# BIOPHYSICS AND BIOCHEMISTRY

# Free-Radial Oxidation of Lipids in Human Serum as a Function of Vitamin E Content

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The relationship between free-radial oxidation of human serum lipids and serum content of vitamin E is studied by the chemiluminescence method. A linearity between chemiluminescence and vitamin E content is established. By approximating experimental data we deduced a set of equations characterizing the chemiluminescence parameters as a function of vitamin E content. The correlation coefficients have been calculated in the 0.7605-0.9671 range.

**Key Words:** *chemiluminescence; vitamin E; free radicals; antioxidants; lipid peroxidation*

Free-radical oxidation (FRO) of lipids has been extensively studied. It is shown that both *in vivo* [1,12] and *in vitro* [3,13] FRO modifies physicochemical properties of cell membranes and, consequently, impairs their functions, leading to the development of various pathologies [8,13].

Previously, we evaluated the role of synthetic and naturally occurring antioxidants (AO) in FRO of individual lipids and blood lipids *in vitro* [3] as well as the role of vitamin E in pathological processes [6]. However, there is no information regarding the relationship between the intensity of FRO of serum lipids and the content of vitamin E, the major fat-soluble serum AO [11], in healthy subjects.

Our goal was to study the kinetics of FRO of serum lipids as a function of vitamin E content in healthy men using the chemiluminescence (CL) method.

## MATERIALS AND METHODS

Sera from 24 healthy male donors aged 28-48 years were used. Blood was collected from the cubital vein in the morning after an overnight fast. Serum was prepared by the standard method. The following CL parameters were measured in the presence of hydrogen peroxide at 37°C [1]: maximum intensity ($I_{max}$) [8], light sum ($S$) for a given time period (2 min) [5], ratio of $I_{max}$ to the time, during which CL intensity decreases 2-fold ($T$) [7], and the tangent of the descending segment of the kinetic curve (tg$\alpha$).

Chemiluminescence was measured in a PKhL-01 device equipped with a FEU 84-3 photomultiplying unit and calibrated in absolute units quantum/sec×4$\pi$. Kinetic curves were recorded and processed with an AkhLG-2-01 analyzer using specially designed software. Serum concentration of vitamin E was determined as described elsewhere [10].

Vitamin E concentration and CL parameters were measured randomly. In each experiment, the mean of three determinations was calculated. Correlations were calculated by approximation using the least squares method.

## RESULTS

The kinetics of serum CL recorded in the presence of hydrogen peroxide agrees with the literature data [5,8]. The fast flash of CL reaches the maximum that

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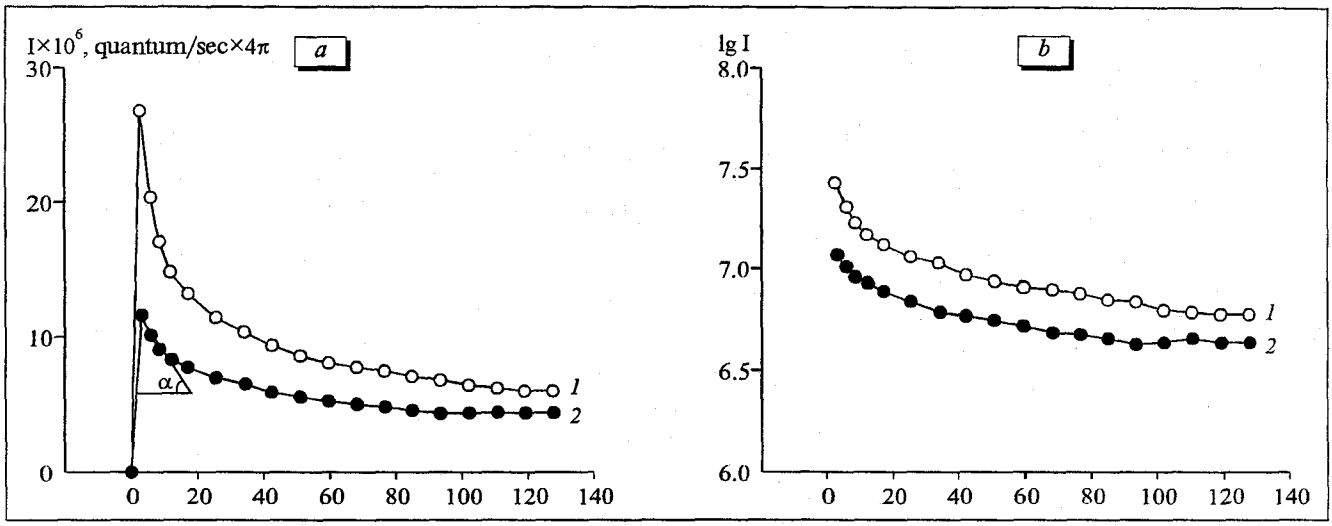


