A CORRELATION BETWEEN MEMBRANE FLUIDITY AND THE CRITICAL TEMPERATURE FOR CELL ADHESION

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The temperature dependence of cell adhesion is a well-known phenomenon seen, for example, in the neuroretinal cells of chick embryos (14, 15, 27) and in cultured chick fibroblasts (28). In addition, physicochemical studies of lipids in biological membranes mainly by means of the spin-label method have revealed dynamic aspects of the lipid structure: anisotropic motion (5), lateral diffusion (9, 25) and thermotropic (26), and ionotropic (6, 16) lateral phase separations. Data which suggest a correlation between cell adhesion, as measured by cell-substrate adhesion, and physicochemical characteristics of the cell membrane, as revealed by the spin-label method, are presented in this paper.

MATERIALS AND METHODS

Measurement of Cell Adhesion

BHK 21 cells in the confluent state were harvested by treatment with trypsin (0.25%, Difco Laboratories, Detroit, Mich., 1:250) for 15 min at 37°C, and washed three times with cold Ca^{2+}- and Mg^{2+}-free modified Puck's saline G (CMFS) (21). The cells were finally suspended at a density of 2×10^5 cells/ml in 3 ml of the assay medium in Falcon plastic dishes (no. 3002, Falcon Plastics, Div. of BioQuest, Oxnard, Calif.), which had been coated with gelatin (0.5%, Merck Chemical Div., Merck & Co., Inc., Rahway, N. J.). The assay medium was Ca^{2+}- and Mg^{2+}-free modified Eagle's MEM (CMF-MEM) buffered at pH 7.4 with 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and

containing 2 mM Ca^{2+} or Mg^{2+} (29). Before addition of the cells, this medium was adjusted to the various temperatures. After an appropriate time, the dishes were gently shaken and the cells were fixed with 2 ml of a 5% glutaraldehyde solution. Further attachment or detachment was not caused by this fixation (29). Unattached cells were counted with a Coulter counter (model Z_B, Coulter Electronics Inc., Hialeah, Fla.) (18). The number of attached cells was obtained by subtracting the measured number of unattached cells from the total.

Measurement of Membrane Fluidity

In the present investigation, membrane fluidity was monitored by measuring the overall splitting of the ESR spectrum of a stearic spin label, 5-SAL (4',4'dimethyloxazolidine-*N*-oxyl derivative of 5-keto stearic acid). The spin label was synthesized by the method of Jost et al. (7). BHK cells at the confluent state in Falcon plastic dishes were washed three times with CMFS. Then, 3 ml of CMF-MEM containing 2 mM Ca^{2+} or Mg^{2+} and 30 μl of an ethanol solution of 5-SAL (2 mM) were added, and the cells were incubated for 10 min at 37°C on a gyratory shaker (model G-25, New Brunswick Scientific Co., New Brunswick, N. J.). The spin-labeled cells were washed three times with cold CMFS, scraped loose with a piece of silicone rubber in cold CMF-MEM (50 mM HEPES) containing 2 mM Ca^{2+} or Mg^{2+}, and collected by centrifugation (160 *g*, 4 min).

ESR spectra were measured with a commercial X-band spectrometer equipped with variable temperature accessories (JEOLCO model ME-2X). The temperature was measured by a thermocouple inserted into the center of the cavity. The thermocouple reading was taken separately from the spectral recording. The magnetic field scan was calibrated by the signal of Mn^{2+} in MgO.

RESULTS

Temperature Dependence of Cell Adhesion

The result of assaying the temperature dependence of cell adhesion to substrate coated with gelatin is shown in Fig. 1. When the assay was made 3 h after inoculation in CMF-MEM with 2 mM Mg^{2+}, the number of cells attached to the plastic substrate increased from 10 to 90% in the temperature range from 4°C to 24°C. In 2 mM Ca^{2+}, the cells did not adhere below 8°C, in contrast with the case in Mg^{2+}. Though the percentage of unattached cells was varied in the other two experiments, the fact that the cell attachment did not occur below 8°C was well confirmed by these experiments. Cell adhesion occurred at temperatures higher than 12°C, the number of attached cells increasing with the rise in temperature.

