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ACCUMULATION OF A PHOTOSENSITIZER IN CELLS WITH ALLOWANCE FOR THE PASSAGE OF ITS CHARGED FORMS ACROSS THE MEMBRANE

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A mathematical model has been constructed and studied of the penetration of hematoporphyrin (HP) ions into cells and their accumulation there. HP is a photosensitizer most frequently used in the photodynamic therapy of tumors. It is shown that the selectivity of HP accumulation in tumors depends on the following factors: (a) the lowered acidity inside malignant cells and in the surrounding medium and (b) the higher concentration in tumor cells of lipids, to which the HP molecules bind.

Some fluorescent dyes introduced into an organism have the property of accumulating predominantly in tumor tissues. These dyes can be used as phototherapeutic agents. The irradiation of malignant tissues containing a dye by visible light may lead to the disappearance of the tumor. This way of detection and treatment of tumors forms the foundation of the method of photodynamic therapy (PDT) [1]. The successful use of PDT largely depends on the elaboration of methods for raising the photosensitizer level in the cell.

In an earlier paper [2] we considered a mathematical model of intracellular accumulation of hematoporphyrin (HP) — a sensitizer most frequently used in PDT. It was assumed in the model that only the neutral HP form passes across the cell membrane. The calculations showed that the intracellular concentration of HP can appreciably exceed its concentration in the surrounding medium when the pH inside the cell is higher than outside. In the present paper the same problem is considered but the penetration of not only the neutral but also charged forms of HP into the cell is taken into account.

First we examine in more detail the formation of HP ions. As is known, HP can exist in the neutral form, and as an anion, a dianion, a cation, and a dication. Depending on the pH of the environment, the protonization-deprotonization reactions of the HP molecule have the form

$$C^{2-} + \mathrm{H}^+ \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} C^-; \quad k_{a2} = k_{-2}/k_{+2}; \quad \mathrm{pK}_{a2}\ 7.2 \pm 0.1;$$

$$C^- + \mathrm{H}^+ \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C^0; \quad k_{a1} = k_{-1}/k_{+1}; \quad \mathrm{pK}_{a1}\ 6.5 \pm 0.1;$$

$$C^0 + \mathrm{H}^+ \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} C^+; \quad k_{N1} = k_{-3}/k_{+3}; \quad \mathrm{pK}_{N1}\ 5.4 \pm 0.1;$$

$$C^+ + \mathrm{H}^+ \underset{k_{-4}}{\overset{k_{+4}}{\rightleftharpoons}} C^{2+}; \quad k_{N2} = k_{-4}/k_{+4}; \quad \mathrm{pK}_{N2}\ 2.9 \pm 0.1.$$

The symbols C^{2-}, C^-, C^0, C^+, and C^{2+} denote the corresponding HP ion types and their concentrations; k_{ai} and k_{Ni} are the equilibrium constants of the reactions; $\mathrm{pK}_j = -\log k_j$; the pK values are taken from [3].

We introduce the total HP concentration by the formula

$$C_\Sigma = C^0 + C^- + C^{2-} + C^+ + C^{2+}.$$

The relative concentrations for the stationary distribution of HP over the ion types are

$$\alpha_0 = \frac{C^0}{C_\Sigma} = \left(\frac{k_{a2}k_{a1}}{[H^+]^2} + \frac{k_{a1}}{[H^+]} + 1 + \frac{[H^+]}{k_{N1}} + \frac{[H^+]^2}{k_{N1}k_{N2}} \right)^{-1},$$
$$\alpha_{a1} = \frac{C^-}{C_\Sigma} = \frac{k_{a1}\alpha_0}{[H^+]}; \qquad \alpha_{a2} = \frac{C^{2-}}{C_\Sigma} = \frac{k_{a1}k_{a2}\alpha_0}{[H^+]^2}, \tag{1}$$
$$\alpha_{N1} = \frac{C^+}{C_\Sigma} = \frac{[H^+]\alpha_0}{k_{N1}}; \qquad \alpha_{N2} = \frac{C^{2+}}{C_\Sigma} = \frac{[H^+]^2\alpha_0}{k_{N1}k_{N2}}.$$

It is clear that for other pK values (see, e.g., [4, 5]) the character of the distribution will change. We also note that in the present paper the dependence of pK on the degree of HP aggregation [6] is not taken into account. Figure 1 shows that for the normal pH the relative concentration of HP cations is very low whereas anions and the neutral form dominate.

