PEROXIDASE ACTIVITY OF INFLUENZA VIRUS

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It was shown previously [3] that the initial stages of interaction between influenza virus and the cell are accompanied by oxidation-reduction reactions catalyzed by cellular and viral enzymes. One results of this may be the intensification of peroxidation of cell membrane lipids, leading to accumulation of hydroperoxides. Inhibition of several oxidoreductases by hydroperoxides [1], and also a decrease in the reproduction of influenza virus as a result of its exposure to unsaturated fatty acids [10], which evidently contain hydroperoxides, suggests that influenza virus possesses a mechanism of defense against lipid peroxidation (LPO) products. Only if this is so can the initial stages of interaction between influenza virus and the cell take place effectively. One type of such defense could be destruction of LPO products in peroxidase-like reactions.

In the investigation described below a chemiluminescence method was used to study the peroxidase activity of influenza virus.

EXPERIMENTAL METHOD

Superoxide desmutase (SOD), antimycin A, and mannitol were obtained from Sigma (USA), horse spleen ferritin and sodium azide from Serva (West Germany), and horseradish peroxidase from Reanal (Hungary). All other reagents were of Soviet manufacture.

Influenza virus A (FPV, Rostock strain), propagated in the allantois of 10-11-day chick embryos, was purified by the method in [9]. The virus was suspended in Dulbecco's buffer, not containing Ca^{++} or Mg^{++}.

Chemiluminescence was measured on the apparatus described previously [2], programmed for single-electron photon counting at 20°C. The composition of the reaction mixture (total volume 2 ml) was: H_2O_2 0.25 mM, luminol 10 μM, influenza virus $\approx$7 log PFU/ml. In the experiments to study peroxidase activity of iron-sulfer-containing proteins (Fe-S-proteins), ferritin (10 μg) or peroxidase (10 μg) was added instead of influenza virus.

To study the action of interceptors of oxygen radicals and complexones on the intensity of chemiluminsecence, SOD (25 μg), mannitol (0.33 mM), ethanol ($2.5\ 10^{-1}$M), sodium azide (0.5 mM), o-phenanthroline (0.2 mM), and antimycin A (0.2 μM) were added to the incubation mixture.

EXPERIMENTAL RESULTS

Luminescence appeared immediately after the addition of H_2O_2 to the virus—luminol system and reached maximal intensity after 1-2 min, after which it decreased for 10-15 min (Fig. 1). Luminescence of similar character was described in [6, 7], in which it was shown that oxidation of luminol by hydrogen peroxide take place if peroxidase is present in the system. Denaturation of the influenza virus by heating in a boiling water bath for 30 min led to a decrease in the intensity of chemiluminescence by an order of magnitude.

These results suggest that influenza virus possesses peroxidase activity.

Luminescence of luminol is known [4, 7] to be initated by a number of excited forms of oxygen, formed in the course of the peroxidase reaction. To discover the molecular nature of the intermediate reaction products fromed in the virus—luminol—H_2O_2 system, the effect of interceptors of excited forms of oxygen on the intensity of chemiluminescence was studied.

Addition of SOD before H_2O_2 to the reaction mixture led to a fall in the intensity of luminscence by 65%. Mannitol and ethanol, interceptors of the hydroxyl radical, reduced the

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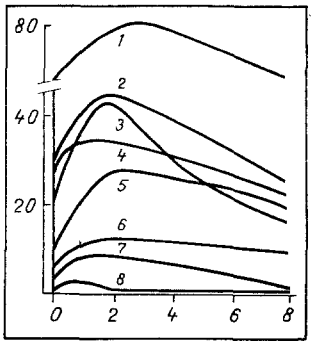

Fig. 1. Kinetics of chemiluminescence in H_2O_2—luminol—influenza virus (1, 2, 4-7 and H_2O_2—luminol—ferritin (3, 8) systems. Abscissa, time after addition of H_2O_2 to system (in min)) ordinate, intensity of luminescence in pulses/sec x (10^{-3}). 1, 3) control; 2) mannitol; 4) ethanol; 5) SOD; 6) antimycin A; 7) sodium azide; 8) o-phenanthroline.

intensity of luminescence by 45 and 55% respectively. Addition of sodium azide, an interceptor of singlet oxygen, to the system reduced the intensity of chemiluminescence by 90%.

These results show that influenza virus, in the presence of hydrogen peroxide and luminol, can generate excited forms of oxygen such as the superoxide anion-radical, singlet oxygen, and the hydroxyl radical.

In the course of the investigation the question of the type of peroxidase contained in influenza virus frequently cropped up. Since peroxidase activity could be due to the presence of Fe ions or Fe-S-proteins, it was necessary to test this hypothesis experimentally by means of inhibitor analysis.

Addition of o-phenanthroline, a specific reagent for bivalent iron, to the reaction mixture was found not to reduce, but to increase chemiluminescence by 60%. It was also found that o-phenanthroline effectively inhibits chemilumescence in H_2O_2—Luminol—ferritin and H_2O_2—Luminol—peroxidase systems. After analysis of the data it was possible to exclude from the number of possible components of influenza virus both Fe ions and proteins containing non hemin and hemin iron.

Besides Fe ions and Fe-S-proteins, we know that decomposition of hydroperoxides, accompanied by generation of active forms of oxygen, can also be initiated by Fe-S-proteins. Destabilization of their structural state by iron chelating agents, such as o-phenanthroline, leads to intensification of LPO, which is inhibited by interceptors of active forms of oxygen [8]. At the same time, it has been shown [5] that antimycin A can bind with Fe-S-proteins and thereby inhibit their oxidation-reduction functions.

The present experiments showed that the yield of chemiluminescence in the influenza virus—luminol—H_2O_2 system was reduced by 80-90% in the presence of antimycin A.

From all the data thus obtained it can thus be concluded that influenza virus possesses peroxidase-like activity, for which Fe-S-proteins are most probably responsible.

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