Title: The effect of elevated temperature on the aggregation of African green monkey kidney cells.

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Abstract

The aggregation kinetics of African green monkey kidney cells CV1 and of the SV40 transformed derivative COS1 cells that had been incubated at 37°C or 43.5°C was studied using the shaking flask system. COS1 cells show a three fold decrease in aggregation rate compared to CV1 cells when both cell types were incubated and aggregated at 37°C. When these cell types were incubated at 43.5°C for 5 hours, then aggregated at 37°C showed a faster aggregtaion kinetics than before. Their aggregation at 43.5°C with prior incubation at 37°C or 43.5°C reached the aggregation kinetics of 43.5°C incubated cells aggregated at 37°C. The addition of serum in the aggregation medium did not influence extensively the aggregation rates of both cell types.

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INTRODUCTION

During the development of an animal the relationships among cells, such as cell adhesion, change extensively. Alterations in cellular adhesiveness reflect changes in cell surface and mutual cell behaviour. Such alterations have been studied in the embryonic as well as in the adult stage of multicellular animals (Collins, 1974; Roos, 1984).

The adhesiveness responsible for aggregation is a property of the cell surface which survives the dispersal procedure (Edwards and Campbell, 1971) and this may give some insight in the mechanism by which the cells adhere in vivo. As noted by others (Edwards and Campbell, 1971; Gingell and Vince, 1980; Lackie and Smith, 1980), cell adhesiveness is temperature dependent (for further discussion see Curtis, 1973). Different cell types do not aggregate at $4^{o}C$ while they do at $37^{o}C$. To our knowledge, however, how elevated temperatures affect the aggregation of cells has not been reported in the literature.

In recent years, elevated temperatures have been applied to malignant tissues in combination with radiotherapy (Scott et al, 1983). Such elevated temperatures subject these malignant tissues as well as their neighboring normal ones to a stress. The result of this stress is the induction of the expression of a set of genes named " heat shock genes " (Lindquist, 1986; Schlesinger et al, 1982).

Our study aims to investigate whether the adhesive properties of normal and transformed cells are altered at elevated temperatures and how this affects their mutual relationships.

MATERIALS AND METHODS

African green monkey kidney cells CV1 clone MA2, and SV40 transformed CV1 cells, COS1 TR8 (Gluzman, 1981) were used in this study. Subconfluent cultures on glass petri dishes, 10 cm I.D., in Dulbecco's modified minimal essential medium supplemented with 7% new born calf serum (Gibco, U.K.) were used to study the adhesive properties of these cells. The cells before being applied to the aggregation system were cultured either for 48 hours at 37°C or after this period the petri dishes were transferred to 43.5°C for 5 hours in a humid chamber with 5% CO_2 atmosphere. The cells were dispersed from the petri dishes using trypsin/versene and finally suspended in Hank's HEPES balanced salt solution pH 7.4, in the absence or presence of 7% new born calf serum at a concentration of $1x10^6$ cells per ml. Care was taken so that cell aggregation begins with single cell suspension.

Cell suspension (4ml) was transferred in siliconized, stoppered 10 ml conical flasks. A reciprocating shaker bath, controlled at 92 strokes per minute, was used to study the initial cell aggregation (Curtis and Greaves, 1965). The water temperature was controlled either at 37°C or 43.5°C (±0.1°C). Samples were removed from the flasks at specific time intervals and the remaining single cells and cellular aggregates were counted with a haemocytometer. The viability of the aggregating cells was examined at the beginning and at the end of the aggregation period using trypan blue. In all cases, their viability was found over 98% after haemocytometer counting.

RESULTS

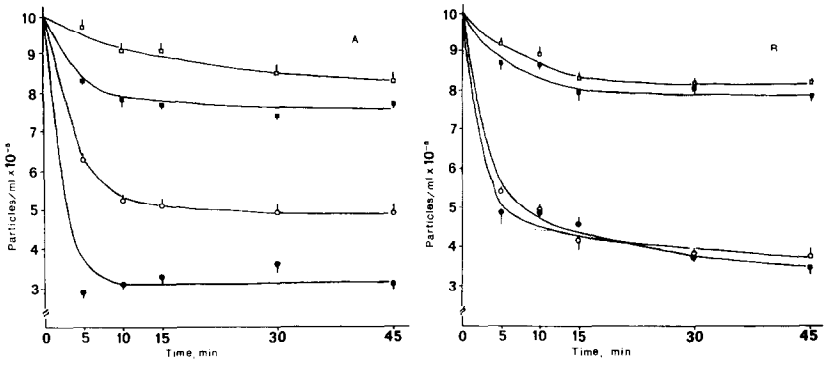
The incubation and aggregation temperatures of 37°C and 43.5°C are selected because they constitute the physiological and a stressing temperature for animal cells. For CV1 and COS1 cells, the 43.5°C and the 5 hrs stressing period constitute a severe stress that it is a period where the cells remain still undamaged. In these cells, the heat shock proteins during this incubation period at 43.5°C are still at their inducible stage (Angelidis et al, 1988).

