Cell Membrane Response on D-Glucose Studied by Dielectric Spectroscopy. Erythrocyte and Ghost Suspensions

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The kinetics of D-glucose influence on spherical human erythrocyte and ghost suspensions, which were prepared from fresh blood provided by healthy donors, was studied by time domain dielectric spectroscopy (TDDS) in a wide physiologic range of glucose concentrations. The dielectric response of cell membranes was found to demonstrate a nonmonotonic behavior in the vicinity of 12 mM concentration of D-glucose in both cell and ghost suspensions. In contrast to spherical cells, where the membrane effect after exposure to D-glucose was reduced after 15−30 min, no reaction was detected in the case of ghosts. The possible mechanisms of glucose transport through the membrane and metabolism are discussed in this paper.

Introduction

An increasing concentration of glucose in blood induces significant reactions in blood and human tissues. It is known that the concentration of sodium decreases and potassium increases due to the active transport of ions and water movement from the tissue and blood cells, such as erythrocytes, into the vascular system.[1] It can be understood on the cellular level that glucose uptake through membrane transporters (GLUTs) requires controlling the electrolyte balance to maintain osmolarity and cell sizes.[2] However, the whole mechanism is still not fully clarified. Such a variation of the electrolyte balance can lead to membrane potential changes as well as to a change of ion concentrations, and consequently to dielectric properties alteration of the cell membrane. This effect can be observed with a noninvasive, real-time monitoring technique such as time domain dielectric spectroscopy (TDDS).[3,4] It is well known that interfacial surface polarization is very sensitive to electric properties of cell membranes.[3] Therefore, TDDS studies are helpful to clarify the mechanism and kinetics of cell reactions to glucose, and it is important for basic research as well as for medical applications (e.g., in the field of diabetes).

In our previous work, a TDDS study for spherical erythrocyte suspensions with different concentrations of D- and L-glucose was performed.[3] It was shown that the spectrum amplitude decreased around 12 mM of D-glucose, followed by a nonmonotonic behavior of the specific cell membrane capacitance (that relates to permittivity and thickness of the cell membrane) versus D-glucose concentration as shown in Figure 1. However, no significant changes were found in the case of L-glucose, which is a biologically inactive optical isomer of D-glucose and hence not metabolized. This study clarified that cell reactions to D-glucose change the electric properties of the membrane significantly. However, the detailed mechanisms, especially the kinetic reaction to glucose, are still not clarified, as technically time dependency was not controlled in the previous measurements.

In the present work, kinetic monitoring of dielectric spectroscopy measurements of spherical human erythrocyte suspensions with different concentrations of D-glucose were performed to clarify time dependence of the cell reactions. Furthermore, similar experiments using erythrocyte ghosts (devoid of cytoplasmic content) were performed to clarify the role of the membrane and cytoplasm. The possible mechanisms of the glucose influence on human erythrocyte membranes are discussed.

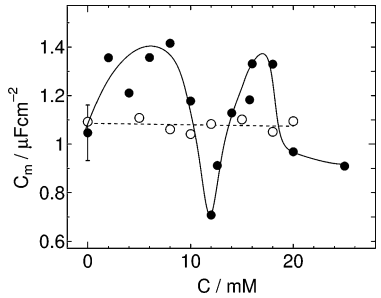

Figure 1. Specific cell membrane capacitance per unit surface area (C_m) of spherical erythrocyte suspensions from outdated red blood cells at 25 °C versus D-glucose (full circle) and L-glucose (open circle) concentrations. In the data analysis, permittivity of the cell membrane (ϵ_m) is estimated by the fitting procedure, where the following parameters were fixed: permittivity of cytoplasm, $\epsilon_{cp} = 60$; conductivity of cell membrane, $\sigma_m = 0$; thickness of cell membrane, $d = 3.1$ nm; and cell radius, $r = 2.75$ μm. Then, C_m was obtained by $C_m = \epsilon_m\epsilon_0/d$, where ϵ_0 is the permittivity of vacuum. Results of 10 measurements were averaged for each data point.

