CELL SURFACE EFFECTS OF POKEWEED OBSERVED BY ELECTROPHORETIC LIGHT SCATTERING

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1. Introduction

Currently it is believed that the stimulation of lymphocytes by mitogens takes the following pathway [1,2]. Binding takes place between the mitogen and the appropriate receptor site on the cell membrane, occurring within seconds or minutes. Binding is followed by an alteration in the chain of metabolic events in the cell which eventually lead to mitosis. Mitosis is always preceded by agglutination. The metabolic changes occur within minutes or hours. The experiments which are described here investigate how the electrophoretic mobility, μ_E, of rat thymus lymphocytes is affected by pokeweed stimulation. Measurements of the electrophoretic drift velocity of the lymphocytes were made by an electrophoretic light scattering apparatus [3]. This new technique has the advantage of being an objective fully instrumental method which requires only a one minute experimental run time during which hundreds of cells are sampled. Furthermore, no concentration gradients are formed in the suspending medium which ensures that the ion atmosphere of the cells does not change during electrophoresis.

2. Materials and methods

The thymus lymphocytes studied were prepared by the following method. The thymus was surgically removed from male rats (25 days old). After making incisions in the thymus the lymphocytes were dispersed and washed three times in Hank's balanced salt solution. Finally the cells were suspended in a salt-sorbitol solution composed of one part 0.145 M. NaCl and nine parts 0.290 M. sorbitol. The concentration of the final suspension was 3×10^6 cells/ml. An aliquot of the lymphocyte suspension was exposed to pokeweed mitogen (50 μg/ml). The viability of the cells was determined to be better than 90% using trypan blue vital stain solution.

The electrophoretic drift velocity for lymphocytes was measured with and without pokeweed in the suspending medium. The light scattering apparatus which was used for these measurements is shown diagramatically in fig.1. Laser light from a krypton ion laser (Coherent Radiation Ltd.), operating at a wavelength of 6471 Å was incident on a lymphocyte suspension which had been placed in an electrophoretic light scattering chamber [4]. Scattered light from the cells and a local oscillator illuminated the surface of a photodetector, positioned at an angle θ with respect to the incident laser light. The photodetector in turn emitted a sequence of photoelectron pulses which were transmitted to a PDP-9 minicomputer for analysis.

3. Discussion and results

During electrophoresis the lymphocytes have a combination of Brownian motion and a constant drift which causes the scattered light to exhibit intensity fluctuations. These cause modulations in the number of photoelectron pulses which are emitted by the photodetector. Information concerning the motion of lymphocytes was obtained by performing an autocorrela-

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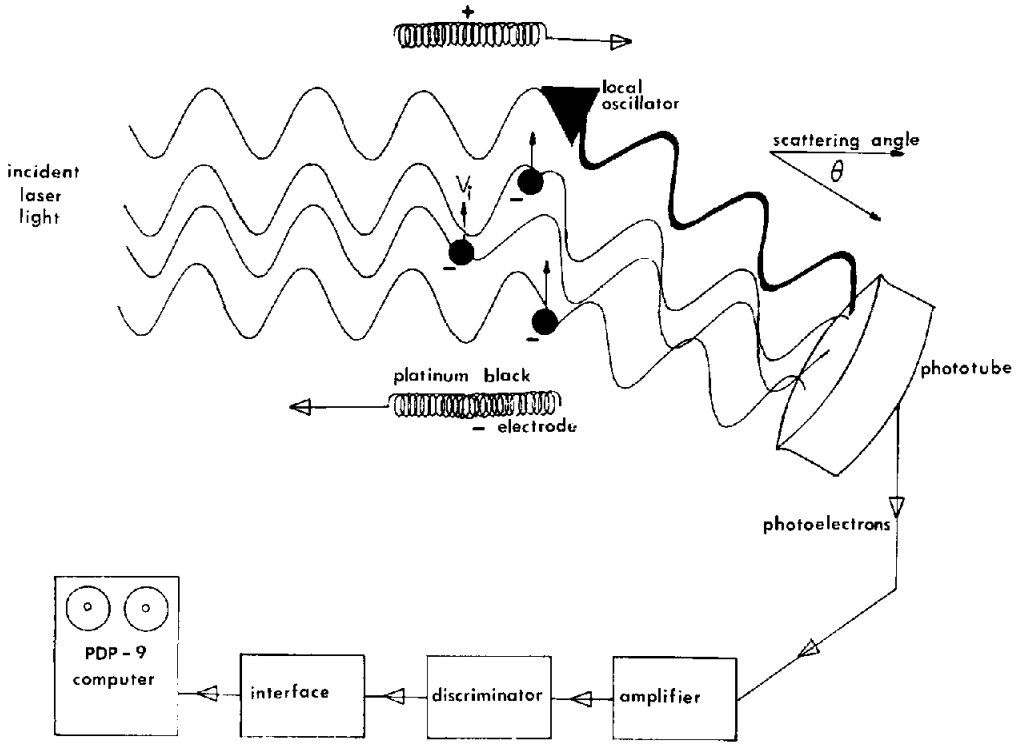

