FRÖHLICH COHERENT STATES: IMPLICATIONS FOR THERMAL SENSITIVITY OF CELLS

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The inactivation rate of cells induced by heat in biological systems exhibits significant variations. The Fröhlich hypothesis of coherent vibrations is used to explain the observed differences between thermal sensitivities of malignant cells and their healthy counterparts. The resistance of the cellular membrane to the thermal fluctuations, pore creation, and membrane permeability increase may depend on the coherent state energy. A higher membrane permeability results in a lower cell survival rate when heated to the hyperthermic range.

INTRODUCTION

Heat treatment in a temperature range of 41 – 46 °C leads to an increased lethality of cells in biological systems. Differences between thermal sensitivities of healthy cells and their malignant counterparts have very often been found, which were the original rationale for the hyperthermic cancer treatment. E.g. the relative values of the surviving fraction of normal melanocytes and malignant melanoma cells after two hours heating at the temperature 43 °C are about 0·7 and 0·4, respectively (in vitro) [1]. Thermal sensitivities of cells depend on many factors (e.g. on the cell type, phase of the cell-cycle, metabolic sources, ambient medium and environmental conditions).

Heat effects on the cellular level are explained as a result of damage to the membrane, inhibition of DNA, RNA and protein synthesis, inhibition of cellular respiration and inactivation of enzymes, as is described e.g. in [2–7]. Heat injury to cells begins with damage to the membrane and with changes in passive membrane permeability as follows from experimental findings [8]. The cell membrane is very likely the main target for heat lesions. Experimental findings suggest the formation of nonspecific aqueous channels (pores) in the membrane [9]. Calcium ion influx through the channels and increase of calcium level inside the cell is connected with the cell inactivation process [10, 11]. Heat induced membrane permeability is explained as a result of integral protein release from the membrane [8].

Cell energy depletion and decrease of adenosine triphosphate (ATP) content following heat treatment were observed [11, 12]. A decrease of ATP level as well as a change in ATP turnover and enhanced specific activity of ATP in melanoma cells after heating at 42 °C were found [13].

We assume that thermal sensitivity of cells depends on the membrane energy. The Fröhlich hypothesis of coherent vibration states is used to explain different

thermal sensitivities of healthy and malignant cells. The Fröhlich coherent states are conditioned by the energy supply to the vibration systems [14]. The electric dipole or multipole structures are connected with the vibration states. The locus of the vibrations is assumed to be the cellular membrane. In this paper we analyse the dependence of the membrane permeability on the coherent state energy.

PORE INDUCTION AND CELL SURVIVAL RATE

Litster [15] analysed the possibility of pore creation in the membrane by thermal fluctuations using a simple energy relation $\Delta E = 2\pi\gamma r - \pi\Gamma r^2$, where ΔE is the change of the energy needed to create a pore, γ is the strain energy per unit length of the pore edge, and Γ is the energy per unit area of the undisturbed bilayer membrane. For energy smaller (greater) than the energy barrier $\Delta E_c = \pi\gamma^2/\Gamma$, corresponding to a critical radius γ/Γ, the pore reseals (expands indefinitely). The energy barrier is determined from the condition $\mathrm{d}(\Delta E)/\mathrm{d}r = 0$ [15]. We assume that an "indefinitely" expanding pore without any other perturbation spreads only over less rigid patches of the membrane and might be treated by a biological repair mechanism even before reaching the boundary of more stable areas. Thermal fluctuations may initiate even the pore resealing process.

Subjected to thermal fluctuations some parts of the membrane exert time dependent displacements. As a rough approximation we substitute bound highly damped particle motion for real local displacements of an appropriate part of the membrane. Then the Brownian motion theory may be used. The density function of highly damped one-dimensional Brownian motion of a bound particle is given by [16]

$$f(s) = \sqrt{\left(\frac{K}{2\pi kT}\right)} \exp\left(-\frac{Ks^2}{2kT}\right), \tag{1}$$

where K is the force constant of the displacement s and k and T have the usual meanings. In harmonic approximation $E_d = Ks^2/2$ and this displacement energy represents the pore creation energy ΔE. We assume that the energy of displacement normal to the membrane surface necessary to create a critical pore is considerably greater than the energy of lateral displacement. Rotational, twist or shear motions are neglected as well as orientation changes of lipid molecules. The density function connected with two-dimensional displacements may be derived from eq. (1) in the form

$$\varphi(E) = \frac{1}{kT} \exp\left(-\frac{E}{kT}\right), \tag{2}$$

where $E = Kr^2/2$ and r is the displacement in the lateral direction. Using eq. (2) the probability of critical pore induction is given by

$$F(\Delta E_c) = \int_{\Delta E_c}^{\infty} \varphi(E)\,\mathrm{d}E = \exp\left(-\frac{\Delta E_c}{kT}\right). \tag{3}$$

