EFFECT OF WHEAT GERM AGGLUTININ AND CONCANAVALIN A ON ERYTHROCYTE MEMBRANE SKELETON. THERMAL DIELECTROSCOPY STUDY

Boyana Paarvanova, Bilyana Tacheva, Miroslav Karabaliev, Ivan T. Ivanov
Department of Physics, Biophysics, Rentgenology and radiology. Medical Faculty of Thracian University, Stara Zagora, Bulgaria.

ABSTRACT

Deformability and stability of erythrocyte membrane (EM) largely depend on spectrin-based membrane skeleton (MS) and its attachment to the lipid bilayer. Concanavalin A binds to band 3 without effect on EM deformability, while wheat germ agglutinin (WGA) binds to Glycophorin A decreasing the EM deformability by unclear mechanism. Here, the binding of these lectins was studied using the dielectric relaxation on MS which involves a piezo effect on spectrin, powered by the electrostriction of EM through the attachment sites of MS (Ivanov and Paarvanova, 2016). To bind lectins, erythrocytes were suspended in working medium of isotonic 10 mM NaCl and mannitol solution, containing the lectin, at 22 °C for 30 min, hematocrit 0.10. The erythrocytes were washed, suspended at hematocrit 0.45 in working medium and heated. At the spectrin denaturation temperature (49.5 °C) the $C^* = C_{re} + jC_{im}$ changed by $\Delta C^* = \Delta C_{re} + j\Delta C_{im}$. The dielectric loss curve of spectrin, $\Delta C''_{im}$, derived from ΔC_{im} data, and the ΔC_{re} vs C_{re} plot were used to study the dielectric relaxation, assuming the dielectric activity of denatured spectrin nil. Up to 0.25 mg/ml, WGA strongly subdued the $\Delta C''_{im}$ curve and ΔC_{re} vs C_{re} plot, while Concanavalin A was without effect. This outcome indicates that WGA, in contrast to Concanavalin A, could sever the bridges between MS and bilayer, such that implicate Glycophorin A.

Keywords: *thermal dielectroscopy, spectrin skeleton detachment, lectin, erythrocyte deformability.*

INTRODUCTION

The plasma membrane of human erythrocytes consists of under-membrane skeletal cortex attached to the lipid bilayer membrane directly **(**Kapus and Janmey, 2013) and via two types of high-affinity protein bridges (Oliveira and Saldanha, 2010). The stability and deformability of erythrocytes are highly dependent on the strength of above mentioned protein bridges (Anong et al., 2009). The band 3 integral protein, coupled with the minor integral glycoprotein Glycophorin A (Fig. 1) is chief participant in the two bridges. The under-membrane skeletal cortex contains chiefly fibrilar protein spectrin and polymerized actin. Spectrin exhibits considerable segmental motion with a correlation time ~ 10^{-6} s which strongly contributes to the mechanical function of membrane skeleton (Fung et al., 1996).

The segmental motion of spectrin was recently studied applying the method of thermal dielectric spectroscopy on suspensions of native erythrocytes and isolated erythrocyte membranes (EMs) (Ivanov and Paarvanova, 2016). At 49.5°C, which is the denaturation temperature of spectrin (Brandts et al., 1977), frequency dependent threshold changes in the complex impedance, ΔZ^*, and complex capacitance, ΔC^*, of erythrocyte and EM suspensions were detected. Based on the assumption that the spectrin skeleton of erythrocytes and EGMs was dielectrically active only at its native state, the ΔZ^* and ΔC^* were considered as contribution of spectrin skeleton to the dielectric properties of native erythrocytes and isolated EMs.

The mathematical processing of ΔZ^* and ΔC^*, according to the methods of dielectric spectroscopy (Klösgen et al., 2011), allowed reveal two dielectric relaxations on the membrane skeleton of erythrocytes and isolated EMs. The second relaxation was detected at higher frequencies which allowed the electric field to penetrate into cytosole and interact directly with the spectrin dipoles.