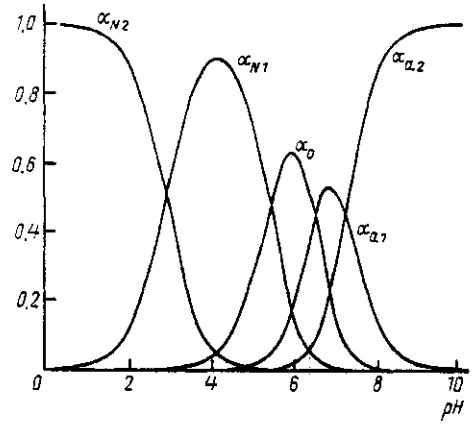

Fig. 1

Stationary distribution of relative concentrations of HP ionic forms.

As in [2], the kinetic model will be constructed for the cell culture used in *in vitro* experiments. Let us consider the main assumptions of the model. The cell membrane has electric and thermodynamic potential barriers. The neutral form of an HP molecule is "indifferent" to the electric potential, and the membrane barrier height for it is $\Delta G^0 = \Delta G$. For charged HP forms this quantity is a sum of the electrostatic and thermodynamic potential barriers, ΔG_e and ΔG, and we have $\Delta G_e = |\psi|F$ for singly charged ions and $\Delta G_e = 2|\psi|F$ for doubly charged ions, where F is the Faraday constant and ψ is the membrane potential (Fig. 2). We shall assume that ΔG is the same for all types of HP ions and that the cytoplasm is an aqueous medium.

The model takes into account the following factors.

1. The diffusion of the five types of HP ions C^+, C^{2+}, C^0, C^-, and C^{2-} across the cell membrane under assuming their complete mixing within the volumes of the cell and the medium with the following input and output constants (see Fig. 2):

$q_0 = \tilde{q}_0 \equiv q_0 = A\sigma_\Sigma \exp\{-\Delta G/RT\}$ for the neutral form;

$q_{a1} = q_0 \exp\{-\psi F/RT\}$, $\tilde{q}_{a1} = q_0$ for the anion C^-;

$q_{a2} = q_0 \exp\{-2\psi F/RT\}$, $\tilde{q}_{a2} = q_0$ for the dianion C^{2-};

$q_{N1} = q_0$, $\tilde{q}_{N1} = q_0 \exp\{-\psi F/RT\}$ for the cation C^+;

$q_{N2} = q_0$, $\tilde{q}_{N2} = q_0 \exp\{-2\psi F/RT\}$ for the dication C^{2+}.

