Formations of amino acids from unsaturated and saturated aliphatic carboxylic acids by CGDE

No.	Starting materials (0.005 moles)	Reaction products (yield percent) Asp	Thr	Ser	Glu	Gly	Ala	αABA	β-Ala	iso-Ser	e-OH-Asp	t-OH-Asp	e-OH-Glu*	t-OH-Glu*
1	CH_2=CHCOOH	+	+	3.1	+	0.1	2.6	0.5	1.8	2.1				
2	CH_3CH=CHCOOH	+	3.9	+	+	0.3	0.3	1.6	0.3	—				
3	HOOCCH=CHCOOH (cis)	4.4	—	0.1	2.2	0.2	0.1	0.1	0.2	—	3.2	2.8	—	—
4	HOOCCH=CHCOOH (trans)	4.5	—	0.1	2.2	0.2	0.1	0.1	0.2	—	3.3	3.0	—	—
5	HOOCCH$_2$CH=CHCOOH	—	0.2	—	1.3	0.1	+	—	+	—	—	—	0.9	0.8
6	CH_3CH_2COOH	0.1	—	0.1	—	0.1	1.2	+	0.9	+				
7	$CH_3CH_2CH_2$COOH	+	0.1	+	+	0.1	+	0.8	0.3					
8	HOOCCH$_2$CH$_2$COOH	1.6	—	+	0.2	+	+	—	0.2		+	+	—	—
9	HOOCCH$_2$CH$_2$CH$_2$COOH	+	—	—	1.8	0.1	+	—	—		—	—	+	+

Voltage 400–600 V; current 50–60 mA; time 1 h; temp. 10–15 °C. * e-OH- or t-OH-Glu: erythro- or threo-β-hydroxy glutamic acid.

identified. The CGDE of the unsaturated carboxylic acid gives more hydroxy amino acids than that of the saturated one. It could be considered that the hydroxy radicals generated from water attack the double bond of the unsaturated carboxylic acid. There is no difference in the species and the amounts of amino acids synthesized from maleic acid and fumaric acid by CGDE

The glow discharge electrolysis could be regarded as a simulation of lightning hitting the primitive sea which contains organic and inorganic compounds. Thus, the above findings suggest one possibility for the prebiotic synthesis of hydroxy amino acids from simple organic compounds under possible prebiotic conditions on the primordial earth.

Possible role of intestinal alkaline phosphatase activity in thiamine transport

T. Matsuda, A. Baba and H. Iwata

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, 133-1, Yamada-kami, Suita-shi, Osaka (Japan), 14 June 1977

Summary. Thiamine deficiency caused a marked decrease of intestinal alkaline phosphatase (al-Pase) activity, but had no effect on the Ca^{++}-ATPase activity and Ca^{++}-absorption in rats. The al-Pase activity was significantly decreased 1 h after oral administration of ethanol at 0.5 and 2.5 g/kg. In contrast, Mg^{++}-, Ca^{++}- and $(Na^+ + K^+)$-ATPase activities did not change after the administration of ethanol. These findings show that the al-Pase activity, unlike the Ca^{++}-ATPase activity, is not related to Ca^{++}-absorption. A possible role of al-Pase activity in the active transport of thiamine in the intestine was discussed.

It is reported that intestinal Ca^{++}-binding protein[1,2], Ca^{++}-ATPase[3–5] and al-Pase[6–10] play functional roles in the transport of Ca^{++} in the intestine. A close relation between the Ca^{++}-ATPase and al-Pase activities under a variety of conditions suggested that activities of these enzymes might represent measure of the same enzyme, though a few reports[11,12] conflict with this idea.

Previously we showed that the al-Pase and thiamine diphosphatase (TDPase) activities of rat duodenum were markedly decreased by thiamine deficiency and suggested that intestinal TDPase activity was identical with the al-Pase activity[13]. These results led us to consider that thiamine deficiency possibly causes decreases of intestinal Ca^{++}-ATPase activity and Ca^{++}-absorption. The present study was carried out to clarify this point.

Finally, this paper reports the effect of ethanol, which is well-known to impair intestinal absorption of thiamine in human[14,15] and animals[16], on the activities of intestinal phosphatases to deduce a possible role of the al-Pase activity in the absorption of thiamine.