The first relaxation, used in this study, took place at frequencies whereat the lipid bilayer was effective insulator of cytosole from outside field. This relaxation depended on the availability

of bridges between the integral proteins and spectrin. It was explained as a direct piezoeffect on the flexible spectrin filaments powered by the electrostriction of lipid bilayer, i.e., by the alternating mechanical force originating from the frequency-dependent electric charging (beta dispersion) of bilayer. (*Fig. 1.)*

The lectin concanavalin A binds to band 3 protein preserving normal deformabilty of erythrocytes (Chasis et al., 1985). Another lectin, wheat germ agglutinin (WGA) specifically binds to the olygosaccharide moiety of Glycophorin A and decreases the deformability of erythrocytes by an unclear mechanism (Evans and Leung, 1984; Danilov and Cohen, 1989; Chassis and Schrier, 1989). In an effort to shed light over this mechanism, we studied the effect on the first dielectric relaxation produced by the WGA binding to erythrocytes. The obtained findings possibly indicate that binding of WGA to glycophorin A resulted in detachment of spectrin skeleton from bilayer membrane.

MATERIALS AND METHODS

Chemicals. Weat germ agglutinin (WGA), concanavalin A, NaCl and mannitol were purchased from Sigma Chemicals Co, St. Louis, MO, USA.

Isolation of human erythrocytes. Human erythrocytes were isolated by centrifugation (250 x g, 5 min) of freshly collected heparinized blood from the clinical laboratory of Thracian University, Medical Faculty, Stara Zagora, Bulgaria. After precipitation of erythrocytes, the plasma and upper layer of white blood cells were removed. Prior to usage, erythrocytes were washed three times in large volumes of isotonic solution of 10 mM NaCl and mannitol (working medium).

Collection of the raw data for complex capacitance, C*. The sample suspension contained erythrocytes (control) or erythrocytes, treated with indicated lectin. The erythrocytes were suspended in working medium at hematocrit 0.45. The sample suspension (volume 70 μl) was injected into a conductometric cuvette, made up of a glass tube (length 120 mm, outside diameter 4 mm, wall thickness 0.5 mm) containing two platinum electrodes spaced at 4 mm. The cuvette was tightly inserted in a hole drilled in an aluminium block which was heated at a constant rate (2.0 °C/min). At this heating rate the erythrocyte membranes retain its impermeability to ions in the temperature range of 37 to 56 °C (Muravlyova et al., 2013).

During the heating, an alternating voltage of 150 mV was applied between the electrodes. The complex capacitance, $C^* = C_{re} - j.\ C_{im}$, of tested suspension was continuously measured and separated into its real (C_{re}) and imaginary (C_{im}) parts using Solartron 1260 Impedance Frequency Analyzer (England) controlled by a computer equipped with Miniscan software. The C* values were measured at 16 frequencies between 7 kHz and 10 MHz, scanned sequentially with an integration time of 0.5 s. Here, j is the imaginary unit, $j^2 = -1$. (*Fig. 2.)*

Thermal and frequency analysis of the complex capacitance, C* of erythrocyte suspension. Real capacitance, C_{re}, represents the ability of suspension to store charges, while imaginary capacitance, C_{im}, is proportional to the power at which the field dissipates energy to move the free ions (conductive loss) and rotate the dipoles (dielectric loss). To remove the conductive loss component from C_{im} and derive the useful dielectric loss component, C''_{im}, we took advantage of their characteristic frequency dependences, as shown in Fig. 2. For human blood at frequencies between 1 kHz and 40 kHz, the dielectric loss is negligible in respect to conductive loss and the log (C_{im}) linearly declines with log (f) reflecting the frequency dependence of pure conductive loss (Gabriel et al., 1996). The latter was extrapolated above 50 kHz and subtracted

from the log (C_{im}) / log (f) dependence thus obtaining C''_{im}, the energy loss due to the stimulated oscillation of dipoles.

With concentrated erythrocyte suspensions (hematocrit value above 0.40) both C_{re} and C_{im} depend entirely on the structure and dynamics of erythrocyte lipid membrane with small contribution of under-membrane spectrin cortex. In order to distinguish the latter, spectrin was thermally denatured and the consequent change in C^* was determined and attributed to the native cortex as the dielectric activity of denatured cortex is nil.

