EFFECT OF MEMBRANE POTENTIAL ON FUSION OF SENDAI VIRUS ENVELOPE WITH CHICK ERYTHROCYTE MEMBRANE AND ON VIRUS-INDUCED HEMOLYSIS*

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KEY WORDS: Sendai virus; electron paramagnetic resonance; virus-induced membrane fusion; virus-induced hemolysis; membrane potential.

In recent years considerable interest during investigation of the molecular mechanisms of the cytopathogenic action of viruses on target cells has been concentrated, not on elucidation of disturbances of protein and nucleic acid synthesis, but on the study of processes taking place in membranes of infected cells. The study of structural changes in the lipoprotein envelopes of viruses and the plasma membranes of cells can be attributed both to their importance in the development of the initial stages of interaction between virus and cell and to their role in the pathogenesis of virus infections. The use of methods of electron-paramagnetic resonance (EPR) and of spin labeling, by means of which objective information can be obtained about changes in the structure of lipoprotein membranes under various conditions and under the influence of various factors can be obtained and the dynamics of fusion of biomembranes, of varied origin, can also be directly observed [5-7].

Until recently the effect of membrane potential on effectiveness of virus-induced membrane fusion has not been studied. This aspect of interaction between virus and cell, in our view, is extremely important because of the increased conductance of the plasma membrane of cells discovered previously during their interaction with Sendrai virus, and its dependence on membrane potential [2].

EXPERIMENTAL METHOD

Sendai virus was grown in the allantoic sac of 10-day-old chick embryos for 72 h. It was concentrated and purified by differential centrifugation [4]. A viral suspension with titer of 1:100,000 in the hemagglutination test and with a protein concentration of 5 mg/ml was used. For selective cleavage of F-protein the viral suspension was treated with trypsin [8]. Chick embryonic erythrocytes, washed twice with physiological saline, buffered with 0.01 M Tris-HCl, pH 7.4, were used. In some experiments a 2% suspension of erythrocytes was treated with valinomycin ($5 \cdot 10^{-6}$ M) in the presence of KCl in different concentrations for 30 min at 37°C. Next, to study virus-induced hemolysis, the erythrocytes were treated with the viral suspension (1:100 v/v) and incubated for 30 min at 37°C. The erythrocytes were then sedimented by low-speed centrifugation and the degree of hemolysis measured spectrophotometrically at a wavelength of 540 nm, and expressed as a percentage, taking total hemolysis of erythrocytes taking place after the addition of 0.045 M NH_4OH as 100% [9].

To study virus-induced membrane fusion by the EPR method, erythrocytes treated as specified above were sedimented and used in the form of a 50% suspension.

Spin-labeled derivatives of stearic acid (Syva, USA), in which the spin-label was located at the 16th carbon atom of the hydrocarbon chain, were used.

EPR spectra were recorded on a Varian E-4 spectrometer (USA), fitted with an attachment for constant-temperature incubation of the specimens, which can be programmed both to record

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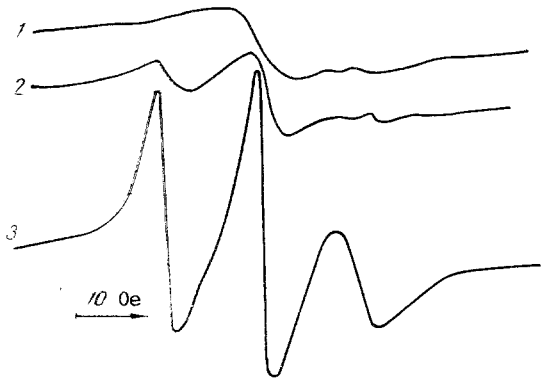

