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Energy metabolism of non-transformed and benzpyrene-transformed 3T3 cells: a microcalorimetric study

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Non-transformed and benzpyrene-transformed 3T3 cells attached to microcarriers were studied with microcalorimetry. The transformed cells manifested a higher rate of heat production and a larger anaerobic contribution to the total catabolism than did the non-transformed cells. Moreover, the transformed cells were characterized by a highly pH-dependent metabolism with a drop in thermal power occurring 0.3 pH units lower than the corresponding drop for the non-transformed cell line. We believe this shift in pH to be due to the higher intracellular pH maintained by the transformed cells. In addition, the study describes the correlation between rate of heat production per cell and the degree of confluency for each cell line, respectively.

Introduction

Tumor cells exhibit a variety of changes in such biophysical properties as expression of new cell surface molecules, membrane pump activity, and plasma membrane mobility. We have recently attempted to study such changes with microcalorimetry. For lymphocytic leukemic cell lines of T-cell type, a depression of metabolism, measured as the thermal power (i.e., the rate of heat production), could be observed upon treatment with the lectin, phytohemagglutinin, which is known to stimulate normal T-lymphocytes [1]. A more direct comparative approach was adopted for studying the temperature sensitivity of simian virus 40 transformed 3T3 cells and that of the non-transformed parental cell line [2]. The 3T3 cell type is mainly characterized by its infinite life and preserved contact inhibition [3]. As varieties of the parental cell line transformed by different means differ in a number of tumorigenic properties, the system is suitable for use in a range of comparative studies. In the present work, benzpyrene-transformed 3T3 cells and the non-transformed parental line were studied calorimetrically with respect to overall metabolic rate and metabolic pH dependence. In addition, anaerobic and aerobic contributions to overall catabolism were determined by measuring lactate production and oxygen consumption, respectively.

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Materials and Methods

Cell culture

Non-transformed 3T3 cells from Balb/c mouse embryo, clone A-31 [3] and benzpyrene transformed 3T3 (BPA-31) cells of the same clone [4] were kindly provided by Prof. A.B. Pardee (Dana-Farber Cancer Institute, Boston, MA, USA) and cultured in Dulbecco's modification of Eagle's medium (DMEM) (Flow Laboratories, Rockville, MD, USA) supplemented with 10% newborn calf serum, 0.05 g/l gentamicin, 0.3 g/l glutamine and 0.85 g/l $NaHCO_3$ (Gibco, Uxbridge, UK). The culture medium was buffered with 20 mM Hepes (pH 7.4). Bicarbonate was excluded in the medium used for experiments. The cells were cultured as monolayers before establishment of microcarrier cultures used for experiments.

The microcarriers (Cytodex 1, Pharmacia/LKB, Uppsala, Sweden) were hydrated and autoclaved in calcium- and magnesium-free phosphate buffered saline, and finally washed with culture medium. A trypsinized monolayer of cells was mixed with the microcarriers in a 100 ml spinner flask (Techne, Cambridge, UK) and the cells were allowed to adhere during a period of 5 h with intermittent stirring every half hour. All glassware used for the microcarriers was siliconized. A detailed description of microcarrier cultures can be found elsewhere [5].

Microcalorimetry

A four-channel microcalorimeter of the heat-conduction type ('Thermal Activity Monitor', LKB/Thermometric, Järfälla, Sweden) equipped with four stirred vessels, was used in all calorimetric experiments [6,7]. Samples were taken from the spinner flasks and the microcarriers were allowed to settle to enable exchange to bicarbonate-free medium. Sample suspensions of 2.7 ml each were transferred to microcalorimetric vessels which were inserted into the calorimeter. The thermal power was measured for a period of 2 h at 37°C; the cells being turbine-stirred at a rate of 90 rpm [7]. At the end of each calorimetric experiment, pH was measured and the number of cells was counted as released nuclei according to van Wezel [8]. Microcalorimetry of cells attached to microcarriers has earlier been described in some detail for Vero cells [9]. All measurements were made at a pH of 7.2, if not otherwise stated.

Determination of thermal power as a function of pH

Prior to measurements, culture medium was prepared at a pH-range of 6.6 to 9.1. All pH measurements were made with a Radiometer G297/62 capillary electrode (Radiometer, Copenhagen, Denmark). The desired pH was obtained by adding either hydrochloric acid or sodium hydroxide to the Hepes buffered culture medium. The microcarrier culture medium was replaced by the standardized pH medium as described above.

Values for thermal power were correlated with pH measured immediately after the calorimetric experiment. All measurements were made on the 2nd and 3rd day (48 h and 72 h after attachment of cells to the microcarriers) for the transformed and non-transformed cells, respectively.