At the denaturation temperature of spectrin, T_A, the real capacitance, C_{re}, measured at a given frequency, changed (Fig. 3). The respective change, ΔC_{re}, was determined by subtracting the suspension capacitance at the denatured state of spectrin (3 °C after T_A) from that in the native state of spectrin (3 °C prior T_A). Next, the ΔC_{re} was corrected by $\Delta C_{re\ correction}$, taking into account its overlapping with the general temperature dependence of capacitance (Fig. 3) (*Fig. 4).*

Similar to C_{re}, the dielectric energy, C''_{im}, of tested suspension also changed at the spectrin denaturation temperature. The latter change, denoted by $\Delta C''_{im}$, was also ascribed to the native spectrin cortex. Fig. 4 shows how the spectrum of dielectric energy, $\Delta C''_{im}$, released on spectrin network of erythrocytes, was determined. The C''_{im} of a suspension, containing native erythrocytes, was determined for 16 frequencies at two temperatures, one just prior to and the other just after the spectrin denaturation. In a recent communication (Ivanov and Paarvanova, 2016) it was shown that the dielectric loss, remaining after the spectrin denaturation, could be associated to the band 3 integral protein, as it disappears at the denaturation temperature of band 3. Hence, the difference between the dielectric loss curves prior to and after the spectrin denaturation, $\Delta C''_{im}$, was ascribed to the dielectric energy lossed on the native spectrin network. The dielectric loss curve of spectrin represents a symmetric bell-shaped peak (Fig. 4), centered at the critical frequency of beta relaxation, determined by the frequency dependence of C_{re}. Exactly the same bell-shaped curve was obtained with suspensions of isolated erythrocyte membranes (Ivanov and Paarvanova, 2016) indicating that erythrocyte membrane was mainly responsible for the detected dielectric loss in the tested suspensions.

Binding of lectins to erythrocytes. 0.15 ml erythrocytes were suspended in two ml working medium with or without the indicated lectin (WGA or concanavalin A) for 20 min at 20°C. Prior to usage the cells were isolated and washed in two ml working medium. Under microscope, no agglutination of lectin-treated erythrocytes was established. (Fig. 5).

RESULTS

The real capacitance, C_{re}, of tested erythrocyte suspension, measured at a temperature several °C below the spectrin denaturation temperature, changed from a high plateau level (static value) at the low frequency end to a very low level at the high frequency end (not shown). This well known frequency dependence reflected beta dielectric dispersion which is due to the charging of lipid membrane (effect of Maxwell-Vagner). We studied the frequency dependence of ΔC_{re} and established that within the frequency interval of beta dispersion (100 kHz – 1000 kHz) the ΔC_{re} also decreased remaining strictly proportional to C_{re} (Fig. 5, left plot). The strict proportionality between ΔC_{re} and C_{re} is in line with the proposed mechanism which generates piezo-electricity on spectrin (ΔC_{re}) as a response to the charging of erythrocyte plasma membrane (C_{re}).

Binding of WGA and concanavalin A to the outside surface of erythrocytes did not substantially decrease the static value of C_{re} indicating preserved barrier function of the lipid membrane. For the case of WGA this is shown in Fig. 5, left plot. However, the static value of ΔC_{re} was strongly decreased with increasing WGA concentration (Fig. 5, left plot). The other lectin,

concanavalin A, was not able to reduce the static value of ΔC_{re} (not shown). In contrast to concanavalin A, WGA strongly decreased the dielectric energy, $\Delta C''_{im}$, released on spectrin (Fig. 5, right plot).

DISCUSSION

The findings, obtained in this study, indicate another difference in the action of WGA and concanavalin on native EM. In contrast to concanavalin A, binding of WGA to erythrocytes diminished the mechanical energy (vibrations) transferred from the electrostricted lipid bilayer to spectrin possibly by severing specific attachment bridges between MS and the lipid membrane. This conclusion is in line with other reports.

Indeed, concanavalin A has been shown to strengthen the bridges between MS and lipid membrane (Gokhale and Mehta, 1987). By contrast, in response to ligand binding glycophorin A has been shown to disassociate from the cytoskeleton of human erythrocytes (Knowles et al., 1994). Binding either a monoclonal antibody or its monovalent Fab fragment to the exoplasmic domain of glycophorin A of erythrocytes immobilized the receptor and rigidified the membrane. However, this immobilization and rigidification did not occur when antibodies were bound to a mutant form of glycophorin A lacking the cytoplasmic domain. These results, combined with here presented findings, imply that the site of the immobilization/rigidification lies within the spectrin skeleton and do not require receptor crosslinking on the outside surface of erythrocyte.

