Thermal Behaviour of Lymphocyte Membrane: ESR Investigation

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The order parameter, *S*, of the plasma membrane of *in toto* human peripheral blood lymphocytes was obtained by electron spin resonance spectroscopy in the temperature range 25–41 °C. This membrane is completely in the liquid crystalline state above 31 °C. In presence of the antigen ETB from *Staphylococcus aureus* at the concentration of 4 μg/3×10^7 cells an overall decrease of the order parameter for this membrane is observed.

The decrease of *S* is followed by an upwards shift at about 35 °C of the temperature of the liquid crystalline state.

Introduction

The structural properties of plasma membrane of human lymphocytes and the induced effects of several antigen substances like Con-A, PHA, coleric toxin have been widely investigated at the physiological temperature of 37 °C [1–4].

Using ESR spectroscopy [2, 5–8] and different spin labels it was shown that exposure of cells to mitogenic agents causes the increase of the membrane fluidity [9–11].

Nevertheless, none of these studies dealed with the characterization of the physical state, *i.e.*, gel or liquid crystalline like, of the plasma membrane of lymphocytes at 37 °C. In this note we report on the thermotropic properties of this cell membrane and on induced ETB mitogenic antigen effects.

Materials and Methods

Human peripheral blood lymphocytes were obtained from single donors by the method of Boyum [12].

The best preparation contained 10^6 small lymphocytes/ml. Before spin labeling cells were washed twice and suspended in the RPMI 1640 medium without serum to avoid the binding of spin labels to serum proteins. For the spin labeling operation the cell concentration was increased to 3×10^7 cells/50 μl. The 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (5-NSA) spin label was purchased from Aldrich and stored at 4 °C as a 3.2×10^{-2} M ethanol solution. To spin label the plasma membrane, the needed volume of spin label solution was placed in a small vial and the solvent evaporated with a stream of dry N_2. Afterwards, 50 μl of concentrated lymphocyte suspension were added and the vial shaken at 20 °C for 10 min. When needed, 4 μg of ETB from Staphylococcus aureus (Sigma product) in PBS were added to the spin labeled cells.

ESR measurements were carried out immediately after sample preparation since reduction of the signal intensity was observed with time and temperature increase (paper in preparation). Care was used to prepare a new sample for each experiment.

ESR spectra were recorded with a Bruker ER 200 D-SRC X-band spectrometer equipped with the ESP 1600 Data System and the ER 4111 VT variable temperature control unit (accuracy ± 0.3 °C) in the temperature range 25–41 °C. Samples were inserted in sealed off capillary tubes accomodated within standard 4 mm quartz ESR tube containing silicon oil to avoid temperature gradient and were positioned in the center of a TE_{102} ESR cavity. To avoid dipolar line broadening the molar concentration between spin label and membrane lipids was held at 1:300. The concentration of 4×10^{-15} M lipids/cell was also used [2].

Abbreviations: Con-A, concanavalin A; PHA, phytohaemagglutinin; ETB, staphylococcal enterotoxin B; PBS, phosphate buffer saline; 5-NSA, 5-nitroxide stearic acid; ESR, electron spin resonance; *S*, order parameter.

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Results and Discussion

In the inset of Fig. 1 the ESR spectrum at 25 °C of 5-NSA located into the plasma membrane of human lymphocytes is shown.

It looks like a powder spectrum characteristic of nitroxide spin labels undergoing tumbling in the slow motional regime, *i.e.*, with rotational correlation time $\tau_c > 10^{-8}$ s. The separation between the outer and inner resonance lines, related to the parallel and perpendicular, $T'_{//}$ and $T'_{\perp}$, components of the motionally averaged axial symmetric nitrogen hyperfine T-tensor were evaluated. These experimental values can be reconduced to the order parameter, S, of the lipid matrix of the plasma membrane by the relation [13, 14]:

$$S = \frac{(T'_{//} - T'_{\perp})}{T_{zz} - (T_{xx} + T_{yy})/2} \cdot \frac{a_{N\times 1}}{a_{Ns}}$$

where T_{zz}, T_{yy} and T_{xx} are the components of the T-tensor for the same label in crystal while, $a_{N\times 1}$ and a_{Ns} are the isotropic hyperfine coupling constants for the label in crystal and membrane, respectively.