Determination of oxygen consumption and lactate production

Oxygen consumption was measured polarographically in a thermostat-regulated oxygraph (Hansatech, Norfolk, UK). The cell/microcarrier suspension was prepared as described above, and samples were taken for calorimetry and for measurements of lactate production and oxygen consumption.

The suspension used with the oxygraph was three times as concentrated as that used in calorimetry. The saturation concentration of oxygen in the medium in equilibrium with air at 37°C was 0.199 mM [10]. Lactate production was measured in parallel to oxygen consumption in a series of Erlenmeyer flasks incubated in a shaking water bath. Samples were taken at intervals of 40 min and lactate was measured enzymatically in the neutralized supernatant after precipitation with perchloric acid [11]. All reactants for the enzymatic assay were from Boehringer Mannheim Scandinavia (Bromma, Sweden).

Results

Degree of confluency

To ascertain the effect of changes in cell density on the thermal power per cell, cultures were monitored calorimetrically. Thermal power per cell as a function of time after culture initiation is shown for non-transformed and benzpyrene-transformed cells in Figs. 1 and 2, respectively (in which time zero corresponds to the time of cell attachment to microcarriers).

In view of the large variation in thermal power per cell as a function of increasing confluency, in every instance the complete set of measurements was obtained during one day. In Figs. 1 and 2, each point on the curves corresponds to a rate of heat production that was either constant or increasing linearly with time. The linear increase in thermal power with time represents the net effect of exponential growth modified by the decreasing pH of the medium [12].

The effect of pH on thermal power

Fig. 3 shows the thermal power per cell as a function of pH for both cell lines. The non-transformed cells manifested a pH-independent metabolism in the pH-range 7.0–8.4. If the points between pH 7 and pH 8 are considered as a straight line, the overall increase in thermal power per cell per pH-unit was 18% (5 pW/cell). By contrast, transformed cells not only manifested much greater thermal power over the whole pH-range but were also characterized by a pH-dependency of about 34% (17 pW/cell) per pH-unit between pH 7 and pH 8. Moreover, the transformed cells manifested a drop in thermal power that occurred at 0.3 pH-units lower than the corresponding drop in thermal power observed for the non-transformed cells.

Calculated versus measured thermal power

Oxygen consumption and lactate production are useful variables for assessing the contributions from

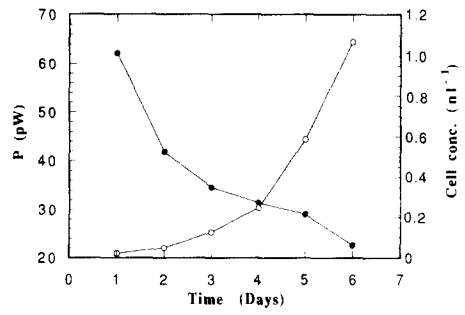

Fig. 1. Thermal power per cell, P (●———●) and cell concentration (○———○) as a function of time for 3T3 cells, clone A-31.

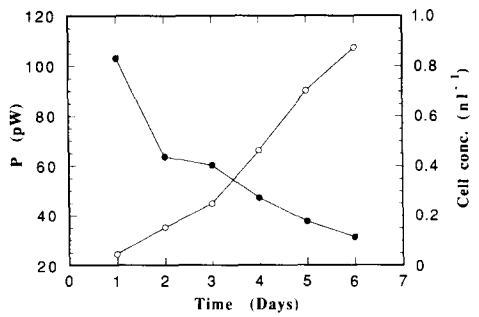

Fig. 2. Thermal power per cell, P (●———●) and cell concentration (○———○) as a function of time for 3T3 cells, BPA-31.

aerobic and anaerobic catabolism to overall heat production. Lactate production from glucose is associated with an enthalpy value of −109 kJ per mol of glucose [13]. The formation of one mole of lactate is associated with the production of one mole of protons which is taken up by the Hepes buffer. The enthalpy value for the buffer reaction is −21.7 kJ/mol [13]. The oxygen consumed is used for oxidation both of carbon sources in the medium, mainly glucose and glutamine, and of different intracellular substrates such as proteins. Thus, an average enthalpy value of −469 kJ/mol of oxygen, as suggested by Gnaiger and Kemp [14], was used in calculations. The calorimetric and calculated thermal power values are given in Table I.

