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Measurements and analyses of electrophoretic mobilities of RAW117 lymphosarcoma cells and their variant cells

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Abstract

The electrophoretic mobilities of the cells of malignant lymphosarcoma cell line RAW117-P and its variant H10 with a highly metastatic property to the liver have been measured at various ionic strengths. The cells of parental cell line (RAW117-P) show higher mobility values in magnitude than those of its variant line (RAW117-H10) in the whole range of electrolyte concentration measured. We have also measured the sialic acid amount carried by cells of both lines. The content of sialic acids in RAW117-H10 cells is observed to be about 27% less than that in RAW117-P cells. The mobility data obtained have been analyzed by a novel mobility formula for colloidal particles with ion-penetrable surface charge layers. The observed mobility difference between RAW117-P cells and RAW117-H10 cells is found to be due to the difference in friction exerted by the cell surface layers on the liquid flow around the cells between these two types of cells and to the difference in fixed-charge density in their surface layers, which is caused by the 27% decrease in sialic acid content. A possible explanation for this mobility difference between these two types of cells is given.

Keywords: Electrophoretic mobility; RAW117 lymphosarcoma cells

1. Introduction

The cell surface modification by carbohydrate chains appears to be an intrinsic part of genetically regulated process of cell differentiation and oncogenic transformation [1–3]. Cellular transformation has been reported to result in an increase of sialic acid of glycoproteins [4,5] and to be associated with blocked biosynthesis of glycolipids and aberrant expression of the developmentally programmed biosynthetic pathway [2,3,6]. To study the details of the change in physicochemical properties of the cell surface responding to the malignant behavior, we measured the amount of sialic acid carried by RAW117-P cells and RAW117-H10 cells and the electrophoretic cell

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mobilities. These data should reflect certain properties of the cell surface and their change due to the malignant behavior.

The cell surface is normally covered with a layer of polyelectrolytes. Previously, our group 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 [7–9]. In the present paper, we report some results of electrophoretic mobility measurements on RAW117-P and RAW117-H10 cells immersed in a phosphate buffer solution as a function of the ionic strength at pH 7.4. The electrophoretic mobility data together with the data on the sialic acid amounts carried by the both types of the cells are analyzed via the above-mentioned theoretical model for soft particles, followed by the estimation of structural change in the cell surface responding to the malignant behavior.

2. Materials and methods

2.1. Tumor cells

The tumor cell line RAW117-P was murine lymphosarcoma, induced by the Abelson leukemia virus. A variant line RAW117-H10 was selected ten times *in vivo* for liver colonization and formed more than 200-fold gross liver tumor than RAW117-P did [10,11]. Both cell lines were grown as suspension culture in plastic petri dishes (Falcon Plastic, Oxnard, CA) with the use of RPMZ-1640 supplemented with 5% heat-inactivated (56°C, 30 min) fetal bovine serum (Cell Culture Laboratories, Cleveland, OH) with antibiotics.

2.2. Determination of sialic acid

RAW117-P (3.2×10^7) and H10 (5.2×10^7) cells were pooled to analyze the amount of sialic acid. The cells were treated with 20 mU of neuraminidase from *C. perfringens* (Sigma, St. Louis, MO) at 4°C over night in 100 μl of 50 m*M* sodium acetate buffer (pH 5.5). After the incubation, the mixture was centrifuged, and aliquots of the supernatant were taken and used for detection of sialic acid by thin layer chromatography followed by densitometric determination. Thin layer chromatography was performed on a HPTLC plate (Merck, Darmstadt, Germany) using a solvent mixture of ethanol/pyridine/butanol/acetic acid/water (100/10/10/3/30 by volume). As the standards, 0.2, 0.5 and 1 μg of sialic acid were applied and bands of sialic acid were visualized by spraying with resorcinol–HCl reagent and scanned with a Shimadzu TLC scanner (CS-930) at 580 nm.

2.3. Measurement of electrophoretic mobility

The electrophoretic mobility of the above-prepared cells was measured in phosphate buffer solutions (pH 7.4) with various ionic strengths by using an automated electrophoresis apparatus, PEN KEM System 3000 at 37°C. Prior to the measurement, the prepared cell suspension was centrifuged and the collected cells were redispersed in the 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 (0.0475 *M* Na_2HPO_4 and 0.0116 *M* KH_2PO_4) with distilled water. All solutions used were made isotonic by addition of sucrose.

3. Results and discussion

The amounts of sialic acid of RAW117-P and RAW117-H10 cells were found to be 2.26 nmol/10^8 cells and 1.65 nmol/10^8 cells, respectively. This means that about 27% of sialic acid was removed by the modification of RAW117-P cells.

The measured values of electrophoretic mobilities of RAW117-P and RAW117-H10 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

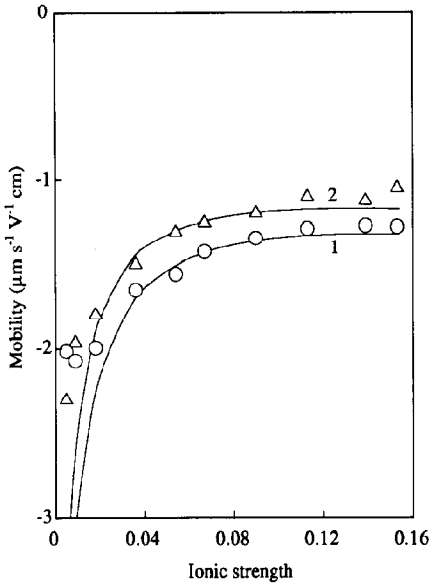

Fig. 1. Electrophoretic mobilities of RAW117-P cells and RAW117-H10 cells. Symbols are experimental data measured as a function of the ionic strength in the suspending medium at pH 7.4 and 37°C: (○) RAW117-P cells, and (△) RAW117-H10 cells. Solid curves are theoretical results calculated with $zN = -0.04$ M, and $1/\lambda = 1.5$ nm (curve 1) and $zN = -0.03$ M, and $1/\lambda = 1.7$ nm (curve 2).