CONCLUSSION

The obtained results indicate that in contrast to concanavalin A, binding of WGA to human erythrocytes could sever the attachment bridges of under-membrane skeleton, such that involve glycophorin A.

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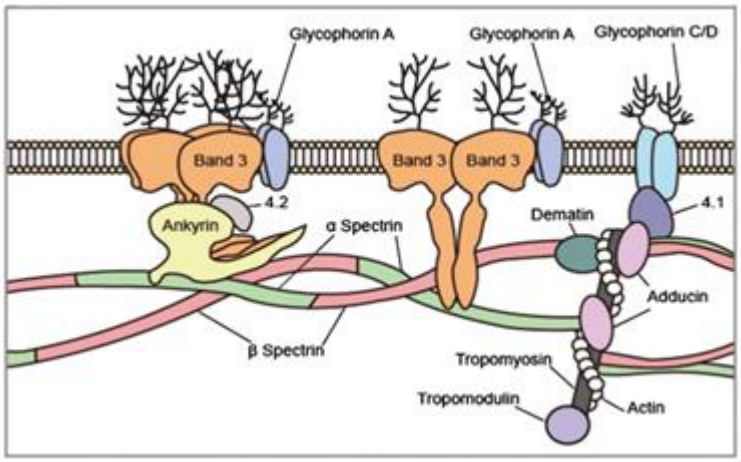

Fig. 1. Schematic cross-section of EM. Shown are the lipid membrane with its major integral proteins, band 3 and glycophorin C, and the under-membrane cortex with its major protein, spectrin.

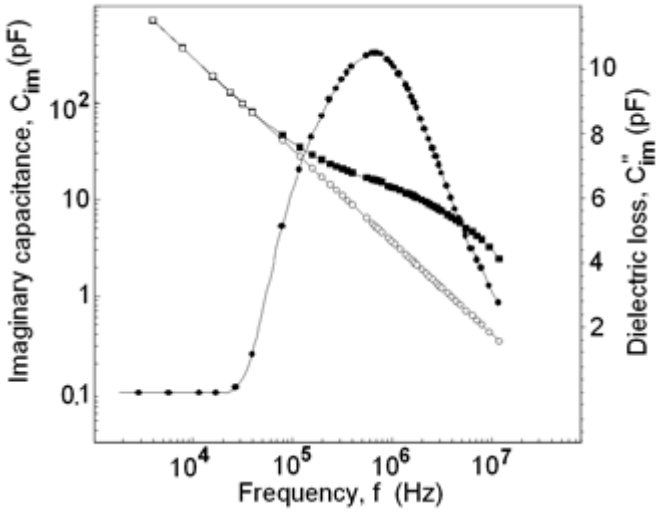

Fig. 2. Derivation of dielectric loss curve, C''_{im}, from the total energy loss rate, C_{im}, of studied erythrocyte suspension. Curved line (■) represents the frequency dependence of C_{im}, which includes conduction loss rate (o) and dielectric loss rate, C''_{im}, (●).

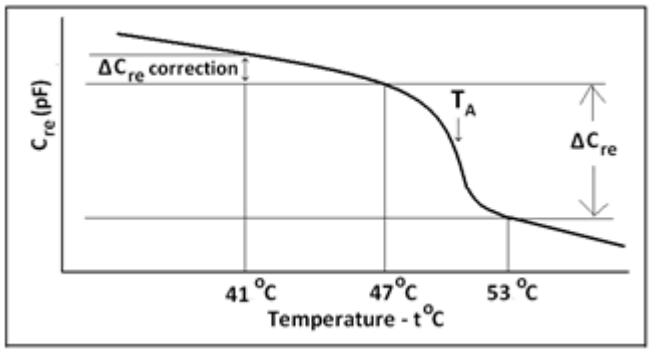

Fig. 3. Effect of spectrin denaturation temperature, T_A, on the thermal profile of real capacitance, C_{re}. The change in capacitance, ΔC_{re}, of erythrocyte suspension is shown at a single frequency only.