Fig. 1

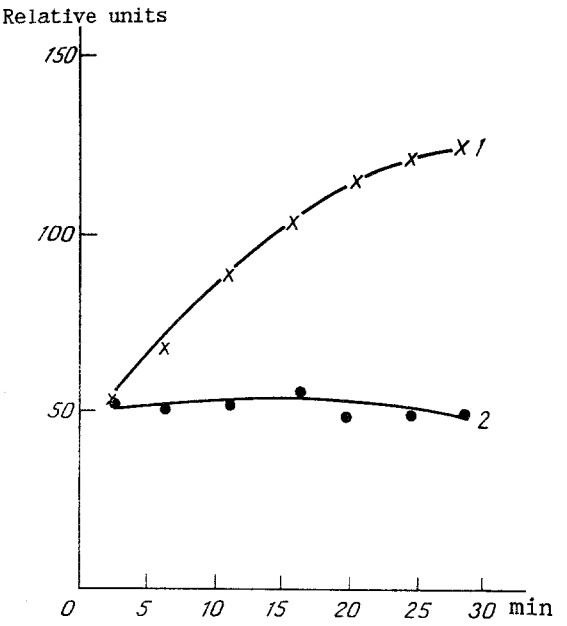

Fig. 2

Fig. 1. Changes in EPR spectra of spin-labeled Sendai virus particles during incubation with chick embryonic erythrocytes. 1) EPR spectrum of spin-labeled virions at 0°C; 2) EPR spectrum of virus particles after addition of chick erythrocytes at 0°C; 3) EPR spectrum 16 min after incubation of erythrocytes and virus at 37°C.

Fig. 2. Time course of increase in amplitude of EPR signal during interaction between control (1) and trypsin-treated (2) Sendai virus with chick erythrocytes.

complete spectra and also for recording "at a point," corresponding to the peak of the midline of the spectrum. This mode of recording allows the dynamics of the membrane fusion process to be compared directly under different experimental conditions.

The spin-labeled analog of stearic acid C_{16} was introduced into Sendai virus particles by the following method. The original solution of the spin label in ethanol was applied to the base and wall of a conical ampul 0.5-0.7 mm in diameter. After evaporation of the solvent, the suspension of Sendai virus was introduced into the ampul and shaken for 3 h at 37°C.

To study fusion of the Sendai virus envelope with the chick erythrocyte membrane, spin-labeled virions were mixed with a 50% suspension of erythrocytes, treated as mentioned above in the ratio of 1:10, and the EPR spectrum was recorded at 0°C. The temperature was then raised to 37°C and either changes in the spectrum were recorded every 5 min or the dynamics of the increase in amplitude of the signal was studied by the "point" mode of recording.

EXPERIMENTAL RESULTS

An important effect used in research by the spin-label method is spin-spin exchange or dipole-dipole interaction between spin-labels, the magnitude of which increases with an increase in concentration of the spin-labels. These exchange effects lead to an increase in width of all three lines in the EPR spectra of the spin-labels or even to their fusion into a single line. What is particularly important is that widening of the lines is accompanied by a simultaneous reduction of the amplitude of the corresponding spectral lines: A change in the width of the lines in this case leads to a decrease in its intensity in the square. Naturally with a decrease in concentration of the spin-labels the width of the lines will be reduced but the amplitude increased. The principles discovered for parameters of EPR spectra of spin-labels provide a basis for the application of this method to analyze the time course of fusion of biological membranes of different origins [1].

In the present investigation we used a similar approach to study the effect of factors altering membrane potential of erythrocytes on fusion of envelopes of Sendai virions with their plasma membranes.

The C_{16} spin label was introduced into the envelope of Sendai virus in a high concentration, so that the EPR spectrum was widened as a result of spin-spin interaction (Fig. 1). When the spin-labeled virions were adsorbed on the surface of the erythrocytes and incubated

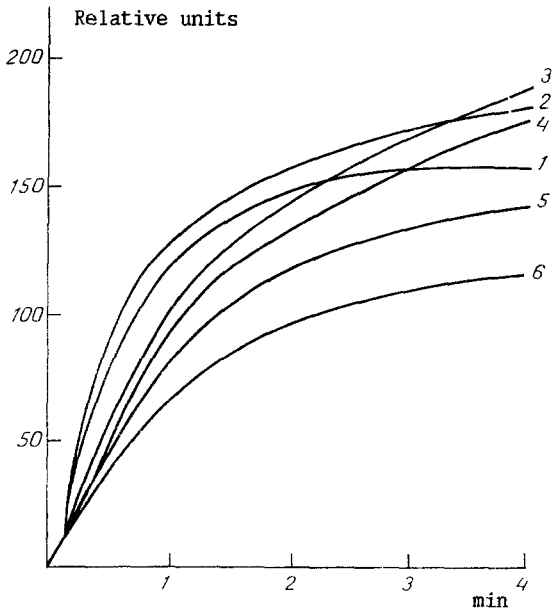

Fig. 3. Effect of changes in membrane potential on kinetics of increase in amplitude of EPR signal during interaction of spin-labeled Sendai virus with chick erythrocytes at 37°C. 1) Control experiment without valinomycin, KCl concentration 1 mM: 2-6) experiments in the presence of $5 \cdot 10^{-6}$ M valinomycin. KCl concentration: 2) 1 mM, 3) 5 mM, 4) 25 mM, 5) 125 mM, 6) 150 mM.

