Do Changes in Cell Shape Affect Suspension Conductivity?

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Abstract. The conductivity of a suspension containing uniformly oriented asymmetric cells depends on the shape of the cells. Whether the shape of cells with random spatial orientation also affects the conductivity of a suspension is not clear. A highly sensitive apparatus was used to register the dynamic changes in conductivity of erythrocyte suspension, upon induced morphological transformation (discocytes $\rightleftarrows$ spherocytes). The results obtained with a sensitivity of up to 0.06% show that the drastic change of cell shape itself did not affect the suspension conductivity.

Key words: Suspension conductivity — Erythrocyte shape — Morphogenic agents

Introduction

The conductivity measurements of cell suspensions are used to investigate the electric parameters of cell membranes, which describe the structure and dynamic behaviour of these membranes. The electrical parameters of membranes provide a useful tool for the study of modifications in the overall arrangement of the membranes in pathological conditions or upon the action of various external agents (Bonincontro et al. 1989).

Several equations can be used to calculate the cell conductivity K_c from the two measurable variables (suspension conductivity K_s and conductivity of the suspension media K_m), (Flutak and Terlecky 1973). The most accepted relationship for this purpose has been the Maxwell – Wagner formula (Fricke 1953):

$$\frac{K_s - K_m}{K_c + f \cdot K_m} = p \cdot \frac{K_c - K_m}{K_c + f \cdot K_m} \tag{1}$$

where p is the suspension hematocrit and f is a dimensionless factor that mainly depends on the cell shape. According to this relation, the K_s value of a suspension

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containing only uniformly oriented (stratified) asymmetric cells depends on the shape of these cells; this makes the calculation of K_c difficult. Therefore, the form factor f is calculated and tabulated for cells with the shape of prolate ellipsoids of revolution (Fricke 1924) or it can be experimentally assessed for other cases (Rabinovich 1972). For oblate spheroids with a 1:4 axial ratio, $f = 1.5$, and for spherical cells $f = 2.0$ (Pauly and Schwan 1966).

Some authors accept that even if the cells are randomly oriented in space, the form factor f affects the value of K_s (Velick and Gorin 1940; Pauly and Schwan 1966). Others claim the opposite and provide experimental data in support. However, these data have been obtained with a low sensitivity (Buevich and Lisogorov 1965; Vransky and Nicolova 1971). The problem of a possible influence of the cell shape factor on the value of K_s and, therefore, on the calculated value of K_c, in real suspensions with randomly oriented cells has still remained unresolved (Miroshnikov et al. 1986). On the other hand, the impact of changes in shape of cells during prolonged tracking of the conductivity of a suspension (Ivanov and Lyutskanov 1988) needs to be estimated. Aiming at this, a conductometer with an increased sensitivity was used to register dynamic variations of erythrocyte suspension conductivity upon inducing reversible morphological transformations (discocytes $\rightleftarrows$ spherocytes).

Materials and Methods

Human erythrocytes were separated by centrifugation from citrated freshly drawn blood. To allow the red cells to assume spherical form, the cells were three times washed in excess volume of NaCl saline (Bessis 1956).

Prior to the conductometric measurements these spherocytes were suspended in the same NaCl saline, pH 7.0, at 0.20 hematocrit. The value of K_s was measured at 1 kHz with an LM - 301 conductometer (Hydromat DDR), equipped with an external generator. A sensitive microvoltmeter V–504 (Meratronic – Poland) with five digital readings was connected to the output of the conductometer. This layout strongly increased the sensitivity of the measurements, and changes in K_s as small as 0.06% were distinguishable. The conductometric cuvette was a vertical glass cylinder with a closed bottom. The platinum electrodes were fixed at 70 mm from each other. The cuvette was placed into a water bath kept at $37 \pm 0.1\,^{\circ}C$. The cuvette held 4.1 ml of suspension under continuous stirring during the measurements.

It has been established that cells in early stages of shape transformation do not change their volume (Deuticke 1968). Morphological transformations of the erythrocytes were induced during the measurements by adding a small volume of solution containing the appropriate morphogenic agent.