2. The kinetics of HP distribution over the ion types inside the cell.

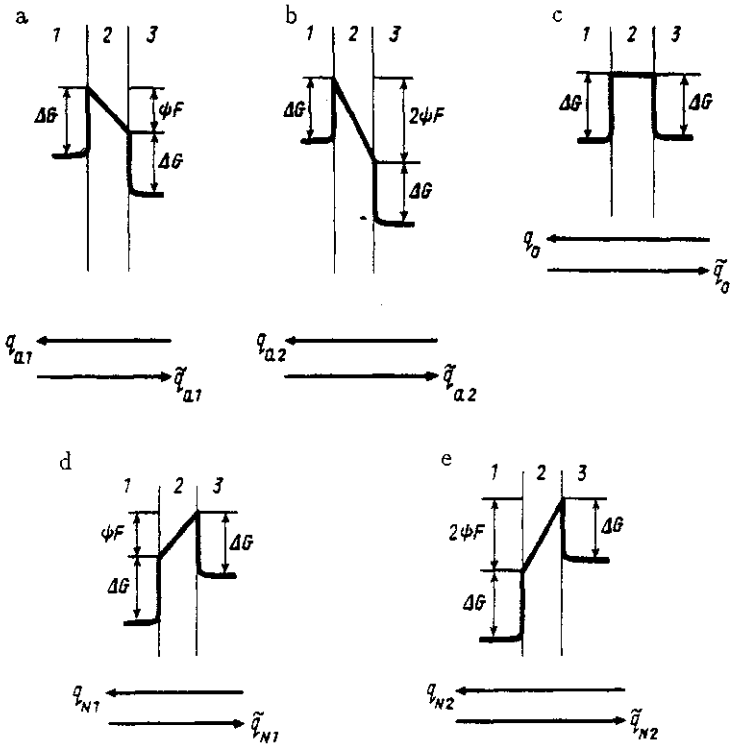

Fig. 2

Potential barrier of the cell membrane for the anion (a), dianion (b), neutral form (c), cation (d), and dication (e): (1) cell; (2) membrane; and (3) surrounding medium.

3. The difference in the pH inside and outside the cell; the pH^{in} is assumed to be constant in the process of HP accumulation in the cell, i.e., the work of the intracellular buffer*) and the pH^{in} variation during the life cycle of the cell**) are not taken into account.

4. The membrane potential ψ, which is negative and constant and which impedes the penetration of the anion HP forms across the cell membrane. Using the Goldman-Hodgkin-Katz equation for the electric potential ψ of the membrane [10–12], it can easily be shown that in the process of HP penetration into and accumulation in the cell the variations of ψ can be neglected. However, the results of our direct measurements of ψ [13] show that the potential ψ changes when HP passes across the membrane but regains its former value after the HP penetration into the cell.

*) The main buffer maintaining a constant pH in the intracellular liquid is the phosphate buffer system $H_2PO_4^-$ — HPO_4^-, pK 6.86 [7, 8]. Our calculations show that the rate of the pH^{in} restoration by the buffer substantially exceeds the rate of HP penetration into the cell and the rate of the related change of the intracellular pH.

**) As is known, the pH^{in} variation over the life cycle of the cell attains 0.3 (with the mean value of the pH^{in} preserved) [9]. However, different cells in the culture are in different phases of their life cycles, and this provides a constant mean value of the pH^{in} in the culture.

Therefore the kinetic equations for HP accumulation in the cell are written as

$$\begin{aligned}
\frac{dC^0_{\text{in}}}{dt} &= q_0 C^0_{\text{ex}} - \tilde{q}_0 C^0_{\text{in}} - k_{-1}C^0_{\text{in}} + k_{+1}C^-_{\text{in}}[\text{H}^+]^{\text{in}} - k_{+3}C^0_{\text{in}}[\text{H}^+]^{\text{in}} + k_{-3}C^+_{\text{in}};\\
\frac{dC^-_{\text{in}}}{dt} &= q_{a1}C^-_{\text{ex}} - \tilde{q}_{a1}C^-_{\text{in}} - k_{-2}C^-_{\text{in}} + k_{+2}C^{2-}_{\text{in}}[\text{H}^+]^{\text{in}} + k_{-1}C^0_{\text{in}} - k_{+1}C^+_{\text{in}}[\text{H}^+]^{\text{in}};\\
\frac{dC^{2-}_{\text{in}}}{dt} &= q_{a2}C^{2-}_{\text{ex}} - \tilde{q}_{a2}C^{2-}_{\text{in}} + k_{-2}C^-_{\text{in}} - k_{+2}C^{2-}_{\text{in}}[\text{H}^+]^{\text{in}};\\
\frac{dC^+_{\text{in}}}{dt} &= q_{N1}C^+_{\text{ex}} - \tilde{q}_{N1}C^+_{\text{in}} + k_{+3}C^0_{\text{in}}[\text{H}^+]^{\text{in}} - k_{-3}C^+_{\text{in}}\\
&\quad - k_{+4}C^+_{\text{in}}[\text{H}^+]^{\text{in}} + k_{-4}C^{2+}_{\text{in}};\\
\frac{dC^{2+}_{\text{in}}}{dt} &= q_{N2}C^+_{\text{ex}} - \tilde{q}_{N2}C^+_{\text{in}} + k_{+4}C^+_{\text{in}}[\text{H}^+]^{\text{in}} - k_{-4}C^{2+}_{\text{in}}.
\end{aligned} \tag{2}$$