In order to test whether the temperature between 8°C and 12°C (t*) is the critical one to permit cell adhesion in the presence of Ca^{2+}, the number of unattached cells was counted at various times after inoculation (Fig. 2). More cells ad-

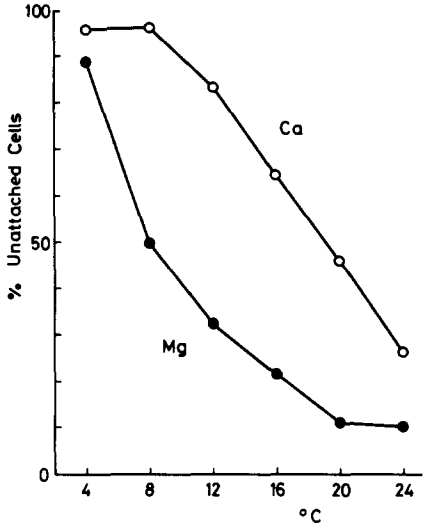

FIGURE 1 Cell adhesion to the gelatin-coated plastic dish at various temperatures. To obtain the number of attached cells, unattached cells were counted 3 h after inoculation in the presence of either 2 mM Ca^{2+} or 2 mM Mg^{2+}. Both experiments were carried out with cells from the same batch. Each circle indicates an average of two measurements giving essentially the same value.

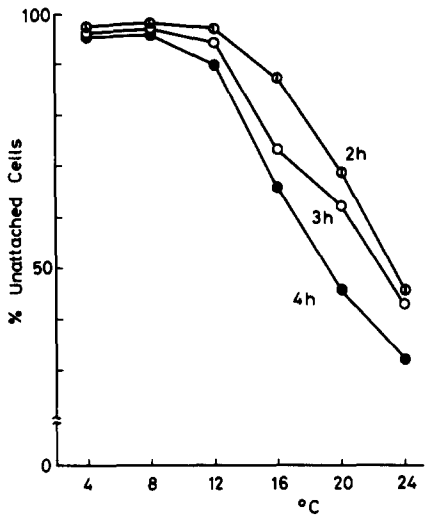

FIGURE 2 Cell adhesion in 2 mM Ca^{2+} at different temperatures 2 h, 3 h and 4 h after inoculation. At each time there is a break at t* (see text).

hered with the increase of the incubation time at temperatures above t*, while hardly any adhered below t*, irrespective of the incubation time. Although the percentage of unattached cells was varied in three separate assays so far conducted, the presence of a critical temperature (t*) was reproducibly recognized in all cases. Microscope observation confirmed that no cell adhesion occurred below 8°C at any incubation time.

Cells previously incubated at 4°C for 3 h in Ca^{2+} attached to the substrate when the medium was warmed to 37°C, indicating that they were viable and had the potentiality of adhesion after such treatment with low temperature.

Temperature Dependence of Membrane Fluidity

The ESR spectrum of BHK cells spin-labeled with 5-SAL showed the characteristics due to the label undergoing anisotropic rotational motions (Fig. 3). This indicates that 5-SAL was incorporated into the lipid bilayer of the cells. The overall splitting, which is equal to twice the parallel principal value $2T_{\parallel}$, was used to monitor the membrane fluidity. A decrease in the splitting value represents an increase in the membrane fluidity. At least three spectra were recorded at each temperature to determine the splitting value, and more than five independent experiments were carried out to obtain the temperature dependence of membrane fluidity. Fig. 4 shows an example of the plot of the overall splitting against the reciprocal temperature for the whole cells in the presence of 2 mM Ca^{2+}. There are breaks at about 10°C, 20°C and 30°C, where the slope of the curve changed. The presence of these breaks was confirmed in four other experiments. This is equivalent to changes in the temperature coefficient of membrane fluidity and may correspond to some change in the physical state of membrane lipid at the characteristic temperatures.