Fig.1. A diagramatic representation of the electrophoretic light scattering apparatus. The scattering angle θ was set a 10°. A RCA C31034 phototube with electromagnetic shielding was employed. An electric field, E, was applied perpendicular to the incident laser light by platinum black electrodes.

tion analysis of the photoelectron pulse train [5]. The normalized autocorrelation function for the case of electrophoresis may be written [3]

$$C_I(\tau) = \exp(-D\,|\,K\,|^2\tau) \sum_i A_i \cos(\bar{K}\cdot\bar{v}_i\tau) \qquad (1)$$

where τ is the time delay, $\bar{K}$ is the scattering wave vector and D is the diffusion constant of the scattering particles. A_i is a constant and $\bar{v}_i$ is the electrophoretic drift velocity for the i^{th} particle. For a population of light scattering particles with a uniform electrophoretic drift $|\bar{v}_i|$, the electrophoretic mobility may be calculated from the period $(2\pi/\bar{K}\cdot v_1)$ in the autocorrelation function. Thus,

$$\mu_E = \frac{v_1}{E} = \frac{2\pi}{E\,\Delta\,K\cos\frac{\theta}{2}} \qquad (2)$$

where E is the applied electric field, θ is the scattering angle and Δ is the oscillation period. Using μ_E, the zeta potential, ψ_z, and the corresponding surface charge density, σ_z, can be calculated employing the Henry equation and the Debey–Huckel theory, respectively [6,7].

The result of an electrophoretic light scattering experiment for lymphocytes suspended in the salt-sorbitol solution is given by the autocorrelation function shown in fig.2. Three equally spaced oscillations indicate that the distribution in μ_E for these cells must have been quite narrow with a mean value of 3.1×10^{-8} m^2/V-sec. Fifteen min after the addition of pokeweed (50 μg/ml) to the suspension of lymphocytes, a light scattering experiment resulted in the autocorrelation function shown in fig.3. Comparing this function with the one in fig.2, there are two features which can be attributed to the presence of pokeweed. First, the period of the oscillation has increased to approx. twice the value obtained for lymphocytes without pokeweed present. This corresponds to a decrease in the mean