Studying the aggregation kinetics of CV1 and COS1 cells, growing at 37°C, as a measure of their adhesiveness, we observe a fast and extensive aggregation for CV1 cells while COS1 cells aggregate at a gradually reduced rate (Fig. 1A). These aggregation kinetics represent the adhesive potential of CV1 and COS1 cells in Hank's HEPES without any external additives followed over a 45 minutes period. We used HEPES as buffer in order to avoid any extensive pH changes during the aggregation period. The observed aggregability of COS1 cells is at least three times lower than that of CV1 cells. We counted the different classes of cellular particles, single cells and cellular aggregates, to exclude the possibility of cell death as a factor for reduction in absolute particle number over the aggregation period. As it is seen in histogram I, the number of single cells decreases as the number of cellular aggregates increases. In CV1 cells these cellular aggregates consist mainly of over five cell particles (His. IB) while in COS1 cells the two cell particles predominate (His. ID). Calculating approximately the total cell number at any time we find that the incorporation of cells into aggregates is the only

parameter that accounts for the reduced number of cellular particles.

The incubation of both cell types at 43.5°C results in higher and faster aggregation rates than that at 37°C (Fig. 1A). Although COS1 cells appear with increased aggregability when incubated at 43.5°C compared to normal temperature, their kinetics show an almost three fold decrease compared to CV1 cells. This increased aggregability of cells incubated at higher temperature is under further investigation. Histogram I shows that even at this higher incubation temperature, the incorporation of cells into aggregates and not cell death is the result of the increased aggregation rates at 37°C for both cell types incubated at 43.5°C. An inportant observation is that the size of cellular aggregates of CV1 cells is so large that they are visible by naked eye in the flasks where aggregation takes place. The size of COS1 cell aggregates was larger when the cells were incubated at 43.5°C than at 37°C but very small to be visible by naked eye in the flasks. In general we could say that both cell types follow a slow aggregation kinetics when incubated at 37°C and a faster one when incubated at 43.5°C. Cell aggregation at the elevated temperature in Hank's HEPES, follows the same kinetics when the cells were pre-incubated at 37°C or 43.5°C (Fig. 1B). The initial aggregation kinetics at 43.5°C of CV1 cells resembles that of cells incubated and aggregated at 37°C. By the end of the aggregation period, it reaches the threshold of cells incubated at 43.5°C and aggregated at 37°C. Similar kinetics is observed for the COS1 cells.

The addition of serum in the aggregation medium resulted in drastic changes on the aggregation kinetics of CV1 cells but not of COS1 cells. These changes are more obvious when cells aggregated at 43.5°C than at


A
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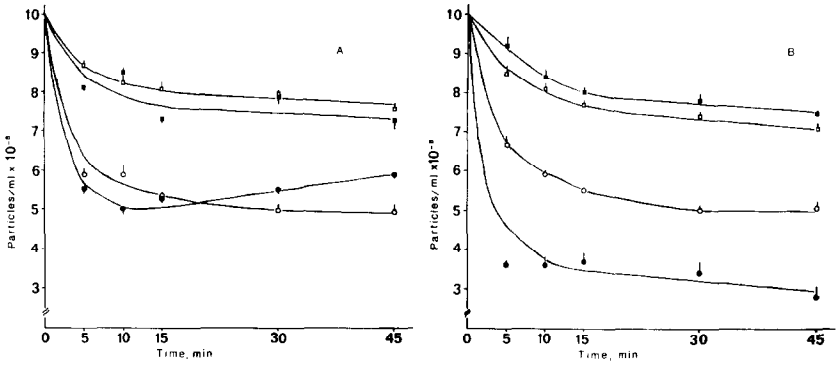
Particles/ml x 10⁻⁵
Time, min
0 5 10 15 30 45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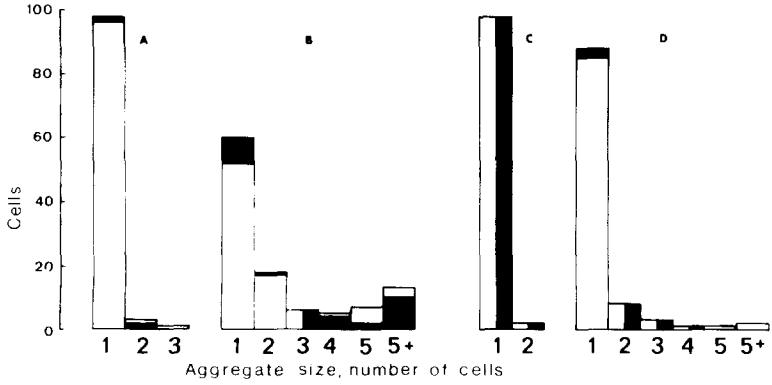
A
B
C
D
Cells
100
80
60
40
20
0
1 2 3
1 2 3 4 5 5+
1 2
1 2 3 4 5 5+
Aggregate size, number of cells

LEGENDS

Figure 1.

