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Change in electrophoretic mobility of HL-60RG cells by apoptosis

K. Makino[1-3]), M. Ikekita[1,3]), T. Kondo[1-3]), S. Tanuma[1,3]), and H. Ohshima[1,2])

[1]) Faculty of Pharamaceutical Sciences, Science University of Tokyo, Shinjuku-ku, Tokyo 162, Japan
[2]) Institute of Colloid and Interface Science, Science University of Tokyo, Shinjuku-ku, Tokyo 162, Japan
[3]) Research Institute for Biosciences, Science University of Tokyo, Noda, Chiba 278, Japan

Abstract: We measured the electrophoretic mobilities of HL-60RG cells and their apoptotic cells triggered by Actinomycin D as a function of the ionic strength of the suspending medium at pH 7.4. Both types of cells showed negative mobilities. The apoptotic HL-60RG cells exhibited larger mobility values in magnitude than intact HL-60RG cells in the whole range of the electrolyte concentration measured. The obtained data were analyzed via a mobility expression for "soft particles", that is, colloidal particles with ion-penetrable surface layers. The observed mobility difference between the intact and apoptotic HL-60RG cells was found to be due mainly to the difference in friction exerted by the cell surface layers on the liquid flow around the cells between these two types of cells rather than the difference in charge density in their surface layers. A possible explanation for this mobility change by apoptosis is given.

Key words: Electrophoretic mobility – apoptosis – HL-60RG cells

Introduction

It is known that apoptosis is a form of cell death characterized morphologically by, for example, condensation of nuclear chromatin, compaction of cytoplasmic organelles, loss of surface microvilli and blebbing of the plasma membrane [1]. Apoptosis is observed in normal physiological processes, i.e., immunologically mediated negative selection of autoreactive T-cell clones [2] and the unwanted cell elimination from tissues during embryogenesis [3]. It is also induced by exposure of cells to some toxins [4]. Biochemically, it is associated with the fragmentation of DNA into internucleosomal fragments. Apoptosis, which can be selectively triggered by cells in response to some stimuli, ultimately results in the degradation of the nuclear DNA into oligonucleosome chains and the fragmentation of the cell into neat "bite-size" pieces for efficient disposal by neighboring cells or marauding macrophages [5]. Despite these histological observations, the mechanisms leading to the recognition and uptake of neutrophils still remain obscure. Since apoptotic cells are considered to interact with phagocytotic cells specifically, the surface of apoptotic cells should be different from that of normal cells. To study the details of the change in physicochemical properties of the cell surface caused by apoptosis, we measured the electrophoretic mobilities of intact and apoptotic human promyelocytic leukemia cell lines (HL-60RG cells), which are expected to reflect certain properties of the surfaces of these cells and their changes due to apoptosis.

The cell surface is normally covered with a layer of polyelectrolytes. Ohshima and Kondo developed a theory of electrophoresis of colloidal particles covered with polyelectrolytes, which we call particles with "soft surfaces" or "soft particles" and demonstrated that soft particles show quite a different electrophoretic behavior from that of usual rigid colloidal particles with no structural surfaces [6, 7]. This theory has been applied to various "soft particles," such as latex particles covered with poly(N-isopropylacrylamide) gel layers [8] and some types of lymphosarcoma

cells [9]. In the present paper, we report some results of electrophoretic mobility measurements on intact and Actinomycin D-induced apoptotic HL-60RG cells immersed in a phosphate buffer solution as a function of the ionic strength at pH 7.4. The electrophoretic mobility data are analyzed via the above-mentioned mobility formula for soft particles, followed by the estimation of structural change in the cell surface induced by apoptosis.

Materials and methods

Materials

Actinomycin D was purchased from Sigma Chemical Company. All other reagents were of analytical grade.

Induction of Apoptosis

A human promyelocytic leukemia cell line, HL-60RG was maintained in RPMI1640 medium supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories). Cells were passaged three times weekly. For all experiments, exponentially growing cells were used. Apoptosis was chemically induced by treatment with 4 μg/ml actinomycin D for 6 hr at 37 °C in a CO_2-incubator [10].

Measurement of electrophoretic mobility

The electrophoretic mobility of the above-prepared cells was measured in phosphate buffer solutions with various ionic strengths by using Automated Electrokinetics Analyzer, PEN KEM System 3000 at 37 °C. Prior to the mobility measurement, the prepared cell suspension was centrifuged and the collected cells were redispersed in the respective phosphate buffer solutions. The measurement was repeated at least 20 times. The ionic strength was adjusted by dilution of the phosphate buffer solution with an ionic strength of 0.154 with distilled water. All solutions used were made isotonic by addition of sucrose.