Taking into account the input and output constants of the HP ionic forms and the equilibrium constants (from the *in vitro* experiments it is known that $q_0 \sim 10^{-3}$ s^{-1}, $k_{-1} = k_{-2} \sim 1$ s^{-1}) and introducing $\varepsilon = q_0/k_{-1,-2} = 10^{-3}$ and the dimensionless time $\tau = tk_{-1} = tk_{-2}$, we rewrite Eqs. (2) in the form

$$\begin{aligned}
\frac{dC^{2-}_{\text{in}}}{d\tau} &= \varepsilon(\exp\{-2\psi F/RT\}C^{2-}_{\text{ex}} - C^{2-}_{\text{in}}) + \left(C^-_{\text{in}} - \frac{C^{2-}_{\text{in}}[\text{H}^+]^{\text{in}}}{k_{a2}}\right);\\
\frac{dC^-_{\text{in}}}{d\tau} &= \varepsilon(\exp\{-\psi F/RT\}C^-_{\text{ex}} - C^-_{\text{in}}) - \left(C^0_{\text{in}} - \frac{C^-_{\text{in}}[\text{H}^+]^{\text{in}}}{k_{a1}}\right.\\
&\quad \left. - C^-_{\text{in}} + \frac{C^{2-}_{\text{in}}[\text{H}^+]^{\text{in}}}{k_{a2}}\right);\\
\frac{dC^0_{\text{in}}}{d\tau} &= \varepsilon(C^0_{\text{ex}} - C^0_{\text{in}}) - \left(C^0_{\text{in}} - \frac{C^-_{\text{in}}[\text{H}^+]^{\text{in}}}{k_{a1}} - C^+_{\text{in}} + \frac{C^0_{\text{in}}[\text{H}^+]^{\text{in}}}{k_{N1}}\right);\\
\frac{dC^+_{\text{in}}}{d\tau} &= \varepsilon(C^+_{\text{ex}} - C^+_{\text{in}}\exp\{-\psi F/RT\}) - \left(C^+_{\text{in}} - \frac{C^0_{\text{in}}[\text{H}^+]^{\text{in}}}{k_{N1}}\right.\\
&\quad \left. - C^{2+}_{\text{in}} + \frac{C^+_{\text{in}}[\text{H}^+]^{\text{in}}}{k_{N2}}\right);\\
\frac{dC^{2+}_{\text{in}}}{d\tau} &= \varepsilon(C^{2+}_{\text{ex}} - C^{2+}_{\text{in}}\exp\{-2\psi F/RT\}) - \left(C^{2+}_{\text{in}} - \frac{C^+_{\text{in}}[\text{H}^+]^{\text{in}}}{k_{N2}}\right).
\end{aligned} \tag{3}$$

In solving system (3), use is made of the HP distribution over the ion types outside the cell in accordance with the pH^{ex} by formulas (1). In the conditions of *in vitro* experiments, the volume of the surrounding medium is much greater than the total volume of the cells, and the number of moles of the dye inside the cells is much greater than outside. This allows the HP concentration outside the cell to be considered constant.

The terms with ε in system (3) are much smaller than the others. By disregarding them we obtain the already solved problem of HP distribution over the ion types inside the cell.

We now consider the dynamics of the total HP concentration. To this end the equations in system (2) are added together:

$$\begin{aligned}
\frac{dC^{\text{in}}_{\Sigma}}{d\tau} &= \varepsilon(C^0_{\text{ex}} + C^-_{\text{ex}}\exp\{-\psi F/RT\} + C^{2-}_{\text{ex}}\exp\{-2\psi F/RT\} + C^+_{\text{ex}} + C^{2+}_{\text{ex}}\\
&\quad - C^{2-}_{\text{in}} - C^-_{\text{in}} - C^0_{\text{in}} - C^+_{\text{in}}\exp\{-\psi F/RT\} - C^{2+}_{\text{in}}\exp\{-2\psi F/RT\}).
\end{aligned} \tag{4}$$