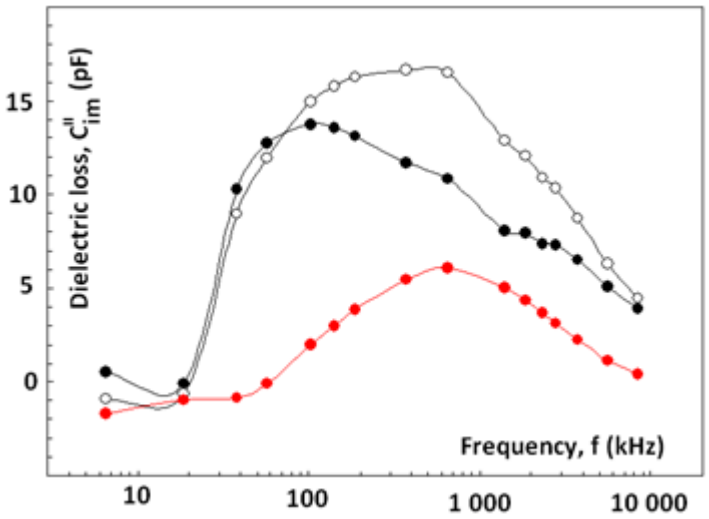

Fig. 4. Effect of temperature on the dielectric loss curve, C''_{im}, of erythrocyte suspension. Shown are the frequency profiles of C''_{im} at the temperature intervals 37-47°C (o) and 51-63°C (●). The subtraction of the latter curve from the former one gives the curve of dielectric loss curve, $\Delta C''_{im}$, released on the intact spectrin skeleton (🔴).

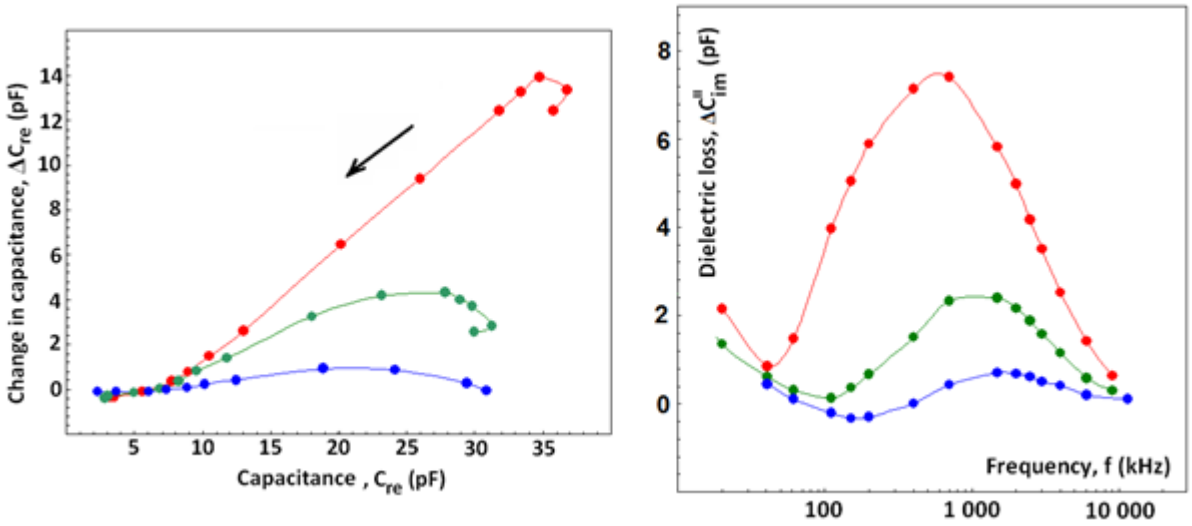

Fig. 5. Left: relationship between the suspension capacitance, C_{re} (pF), and its change, ΔC_{re} (pF), at the spectrin denaturation temperature. Arrow indicates the increase in the measuring frequency. Right: effect of WGA on the dielectric loss curve, $\Delta C''_{im}$, of spectrin. The suspension contained native erythrocytes (🔴), or erythrocytes treated with WGA at the concentration 0.125 mg/ml (🟢) and 0.250 mg/ml (🔵).