MEASUREMENT OF CHEMILUMINESCENCE OF BLOOD SERUM COMPONENTS IN THE PRESENCE OF Fe^{++} IONS

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Measurement of chemiluminescence (CL) of blood serum in the presence of bivalent iron (Fe^{++}) is a method of assessing the state of lipid peroxidation (LPO) in the blood [2]. At the present time the attention of investigators has been drawn to the study of the state of LPO in the components of blood serum and, in particular, in atherogenic lipoproteins — the low-density and very low-density lipoproteins (LDL and VLDL respectively), due to their definite role in the development of atherosclerosis [3, 7, 9].

In order to study LPO in blood serum components the writers developed a method of recording CL of the total LDL + VLDL fraction in the presence of Fe^{++} and the quenching effect of the supernatant left after sedimentation of this lipoprotein fraction by the action of heparin and $CaCl_2$.

EXPERIMENTAL METHOD

Blood for testing was taken from 14 intact male chinchilla rabbits weighing 3.6 ± 0.5 kg. Blood serum was obtained by centrifugation for 15 min at 2400g.

Serum lipoproteins were isolated by precipitation in the presence of heparin and $CaCl_2$ [4]. To 2 ml of 15 mM $CaCl_2$ solution 0.2 ml of blood serum and 0.04 ml of a 1% solution of crystalline heparin were added. The tube was carefully shaken. The resulting suspension formed after incubation for 5 min at room temperature was centrifuged for 5 min at 2000g. The supernatant was removed with a pipet. The residue consisted of the combined LDL + VLDL fraction, including apoprotein-B [3], and this combined fraction was accordingly described as apo-B-containing lipoproteins (apo-BL). The residue was treated with 1 ml of 0.85% NaCl solution and shaken until a homogeneous, slightly opalescent suspension of apo-BL had formed. If it was necessary to study large quantities of apo-BL, the quantities of all ingredients were proportionally increased.

The apo-BL suspension was transferred to the cuvette of a chemiluminescence apparatus and 8 ml phosphate buffer (20 mM KH_2PO_4, 10 mM KCl, pH 7.4) was added. To initiate CL, 1 ml of 25 mM $FeSO_4 \cdot 7H_2O$ solution was added. A flash of CL was observed (Fig. 1), in which 5 principal parameters could be distinguished [1]: h) the height of the rapid flash (in relative units), depending on the content of lipid hydroperoxides in the test system; H) the height of the slow flash (in relative units), reflecting the ability of the lipids to undergo peroxidation, i.e., the highest possible intensity of LPO after addition of Fe^{++}; S) the light sum of the slow flash (in relative units), determined as the area below the curve from the beginning of development of the slow flash until it reached its maximal value, giving an estimate of the number of branching side chains, i.e., the number of peroxide radicals (ROO°) formed per Fe^{++} ion; tan α) the tangent of the angle of slope of the slow flash, determining the rate of lipid oxidation; τ) the latent period (in sec) from the time of addition of Fe^{++} to the beginning of development of the slow flash, indicating the rate of oxidation of Fe^{++} until the critical Fe^{++} concentration is reached; the latent period depends on the ratio

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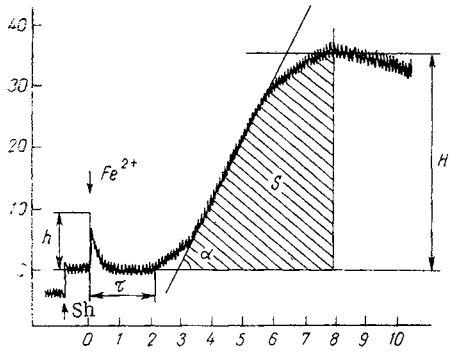

Fig. 1. Kinetics of CL flash of apo-BL after addition of Fe^{++}. Sh) Time of opening shutter separating end of FÉU-127 photomultiplier from cuvette. Abscissa, time (in min); ordinate, intensity of CL (in relative units).

between pro- and antioxidants in the system. To rule out any concentration effects, values of CL of apo-BL determined were normalized for apo-BL.