Eq. (3) yields the Boltzmann factor which may be introduced directly as in [17, 18]. Multiplying the Boltzmann factor $F(\Delta E_c)$ in eq. (3) by some fundamental frequency $1/\tau$ of events per unit area and by the undisturbed area $S - S_p$ of the membrane (where new pores may be created) we get the rate equation

$$\frac{dS_p}{dt} = (S - S_p)\left[\frac{\tau}{F(\Delta E_c)}\right]^{-1}, \tag{4}$$

where t is time, S and S_p are the total area and the area of pores, respectively. If S is a constant, we get from eq. (4) that

$$\frac{(S - S_p)_t}{(S - S_p)_0} = \exp\left\{-\left[\frac{\tau}{F(\Delta E_c)}\right]^{-1} t\right\}, \tag{5}$$

where the index 0 denotes the value at the initial time. The pore creation analysis does not involve the backward process. i.e. critical and overcritical pore resealing.

Pore creation disturbs the separation of the cell interior from the ambient medium. The flow of charged and neutral molecules and particles is mediated by the pores. The flow across the membrane causes e.g. changes of the electric potential of the membrane, of the inner cell pressure and of the cytoplasm content. In accordance with the experimental findings we assume the increased membrane permeability to be connected with higher cell mortality. The number of surviving cells may be assessed from the total area S_p of the pores. The relative number n_s of surviving cells is proportional to $(S - S_p)_t/(S - S_p)_0$ in eq. (5). A delay of cell inactivation may be expected during a short period after $t = 0$ before the cytoplasm content is considerably changed.

In a narrow temperature range cell inactivation mechanisms are described by a general expression similar to (5) [19].

INHIBITION OF PORE INDUCTION BY COHERENT VIBRATIONS

Radial polar coherent vibrations generate dipoles in both the layers of the bilayer membrane. The dipoles located in one layer of the membrane interact with the dipoles in the other layer. Interaction energy of two dipoles located axially along the same radius is given by

$$E_i = \frac{q^2}{4\pi\varepsilon T_0}\int_0^{T_0}\left(\frac{2}{a+b} - \frac{1}{2a+b} - \frac{1}{b}\right)dt = \frac{q^2}{4\pi\varepsilon}\left[\frac{2}{a_0+b_0} - \frac{2}{\sqrt{[(a_0+b_0)^2 - a_0^2]}}\right], \tag{6}$$

where $a = a_0\cos(\omega t)$, $b = b_0 + a_0[1 - \cos(\omega t)]$ (fig. 1), $T_0 = 2\pi/\omega$ is a period of coherent vibrations, ε is permittivity, and q is the charge. (The "static" field term given by eq. (6) predominates in the close vicinity of a source.) The interaction energy multiplied by the effective number of dipoles (quadrupoles) per unit area yields the

increment of Γ. The negative value of E_i signifies that higher values of energy stored in the coherent vibration states make the membrane more resistant to pore creation. The interaction energy E_i may decrease the mobility of lipid molecules and/or increase the strain energy of the pore edge.

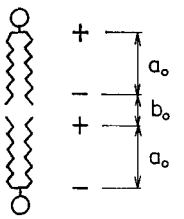


Fig. 1. Assumed dipoles in a lipid bilayer membrane.

The external electric field applied to a cell forms a transmembrane potential. The transmembrane potential tends to decrease the stability of the membrane against thermal fluctuations. The energy E_f associated with the transmembrane potential U is given by [18]

$$E_f = \frac{\varepsilon_0 \varepsilon'_m}{2d}\left(1 - \frac{\varepsilon'_m}{\varepsilon'_w}\right) U^2 = AU^2 , \tag{7}$$

where d is the membrane thickness, ε'_m and ε'_w are the relative permittivities of the membrane and of the cytoplasm, respectively, and ε_0 is the permittivity of the free space.