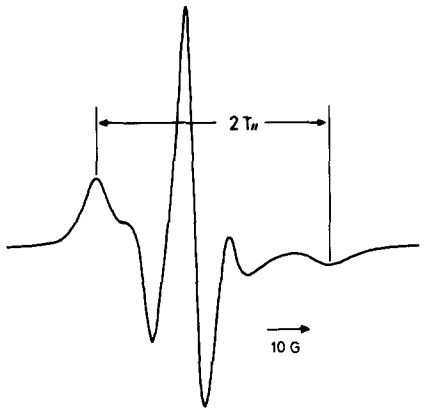

FIGURE 3 ESR spectrum of 5-SAL incorporated into BHK cells in 2 mM Ca^{2+}. The spectrum was measured at 23°C. The overall splitting value, $2T_{\parallel}$, was used to monitor the membrane fluidity.

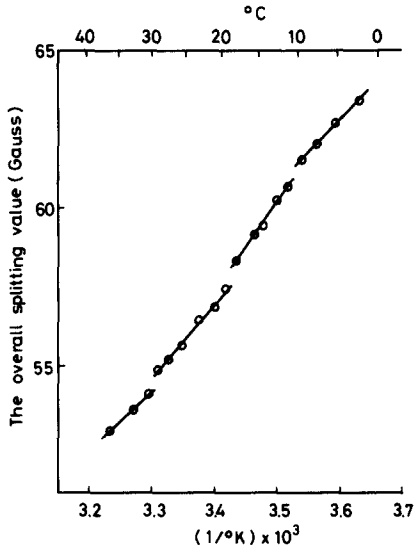

FIGURE 4 The overall splitting of the ESR spectrum of 5-SAL incorporated into BHK cells against a reciprocal of the absolute temperature (1/T). The size of the experimental points represents an approximate limit of error.

The temperature dependence of the spectral parameter in the presence of 2 mM Mg^{2+} was essentially the same as that in the presence of 2 mM Ca^{2+}.

Since the whole cell was spin-labeled in the present experiment, a possibility remains that the ESR signal is not due solely to the label in the cell surface membrane, but also to label incorporated in the membranes of other organelles. Surface membranes of BHK cells were isolated by the method of Lelievre (11). The fraction isolated was checked for purity using 5'-nucleotidase (EC 3.1.3.5) as a marker for surface membrane. Isolated surface membranes labeled with 5-SAL indicated the same ESR spectrum as that of the whole cells shown in Fig. 3.

DISCUSSION

The present results show that the critical temperature (t*) allowing the attachment of BHK cells in 2 mM Ca^{2+} to protein-coated plastics coincided well with one of the characteristic temperatures

(10°C) for the membrane fluidity change of the cells, and suggest the possibility that the physical state of the membrane lipid can be one of the governing factors permitting cell adhesion. Although further studies are required to clarify the molecular mechanism underlying the correlation, some speculations can be made at present. On raising the temperature above 10°C, there occurs some change in the physical state of the membrane lipid. This change may increase the flexibility or deformability of the cell membrane (1, 10), which facilitates cell spreading or pseudopodia formation. It has been pointed out that cellular motility may be a critical factor in cell adhesion (2, 17, 19, 22, 28). The change in the physical state of the lipids may also affect the insertion (13) and distribution (3, 4, 8, 20, 23) of proteins in the membrane, and the activity of membrane-bound enzymes (31). These molecular events would also be relevant to cell adhesion (12, 24, 27, 30).

The correlation between the characteristic temperature for cell adhesion and membrane fluidity was not observed in the presence of Mg^{2+}. Since the two divalent cations, Ca^{2+} and Mg^{2+}, are considered to act in qualitatively different ways in cell adhesion (27, 29), it is probable that Mg^{2+} is effective in cell adhesion in such a manner that is independent of the change in membrane fluidity at about 10°C.

SUMMARY

BHK 21 cells can adhere to a protein-coated plastic dish in the presence of Ca^{2+} at temperatures above 12°C. However, they cannot adhere below 8°C. The ESR spectrum of cells spin-labeled with a stearic acid label indicated that the membrane fluidity changed characteristically at 10°C, 20°C, and 30°C. The critical temperature for cell adhesion coincided well with one of the characteristic temperatures for the membrane fluidity change. In the case of adhesion in the presence of Mg^{2+}, no such correlation was observed.

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