CHEMILUMINESCENCE OF AN H_2O_2 CONTAINING SYSTEM IN THE PRESENCE OF IRON COMPOUNDS

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One of the mechanisms initiating injury during hyperoxia is activation of radical formation [6, 8-10]. An important step in the triggering of free-radical reactions is the formation of "active" forms of oxygen: $HO_2^{\cdot}$, $H_2O_2^{\cdot}$, $OH^{\cdot}$, and $^1O_2$. The most effective catalysts for their generation are compounds of metals of variable valency and, in particular, Fe^{++} [4, 5, 7, 10]. Compounds of hemin and nonhemin iron are widely distributed in the body and are evidently potential centers of radical formation. Maximal iron concentrations are to be found in the hemoglobin of the blood which, during hemolysis of erythrocytes in the plasma, may undergo partial destruction with the formation of hemin derivatives.

The H_2O_2—iron compounds system is a model of Fenton's reagent and its ability to produce radical forms of oxygen can be estimated on the basis of intensity of chemiluminescence of the reaction between peroxide and a metallic compound.

The aim of this investigation was to study the relative effectiveness of radical formation from H_2O_2 by various concentrations of hemoglobin, hemin, and Fe^{++} ions, based on measurement of the intensity of chemiluminescence.

EXPERIMENTAL METHOD

In a constant-temperature (37°C) cuvette, stirred with a teflon pestle, 8.9 ml of a solution of 20 mM KH_2PO_4 in 100 mM KCl, pH 7.4, and 1 ml of 3% H_2O_2, 0.1 ml of bovine hemoglobin solution (from Koch-Light, England), hemin chloride (from Calbiochem, USA), or $FeSO_4$ was added to the cuvette to produce a final concentration of between 10^{-3} and 10^{-8} M.

The intensity of chemiluminescence was recorded on an apparatus [1] in which a photoelectronic multiplier (FEU-39A) served as detector. The intensity of the quick flash was determined in relative units. The light sum of the flash in 5 min was measured by gravimetric analysis of the graph. Measurement of each concentration was repeated from 5 to 8 times. The reproducibility of the results was within limits of 20%.

EXPERIMENTAL RESULTS

The most effective of the iron-containing components tested for generation of luminescence with H_2O_2 was blood hemoglobin. In a concentration of 10 μM it was 5 times, and in a concentration of 1 μM 7 times more effective for generation of the fast flash than free hemin (Table 1). The maximum of effectiveness according to the light sum occurred with a concentration of 10 μM. With a decrease in hemoglobin concentration to 1 μM the light sum of the flash was reduced by about 60 times.

During interaction of organic iron derivatives with hydrogen peroxide luminescence is evidently generated as a result of several processes: the formation of an activated complex of the iron compound with H_2O_2, its decomposition and production of radicals, primarily $OH^{\cdot}$ radicals, recombination of radicals and their interaction with the organic part of the compound, in the case of protein — oxidative destruction of tryptophan residues with the formation of

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TABLE 1. Dependence of Intensity of Fast Flash (I_{max}) and Light Sum (S) of Chemiluminescence in System of H_2O_2—Compounds of Nonhemin and Hemin Iron on Concentration

Hemoglobin			Hemin chloride			$FeSO_4$		
C, M	I_{max}	S	C, M	I_{max}	S	C, M	I_{max}	S
$4 \cdot 10^{-5}$	1550±98,7	720	10^{-4}	65,0±12,3	42	10^{-3}	230,0±21,1	33
$2 \cdot 10^{-5}$	1263±101,5	1020	10^{-5}	205,3±34,8	51	$8 \cdot 10^{-4}$	86,2±10,1	12
10^{-5}	1038±75,3	1480	10^{-6}	180,7±26,1	49	$6 \cdot 10^{-4}$	65,0±9,8	10
10^{-6}	165±17,4	25	10^{-7}	50,3±7,1	27	$4 \cdot 10^{-4}$	41,3±9,3	7
10^{-7}	23,8±3,8	10	10^{-8}	5,5±1,2	2	$2 \cdot 10^{-4}$	13,8±3,1	9
10^{-8}	5,5±41	2	—	—	—	10^{-4}	7,2±1,2	—

Legend. C) Concentration.