The effect of the coherent vibrations and of the external electric field on the membrane permeability may be assessed from the changes of Γ and γ. We assume that the total value of Γ is given by

$$\Gamma = \Gamma_0 + E_i + E_f , \tag{8}$$

where Γ_0 is the energy per unit membrane area without the external electric field and the coherent vibrations.

THEORETICAL RESULTS AND DISCUSSION

The dependence of the relative number of surviving cells on temperature and time of treatment is shown in fig. 2a. The thick solid curves correspond to experimental data on the Chinese-hamster ovary cells [20] and are given by the relation (fitting the experimental curves) [21]

$$n_s = 1 - \left[1 - \exp\left(-\frac{t}{\tau_0}\right)\right]^N , \tag{9}$$

where N and τ_0 are constants. N is equal to about 100 and τ_0 is in a range of about

10–1 min for 43 °C up to 46 °C (Chinese-hamster ovary cells) [21]. τ_0 is about one order greater for pig kidney cells [20]. Eq. (9) coincides with a simple exponential dependence for $n_s \leqq 0{\cdot}1$; comparing (5) and (9) we get $\tau_0 = \tau/F(\Delta E_c)$. If the energy of the coherent vibration states increases, the energy Γ per unit membrane area decreases, and the number of surviving cells increases (the thin curves in fig. 2a). The Γ value of these curves differs from the corresponding experimental value of the thick curves by the interaction energy E_i given by eq. (6). In this case E_i represents

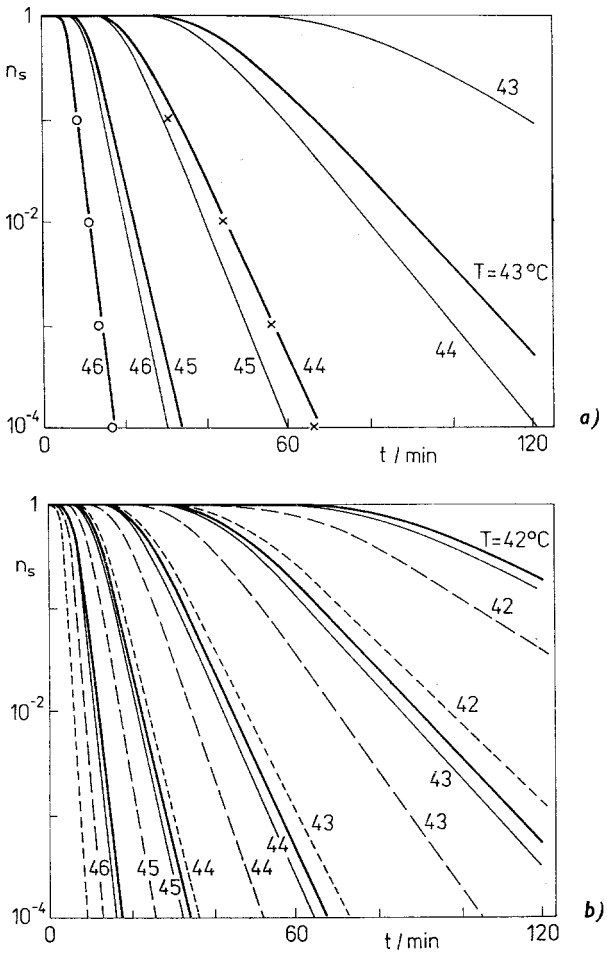


Fig. 2. Computed survival curves for cells heated at different temperatures as a function of time. The thick solid curves correspond to experimental findings (Chinese-hamster ovary cells [20]). $\Gamma = 10^{-3}$ J/m^2, $N = 66{\cdot}3$, $\gamma = 1{\cdot}77 \times 10^{-11}$ J/m, $1/\tau = \exp(\alpha)$ and α is about 223. a) The thin curves denote the effect of coherent vibration for $q = 0{\cdot}00375\, e$ (where e is the elementary charge), $a_0 = 1{\cdot}5$ mm, $b_0 = 1$ nm and the effective number of elementary dipoles per unit area is 10^{18} m^{-2}. Experimental values: o — 46 °C; × — 44 °C [20]. b) The thin solid, dashed, and dotted curves denote heat effects together with the external electric field generating a transmembrane potential drop of 0·01, 0·025 and 0·04 V, respectively. $A = 1{\cdot}7 \times 10^{-3}$ F/m^2.

an increment of the total coherent vibration interaction energy. A basic value of E_i is very likely included in the Γ of the thick curves in fig. 2a.