Results and discussion

By the treatment of HL-60RG cells with actinomycin D, DNA cleavage to soluble fragments, condensation of chromatin and shrinkage of cells were observed to confirm the induction of apoptosis [11]. It was microscopically observed that in solutions with ionic strengths between 0.005 and 0.154, no morphological changes were induced by ionic strength change for intact and apoptotic cells. This implies that the presence of ions and of sucrose little affects the cell structure during the measurement of their electrophoretic mobilities.

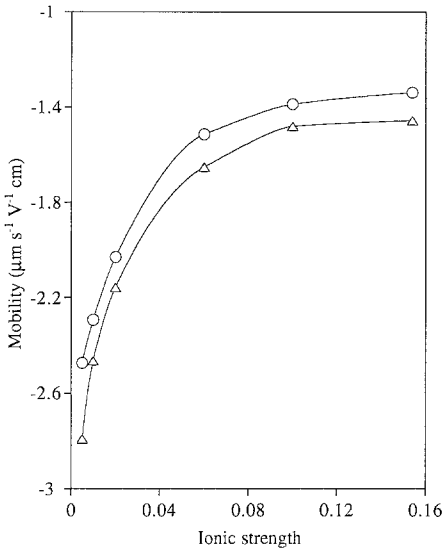

Fig 1. Electrophoretic mobility of intact HL-60RG cells and their apoptotic cells measured as a function of the ionic strength in the suspending medium at pH 7.4 and 37 °C: ○, intact HL-60RG cells; △, apoptotic HL-60RG cells

The measured values of electrophoretic mobilities of intact and apoptotic HL-60RG cells are plotted against the ionic strength of the dispersing medium in Fig. 1. Both types of cells exhibit negative mobility values, implying that the surfaces of these cells have a net negative charge. In solutions of lower ionic strengths, the electrophoretic mobility of both types of cells becomes more negative. The mobility of apoptotic HL-60RG cells is more negative than that of intact HL-60RG cells at ionic strengths between 0.005 and 0.154.

As mentioned in the introduction, we employ the mobility formula for "soft particles" to analyze the measured mobility data. This is because the electrophoretic mobilities for both intact and apoptotic HL-60RG cells tend to non-zero values

even in the solution with the ionic strength of 0.154, which means these cells are soft particles [6–9]. The electrophoretic mobility μ of a colloidal particle covered by a layer of polyelectrolyte chains (i.e., soft particles), in which ionized groups of valency z are uniformly distributed at a number density of N (m^{-3}), moving in a liquid containing a symmetrical electrolyte of valency ν and bulk concentration (number density) n (m^{-3}) in an applied electric field, is expressed as [6, 7]

$$\mu = \frac{\varepsilon_r \varepsilon_0}{\eta} \frac{\psi_0/\kappa_m + \psi_{\mathrm{DON}}/\lambda}{1/\kappa_m + 1/\lambda} + \frac{zeN}{\eta\lambda^2}, \tag{1}$$

with

$$\psi_{\mathrm{DON}} = \frac{kT}{ve} \ln\left[\frac{zN}{2\nu n} + \left\{\left(\frac{zN}{2\nu n}\right)^2 + 1\right\}^{1/2}\right], \tag{2}$$

$$\psi_0 = \frac{kT}{ve}\left(\ln\left[\frac{zN}{2\nu n} + \left\{\left(\frac{zN}{2\nu n}\right)^2 + 1\right\}^{1/2}\right] + \frac{2\nu n}{zN}\left[1 - \left\{\left(\frac{zN}{2\nu n}\right)^2 + 1\right\}^{1/2}\right]\right), \tag{3}$$

$$\lambda = (\gamma/\eta)^{1/2}, \tag{4}$$

$$\kappa_m = \kappa\left[1 + \left(\frac{zN}{2\nu n}\right)^2\right]^{1/4}, \tag{5}$$

$$\kappa = \left(\frac{2ne^2\nu^2}{\varepsilon_r \varepsilon_0 kT}\right)^{1/2}. \tag{6}$$

Here, η is the viscosity, γ is the frictional coefficient of the polyelectrolyte layer, ε_r is the relative permittivity of the solution, ε_0 is the permittivity of a vacuum, ψ_{DON} is the Donnan potential of the particle surface layer, ψ_0 is the potential at the boundary between the particle surface layer and the surrounding solution, κ is the Debye–Hückel parameter of the surrounding solution, k is the Boltzmann constant, and T is the absolute temperature. If the unit of the concentration n is converted to M (mole/l), then n becomes practically equal to the ionic strength, since the counterions in the present measurement are monovalent (Na^+ and K^+), and the coions, less important, are regarded approximately as monovalent. We call ψ_0 the surface potential of the polyelectrolyte-coated particle and κ_m can be interpreted as the Debye–Hückel parameter of the polyelectrolyte layer. The parameter λ, the reciprocal of which $1/\lambda$ has the dimension of length, characterizes the degree of friction exerted on the liquid flow in the polyelectrolyte layer.