Experimental Section

Fresh human erythrocytes from healthy donors were obtained from the Hadassah University Hospital, Jerusalem. The erythrocytes were washed in phosphate-buffered saline as standards for saline (PBS, 290 mOsm) and centrifuged 3 times for 5 min at 300 × g. In the cases of experiments for spherical erythrocytes, the erythrocytes were resuspended in 63% PBS (183 mOsm) and kept for 20 min to form a spherical shape, and then were centrifuged again. They were then resuspended in PBS buffers with D-glucose, where the osmolarity was kept the same as the 63% PBS. The glucose concentrations were changed between 0 and 20 mM. After resuspending the erythrocytes, dielectric spectroscopy measurements at 25 °C were started immediately at controlled temperature (Heto thermostat, Allerod, Denmark).

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Ghosts were prepared from fresh human erythrocytes according to the method of Heard et al.[5] Dielectric spectroscopy measurements for ghost suspensions with different concentrations of D-glucose (0−20 mM) were performed at 25 °C.

The size and shape of erythrocytes were measured by microscope (IX70 Olympus) and captured with a DVC-1300 digital camera. No size differences were found for spherical erythrocytes and ghosts, treated in different D-glucose concentrations and with different time intervals. Volume fractions of erythrocytes were measured in heparinized capillary tubes, which were centrifuged in a Clay Adams centrifuge for 20 min. The volume fraction was corrected to be 80% of the obtained value from the centrifuge method to take into account intercellular space of packed spherical erythrocytes. The same correction was applied as for lymphocyte suspensions reported in our recent paper,[6] where the intercellular space was determined with Dextran blue. In the present work, the volume fraction after the correction was 4.0% for all suspensions of spherical erythrocytes with different concentrations of glucose, and 2.8% for all ghost suspensions, and those were not changed during our TDDS measurements.

TDDS measurements were performed by a time domain dielectric spectrometer (Dipole TDS Ltd., Jerusalem; total inaccuracy is 5%) using a 0.19-pF sample holder with a recording system at nonuniform time scale-up to 5 μs (frequency range from 200 kHz up to 2 GHz).[3,4] Temperature was controlled and held constant at 25 °C by a thermostat (Heto, Allerod, Denmark). The kinetic experiments were performed in the following way: each measurement for a specific glucose concentration at 0, 2, 5, 10, 15, 30, 45, and 60 min was provided with a new portion of suspension, injected into the sample holder from the stock to protect a sedimentation of erythrocytes.

At the first stage of the data analysis, the effects of electrode polarization were subtracted in a time domain according to the fractal approach as presented in detail in previous papers.[3,7,8] This correction is trustworthy even for extremely highly conductive solution as reported in Feldman et al.[7] where static dielectric permittivity of 78 and a good plateau through a frequency range of 0.15 to 70 MHz are obtained for a simple electrolyte solution (KCl 158 mM, dc conductivity 1.73 S/m, 25 °C). Then, the Fourier transformation was carried out to obtain the dielectric spectra.[3,4] The single-shell model,[3,9] which is based on the Maxwell−Wagner mixture formula,[9−11] was applied for a fitting procedure of the spectra. According to the Maxwell−Wagner mixture formula, the observed dielectric spectrum of the cell suspension (ϵ^*) is described by

$$\epsilon^* = \epsilon^*_b \frac{(2\epsilon^*_b + \epsilon^*_c) - 2p(\epsilon^*_b - \epsilon^*_c)}{(2\epsilon^*_b + \epsilon^*_c) + p(\epsilon^*_b - \epsilon^*_c)} \quad (1)$$

$$\epsilon^*_c(\omega) = \epsilon^*_m(\omega)\frac{2(1-\upsilon) + (1+2\upsilon)E(\omega)}{(2+\upsilon) + (1-\upsilon)E(\omega)} \quad (2)$$

$$\upsilon = (1 - d/r)^3,\ E(\omega) = \epsilon^*_{cp}(\omega)/\epsilon^*_m(\omega) \quad (3)$$

and

$$\epsilon^*_i(\omega) = \epsilon_i - j\frac{\sigma_i}{\epsilon_0\omega},\ (i = b, m, \text{or } cp) \quad (4)$$