The chemiluminescent properties of the residual supernatant, consisting of high-density lipoproteins [4] and blood serum proteins, were studied as follows. Into the cuvette of the chemiluminescence apparatus were poured 6.5 ml of phosphate buffer and 0.5 ml of lipoprotein suspension from egg yolk, containing 5 mg phospholipids in chloroform-methanol extract [6]. The dilution of the egg yolk lipoprotein in distilled water was about 1 : 100. The suspension was prepared immediately before the experiment. Since, on addition of the phosphate buffer to the suspension of yolk lipoprotein a flash of CL appeared, this system was called a standard chemiluminescence system (SCS). To study the properties of the supernatant, it was added in a volume of 2 ml to the SCS. To initiate LPO, 1 ml of a 25 mM iron solution was added. In order to add Fe^{++} to the SCS, and after CL of the SCS had reached its maximal value (after about 15 min), the concentration of malonic dialdehyde (MDA) was determined [8]. The quenching effect was determined as the decrease, in percent, of height of the slow flash (or of any other parameter of CL) relative to the control SCS not containing supernatant. The antioxidant effect of the supernatant was determined as the decrease, in percent, in the rise in MDA concentration in the SCS with supernatant relative to the increase in MDA in the control SCS.

The apo-BL concentration in the blood serum [5] and the MDA concentration [8] in the apo-BL suspension in the cuvette of the apparatus before addition of Fe^{++} and after the slow flash of CL had reached its maximal value were determined.

EXPERIMENTAL RESULTS

The method of measurement of CL occupies a special place among existing methods of determining LPO activity or antioxidant activity of a system. Whereas methods of determining primary, intermediate, and secondary LPO products record the existing level of lipid oxidation, CL in the presence of Fe^{++} provides evidence of the capacity of lipids for peroxidation, i.e., the rate at which LPO would take place in the body if it were initiated.

With an increase in apo-BL concentration in the cuvette of the apparatus the intensity of CL increased (Fig. 2). To increase the sensitivity of the method, all that was necessary was to increase the concentration of apo-BL by increasing the volume of blood serum treated. There was a parallel increase in MDA accumulation.

The study of correlations showed that a direct correlation exists between normalized parameters of CL of apo-BL — H, S, and tan α — and the increase in MDA ($r = +0.63$, $r = +0.55$, and $r = +0.57$ respectively). However, correlation between the increase in MDA and the non-normalized parameters H, S, and tan α was higher still ($r = +0.76$, $r = +0.74$, and $r = +0.63$ respectively). This means that the ability of lipids to undergo peroxidation can be estimated in the presence of Fe^{++} without the need for recalculation for the apo-BL con-

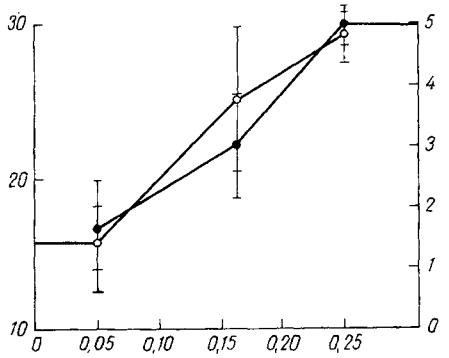

Fig. 2. Effect of apo-BL concentration on slow H flash of Cl of apo-BL and on increase in MDA concentration. Abscissa, apo-BL concentration (in mg/ml); ordinate: on left — H (in relative units); on right — increase in MDA concentration (in $M \times 10^8$).

centration, i.e., with the use of non-normalized parameters. Allowing for the highest value of the coefficient of correlation, it is preferable to use the parameter H. The normalized parameter h is independent of MDA accumulation during LPO ($r = -0.14$). Negative correlation exists between the latent period and MDA accumulation ($r = -0.37$ for non-normalized and $r = -0.39$ for normalized CL).

On the addition of the supernatant to the SCS the intensity of CL fell in the presence of Fe^{++}. There was a parallel decrease in MDA accumulation. This suggests that the quenching and antioxidant action of the supernatant was connected with the presence of the same substance or class of substances in it. This suggestion was confirmed by the statistically significant ($P < 0.05$) positive coefficient of correlation between the ratio between CL of the SCS and the ratio between the increases in MDA ($r = + 0.82$, $n = 14$). Moreover, this provides a basis, when determining antioxidant activity of blood serum, for not measuring MDA, but estimating antioxidant activity purely on the basis of a change in CL of SCS. A separate study of antioxidant activity in the supernatant enables any effects of co-oxidation of apo-BL to be ruled out if the antioxidant activity of whole serum were tested.

By recording CL of apo-BL in blood serum it is thus possible to estimate the ability of the lipoproteins to undergo peroxidation under the influence of Fe^{++}. Measuring changes in CL of SCS in the presence of Fe^{++}, with the solution of the supernatant left after precipitation of apo-BL, can be used as a simple test for estimating the antioxidant activity of blood serum.

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