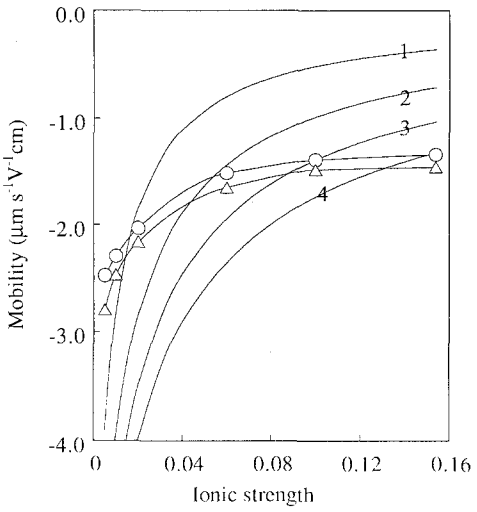

Fig. 2. Electrophoretic mobility of intact HL-60RG cells and their apoptotic cells. Symbols are experimental data: ○, intact HL-60RG cells; △, apoptotic HL-60RG cells. Solid curves are theoretical ones calculated from Eq. (7) with $zN = -0.1$ M (curve 1), $zN = -0.2$ M (curve 2), $zN = -0.3$ M (curve 3) and, $zN = -0.4$ M (curve 4)

Before analyzing the mobility data via Eq. (1), we show that the data cannot be explained by the conventionally-used mobility formula for rigid colloidal particles, that is, the following well-known Smoluchowski's formula:

$$\mu = \frac{\varepsilon_r \varepsilon_0}{\eta} \psi_0 . \tag{7}$$

Equation (7) can be derived by taking the limit $\lambda \to \infty$ in Eq. (1). (In the limit of large λ, "soft" particles become rigid.) In Eq. (7), ψ_0 is the zeta potential of the particles and is practically equal to the surface potential. Figure 2 shows the results calculated via Eq. (7) for "rigid" particles in comparison with the experimental data. Here, Eq. (3) has been employed for the relationship between ψ_0 and zN. The usage of Eq. (3) together with Smoluchowski's formula (Eq. (7)) corresponds to the assumption that the cell surface layer is ion-penetrable but the liquid inside the cell surface layer moves as a whole with the cell itself. On the other hand, Eq. (1) assumes that there is a flow of the liquid inside the cell surface layer relative to the cell itself. Figure 2 indeed shows that Eq. (7) cannot explain the experimental results, implying

that the cells (both the intact and apoptotic HL60-RG cells) do not behave as "rigid" particles.

We, therefore, tried to employ mobility formula, Eq. (1) for "soft" particles. Equation (1) directly relates the measured values of electrophoretic mobility to the density of fixed-charges zN and the parameter $1/\lambda$. Since Eq. (1) contains two unknown parameters, zN and $1/\lambda$, we calculated the electrophoretic mobility as a function of the electrolyte concentration n in the suspending medium for various zN and $1/\lambda$ in order to determine the values of zN and $1/\lambda$ by curve-fitting procedure. As will be seen later in Fig. 5, the mobility formula assuming uniform fixed-charge distribution failed to explain the mobility data at very low ionic strength and the fixed-charges that contribute to the mobility vary with the ionic strength. Therefore, the statistical weight cannot be introduced properly into each data point. This makes difficult to employ the usual least-square method to determine zN and $1/\lambda$ by curve-fitting procedure. Instead, we proceed as follows. First, we note that at a given ionic strength, there are many possible pairs of zN and $1/\lambda$ to yield the same mobility value. In other words, we have a curve $1/\lambda$ against zN on a $1/\lambda - zN$ plane at this ionic strength. At different ionic strengths, we have different curves. As should be expected, these theoretical curves intersect with each other only at one point, as is seen in an example given in Fig. 3, in which the intersection is located at $(zN, 1/\lambda) = (-0.04\,\mathrm{M}, 1.4\,\mathrm{nm})$. For the actual experimental data measured at various ionic strengths, curves of zN against $1/\lambda$ thus calculated will never intersect with each other at one point. Instead, we have regions of zN and $1/\lambda$ where these curves lie closest to each other are shown in Fig. 4. These regions can be considered to be those for the best-fit values of zN and $1/\lambda$. The area of each square corresponds to the deviation of zN and $1/\lambda$. We found that the best-fit regions (shown as shaded squares in Fig. 4) locate around $-0.0330\,\mathrm{M} \leqq zN \leqq -0.0341\,\mathrm{M}$ and $1.694\,\mathrm{nm} \leqq 1/\lambda \leqq 1.717\,\mathrm{nm}$ for intact HL60-RG cells and $-0.0338\,\mathrm{M} \leqq zN \leqq -0.0354\,\mathrm{M}$ and $1.752\,\mathrm{nm} \leqq 1/\lambda \leqq 1.775\,\mathrm{nm}$ for apoptotic HL60-RG cells. The mean values of zN for intact and apoptotic HL-60RG cells are $-0.0336\,\mathrm{M}$ and $-0.0346\,\mathrm{M}$, respectively. From the mean values of zN, one may calculate the volume of a cube occupied by one unit charge. The length of a side of the cube is calculated to be about 37 Å for intact cells and about 36 Å for apoptotic cells.