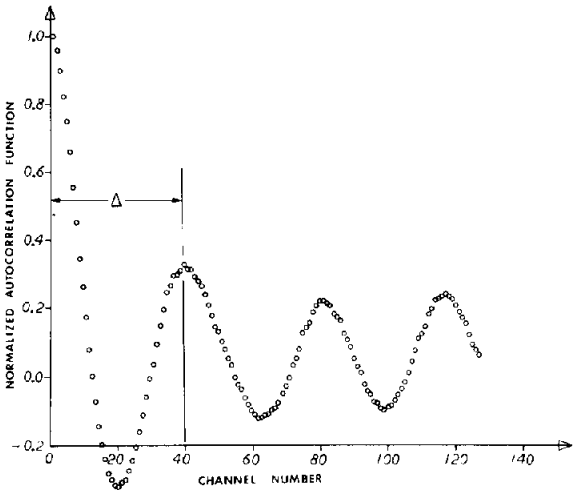

Fig.2. Normalized autocorrelation function for rat thymus lymphocytes in a salt-sorbitol solution as pH 7.4. The time per channel is 3000 μsec. The temperature of the solution was measured to be 21°C using a thermistor. An electric field of 7.7×10^2 V/m was applied to the solution.

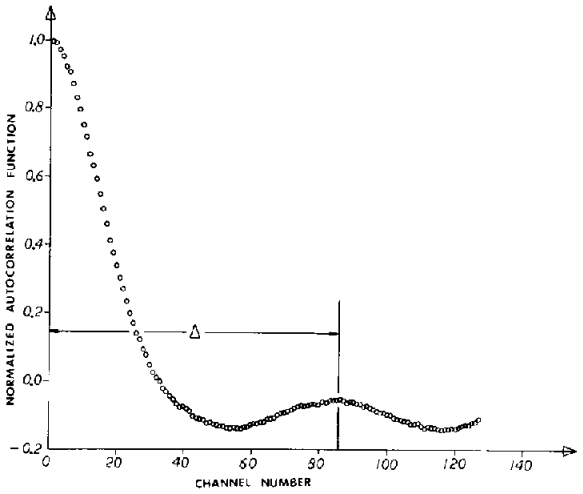

Fig.3. Normalized autocorrelation function for rat thymus lymphocytes in salt-sorbitol solution with a pokeweed concentration of 50 μg/ml and a pH of 7.4. All other parameters were the same as for the experiment described in fig.2.

value of μ_E to a value of 1.4×10^{-8} m^2/V-sec. The effect of binding between pokeweed and the cell membrane is to decrease the surface charge of the cell. The other significant feature of the autocorrelation function is that the amplitude of the oscillation has decreased for pokeweed stimulated lymphocytes. This can be attributed to a broadening in the distribution of μ_E which results in a corresponding broadening of the distribution for the electrophoretic velocity. In this case the autocorrelation function is described by a sum of cosine functions, each of which has a frequency associated with one value of v_i. It is the inter-

Table 1
Cell surface effects of pokeweed

Specimen[a]	Zeta potential ψ_z (mV)	Surface Charge density σ_z $\left[\frac{\text{coulombs}}{m^2}\right]$	Electrophoretic mobility μ_E $\left[\times 10^{-8} \frac{m^2}{\text{V-sec}}\right]$
Rat thymus Lymphocytes Unstimulated	39	2.3×10^{-2}	3.1
Rat thymus lymphocytes Pokeweed[b] stimulated	18	8.5×10^{-3}	1.4

[a] Lymphocytes were prepared in a salt-sorbitol solution at pH 7.4. The cell concentration was 3×10^6 cells/ml.

[b] The electrophoretic light scattering experiment was performed 15 min after the addition of 50 μg/ml pokeweed.

ference resulting from such a summation which could have contributed to the decreased amplitude of the oscillation. Such an interpretation suggests that the individual cells in the lymphocyte population bind a different number of pokeweed molecules to their surfaces.

A summary of the electrophoretic light scattering experiments for lymphocytes with and without pokeweed stimulation is given in table 1. The values of of μ_E, ψ_z and σ_z for cells exposed to pokeweed decreased to approx. half the values for cells without pokeweed. The light scattering experiments also suggest that the presence of pokeweed broadens the distribution of lymphocyte electrophoretic mobilities. This supports the notion that thymus lymphocytes are heterogeneous with respect to the number of pokeweed mitogen receptor sites on the plasma membrane. An extension of these findings will be to determine the shape of the distribution function for μ_E. This would provide information concerning the relative number of cells binding different quantities of pokeweed.

Acknowledgement

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