Materials and methods. Male Sprague-Dawley rats, weighing 80–100 g, were used throughout. Thiamine-deficient and pair-fed rats were prepared as described previously[17]. Rats were fasted overnight but allowed free

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Table 1. Effect of thiamine deficiency on the activities of the brush border phosphatases of rat duodenal mucosa

	al-Pase	TDPase	ATPases activated by Ca^{++}	$Mg^{++} + Ca^{++}$	Mg^{++}
Pair-fed control	5.87 ± 0.90	0.63 ± 0.16	0.21 ± 0.02	0.22 ± 0.03	0.24 ± 0.02
Thiamine-deficient	1.60 ± 0.23**	0.26 ± 0.06*	0.16 ± 0.02	0.16 ± 0.02	0.24 ± 0.02

ATPase activities were determined in the presence of 10 mM $CaCl_2$, 5 mM $MgCl_2$ + 10 mM $CaCl_2$ or 5 mM $MgCl_2$. Activities of al-Pase, TDPase and ATPases are expressed as μmoles p-nitrophenol/mg protein/min, μmoles Pi/mg protein/min and μmoles Pi/mg protein/min, respectively. The value is the mean ± SE of values in 5 separate experiments. * $p < 0.025$, ** $p < 0.005$.

Table 2. Effect of thiamine deficiency on intestinal Ca^{++}-absorption and serum Ca^{++}-concentration

	Ca^{++}-absorption cpm ^{45}Ca/0.2 ml plasma	Serum Ca^{++} (mM)
Pair-fed control	3909 ± 586 (11)	1.80 ± 0.02 (8)
Thiamine-deficient	3359 ± 404 (10)	1.76 ± 0.03 (8)
Thiamine-deficient + Thiamine-HCl (4 mg/kg, s.c., 48 h)	4383 ± 477 (9)	1.82 ± 0.04 (4)

The value is the mean ± SE and numbers of animals are given in parentheses.

access to water and killed 1 h after oral administration of ethanol at doses of 0.5 and 2.5 g/kg, given as 5 and 25% (w/v) ethanol solution, respectively. The crude brush border fractions were prepared from the homogenate of the duodenum, the proximal 12 cm of the small intestine, according to the procedure of Forstner et al.[18] and the final pellet was suspended in 0.5 mM EDTA (pH 7.4).

Al-Pase activity determined by the method of Russell et al.[10] except that concentration of p-nitrophenylphosphate as substrate was decreased to 5 mM. TDPase and ATPase activities were measured as previously reported[19,20]. Protein was determined by the method of Lowry et al.[21].

Ca^{++}-absorption was assayed by the procedure of McNutt et al.[22]. Oral ^{45}Ca-^{40}Ca (2 μCi ^{45}Ca and 1.9 mg Ca^{++} as $CaCl_2$) was administrated 40 min before collection of blood by decapitation and aliquots (0.2 ml) of plasma were counted in a liquid scintillation spectrometer. Serum Ca^{++}-concentration was measured with the aid of an atomic absorption system.

Results and discussion. Thiamine deficiency caused great decrease of al-Pase and TDPase activities in the brush border fraction of the duodenum in agreement with our previous finding[13] with the mucosal homogenate (table 1). On the other hand, the activities of ATPase and Ca^{++}-absorption were not influenced significantly by thiamine deficiency (tables 1 and 2). These observations conflict with the previous reports[7,8,10] that intestinal al-Pase activity is closely related to the Ca^{++}-ATPase activity in the transport of Ca^{++}.

Table 3 shows the effect of ethanol administration on the activities of phosphatases in the duodenal brush borders. Oral ethanol at 0.5 and 2.5 g/kg caused a significant decrease of al-Pase activity. But, Ca^{++}-ATPase and $(Na^+ + K^+)$-ATPase activities were not influenced by the ethanol. On the other hand, in vitro experiments with the brush border showed that 2% (v/v) ethanol decreased the $(Na^+ + K^+)$-ATPase activity by 50%, but did not affect on the al-Pase activity (data not shown). The results in this paper suggest that the activity of intestinal al-Pase is different from that of the Ca^{++}-ATPase. This is also supported by our findings that thiol-containing reagents stimulate the Ca^{++}-ATPase activity, but not the al-Pase activity[23].

Schaller et al.[24] have showed, using an everted sac, that L-phenylalanine, an inhibitor of intestinal al-Pase, decreases the transport of thiamine. Hoyumpa et al.[16] reported that oral ethanol at 0.5–7.5 g/kg decreased the active, but not the passive, component of thiamine transport. They also suggested that this effect could be due to an inhibition of $(Na^+ + K^+)$-ATPase from their finding that ouabain at 100 μM markedly reduced the active transport of thiamine. The present findings suggest

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Table 3. Effect of ethanol administration on the activities of the brush border phosphatases

	Dose (g/kg)	al-Pase	ATPases Mg^{++}	Ca^{++}	$Na + K^+$
Control (11)		3.78 ± 0.02	0.17 ± 0.01	0.20 ± 0.03	0.14 ± 0.01
Ethanol (8)	0.5	2.83 ± 0.23*	0.18 ± 0.02	0.14 ± 0.01	0.11 ± 0.01
Ethanol (8)	2.5	2.34 ± 0.31*	0.20 ± 0.02	0.18 ± 0.02	0.14 ± 0.02

The value is the mean ± SE and numbers of animals in each group are given in parentheses. Activities of al-Pase and ATPase are expressed as μmoles p-nitrophenol/mg protein/min and μmoles Pi/mg protein/min, respectively. * $p < 0.001$.

that intestinal al-Pase activity play a functional role in the active transport of thiamine. On the other hand, we failed to detect any effect of ethanol administration on ($Na^+ + K^+$)-ATPase activity. Our results are therefore in conflict with the suggestion by Hoyumpa et al.[16].