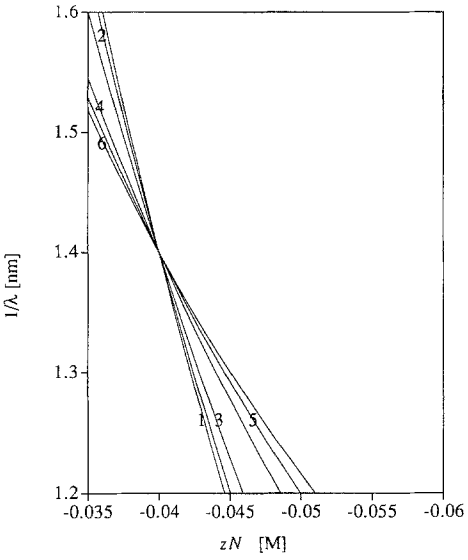

Fig. 3. Curves of $1/\lambda$ against zN that are calculated from theoretical values of μ with $zN = -0.04\,\mathrm{M}$ and $1/\lambda = 1.4\,\mathrm{nm}$ at ionic strengths of 0.005 (curve 1) 0.01 (curve 2), 0.02 (curve 3), 0.06 (curve 4), 0.1 (curve 5) and 0.154 (curve 6) intersect with each other $zN = -0.04\,\mathrm{M}$ and $1/\lambda = 1.4\,\mathrm{nm}$

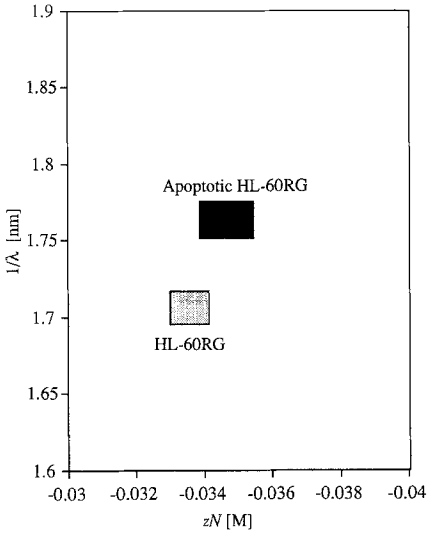

Fig. 4. Regions of zN and $1/\lambda$ values giving best-fit mobilities for HL-60RG cells and their apoptotic cells

In Fig. 5, curves are given which are calculated with the values of zN and $1/\lambda$ corresponding to

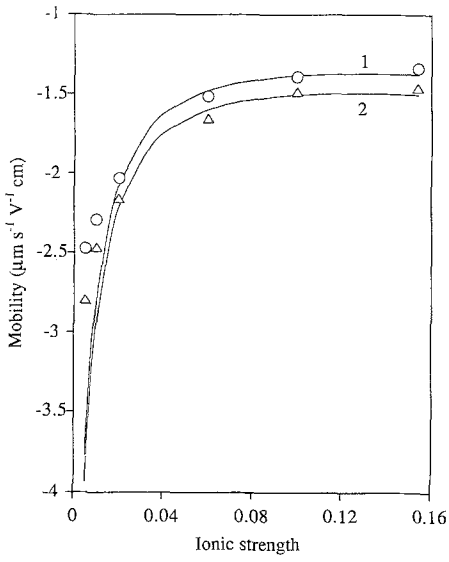

Fig. 5. Electrophoretic mobility of intact HL-60RG cells and their apoptotic cells. Symbols are experimental data measured as a function of the ionic strength in the suspending medium at pH 7.4 and 37 °C: ○, intact HL-60RG cells; △, apoptotic HL-60RG cells. Solid curves are theoretical ones calculated with $zN = -0.0336$ M, and $1/\lambda = 1.7055$ nm (curve 1) and $zN = -0.0346$ M, and $1/\lambda = 1.7635$ nm (curve 2)