where subscripts *b*, *m*, and *cp* indicate buffer, cell membrane, and cytoplasm, respectively, ϵ_i and σ_i are the static permittivity and conductivity of each component, *j* is the imaginary unit, ω is the angular frequency, and ϵ_0 is the permittivity of vacuum. Since conductivity of the cell membrane is very small in general,[12] it is allowable to fix the value to be zero in the data-fitting routine;[9] this will make the fitting results more stable. The geometric parameters, such as thickness of the erythrocyte membrane (d = 3.1 nm, from Lisin et al.[9]) and radius of the cells (r = 2.75 μm, observed by a microscope with a digital camera), were also fixed, as well as the permittivity of cytoplasm, ϵ_{cp} = 60, from the reference value[13] (the suspension spectrum is almost insensitive to changes of the ϵ_{cp} if the volume fraction is small[3,6]). Finally, the specific cell membrane capacitance per unit surface aria was obtained by $C_m = \epsilon_m\epsilon_0/d$.

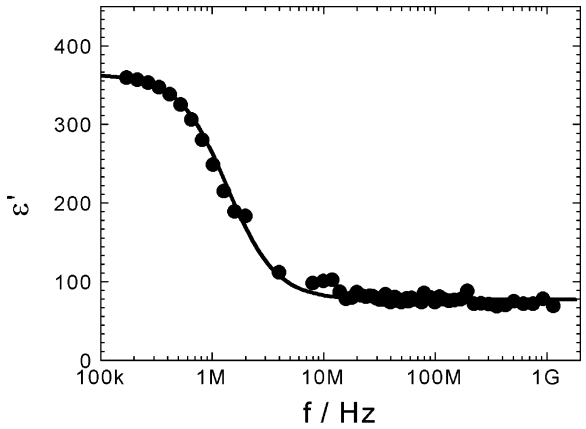

Figure 2. Real part of complex dielectric permittivity for a 4.0% spherical erythrocyte suspension in diluted PBS buffer with 2 mM of D-glucose (total: 183 mOsm) at 25 °C after 10 min resuspension (addition of D-glucose). The solid line indicates one Debye process due to the surface polarization on the membrane, that is, complex permittivity of the suspension, $\epsilon^*(\omega)$, was described by $\epsilon^*(\omega) - \epsilon_\infty = \Delta\epsilon/(1 + j\omega\tau)$, where ϵ_∞ = 77, $\Delta\epsilon$ = 286, and τ =115 ns, respectively. From this spectrum, we obtained the specific cell membrane capacitance C_m = 1.07 by the fitting procedure according to Eqs. 1 − 4 and C_m = 1.03 by a direct estimation from $\Delta\epsilon$ (see Polevaya et al.[6] and Asami et al.[12]), that is, $C_m = 0.667\epsilon_0\Delta\epsilon r^{-1}(1 - (1-p)^{1.5})^{-1}$.

Results and Discussion

Maxwell−Wagner surface polarization causes a single Debye process for spherical erythrocytes and ghosts,[9−11] as shown in Figure 2. It is also known that a contribution of glucose molecules in this frequency range is negligible, because the relaxation strength and the relaxation time due to the molecular orientation of glucose are much smaller than the surface polarization.[3,14]

Figures 3 and 4 show time and D-glucose concentration dependencies of the specific membrane capacitances of erythrocytes in spherical and ghost conditions. The nonmonotonic behavior of C_m against D-glucose concentrations was found in both cases; in other words, a significant decrease of C_m was observed in the region of 10−15 mM D-glucose. A tendency of this change was similar to our primary results without the controlling of time dependence,[3] although detailed shape could be different. For example, a minor maximum can be seen on 12 mM of D-glucose in the case of fresh cells.

In the case of erythrocytes, as shown in Figure 3, time-dependent changes of C_m (such as excitation in the first 15 min with a followed decay) could be seen, except for the case of zero concentration of D-glucose. In contrast, the parameters of the ghost membranes were almost unchanged with time for all D-glucose concentrations (Figure 4). Such a difference in the membrane reaction kinetic on the glucose influence on cells

