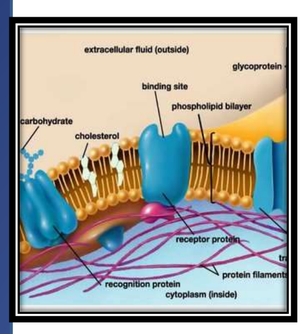
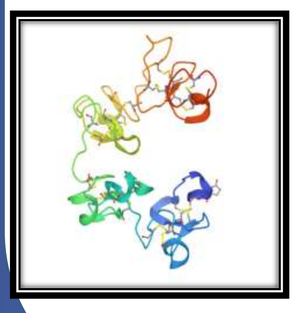
Membrane-protein biophysical interactions: Electrokinetic and light scattering studies in the presence of Polylysine and Wheat Germ Agglutinin in model membranes

Virjinia Doltchinkova, Department of Biophysics and Radiobiology,
Evgenia Sotirovska and Yoana Dikova , Faculty of Biology,
Sofia University “St. Kliment Ohridski”

Introduction



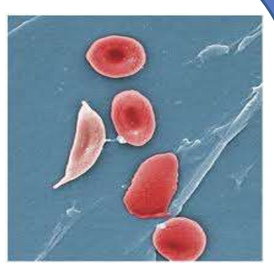


The mechanism of interaction between proteins and phospholipids in biological membranes has been shown to play an important role in the regulation of both the structural and dynamic properties of biomembranes and of the biological function of membrane proteins.

In an attempt to characterize the electrokinetic properties of human erythrocyte membranes and extrinsic proteins, the action of poly-L-Lysine (PL) and wheat germ agglutinin (WGA) to erythrocyte ghosts has been investigated. Polylysine-lipid molecular interactions are mainly due to the electrostatic binding between the polar headgroups of phospholipid and polylysine molecules, according to the literature data. Lectin (WGA) is often used as a biological probe for membrane stability, as well as for analyzing the surface components of the biological membrane with considerable potential to improve biomedical field (Balčiūnaitė-Murzienė and Dzikaras, 2021).

The determination of the electric charge and its changes after the lectin-membrane contact can serve as a manner to register changes in the erythrocyte membrane.

Methods



- The electrophoretic mobility (EPM) measurements are performed using microscopic (Visual) microelectrophoresis with a Cytopherometer (OPTON), using a rectangular cell and platinum electrodes. Zeta potential is calculated from EPM, using Helmholtz-Smoluchowski equation.
- Erythrocyte ghosts are isolated according to Burton et al. (1981) with modifications. Poly-L-Lysine (5 000-10 000) (Sigma) is added to the probes in final concentration of pg/ml. The electrophoretic mobility of intact and treated ghosts is determined in 5 mM Sodium Phosphate Buffer, pH 7.4, with conductivity (0,79 mS/cm). Lipid peroxidation assay is carried out with lyophilized ghosts with protein concentration of 7.3 mg/ml per probe .
- The volume changes of the erythrocyte suspension are studied by intensity of the light scattering at 90 degree and wavelength of 480 nm. For this purpose, Specol 10 Spectrophotometer (Carl Zeiss, Jena, Germany) with Ti attachment is used.
- The extent of lipid peroxidation is estimated by analysis of thiobarbituric acid-reactive substances produced (Halliwell and Gutteridge, 1989) with modifications.

Conclusion

- The electrostatic effect is significantly reduced in the presence of 20-60 ng PL/ml, accompanied by a sharp decrease in aggregation of erythrocyte membranes.
- Polyvalent ions of WGA have a strong electrostatic effect on the outer surface of erythrocyte ghosts at 0.20-2.0 pg WGA/ml with alteration on ζ potential. Higher dose of 2.0 pg/ml of WGA increases the electrokinetic potential of erythrocyte ghosts and decrease the aggregation values.
- No significant change in lipid peroxidation of the lyophilized erythrocyte membranes is demonstrated by Polylysine and Wheat Germ Lectin treatment.