the center of the respective regions. We observe that the theoretical curves and the experimental data points are in good agreement with each other over a wide range of the electrolyte concentration. The observation that as the ionic strength decreases the mobility becomes more negative can be explained as follows. The surface potential as well as the Donnan potential increase in magnitude as the ionic strength decreases due to the decreased shielding effect of electrolyte ions in the medium. As is also seen in Fig. 5, theoretical curves deviate from the experimental data at very low electrolyte concentrations, that is, Eq. (1) overestimates the magnitude of the mobility. This can be explained as follows. The fixed-charges located over the depth of order $1/\kappa_m$ ($\approx 1/\kappa$) measured inward from the surface contribute to the mobility. The parameter N can thus be considered to be an average density over the depth $1/\kappa$. Therefore, as the ionic strength decreases ($1/\kappa$ increases), one can get information on the fixed charges in the deep interior of the surface layer. This previously-mentioned overestimation of the mobility at very low ionic strengths suggests that

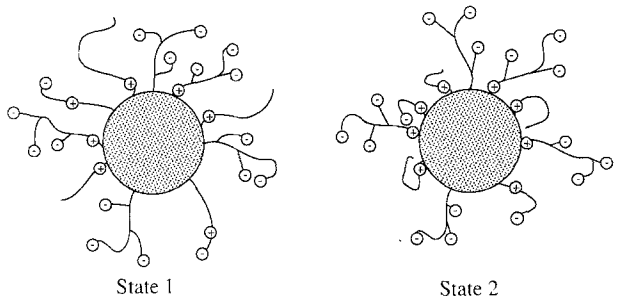

Fig. 6. Schematic representation of the structural change in the cell surface from state 1 to state 2 during apoptosis

positive fixed charges possibly arising from dissociation of amino groups, for example, are present in the deep interior of the cell surface charge layer.

As shown in Fig. 4, the ranges of zN of the two shaded squares overlap while those for $1/\lambda$ do not. The obtained values of zN are thus found close for both types of cells, while the values of $1/\lambda$ increases by apoptosis. That is, the mobility difference between intact and apoptotic HL-60RG cells is attributed mainly to the difference in $1/\lambda$ rather than the difference in charge density in their surface layers. The increase in $1/\lambda$ means the decrease of friction exerted by the polymer segments on the liquid flow. This increase in $1/\lambda$ can be caused by the increase of the "softness" of the polymer chains or by the decrease in their density. Further studies will be needed to determine which mechanism actually accounts for the change in $1/\lambda$ and the possibility of the change in the charge density in the surface layer cannot be ruled out. In the following, we would like to propose one possible mechanism for the increase in $1/\lambda$ caused by the decrease in the density of polymer chains. That is, the change in $1/\lambda$ is due to a change of packing state of polymer chains in the cell surface layer. This is schematically shown in Fig. 6, in which the surface layer is composed of charge-rich polymer chains and charge-poor polymer chains. When apoptosis occurs, most of the charge-poor polymer chains are entangled inward into the interior of the surface charge layer, while the charge-rich polymer chains almost remain at their original positions, leading to a reduction in the polymer chain density in the surface layer. This decrease in the density of polymer chains in the surface layer

may cause a decrease of friction (in other words, an increase in $1/\lambda$) in the surface layer, with less change in the charge density (zN) in this region. This change, in Fig. 6, is shown as a structural change in the surface region from state 1 to state 2 caused by apoptosis. Here, we again mention that the observed mobility change can be induced also by the structural change in charge-rich portions in the polymer chains. Nevertheless, we would like to stress that the faster mobility of apoptotic cells corresponds to the increase in "softness" in their surface layer, as suggested from the larger change in $1/\lambda$ than zN. The predicted increase in "softness" of the surface of apoptotic cells should be correlated with the observation that apoptosis enhances the cell deformability prior to the fragmentation into smaller-sized pieces.

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Authors' address:

Dr. H. Ohshima
Faculty of Pharmaceutical Sciences
Science University of Tokyo
12 Ichigaya Funagawara-machi
Shinjuku-ku, Tokyo, 162, Japan