at 37°C, the amplitude of the signal at first was substantially increased as a result of diffusion of the spin label from the virus envelope into the erythrocyte membrane, leading to a marked decrease in its concentration. The rapid rise of amplitude of the signal led to its 4-6-fold increase after 16 min. Meanwhile, when the labeled virus particles were treated with trypsin for selective cleavage of the protein fusion factor [8], on interaction between them and the erythrocytes no increase was observed in amplitude of the EPR signal (Fig. 2). It was concluded from this fact that dilution of the spin label in these experiments took place mainly as a result of fusion of the virus envelope with the erythrocyte membrane and not due to exchange through the aqueous phase.

The next experiments were aimed at studying the effect of membrane potential on the time course of fusion of the spin-labeled virus particles with chick embryonic erythrocytes, and on their hemolysis.

In the presence of the antibiotic valinomycin, a specific carrier of K^+ ions through lipid membranes, the cell membrane potential depends on the concentration of these ions in the external medium [3]. If the KCl concentration is low the cells are hyperpolarized because of the outflow of K^+ from them, but if it is high they are depolarized. The effect of KCl in different concentrations on the time course of the increase in the EPR signal of spin-labeled Sendai virus in the cource of its interaction with chick erythrocytes in the presence of valinomycin at 37°C is shown in Fig. 3. It will be clear from Fig. 3 that the rate of rise and the final amplitude of the signal decreased with an increase in the K^+ concentration in the extracellular medium and, correspondingly, with reduction of the erythrocyte membrane potential.

The study of virus-induced hemolysis of erythrocytes showed it to be increased by 1.45 ± 0.21 times during an increase in membrane potential with the aid of valinomycin in the presence of 1 mM KCl. An increase in the K^+ ion concentration to 5 mM led to reduction of the degree of hemolysis to the level observed in the control experiment without valinomycin. A further increase in the KCl concentration to 125 mM caused no significant change in hemolytic activity of the virus, by contrast with the reduction of its fusion-promoting activity described above. These differences may perhaps be due to a difference in the concentration of erythrocytes and to a corresponding difference in the ratio between virus particles and cells in these experiments.

Dependence of virus-induced membrane fusion on membrane potential revealed by these experiments confirms the writers' previous hypothesis [2] that electrical breakdown of the cell membrane plays an important role in the initiation of membrane fusion.

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TRANSPOSON CONTENT OF NONCONJUGATIVE PLASMIDS OF *Escherichia coli*

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Cells of strains of *Escherichia coli* isolated from different natural sources may contain both conjugative and nonconjugative plasmids, and often in the form of complexes consisting of more than one plasmid [2]. Meanwhile, if several different plasmids are present in the same bacterial cell, the question of their transposon content arises, i.e., whether all plasmids in the complex contain the same transposon.

To answer this question we made a search for nonconjugative plasmids in antibiotic-resistant natural strains of *E. coli* and then studied the transposon content of these plasmids, contained in complexes.

EXPERIMENTAL METHOD

Strains C-6, C-8, C-19, and C-25 of *E. coli*, isolated from different natural sources and possessing resistance to different antibiotics and to trimethoprim, and also standard strains *E. coli* AP115 Lac$^-$, resistant to nalidixic acid, and *E. coli* C600 Lac$^-$, resistant to streptomycin and rifampicin, were used. The ability of nonconjugative plasmids to be mobilized for transfer to conjugative plasmids was studied in "three-parent" crosses [1]. The search for transposons in the plasmids was undertaken by a method in which bacterial cells containing one of the nonconjugative plasmids were treated with sodium dodecylsulfate in a concentration of 5% for 18 h, after which they were seeded on media with the addition of antibiotics, and clones characterized by monoresistance were then selected. Cells of these clones were used as intermediate recipients in the "three-parent" crosses, in which the donors were cells containing conjugative plasmid pAP42 or pAP43, and the final recipients were *E. coli* C600 Rif^r Lac$^-$ cells. In similar experiments cells with nonconjugative plasmids were introduced into "three-parent" crosses without preliminary treatment of the cultures with sodium dodecylsulfate. The sensitivity of the bacteria to phage MS2 was determined by the agar layers method and treatment with ethidium bromide was carried out by the usual method [3]. DNA was isolated from plasmids pAP42 and pAP42-1 by the method in [10] with some modifications. Plasmid DNAs were restricted by endonucleases Eco RI and Hind III. The restriction fragments were frac-

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