Effect of Poly-L-Lysine and WGA on Zeta potential of Human Erythrocyte Ghosts

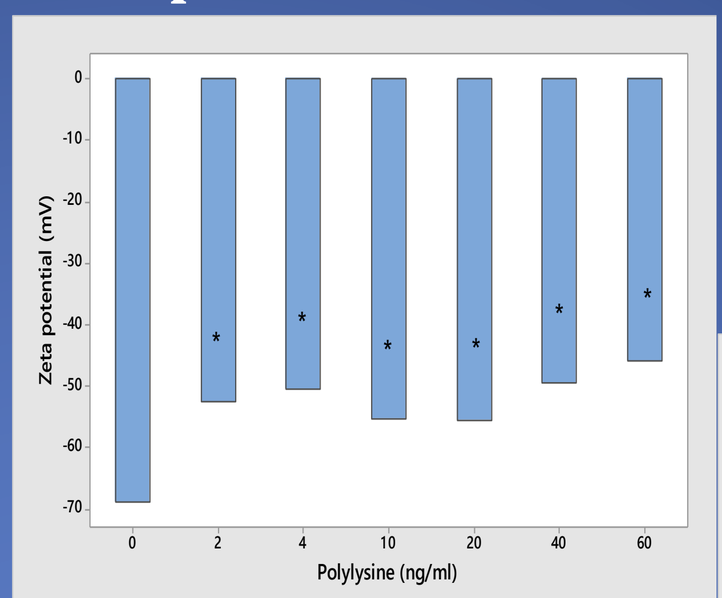


Fig. 1 Zeta potential of human erythrocytes treated with ***Poly-L-Lysine***. Medium contained Sodium Phosphate Buffer (5 mM sodium phosphate, Na_2HPO_4/NaH_2PO_4, pH 7.4). Data are means of 15-30 cells. Electrophoretic migration is measured at a constant electric field of 0.4 mA and 25 ^oC.

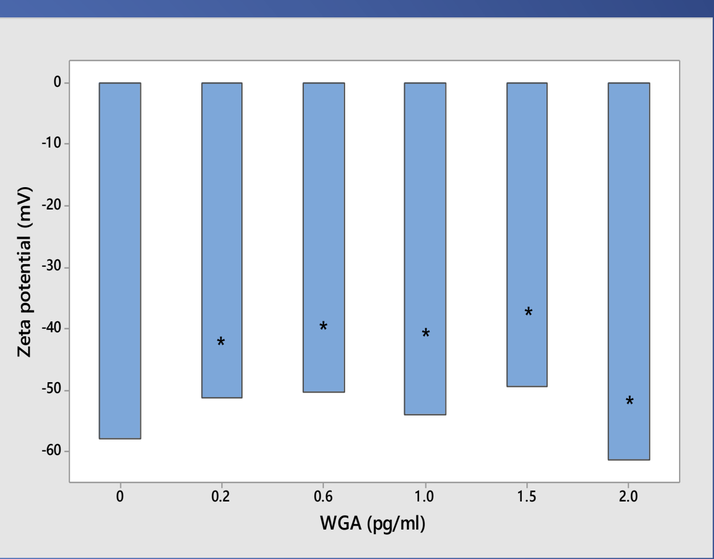


Fig. 2 The effect of Wheat Germ Agglutinin (WGA) on the zeta potential of human erythrocyte ghosts. The solution contained Sodium Phosphate Buffer (5 mM Sodium phosphate Na_2HPO_4/NaH_2PO_4, pH 7.4). Data are means of 15-30 cells.

