Antioxidant Properties of Thiamine

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Thiamine (10^{-4}-10^{-6} M) inhibits lipid peroxidation in rat liver microsomes and free radical oxidation of oleic acid *in vitro*. Thiamine interacts with free radicals and hydroperoxides and is oxidized to thiochrome and thiamine disulfide. The antioxidant effect of thiamine is probably related to successive transfer of $2H^+$ from the NH_2 group of the pyrimidine ring and H^+ from the thiazole ring (after its opening) to reactive substrates.

Key Words: *thiamine; antioxidants; microsomes; iron; hydroperoxides*

Despite a great body of data suggesting antioxidant properties of vitamin B_1 (thiamine), its effect on lipid peroxidation (LPO) in cells is poorly understood. Thiamine produces antioxidant effects on aldehydes, polyphenols, and ascorbic acid [3,9,11] and, similarly to ascorbic acid and cysteine, prevents oxidation of SH groups in cells [12]. The thiamine-ascorbic acid complex inhibits free radical oxidation of dopamine [10]. High levels of free radicals in the brain [13] and LPO products in the liver [8] were found in rats with thiamine deficiency. At the same time, thiamine is rapidly oxidized in media containing Cu(II) and ascorbic acid [7], as well as in peroxidase reactions [14].

Here we studied the effects of thiamine on LPO in rat liver microsomes and free radical oxidation of unsaturated fatty acids and its interaction with reactive oxygen species and hydroperoxides.

MATERIALS AND METHODS

Liver microsomes were obtained from 3-4-month-old male mice by differential centrifugation [4]. Thiamin in final concentrations of 10^{-4} and 10^{-6} M was added to microsomal suspension (3 mg protein/ml) in 0.1 M Tris-HCl buffer (pH 7.4). The mixture was incubated in the presence of O_2 at 37°C for 30, 60, and 120 min. The intensity of LPO was estimated by accumulation of malonic dialdehyde (MDA) [1].

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In model experiments, oleic acid was preliminary oxidized with O_2 at 80°C for 20 min. The reaction was initiated by adding Fe^{2+} ($FeSO_4$ in a final concentration of 10^{-4} M) to 0.15% ethanol solution of oleic acid. LPO intensity was estimated on a KhLM-1Ts-01 chemiluminometer [2]. Antioxidant effect of thiamine was estimated by chemiluminescence quenching.

To evaluate the mechanism of antioxidant effects of thiamine, we studied its interaction with reactive oxygen species and hydroperoxides (t-butyl and oleic acid hydroperoxides). Reactive oxygen species ($HO_2^\bullet$ and $O_2^{\bullet-}$) were initiated in 0.1 M potassium phosphate buffer (pH 7.4) by divalent iron ($FeSO_4$, 10^{-3} M) at 37°C ($Fe^{2+}+O_2+H^+ \rightarrow Fe^{3+}+HO_2^\bullet$; $HO_2^\bullet \rightarrow H^+ + O_2^{\bullet-}$) [1,15]. Generation of peroxide radicals and oleic acid hydroperoxides was induced by partial oxidation of this acid in the presence of O_2. Free radical activity was estimated by the intensity of chemiluminescence [2].

t-Butyl hydroperoxide was dissolved in 0.1 M phosphate buffer (pH 7.4) to a concentration of 10^{-2} M. Oleic acid was dissolved in chloroform to a concentration of 2×10^{-2} M. Thiamine (final concentration 10^{-4} M) was added to the reaction mixture and incubated at 37°C for 1 h-5 days. Thiamine oxidation products, thiochrome and thiamine disulfide, were assayed. Thiochrome was identified fluorometrically at excitation and emission wavelengths of 360-375 and 420-440 nm, respectively. Thiamine disulfide was detected by thin-layer chromatography [6].

The results were analyzed by Student's *t* test.

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RESULTS

Thiamine in a concentration of 10^{-6} M incubated with the suspension of intact microsomes for 30, 60, and 120 min inhibited MDA accumulation by 31, 18, and 16%, respectively; in a concentration of 10^{-4} M, thiamine inhibited this process by 24, 41, and 21%, respectively (Table 1).

The addition of 10^{-4} M thiamine to a solution of oxidized oleic acid containing peroxide radicals and

TABLE 1. MDA Accumulation (nmol/mg protein) in Suspension of Rat Liver Microsomes Incubated with Thiamine ($M \pm m$, $n=4$)

Incubation, min	Without thiamine (control)		With thiamine			
			10^{-6} M		10^{-4} M	
	abs.	%	abs.	%	abs.	%
30	0.97±0.07	100	0.64±0.07	69**	0.74±0.03	76**
60	1.52±0.10	100	1.25±0.03	82**	0.90±0.12	59*
120	3.27±0.08	100	2.76±0.07	84**	2.59±0.16	79**

Note. Here and in Table 2: *$p<0.01$ and **$p<0.05$ compared to the control.

