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THERMOTROPIC BEHAVIOUR OF INTACT HUMAN ERYTHROCYTE MEMBRANES REVEALED BY DIFFEREN- TIAL SCANNING CONDUCTOMETRY

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Abstract The method of differential scanning conductometry (DSC) was applied for investigation of thermoinduced phase transitions in membrane of intact human erythrocytes. This method was found to be sensitive to all phase transitions in erythrocyte membrane studied until now in the interval 5-53°C. Some new results were obtained. The point transition at 18-20°C is discovered to be a duplex one and insulin sensitive. The spectrin denaturation at 48-50°C gives a powerful peak at the end of the thermogram.

INTRODUCTION

The gel-liquid crystal phase transitions in cholesterol depleted membrane of human erythrocytes have been studied by many methods (1)-(4) because of their importance for our knowledge of membrane structure and its biological activity.

There are quite a few methods, however, for such an investigation of intact cells. Our experiments have shown that the method of differential scanning conductometry (DSC) presents essen-

tial information for the thermotropic behaviour of their membranes.

MATERIALS AND METHODS

We have used the DSC for temperature scanning of the cell conductivity K_c towards the electrical conductivity K_m of the suspension medium. This method combines high sensitivity with temperature independence of the measurement from the usual processes, taking place in the electrolyte solutions.

The cell conductivity K_c has been computed by the Maxwell-Wagner equation

$$\frac{K_s - K_c}{K_s + 2K_c} = p \cdot \frac{K_m - K_c}{K_m + 2K_c}$$

where K_s is the cell suspension conductivity, and p is the cell volume density (5). The DSC method may be applied in two ways: scanning the K_c/K_m over the temperature interval, or scanning the relative temperature rate of conductivity

$$\frac{dK_s/dT}{dK_m/dT} = \frac{dK_s}{dK_m} \approx \frac{\Delta K_s}{\Delta K_m} = \frac{K_s(T) - K_s(T-1)}{K_m(T) - K_m(T-1)}$$

over the same temperature interval. Here T is temperature. The two ways give identical results.

When the frequency is low the electrical current bypasses the cell membrane in the frame of the cell electrical double layer. In such a case K_c is called cell surface conductivity and one can expect that this conductivity may be sensitive to some biophysical phenomena, taking place in or near the cell membrane. Experimentally we

have confirmed that the temperature curve of the relative surface conductivity K_c/K_m is sensitive to the phase transitions in the intact human erythrocyte membranes.

The suspension of fresh, twice washed human erythrocytes was incubated at 25°C for one hour and then overnight at 4°C for inhibition of the active transport and establishment of Donan equilibrium. Conductivity of both the suspension and medium was measured with high sensitivity at 75 Hz. The temperature was scanned with 0.5°C per min., and measured with 0.05°C accuracy. During the heating the suspension was slowly stirred. The most favourable volume density was 0.2.

The suspension medium was 0.145 M NaCl in ddH₂O with no buffers or other substances, especially surfactants, in order to avoid adsorption on the cell membranes.

The washing procedure is also important. First we mixed equal amounts of fresh blood with 2% solution of sodium citrate in 0.145 M NaCl. After free sedimentation the plasma was removed. Then the erythrocytes were twice washed in the medium for 10 min by 500 × g. If the washing is done 5 times or more only the last transition may be observed.

RESULTS AND DISCUSSIONS

All cited and investigated so far thermoinduced transitions in human erythrocyte membranes (at 8-12°C, 18-20°C, 28-32°C, 40-43°C and 48-50°C)

are registered on our thermogram, shown below.

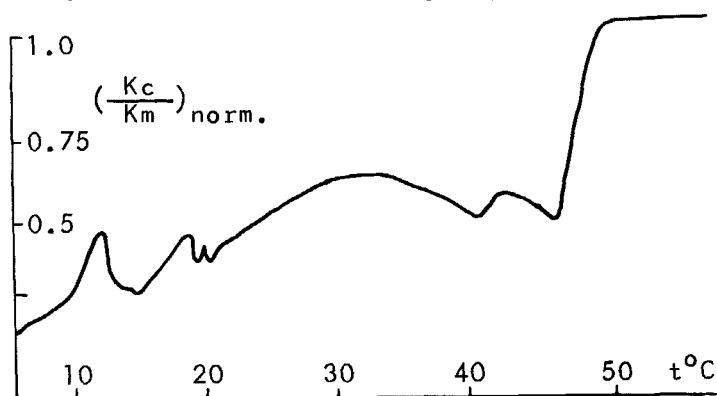


FIGURE 1. DSC thermogram of phase transitions in membranes of intact human erythrocytes.

The water transport activating transition at 8–12°C is very well expressed. It presents gel-to-liquid crystal transition in free lipids. The same transition in the anular lipids occurring at 18–20°C is followed by activation of many enzyme and transport processes in the erythrocyte membrane (3). In (2) it is pointed out that the rate of glucose transport across erythrocyte membranes sharply increases at 20°C.

On our thermogram the well known point transition at 18–20°C (3) is expressed as a duplex one. Our experiments have shown that the height of its two peaks increases several times if the suspension medium contains insulin at concentration about 0.2 µg/ml during the first incubation. Also the distance between two peaks increases

after insulin treatment from 2°C to 4-5°C. The other peaks are not touched by the presence of insulin.

Probably the two peaks of 18-20°C transition are connected with conformational transition of glucose transport mechanism in the erythrocyte membranes, which is activated both by the temperature (2) and by the insulin-receptor interaction.

We have got some results pointing out that the transition at 8-12°C may be sensitive to the presence of human albumin, probably due to cholesterol depletion of erythrocyte membranes.

The transition at 48-50°C, corresponding to the spectrin denaturation (4) is the most powerful. This implies influence of the inner membrane surface proteins on the cell electrical double layer.

The last two weak transitions at 28-32°C and 40-43°C are also present by broad and weak peaks about the same temperatures. This two transitions occur in the protein phase of the erythrocyte membrane.

These results are pointing out that the method of DSC may be applied for investigation of thermotropic behaviour of the membranes of intact human erythrocytes.

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