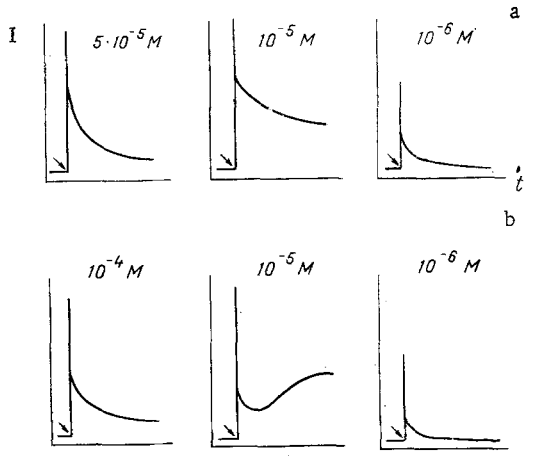

Fig. 1. Chemiluminescence reactions induced by different concentrations of hemoglobin (a) and hemin (b). I) Intensity of chemiluminescence, t) 5-min time interval. Arrow indicates time of addition of compound to H_2O_2.

formylkynurenin derivatives kynurenic, xanthurenic, and anthranylic acids. The trigger is evidently the stage of radical formation, and hemoglobin is the most effective radical former with H_2O_2 of the compounds tested.

Hemoglobin concentrations encountered normally in blood plasma, produced as a result of intravascular hemolysis, amount incidentally to between 1 and 5 μM approximately, and in extreme situations they may increase to 8-10 μM [3]. It will be clear from Table 1 that it was with an increase in hemoglobin concentration from 1 to 10 μM that the greatest increase in the light sum and intensity of the fast flash of chemiluminescence took place during interaction with H_2O_2. Hemoglobin-induced radical formation, especially under conditions facilitating the formation of peroxides in the plasma, is evidently one mechanism of intensification of the harmful action of extremal factors on the body. During exposure to extremal factors, activity of lysosomal peptide-hydrolases in the blood plasma increases, and this creates the conditions for partial destruction of hemoglobin [2]. Accordingly the chemiluminescence of H_2O_2 was tested in the presence of hemin and bivalent iron ions, as possible breakdown products of hemoglobin.

The maximum of luminescence for hemin is found with a concentration of 10 μM (Table 1). In the presence of higher concentrations, inhibition of the flash was observed, probably as a result of inactivation of $OH^{\cdot}$ radicals during interaction with the porphyrin structure of hemin and an increase in color quenching. With hemin concentrations of over 10 μM a slow flash of chemiluminescence was observed, evidently due to Haber—Weiss reactions (Fig. 1).

When a solution of ionic iron was injected into the cuvette only a rapidly decaying flash was observed, evidently due to decomposition of hydrogen peroxide with the formation and subsequent recombination of hydroxyl radicals. A decrease in the $FeSO_4$ concentration to 10 μM was accompanied by a marked decrease in the intensity of chemiluminescence of the fast flash. Comparison of the relative effectiveness of iron ions and hemin shows that the hemin concen-

tration necessary for radical formation with generation of luminescence is two orders of magnitude less than the required concentration of ionic iron (Table 1).

Extraerythrocytic hemoglobin and its destruction products as far as hemin are thus evidently among the most effective pro-oxidants and are potential centers for radical formation in the blood.

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CHROMATO-MASS-SPECTRAL INVESTIGATION OF SECRETION OF THE RABBIT SMALL INTESTINE INDUCED BY CHOLERA AND SALMONELLA TOXINS

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The study of the dynamics of the diarrhea syndrome by gas–liquid chromatography [1] showed that development of the secretory process under the influence of bacterial toxins (cholera enterotoxin and the salmonella lipopolysaccharide complex) in experimental animals is accompanied by marked changes in the time course of entry of low-molecular-weight organic compounds into the intestinal lumen. However, the results were made difficult to interpret because of the absence of data on chemical structure of the metabolites. To solve this problem, a special chromato-mass-spectral study was required, and its results were described below.

EXPERIMENTAL METHOD

Silylated samples of secretion, treated as described in [1], were analyzed on the Finnigan-4021 electron impact mass-spectrometer, connected to a Nara-3 computer. The 6 × 2 mm glass column was packed with 3% methylsilicone OV-1 on Chromosorb-HP (80–100 mesh). The carrier gas was helium (20 ml/min). The temperature of the injector, separator, and source of ions was 285°C. The conditions for programming temperature of the thermostat were: initial 100°C (6 min), final 280°C (15 min), velocity 5°C/min. The spectrum recording time was 2 sec. The ionizing voltage was –70 eV. The reacting gases were isobutane and ammonia (Finnigan).

EXPERIMENTAL RESULTS

Preliminary investigation of the composition of the diarrhea fluid by electron-impact impact chromato-mass spectrometry showed that metabolites secreted into the intestinal lumen belonged to different classes of chemical substances, and in some cases one chromatographic

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