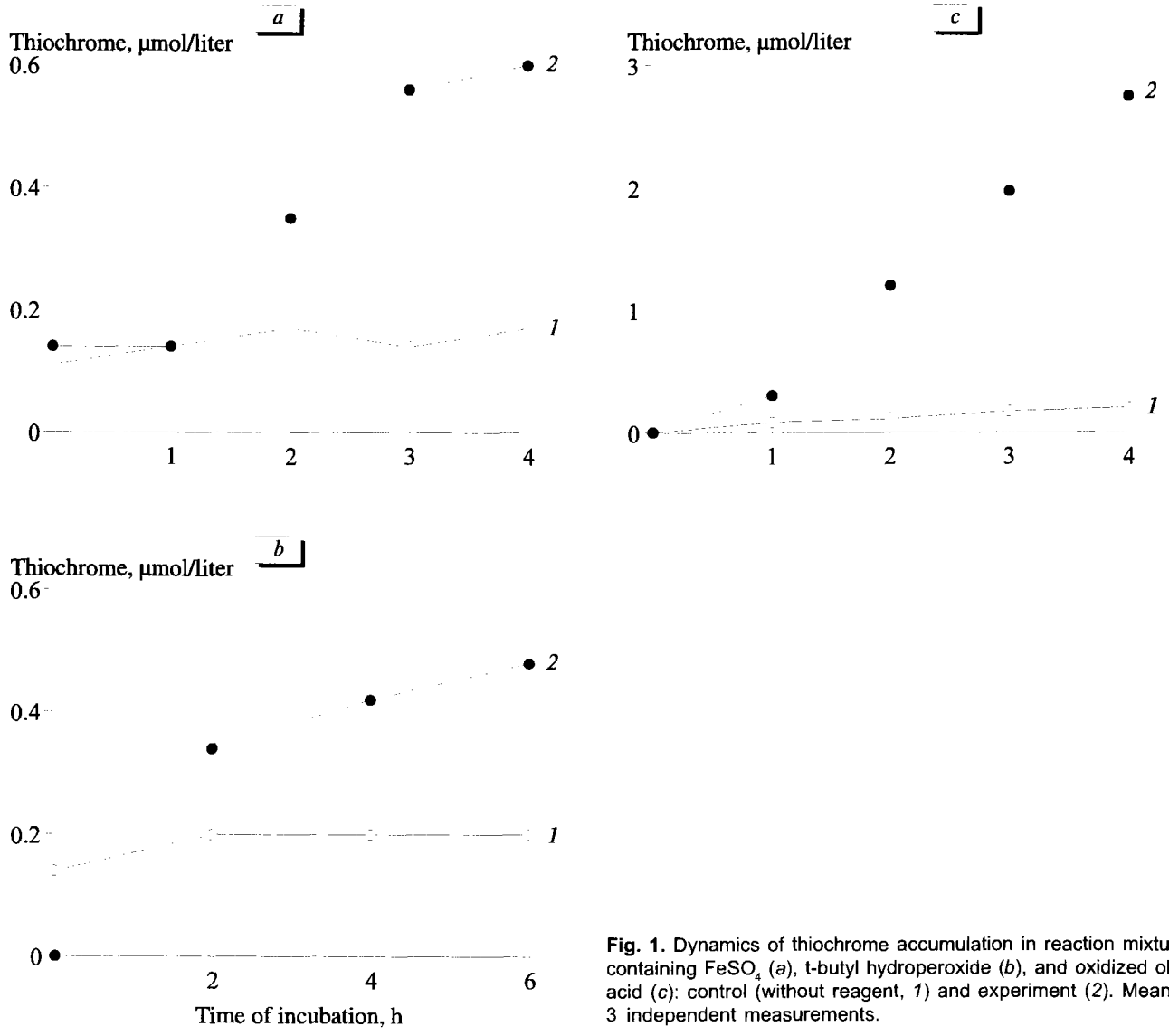

Fig. 1. Dynamics of thiochrome accumulation in reaction mixtures containing $FeSO_4$ (*a*), t-butyl hydroperoxide (*b*), and oxidized oleic acid (*c*): control (without reagent, *1*) and experiment (*2*). Mean of 3 independent measurements.

TABLE 2. Effects of Thiamine on Chemiluminescence of Ethanol Solution of Oxidized Oleic Acid ($M \pm m$, $n=7$)

Parameter	Control		Thiamine, 10^{-4} M	
	abs.	%	abs.	%
Total chemiluminescence, imp/3 min	29 280±698	100	24 590±531	83**
Fast flash amplitude after addition of 10^{-4} M $FeSO_4$, rel. units	12.69±1.25	100	6.92±0.94	45*

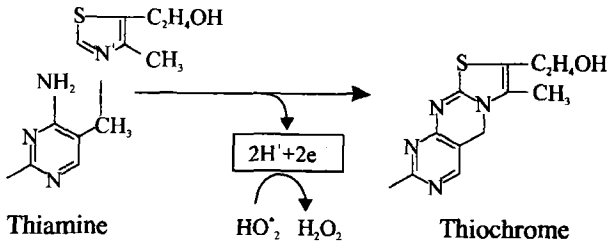

Fig. 2. Formation of tricyclic thiamine and its conversion into thiochrome.

hydroperoxides decreased the intensity of Fe^{2+}-induced chemiluminescence by 17%. The fast flash amplitude decreased 2-fold compared to the control (Table 2).

Thiamine was oxidized after 2-h incubation with reaction mixtures containing oxygen radicals generated by Fe^{2+}, t-butyl hydroperoxide, or oleic acid hydroperoxides (Fig. 1). After incubation for 2 and 4 h, the content of thiochrome increased by 1.5-2 and 4.2-10 times, respectively, compared to the control. Apart from thiochrome, the incubation medium contained trace concentrations of thiamine disulfide. The presence of these thiamine transformation products indicates that under these conditions thiamine oxidation proceeds via 2 independent pathways: 1) ($2H^+ + 2e^-$) splitting from the NH_2 group of the pyrimidine ring with the formation of tricyclic thiamine, which is then converted into thiochrome (Fig. 2); 2) opening of the thiazole ring and loss of $H^+ + e^-$. In the later case, OH^- can be integrated into the thiazole component of thiamine [5].

These results indicate that thiamine directly interacts with free radicals and hydroperoxides, undergoes oxidation, and produces antioxidant effects related to the transfer of ($2H^+ + 2e^-$) from the NH_2 group of the pyrimidine ring to radicals.

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