The non-transformed cells were characterized by a thermal power of 29 pW per cell. Power values calculated from oxygen consumption and lactate production rates show that 25% of the total power was associated with lactate formation and 75% with the oxidative processes. The transformed cells were metabolically much more active, with an overall thermal power of 53 pW per cell; and the contributions from lactate production and oxygen consumption to the total thermal power per cell were 35 and 65%, respectively.

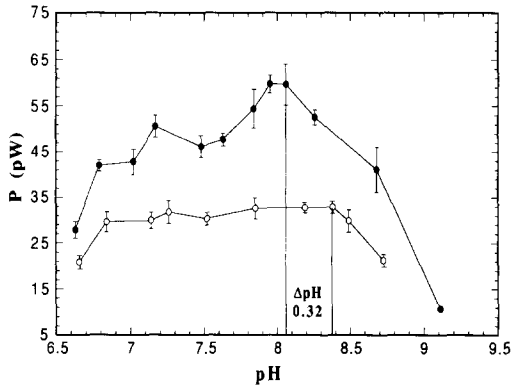

Fig. 3. Thermal power per cell as a function of pH, for 3T3 cells of clone A-31 (○———○) and BPA-31 cells of the same clone (●———●). The ΔpH = 0.3 indicates the pH-shift due to the higher intracellular pH maintained by the transformed cell line. The cell concentration was on the order of $3 \cdot 10^5$ cells per ml.

TABLE I

Rates of lactate formation and oxygen consumption, with power values

Results from measurements of rates per cell of lactate formation (dn_{lac}/dt) and oxygen consumption (dn_{O2}/dt) together with calorimetrically determined power values (P_{cal}) per cell for normal cells (A-31) and for transformed cells (BPA-31). In the table it is also given the calculated power values per cell for lactate formation (P_{lac}) and oxygen consumption (P_{O_2}). Figures in brackets denote the number of determinations. The cell concentration was on the order of $3 \cdot 10^5$ cells per ml except for the oxygen measurements where the cell concentration was about 3-times higher.

Cell line	$10^{17} dn_{lac}/dt$ (mol s^{-1})	$10^{17} dn_{O_2}/dt$ (mol s^{-1})	P_{lac} (pW)	P_{O_2} (pW)	P_{Cal} (pW)
A-31	9±1.3 (4)	5±0.8 (10)	7±1 (4)	21±4 (10)	29±3 (8)
BPA-31	23±2.0 (4)	7±1.3 (10)	17±2 (4)	31±6 (10)	53±7 (6)

Discussion

Non-transformed 3T3 cells are characterized by their capacity for contact inhibition [3]. Cell lines derived from the original 3T3 cells by transformation with different agents have been shown to acquire a variety of characteristics [15–17]. As the most obvious features of the transformed cells are loss of contact inhibition and increased growth rate, it would be reasonable to suppose that the heat production rate would decrease with increasing cell density much more drastically for the transformed than for the non-transformed cell line. However, as can be seen in Figs. 1 and 2, there is no significant difference between the two cell lines with respect to metabolic activity as a function of cell density. The fact that the thermal power decreases with the degree of confluency might be attributed to decreased cell volume [18]. It is also reasonable to expect a decreased metabolic activity per cell when a smaller cell surface area is exposed to nutrients. In order to exclude the contribution of cell volume to metabolic activity, all parameters should however be correlated to both cell number and protein content.

The transformed cells manifested a much higher metabolic rate in general and the thermal power being about 1.8 times greater than that of the non-transformed cells at pH 7.2 (see Table I). In addition, the anaerobic contribution to total thermal power was greater for transformed cells than for non-transformed cells (35% vs. 25%), a finding in agreement with earlier observations of high lactic acid production in tumorigenic tissue (see, for example, Pitot [19]). Our studies of metabolic pH-dependence show that the transformed cells with their higher metabolic rate and, in particular, larger anaerobic metabolic contribution to

the overall metabolism, have a more pronounced dependence on pH. It is well known that the rate of glycolysis is highly pH-dependent, owing to the properties of the regulatory enzyme, phosphofructokinase [20,21]. It is thus reasonable to suppose the pH-dependence to be entirely attributable to the glycolytic pathway. Hence, the difference in pH-dependence of the two cell lines studied here can partly be explained by their different glycolytic rates. Moreover, in the transformed cell line the drop in thermal power after the peak value occurred 0.3 pH-units below the corresponding point for the non-transformed cells. This shift in pH can be explained by the higher activity of the Na^+/H^+ antiport in tumor cells [22]. Thus, for a given external pH, the internal pH is higher for the transformed cells, and the two curves might overlap if the thermal power was measured as a function of internal pH. An increased Na^+/H^+ antiport activity has also been suggested to explain a high lactate production [22].

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