**Fig. 1.** Kinetics of serum chemiluminescence in the presence of hydrogen peroxide (*a*) and in semilogarithmic plots (*b*). *1*) Serum concentration of α-tocopherol is 0.75 mg/dl, 2) 1.31 mg/dl; *a*) ordinate: chemiluminescence intensity (I); abscissa: time after the addition of hydrogen peroxide, sec.

is followed by a decline and a plateau (Fig. 1, *a*). The kinetics is nearly linear in semilogarithmic plots, as shown in [1]. The curves in Fig. 1, *b* are characterized by linear dependence with the correlation coefficients (*r*) equal to 0.9209 and 0.9192. As the blood content of vitamin E increased, all CL parameters decreased (Table 1). Bearing in mind that CL is proportional to the concentration of peroxide lipid radicals ($ROO^{\cdot}$) [1,9], which reflects the intensity of lipid FRO, and that vitamin E is the major fat-soluble serum antioxidant [11], it can be suggested that vitamin E modifies the CL parameters. Antioxidant activity of similar concentrations of vitamin E was demonstrated *in vitro* by other researchers [2].

It should be noted that hydroquinone, a synthetic AO, also reduces FRO of serum lipids. At a concentration of $4\times10^{-5}$ mol/liter hydroquinone lowered tgα from 2.90±0.46 to 0.99±0.06 ($p\leq0.01$) and $I_{max}$ from $(1.17\pm0.06)*10^7$ to $(0.99\pm0.03)\times10^7$ quantum/sec×4π ($p\leq0.05$).

**TABLE 1.** Correlation between Serum Chemiluminescence and Serum Content of Vitamin E (Coefficients of Equation $y=(A\pm a)-(B\pm b)\times$[vitamin E] and Correlation Coefficients)

| Serum concentration of vitamin E, mg/dl | tgα | $I_{max}\times10^{-7}$, quantum/sec×4π | $T\times10^{-5}$, quantum/sec²×4π | $S\times10^{-9}$, quantum/4π |
|---|---|---|---|---|
| 0.75 | 8.93 | 2.68 | 14.74 | 1.93 |
| 0.89 | 7.19 | 2.36 | 9.69 | 1.79 |
| 1.01 | 6.35 | 1.89 | 5.01 | 1.69 |
| 1.03 | 5.49 | 1.95 | 4.67 | 1.69 |
| 1.11 | 4.72 | 1.85 | 4.00 | 1.70 |
| 1.11 | 4.67 | 1.87 | 4.79 | 1.92 |
| 1.16 | 5.08 | 2.13 | 8.46 | 1.58 |
| 1.22 | 4.89 | 1.69 | 5.14 | 1.67 |
| 1.24 | 4.62 | 1.34 | 2.44 | 1.32 |
| 1.29 | 3.93 | 1.26 | 3.66 | 1.09 |
| 1.31 | 3.84 | 1.16 | 2.06 | 1.36 |
| 1.39 | 2.78 | 1.42 | 2.01 | 1.45 |
| *A* | $1.49\times10^6$ | $4.35\times10^7$ | $2.49\times10^6$ | $2.77\times10^9$ |
| *a* | $1.80\times10^5$ | $8.06\times10^6$ | $8.44\times10^5$ | $7.10\times10^8$ |
| *B* | $8.60\times10^5$ | $2.27\times10^7$ | $1.72\times10^6$ | $1.04\times10^9$ |
| *b* | $1.59\times10^5$ | $7.08\times10^6$ | $7.41\times10^5$ | $6.23\times10^8$ |
| *r* | 0.9671 | 0.9139 | 0.8530 | 0.7594 |