Now, it is possible to speculate from our findings that the active transport of thiamine in the intestine is decreased by thiamine deficiency. However, we previously observed that glucose-intolerance in thiamine-deficient rats was restored by thiamine at higher dose than 0.05 mg/kg, irrespectively of whether it was given orally or s.c., suggesting that the absorption of thiamine in thiamine-deficient rats was the same in the control[25]. This discrepancy may be explained by an adaptative mechanism to facilitate absorption of meager supples of thiamine in thiamine-deficient rats as suggested by Hoyumpa et al.[26].

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Serum tyrosinase in human lung carcinoma[1]

Y. M. Chen and W. Chavin[2]

Department of Biology, Wayne State University, Detroit (Michigan 48202, USA), 6 June 1977

Summary. Compared to normal humans, lung carcinoma patients show increased tyrosinase activity. 7 serum enzymic fractions or carriers were present in the diseased state. Further, serum tyrosinase inhibitory factors generally were decreased in lung carcinoma patients compared to normal individuals.

Serum tyrosinase activity appears associated with some malignant diseases[3]. Serum factors affecting tyrosinase activity have been demonstrated in melanoma patients and may be markers for the degree of malignancy[4]. The present report deals with these parameters in patients with lung carcinoma.

Materials and methods. Sera of 11 normal humans and 41 lung carcinoma patients prior to treatment were obtained (table 1). In addition, blood bank plasma also was used. The sera and plasma were fractionated with ammonium sulfate. The proteins were salted out at 60% saturation from the 50% saturation supernatant. These proteins were assayed for tyrosinase activity and for electrophoretic separations producing dopa-melanin bands[3,5]. The 60% saturation supernatant was utilized for separation of serum or plasma fractions affecting tyrosinase activity. These supernatants were desalted by dialysis and ultrafiltrated (Amicon) at 4 mol. wt ranges. The effects of these fractions on the melanogenic activity of mushroom tyrosinase were evaluated[6].

Results and discussion. Sera from normal individuals and lung carcinoma patients did not show statistically significant sex differences in serum tyrosinase activity. The data of each group, therefore, were consolidated (table 1). The tyrosinase activity present in the sera of patients with lung carcinoma was 202% of the normal. However,

Table 1. Serum tyrosinase activity in lung carcinoma.

Category	Age (years)[a] Male	Female	Tyrosinase activity[b] pmole/ml	% normal
Normal	30–50 (6)	25–46 (5)	501 ± 61[c]	–
Lung carcinoma	31–79 (31)	48–81 (10)	1011 ± 87	202

[a]Number of individuals in parenthesis. [b]L-[U-^{14}C] tyrosine conversion to melanin (30 °C, 16 h). [c]Mean ± SEM.

Table 2. R_f-values of narrow dopa-melanin electrophoretic band in sera of patients with lung carcinoma

Band	R_f-values ($\times 10^{-2}$)
1	2.53 ± 0.12[a]
2	3.46 ± 0.15
3	6.00 ± 0.17
4	10.23 ± 0.19
5	11.32 ± 0.11
6	12.99 ± 0.13
7	14.70 ± 0.23

[a]Mean ± SEM.

1 This investigation was supported by NIH grants CA-16563-01 and CA-16563-02 from National Cancer Institute.
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Table 3. Serum inhibitory factors[a] of tyrosinase activity in lung carcinoma

Category	Tyrosinase activity (% control)[b] I_{300}	I_{100}	II	III
Normal[c]	10.93 ± 0.12[d]	8.95 ± 0.11	24.83 ± 0.34	83.01 ± 1.29
Lung carcinoma	6.99 ± 0.06	10.97 ± 0.11	61.70 ± 2.87	98.10 ± 1.83

[a]Mol.wt of fractions: I_{300}, greater than 300,000; I_{100}, 100,000–300,000; II, 50,000–100,000; III, 30,000–50,000. Each fraction added was equal to the amount obtained from 0.2 ml serum or plasma. [b]Mushroom tyrosinase (20 µg) was incubated with L-[U-^{14}C]tyrosinase with and without serum fractions at 30 °C for 1 h. L-tyrosine conversion for control (no serum factor) was calculated as 100%. [c]Blood bank plasma. [d]Mean ± SEM.