The decrease of $|E_i|$ enhances the thermal sensitivity of the cells and vice versa. The interaction energy E_i depends on the energy stored in the coherent vibration states. This energy depends on the energy supply from metabolic sources and energy losses from the vibration system to the ambient medium. If the energy of the coherent vibration states increases with increasing temperature, the tendency of membrane destabilization at higher temperatures may be compensated or overcompensated by the decrease of Γ.

Besides the value of the total coherent state energy, distribution of the energy to the particular normal modes and characteristic features of the modes may be important. Several normal modes may be coherently excited [22]. Dipole-multipole transitions of the vibration modes have significant effect on the interaction energy E_i. Our estimations of the coherent state excitation have to involve the long-range interaction and energy channeling mediated by the generated electromagnetic field. The increased conductivity of the ambient medium connected with the decrease of the pH diminishes the space dependent energy channeling and may result in a lower value of $|E_i|$. The coherent vibration energy may have a nonuniform space distribution. Regions with a lower energy content are more liable to pore creation.

Fröhlich conjectured that a malignant process may be connected with coherent vibration energy changes [14]. Preliminary experimental findings on the leukocyte mediated immunity support this hypothesis [23].

The effect of the external electric field on n_s is shown in fig. 2b. The potential drop across the membrane enhances the thermal sensitivity of the cells. The external electric field induces the polarization of both the membrane lipid layers. Interaction between the polarized layers decreases the effect of the external electric field. Voltages higher than those in fig. 2b should be used to destabilize the membrane.

The energy barrier ΔE_c (the activation energy) is assumed to depend only on the lateral motion of the lipid molecules in the membrane. Rotation (or torsional motion) and orientation changes may also be significant. These motions may loosen the lateral bonds between the lipid molecules (e.g. bonds mediated by positively charged ions). The activation energy is considerably greater in the temperature range below 43 °C than in that above 43 °C [21]. Inactivation thus appears to involve different processes, or at least a change in the fundamental process, when the temperature is below 43 °C.

CONCLUSION

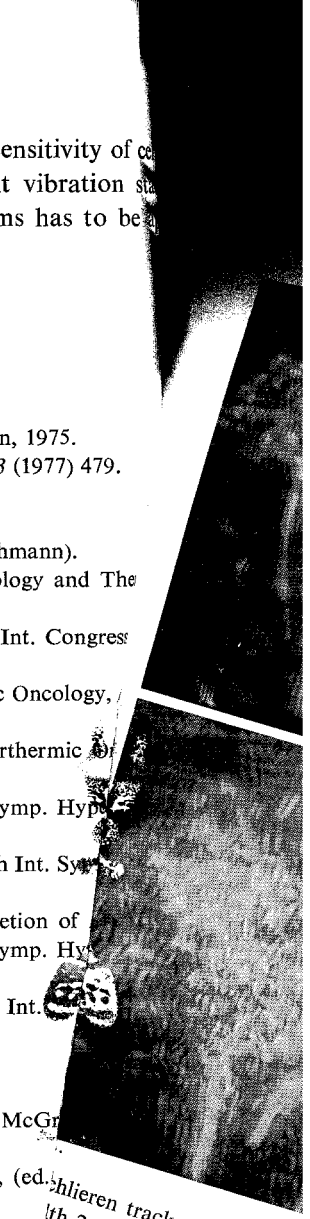
Induction of pores in biological membranes is one of the agents essential for cell killing by heat. Energy of the coherent states located in the membranes contributes to the membrane stabilization and acts against pore induction. If the energy of the coherent states in malignant cells is smaller than in the healthy cells, the former are

more liable to heat damage than the latter. Nevertheless, thermal sensitivity of ce
is a complex problem. The role and significance of the coherent vibration st
mechanism with respect to other physical and chemical mechanisms has to be
preciated on the experimental basis.

Received 13. 6. 1984.

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