Effect of Polylysine and WGA on Light Scattering of Human Erythrocyte Ghosts

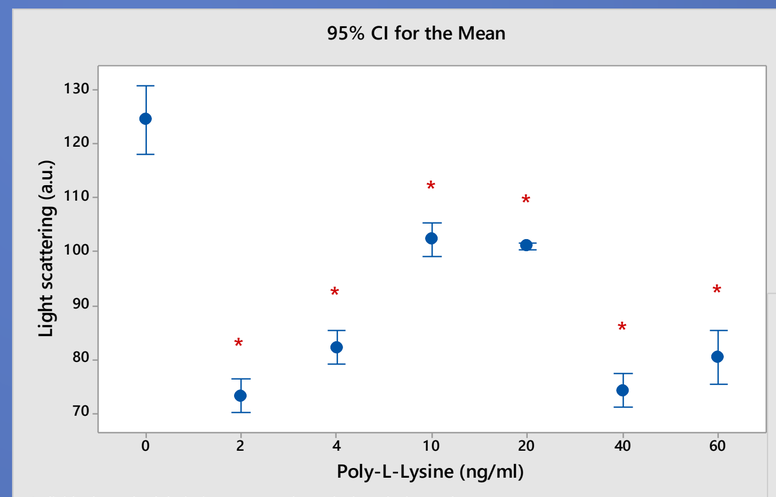
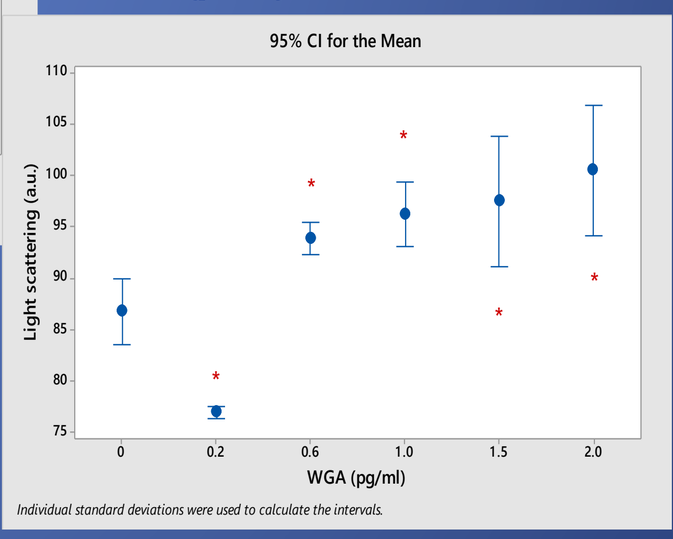


Fig. 3 Light scattering (arbitrary units) of human erythrocytes in the presence of various concentrations of **Poly-L-Lysine and Wheat Germ Agglutinin (WGA).** Erythrocytes are suspended in Sodium Phosphate Buffer (pH7.4).



Lipid peroxidation of lyophilized erythrocyte membranes in the presence of PL and WGA

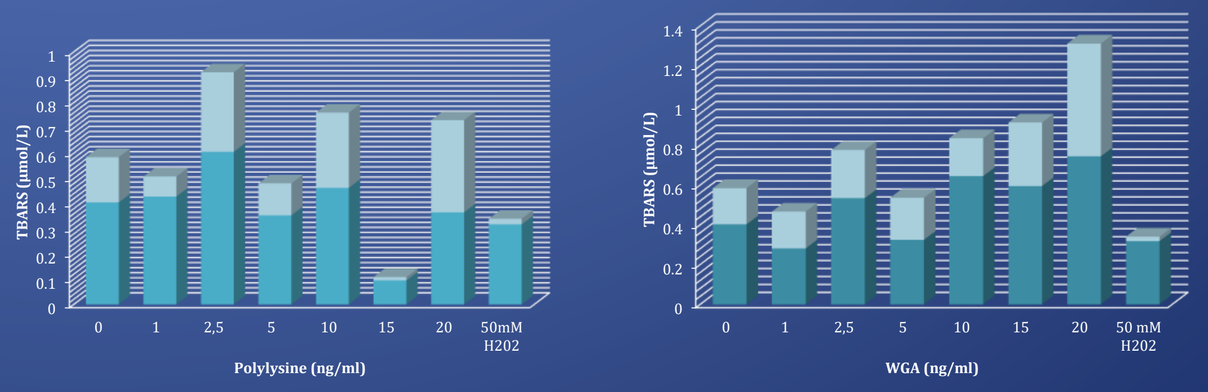


Fig. 4 Lipid peroxidation of lyophilized erythrocyte membranes pre-treated with the Poly-L-Lysine and Wheat Germ Agglutinin (WGA) after 30 min of incubation at 37 ^oC. Lyophilized ghosts were suspended in phosphate buffered saline (PBS: 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 0.2 mM KCl, pH7.4).

.

This work is supported by a Grant KP-06-N38/14/2019 from the Bulgarian National Scientific Fund (BNSF), Ministry of Education and Science of the Republic of Bulgaria. Thanks to the technical assistance of Nadya Hristova-Avakumova and Maria D. Misheva.