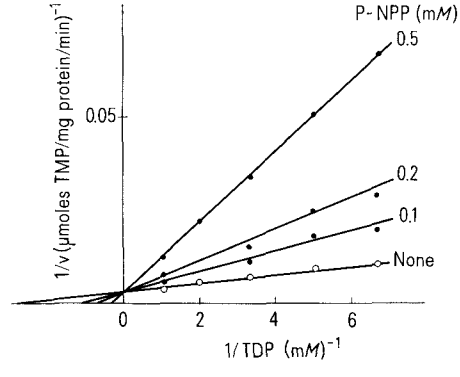
		μmoles Pi/mg protein/h
Intestine	Duodenum	25.05 ± 1.51 (8)
	Jejunum	4.44 ± 0.36 (4)
	Ileum	2.18 ± 0.11 (6)
Kidney		2.69 ± 0.10 (4)
Adrenal		1.83 ± 0.14 (6)
Testis		1.66 ± 0.07 (5)
Liver		1.31 ± 0.09 (4)
Thyroid gland		0.99 ± 0.09 (8)
Spleen		0.46 ± 0.03 (4)
Heart		0.43 ± 0.01 (4)
Brain		0.38 ± 0.02 (4)
Thymus		0.27 ± 0.03 (5)
Skeletal muscle		0.09 ± 0.00 (4)
Brain	Pituitary	1.20 ± 0.08 (5)
	Olfactory lobe	0.59 ± 0.03 (4)
	Cerebral cortex	0.53 ± 0.02 (3)
	Medulla oblongata	0.49 ± 0.04 (3)
	Brain stem	0.46 ± 0.02 (3)
	Cerebellum	0.37 ± 0.02 (3)

Values are given as means (± SE). Numbers of experiments are indicated in brackets. After decapitation, various tissues were homogenized with 10 vol. of ice-cold 0.25 *M* sucrose.

Table II. Effect of thiamine deficiency on TDPase and ALPase activities in the intestinal mucosa of rats

		Specific activity (μmoles/mg protein/min)	
	N	TDPase	ALPase
Experiment 1			
Control	15	0.42±0.04	1.41±0.08
Thiamine-deficient	15	0.24±0.02[c]	0.38±0.03[c]
+ Thiamine[a] (24 h)	6	0.36±0.07	0.67±0.08[c]
+ Thiamine[a] (48 h)	6	0.35±0.06	1.23±0.19
+ Thiamine[a] (72 h)	5	0.26±0.01[c]	1.14±0.10
Experiment 2			
Control	8	0.82±0.05	1.65±0.17
Thiamine-deficient	8	0.51±0.05[c]	0.46±0.06[c]
+ Vitamin D_3[b] (40 h)	9	0.75±0.09	0.76±0.09[c]

Values are given as means (± SE) *N*, number of experiments. [a]4 mg/kg, s. c., [b]500 IU, p. o., [c]$P < 0.01$ from pair-fed control (Student's *t*-test). Pair-fed rats were used as control.



Double reciprocal plot of TDP hydrolysis in the presence and absence of P-NPP. TDPase activity was determined by measuring the rate of formation of thiamine monophosphate (TMP).

Table II shows the effect of thiamine deficiency on these enzyme activities in intestinal mucosa. Both enzyme activities decreased markedly in thiamine deficiency and were restored to the control level after thiamine-HCl (experiment 1). In addition, TDPase was restored completely and only ALPase was restored partly after vitamin D_3 which is known to induce an increase of Ca^{++} absorption and intestinal ALPase in rachitic animals[10], (experiment 2). The corresponding changes of TDPase and ALPase activities in response to thiamine deficiency, coupled with the similar distribution of two enzymes in small intestine, led us to consider that the two enzyme activities represented different measures of the activity of a single enzyme. To investigate this point, we carried out the purification of ALPase from intestinal mucosa in normal rats by the method of SAINI et al.[11], modified in that enzymically active fractions from each column chromatography were concentrated by ultrafiltration instead of ethanol precipitation.

Table III. Results of the enzyme purification steps

Steps	Protein (mg)	Specific activity (μmoles/mg protein/min)		Ratio a/b
		ALPase[a]	TDPase[b]	
Mucosal homogenate	7260	1.73	0.95	1.82
n-Butanol extract	537	8.73	8.50	1.03
Ethanol precipitate	245	30.7	11.8	2.60
DEAE cellulose fraction	81	148	76.2	1.94
Sephadex G-200 fraction	28	175	190	0.92
DE-32 cellulose fraction	19	187	217	0.86
DE-32 cellulose fraction	2	588	482	1.22

TDPase was purified together with ALPase (Table III) and *p*-nitrophenylphosphate (P-NPP) behaved as a competitive inhibitor of TDP hydrolysis (Figure); Ki value obtained by the method of DIXON[12], using data shown in the Figure, was about 0.1 m*M*. The value is in agreement with K_m value of P-NPP hydrolysis (K_m 0.14 m*M*). Furthermore, two enzyme activities in the purified material showed a similar response to heat denaturation and to L-phenylalanine, a stereospecific inhibitor of intestinal ALPase[13] (data not shown). These results strongly suggest that TDPase is identical with ALPase in the intestine.

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Reactions of the Goldfish (*Carassius auratus auratus* L.) to Quantified Mechanical and Thermal Stimuli[1]

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Summary. Goldfish can differentiate between mechanical and thermal stimuli components in combined stimulations. Reactions to heated thermode ($\Delta T = +3\,°C$) differ from those to isotherm thermode by up to 30%. Additional pretension (0.5 *p*) increases the reaction more than double; at simultaneously varied temperature ($\Delta T = +3\,°C$ and $+5\,°C$), there is a further increase in reaction of 25% and 35%. The significance for the organism of the two stimuli components is discussed.

Bony fish can be conditioned with different methods to react to temperature. Besides, fish are capable of an exact temperature selection within a temperature gradient[3]. In addition, DIJKGRAAF[4] found out that minnows (*Phoxinus laevis*) and catfish (*Ameirus nebulosus*) can be conditioned to water jets of different temperatures, even if the lateral line system is eliminated. During further investigations of the distribution of thermal sensitivity over the skin, BARDACH[5] and SERBENYUK and MANTEIFEL[6] showed, by means of stimulation with thermodes, that the entire body surface of the fish is involved in the sensation of temperature.

In general, thermal investigations of aquatic vertebrates had thermal stimuli coupled with mechanical stimuli. Up to now, research on the temperature sense has not sufficiently taken into account that, in this combination, mechanical stimulation becomes effective simultaneously. The obvious connection of the two stimuli modalities has also been confirmed by our investigations where no specific thermoreceptors could be found in fish

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