solutions of lower ionic strengths, the electrophoretic mobility of both types of cells becomes more negative. The reason for this is that as the ionic strength decreases, the shielding effect of electrolyte ions in the medium decreases. It is of particular interest to note that the mobility of RAW117-P cells is more negative than that of RAW117-H10 cells at ionic strengths between 0.005 and 0.154.

The electrophoretic mobility μ of a colloidal particle covered with a layer of polyelectrolyte chains, in which the 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 υ and bulk concentration (number density) n (m^{-3}) in an applied electric field, is expressed as [7,8]

$$\mu = \frac{\epsilon_r \epsilon_0}{\eta} \frac{\Psi_0/\kappa_m + \psi_{DON}/\lambda}{1/\kappa_m + 1/\lambda} + \frac{zeN}{\eta\lambda^2}, \quad (1)$$

with

$$\Psi_{DON} = \frac{kT}{ve} \ln\left[\frac{zN}{2\upsilon n} + \left\{\left(\frac{zN}{2\upsilon n}\right)^2 + 1\right\}^{1/2}\right], \quad (2)$$

$$\Psi_0 = \frac{kT}{ve}\left(\ln\left[\frac{zN}{2\upsilon n} + \left\{\left(\frac{zN}{2\upsilon n}\right)^2 + 1\right\}^{1/2}\right] + \frac{2\upsilon n}{zN}\left[1 - \left\{\left(\frac{zN}{2\upsilon n}\right)^2 + 1\right\}^{1/2}\right]\right), \quad (3)$$

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

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

$$\kappa = \left(\frac{2ne^2\upsilon^2}{\epsilon_r \epsilon_0 kT}\right)^{1/2}. \quad (6)$$

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. 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.

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 a curve-fitting procedure. We have found

that the best-fit curves (shown as solid lines in Fig. 1) are obtained with $zN = -0.04$ M and $1/\lambda = 1.5$ nm for RAW117-P cells and $zN = -0.03$ M and $1/\lambda = 1.7$ nm for RAW1I7-H10 cells. The values of zN for both cells have been chosen in such a way that the ratio of zN values for these two types of cells equals the ratio of the observed sialic acid contents in these cells. Here, we have assumed the negative fixed-charges carried by the cells arise mostly from sialic acids. It is observed that the theoretical curves and the experimental data points are in good agreement with each other over a wide range of electrolyte concentration. At very low electrolyte concentrations, however, theoretical curves deviate from the experimental data, that is, eq. (1) overestimates the magnitude of the mobility. This can be explained as follows. The particle fixed-charges located over the depth of order $1/\kappa_m$ ($\approx 1/\kappa$) from the surface layer/solution boundary contribute to the mobility. Thus, as the ionic strength decreases ($1/\kappa$ increases), one can obtain information on the fixed charges in the deeper interior of the surface layer. This suggests that positive fixed charges possibly arising from dissociation of amino groups, for example, are present in the deep interior of the surface charge layer.

Our analysis demonstrates that the mobility difference between RAW117-P and RAW117-H10 cells is attributed to the differences both in $1/\lambda$ and in charge density zeN in their surface layers. Among these differences, the difference in N is corresponds to the difference in sialic acid contents in both cells. The difference in $1/\lambda$ may possibly reflect a change of packing state of polymer chains in the cell surface layer. The result (obtained by the curve-fitting procedure) that $1/\lambda$ is greater for RAW117-H10 cells than for RAW117-P cells implies that the friction exerted by the surface layer on the liquid flow around the cells is less for RAW117-H10 than for RAW117-P. In other words, the surface layer of RAW117-H10 cells is "softer" than that of RAW117-P cells. Also the charge density, zeN, of RAW117-H10 cells is found to be 27% lower than that of RAW117-P cells, assuming that the membrane fixed charges arise mostly from sialic acids and that the charge-density ratio of P cells to H10 cells is the same as the ratio of the total amount of sialic acids.

Difference of the electrophoretic mobility is thought to be attributed to the cell surface modification which is mostly due to the extent, type and site of glycosylation of membrane proteins and lipids. This modification includes the extent of sialylation of the carbohydrate moieties of glycoconjugates. Recently, we examined the glycolipid composition of these two cell lines (unpublished data). The result showed that the total amount of sialic acid-containing lipids was higher in the parental cell line than in the metastatic variant, whereas the concentration of highly sialylated glycolipids was higher in RAW117-H10 cells than in the parental cell.

Dennis et al. [12] have shown that a highly metastatic mouse cell line tumor MDAY-D2 expresses sialylated asparagine-linked complex oligosaccharides, whereas the non-metastatic mutant MDW4 synthesizes prematurely truncated oligosaccharides. This observation together with our present result suggest that carbohydrate modification of cell surfaces of metastatic variant cells appears to be more complex than their non-metastatic counterpart.

The "softness" of RAW117-H10 cell surface, which was elucidated by the present experiment, caused by the modification may be an advantage for metastatic tumor cells to survive or adapt in the capillary with higher blood pressure and to invade the endothelial cells of the target tissue.

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