Aggregation kinetics of shaken suspensions of CV1 and COS1 cells. o CV1 and ◘ COS1 cells incubated at 37°C and • CV1 and ■ COS1 cells incubated at 43.5°C for five hours, were dispersed with trypsin/versene. The cells were suspended in Hank's HEPES at $1x10^6$ cells/ml. 4ml suspensions were reaggregated in a shaker bath controlled: A, at 37°C and B, at 43.5°C. Each point represents the mean value of five experiments ± SD.

Figure 2.

Aggregation kinetics of shaken suspensions of CV1 and COS1 cells. o CV1 and ◘ COS1 cells incubated at 37°C and • CV1 and ■ COS1 cells incubated at 43.5°C for five hours, were dispersed with trypsin/versene. The cells were resuspended in Hank's HEPES supplemented with 7% new born calf serum at $1x10^6$ cells/ml. 4ml suspensions were reaggregated in a shaker bath controlled: A, at 37°C and B, at 43.5°C. Each point represents the mean value of five experiments ± SD.

Histogram I.

Distribution of cells into aggregates.
The histograms represent the percentage of cells in aggregates of each size class. A, CV1 at time 0, B, CV1 at 30 minutes, C, COS1 at time 0 and D, COS1 cells at 30 minutes of reaggregation. ▭ cells incubated at 37°C and ▬ cells incubated at 43.5° C and reaggregated at 37°C.

37°C (Fig. 2A,B). When CV1 cells aggregate at 37°C with prior incubation at either temperature, they follow almost the same aggregation kinetics as the cells incubated and aggregated at 37°C in the absence of serum (Fig. 2A). This indicates that in the presence of serum the adhesive potential of CV1 cells is not affected by prior incubation at 43.5°C. 37°C incubated CV1 cells aggregated at 43.5°C followed the slower aggregation kinetics while 43.5°C incubated cells followed the faster one (Fig. 2B). This faster kinetics reaches the kinetics level of cells being incubated at 43.5°C and aggregated at either temperature (Fig. 1A,B). The kinetics of COS1 cells is of the faster type for either incubation and aggregation temperatures (Fig. 2A,B). It must be noted here that when the cells aggregate in the presence of serum the incorporation of cells into aggregates is the only parameter that accounts for the decreased number of cellular particles at any time.

DISCUSSION

The kinetics of cell aggregation over short times can provide a measure of the instantaneous adhesiveness of the cell (Curtis and Greaves, 1965). Comparative studies on the aggregation kinetics and adhesiveness of normal and transformed cells showed that transformation by viruses results in reduced cellular adhesiveness (Coman, 1944; Easty, 1974; Edwards et al, 1971; Roos, 1984). Altered adhesion is considered as one of the factors causing tumor cell dissemination (Roos, 1984). Thus, if it would be possible to manipulate the adhesive potential of tumor cells it would probably be possible to influence their metastatic process. Reduction of the temperature, below 25°C, has as a result a sharp decrease

of the adhesiveness of rabbit leukocytes onto glass coverslips (Lackie and Smith, 1980). On the other hand, it has been reported that BHK fibroblasts that are disperesed by trypsin adhere variably onto culture dishes at 4°C depending on whether the cells are exposed to serum after trypsinization (Curtis and McMurray, 1986). Membrane fluidity which depends on its lipid constitution, is responsible for this reduction due to temperature (Gingell and Vince, 1980). If low temperatures can negatively influence membrane fluidity, then temperatures higher than 37°C could also influence it.

For many years hyperthermia has been used in combination with radiotherapy for tumor treatment (Scott et al, 1983). The effect of hyperthermia on cellular activities is not yet well established. Our results show that cellular adhesiveness is increased when incubation or aggregation of cells takes place at elevated temperatures. The increase of adhesive potential of CV1 appears greater than that of COS1 cells. These adhesive changes at elevated temperatures may represent either alterations on membrane constitution or variations on membrane fluidity which are known to affect cellular adhesion.

Serum proteins are known to affect variably the adhesive properties of cells. It is reported that some cell types require serum proteins while other cell types do not for adhesion and spreading. This is the case for both normal and neoplastic cells (Rajaraman and MacSween, 1980). In the present work, our main observation is that elevated temperature increases initial cellular aggregation regardless of the absence or presence of serum. This then seems to be due to the increase in cellular adhesiveness.

To what extent hyperthermic treatment may affect

tumor cell dissemination may be evaluated by the study of mutual cell adhesiveness at elevated temperatures. The present observations are the basis for the study of possible structural changes of preformed cellular aggregates incubated at elevated temperatures.

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