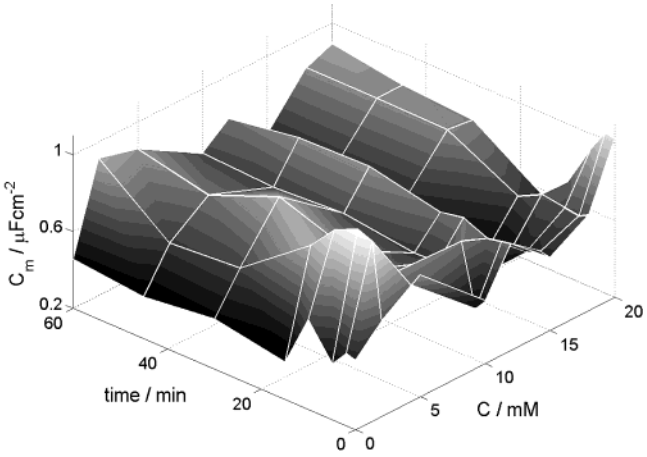

Figure 3. Specific cell membrane capacitance per unit surface area of the spherical erythrocyte membrane against D-glucose concentration and time. Note that no significant changes of cytoplasm conductivity ($\sigma_{cp} \sim 0.7$ S/m) were obtained in the present concentration and time intervals. Suspensions were prepared from fresh human blood. (SD: $\sim \pm 0.09$; estimated from 10 measurements of suspensions with 0 mM glucose).

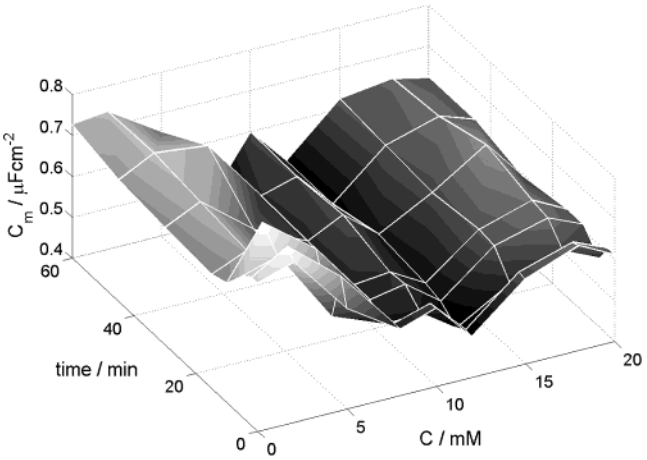

Figure 4. Specific cell membrane capacitance per unit surface area of the ghost membrane against D-glucose concentration and time. Note that no significant changes of cytoplasm conductivity ($\sigma_{cp} \sim 1.3$ S/m) were obtained in the present concentration and time intervals. Suspensions were prepared from fresh human blood. (SD: $\sim \pm 0.06$; estimated from 8 measurements of suspensions with 0 mM glucose).

and not in the ghosts, indicates that the glucose metabolism in the cytoplasm can also be involved in the glucose transport regulation.

It is known that glucose is transported through GLUT1 and is mostly used through glycolysis by the membrane enzymes in human erythrocytes.[15] The electric properties of membranes relate to charges on the membrane in general. Therefore, it can be summarized that glucose uptake and the following biochemical reactions finally change the properties of charges on the membrane (e.g., concentration of ions). This consideration is in agreement with results by Zavodnik et al[16] reporting about significant glucose-stimulated erythrocyte membrane hyperpolarization. The observed nonmonotonic dielectric response of the membrane under glucose influence can be considered as a result of the cell activity on the membrane level, as it was also observed for ghosts. On the other hand, the time dependence that could be seen only for cells and not for ghosts requires the involvement of intracellular factors. These factors should be related to glucose uptake,[17−19] to transport regulation,[20−25] and should be followed by the metabolic cascade.[26,27] The glucose metabolism produces adenosine triphosphate (ATP), and actually ATP regulates glucose uptake,[18,28,29] followed by ion transport through the membrane,[30−32] and other reactions. Therefore, ATP would be one of the key molecules responsible for the changes of the specific cell membrane capacitance found in this work. This is also most probably related to modifications of the ionic condition (concentrations or varieties) on the membrane, because TDDS is very sensitive to such electric changes on membranes. Interestingly, Roe et al.[33] reported ATP-dependent changes of intracellular Ca^{2+} with glucose concentration and time, although the test subjects were mouse islets, not human erythrocyte cells. We expect further studies on the involvement of ATP in electric properties of human cell membranes. These studies will allow further growth in the understanding of the mechanism of the cell reaction to glucose.

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