1. The spherocyte $\longrightarrow$ discocyte transformation was induced by adding 0.15 ml NaCl saline containing human albumin to a final concentration of 0.1% (w/v) (Jay 1975).

2. The discocyte $\longrightarrow$ spherocyte transformation was induced by addition of 0.15 ml NaCl saline supplemented with SDS (sodium dodecyl sulfphate) to a final concentration of 30 μmol/l. At this concentration, SDS transforms discocytes into smooth spherocytes

within 30 s without inducing volume changes or leakage of K^+ through the cell membranes (Isomaa and Paatero 1981).

The change of K_s upon mere dilution of suspension was assessed by adding 0.15 ml NaCl saline to every suspension. The suspension media (NaCl saline) and the solutions of the morphogenic agents had the same conductivity K_m, which was achieved by additions of tiny amounts of NaCl. Prior to the additions, all solutions were heated to 37 °C. The shape of the cells was observed microscopically at 650x. The hemolysis degree was measured spectroscopically at 415 nm, and no statistically significant changes could be established during the shape transformations.

Results and Discussion

The variations in K_s induced by the addition of the morphogenic agents in solution are given in Table 1. The cells underwent shape transformations within 3 min after the addition of the agent, as observed microscopically (data not shown). During the same time the solutions added were equally distributed within the suspension as could be established in a separate experiment using a dye. Also, the conductivity changed during this time interval, whereas subsequently it remained constant for more than 15 min.

Table 1. Dynamic changes of erythrocyte suspension conductivity produced by the addition of morphogenic agents (albumine or sodium dodecyl sulphate) dissolved in NaCl saline. The suspension hematocrit and volume were 0.20 and 4.1 ml. The volume of the added solutions was 0.15 ml. The changes in suspension conductivity were measurable with a sensitivity of up to 0.06% of the initial values. The conductivity changes are presented relative to the initial values. Means ± S.E.M. are given, the number of experiments are shown in the parentheses.

Initial cell shape	Agent added (final concentration)	Final cell shape	Change of suspension conductivity, %
Spherocytes	Albumine (0.1 %)	Discocytes	0.70 ± 0.07 (5) increase
Spherocytes	NaCl saline (control media)	Spherocytes	0.71 ± 0.08 (10) increase
Discocytes	S D S (30 μmol/l)	Spherocytes	0.69 ± 0.07 (8) increase
Discocytes	NaCl saline (control media)	Discocytes	0.70 ± 0.07 (10) increase

Table 1 shows that the addition of the solutions of both agents increased the value of K_s by about 0.70 % of its initial value, independently of the cell shape transformation. In both cases, the addition of control media gave the same increase in K_s, which was obviously due to the dilution of the suspension alone, since it was

not accompanied by any change in the cell shape (not shown). The conductivity of the suspension media K_m remained unchanged upon the addition of any of the probes or media shown in Table 1. This was achieved by preliminary equalization of all conductivities.

Considering that at low frequencies the cell conductivity K_c is more than ten times smaller than the value of K_m (Bothwell and Schwan 1956), relation (1) is usually reduced to:

$$K_s = K_m \cdot \frac{1-p}{1+p/f} \tag{2}$$

It may be calculated from (2) that dilution alone of the suspension by 0.15 ml media increases K_s by 0.75% which is close to the experimentally obtained value. If the form factor f affects the value of K_s of a suspension of disorderly oriented cells, equation (2) can be used to calculate the change in K_s due to change in f from $f = 1.5$ (discocytes) to $f = 2.0$ (spherocytes). This gives an increase in K_s by 2.5 % after discocyte $\longrightarrow$ spherocyte transformation or equal decrease after inverse transformation. These variations in K_s are three times greater than the conductivity changes due to the dilution alone (Table 1).

A dynamic approach was used here to verify the possible impact of a rapid simultaneous change in cell shape alone over the instant value of the suspension conductivity. The results obtained with erythrocytes show that reversible transformation of cell shape does not influence, in measurable terms, the conductivity of a suspension containing large numbers of disorderly oriented asymmetric cells.

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Final version accepted June 25, 1993