Equation (4) has a stationary solution

$$\begin{aligned}
\frac{(C^{\text{in}}_{\Sigma})_{\text{sat}}}{C^{\text{ex}}_{\Sigma}} &= \frac{\alpha^{\text{ex}}_0 + \alpha^{\text{ex}}_{a1}\exp\{-\psi F/RT\} + \alpha^{\text{ex}}_{a2}\exp\{-2\psi F/RT\} + \alpha^{\text{ex}}_{N1} + \alpha^{\text{ex}}_{N2}}{\alpha^{\text{in}}_0 + \alpha^{\text{in}}_{N1}\exp\{-\psi F/RT\} + \alpha^{\text{in}}_{N2}\exp\{-2\psi F/RT\} + \alpha^{\text{in}}_{a1} + \alpha^{\text{in}}_{a2}}\\
&= \frac{f_1(\text{pH}^{\text{ex}})}{f_2(\text{pH}^{\text{in}})}
\end{aligned}$$

ultimately determined by the membrane potential ψ and the pH^{ex} and pH^{in}. Figure 3 demonstrates the dependence of the ratio $(C_\Sigma^{in})_{sat}/C_\Sigma^{ex}$ on the pH^{ex}. Figure 3 shows that the lower the pH^{ex} value and the greater the difference between the pH^{in} and pH^{ex}, the greater is the ratio of the internal and external concentrations. A sharp increase in HP accumulation is observed with decreasing pH^{in} and pH^{ex} in the region of pH^{ex} values lower that 7. The location of the inflection in the curves substantially depends on the pK values. For a pH^{ex} exceeding 7.5 the total concentration of the dye in the cell is always lower than its concentration in the surrounding medium. Similar dependence of the accumulation of the HP derivative was found experimentally in [14]. The important role of low acidity in tumor cells and their environment during the photosensitizer accumulation in the cell was also noted in [3, 5, 6].

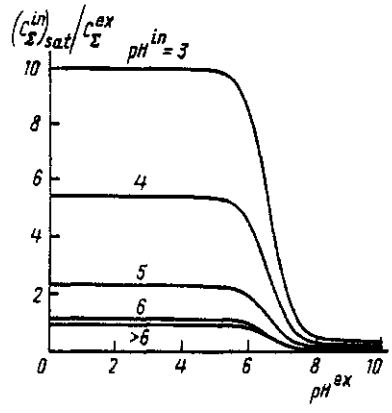

Fig. 3

Dependence of the $(C_\Sigma^{in})_{sat}/C_\Sigma^{ex}$ ratio on the pH^{ex} for different pH^{in} values.

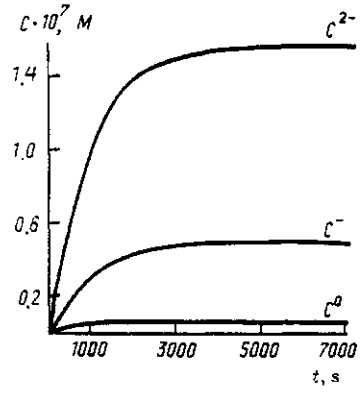

Fig. 4

Kinetics of HP accumulation in the cell: $C_\Sigma^{ex} = 10^{-5}$ M, $pH^{ex} = 7.2$, $pH^{in} = 6.0$, and $\psi = 50$ mV.

At normal pH values the anion and neutral HP forms dominate (see Fig. 1). Therefore one can neglect the presence of cations in the cell. Then, according to system (3), the kinetics of the accumulation of the anion and neutral HP forms in the cell is described by the curves in Fig. 4. In about 7 000 s the accumulation curves attain the level of saturation. This level is different for different pH^{ex}, pH^{in}, and ψ. The HP distribution

inside the cell that corresponds to the exact stationary solution of system (3) ($d/d\tau = 0$) differs little from the distribution calculated by formulas (1) (the terms of the order of ε are added).

It follows from our results that the effect of selective HP accumulation can occur when the pH in the cell cytoplasm is lower than in the surrounding medium. Precisely such situation with pH values is observed *in vivo*. It is well known that glycolysis in malignant cells is intensified to satisfy the increased energy demands. This is accompanied by the release of the glycolysis by-product, lactic acid, into the pericellular space, which reduces the pH there.

The other possible factor causing the selectivity of HP accumulation in tumor cells can be a relation between the hydrophobic dye and the intracellular lipids [15], whose content in tumor cells is higher than in normal cells [2].

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