We have established a linear relationship between CL parameters and serum content of vitamin E which is described by the following equation $y=(A\pm a)+(B\pm b)\times$[vitamin E] and calculated the confidence intervals ($a$ and $b$) for $A$ and $B$ coefficients at a 95% significance level. The equations characterize the kinetic parameters of CL as a function of the vitamin E concentration in serum. Using these equations, we have calculated the CL parameters for the normal vitamin E content (1.04±0.06 mg/dl [6]): $tg\alpha=6.30\pm0.21$ and $I_{max}=(2.08\pm0.10)\times10^7$ quantum/sec$\times4\pi$; $T=(7.70\pm1.03)\times10^5$ quantum/sec$^2\times4\pi$, $S=(1.73\pm0.09)\times10^9$ quantum/$4\pi$.

The correlation coefficients are summarized in Table 1. It should be noted that they are rather high for $tg\alpha$ and $I_{max}$. The effect of tocopherol on CL kinetics was not observed in experiments employing another system of lipid FRO induction [4]. However, these researchers have mentioned that the effect of α-tocopherol can be observed in other systems of lipid FRO induction, since this compound has both antioxidant and antiradical activities [2]. This is consistent with our results obtained in the study of lipid FRO in peptic and duodenal ulcera and accompanying diseases [6]. In the present study we investigated FRO of lipids using four CL parameters and observed stronger correlation that in a previous study [6].

Thus, we have demonstrated for the first time a strong correlation between kinetic parameters of CL and serum content of vitamin E in healthy subjects. These results indicate that our model system is sensitive to serum content of vitamin E and adequately characterizes free-radical oxidation of lipids. Recently, the incidence of diseases associated with lipid FRO has increased, and there are no correct clinical methods for the control of lipid FRO. Since the determination of blood vitamin E content is a laborious procedure requiring considerable blood volumes, the CL method can be employed in clinical practice for predicting the vitamin E content in blood serum.

## REFERENCES


1. V. A. Baraboi, V. E. Orel, and I. M. Karnaukh, *Peroxidation and Radiation* [in Russian], Kiev (1991).
2. E. B. Burlakova and N. G. Khrapova, *Usp. Khimii*, **54**, No. 9, 1540-1558 (1985).
3. Yu. A. Gubskii, A. I. Kuz'menko, and T. G. Voloshenyuk, *Ukr. Biokhim. Zh.*, **65**, 83-88 (1993).
4. E. S. Dremina, V. S. Sharov, and Yu. A. Vladimirov, *Biofizika*, **38**, No. 6, 1047-1052 (1993).
5. A. V. Kozlov, A. N. Osipov, and Yu. A. Vladimirov, *Ibid.*, **35**, No. 2, 347-349 (1990).
6. M. Yu. Kolomoets, I. V. Kuz'menko, L. A. Chernukhina, *et al.*, *Ukr. Biokhim. Zh.*, **64**, No. 2, 79-84 (1992).
7. L. G. Korkina, T. B. Suslova, S. I. Nikolova, *et al.*, *Gig. Truda*, No. 12, 49-50 (1984).
8. V. I. Kuznetsov and O. B. Stanishevskaya, *Lab. Delo*, No. 2, 54-56 (1991).
9. V. Ya. Slyapintokh, O. N. Karpukhin, L. M. Postnikov, *et al.*, *The Chemiluminescence Methods in the Investigation of Slow Chemical Processes* [in Russian], Moscow (1966).
10. J. G. Biem, L. Teets, B. Belavary, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **117**, No. 1, 131-133 (1964).
11. L. W. Burton, A. Joyce, and K. U. Jngold, *Lancet*, No. 8293, 327 (1982).
12. E. A. Dratz, C. C. Farnsworth, E. C. Loew, *et al.*, *Ann. Acad. Sci.*, **570**, 46-60 (1989).
13. H. Wiseman, *Biochem. Pharmacol.*, **47**, No. 3, 493-498 (1994).