The S-value varies from 0.72 at 25 °C to 0.58 at 41 °C. Moreover, when the S-values are plotted *vs.* temperature a single discontinuity is found in the linear regression analysis at about 31 °C. Such a temperature, of course, is not the temperature of the gel → liquid crystalline phase transition of the lymphocyte membrane but, rather, it seems to be the temperature from which the membrane is completely in the liquid crystalline state. In this state, a high degree of rotational isomers of the lipid hydrocarbon chains very likely exists [15]. In fact, real membrane systems show only seldom a single sharp phase transition and the phase changes occur generally over a wide temperature range [16]. This diffusiveness is due to the complex and heterogeneous composition of the membrane system.

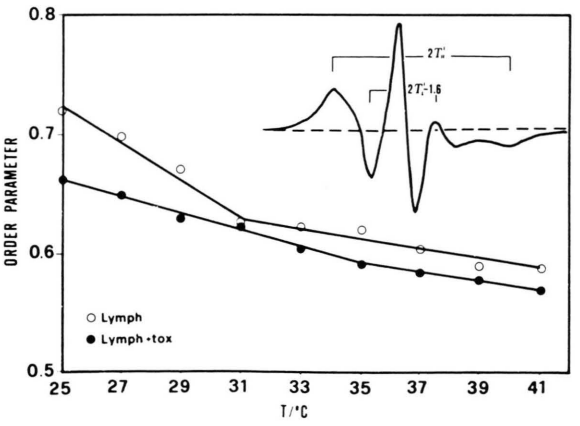

Fig. 1. Order parameter *vs.* temperature of plasma membrane of (−○−) normal and (−●−) ETB treated human lymphocytes. Each point represents the mean value of three measurements with a S.D. of less than 1% of the value indicated. In the inset the powder-like ESR spectrum recorded at 25 °C with its parallel and perpendicular, $T'_{//}$ and $T'_{\perp}$, hyperfine components is shown. The experimental set-up was: 10 mW microwave power, 1.0 G_{pp} modulation amplitude, 1.28 ms time constant. The ESR signal is the average over 20 scans taken with the ESP 1600 Data System.

It is noteworth that the value of ≅ 31 °C found for the fluid state of plasma membrane of human lymphocytes is different from the ones of ≅ 33 and ≅ 38 °C found for *in toto* red blood cells [17] and auxotropic mutants of *E. coli* [18]. Of course, this low value should be related to the function of this membrane which is known to reorganize its lipid components in presence of antigens to form receptor structures [11].

The effect of ETB antigens [19] on thermotropic properties of lymphocyte plasma membrane is shown in Fig. 1. The ESR measurements show that in presence of 4 μg toxin/3×10^7 cells the order parameter is lower than in untreated cells for each investigated temperature. Moreover, the temperature of the crossing point of the two straight lines is shifted upwards to ≅ 35 °C.

At present we only suggest that the reduction of the S-value observed in presence of ETB could be due to the perturbation of electric interactions and/or hydrogen bond network occurring on membrane surface when the formation of the ETB-receptor complexes takes place. These processes seem also to be accompanied by lipids redistribution in the bilayer. In fact, the temperature increase of the fluid phase of the membrane suggests that 5-NSA investigates the hydrogen belt region of lipid domains with chemical composition and structural properties different from those observed in untreated cells.

This hypothesis is in agreement with Curtain's results on the clustering of glycosphingolipids during activation of lymphocytes [3, 9]. Such a clustering, which with glycoproteins form the specific receptor, induces, at the same time an enrichment of

the other region of the membrane in remaining lipids. Moreover, the value of the S parameter at 35 °C, *i.e.*, at the liquid crystalline state of the membrane, corresponds very closely to the one obtained at the same temperature in vesicles made with 1,2-dipalmitoyl, sn-glycero-3-phosphocholine [20]. This fact, gives the best evidence that when ETB interacts with lymphocyte membrane receptors, phosphocholine, *i.e.*, the most abundant phospholipid in plasma membrane of human lymphocytes [21], it segregates to form domains. ETB-receptor complexes are very likely embedded in these domains.

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