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Rapid method for comparing the cytotoxicity of organic solvents and their ability to destabilize proteins of the erythrocyte membrane

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Cytotoxicities of a group of frequently used organic solvents were assessed by their effect on thermal stability of erythrocyte membrane proteins. The denaturation temperatures T_m of membrane proteins, peripheral and intrinsic, were detected by the increase in the derivative of suspension impedance during heating. These T_m linearly changed by ΔT_m in the presence of organic solvents indicating labilization (negative ΔT_m) or stabilization (positive ΔT_m) of the structure of respective membrane protein. The potency P of the solvent with molar concentration C_{ex} to affect the conformation stability of membrane protein was defined as $\Delta T_m/C_{ex}$. This potency decreased as both polarity of solvent and its capability to form hydrogen bonds increased. In some solvents (dimethyl sulfoxide and dimethyl formamide) the potencies to destabilized peripheric and intrinsic proteins were equal. Formamide destabilized selectively peripheral proteins. Some solvents (glycerol, especially erythritol) stabilised termally proteins. As the hydrophobicity of the solvents increased (ethylene glycol, methanol, ethanol, acetone, pyridine, ethyl acetate, diethyl maleate) the potency for destabilization of intrinsic proteins strongly increased. Thus, the use of more polar solvents capable of forming more hydrogen bonds appears preferable when low cytotoxicity should be attained.

1. Introduction

Although displaying cytotoxicity, many organic solvents are frequently used as drug solvent media, cryoprotectants, preservants, additives in blood conservation media [1], inductors of whole cell biotransformation [2] and differentiation [3, 4], and even medicines in some cases [5]. There is potential use of such solvents as virus inactivating agents in human blood for transfusion [6].

The acute toxic effect of organic solvents is now under intense study [7]. The toxicity of organic solvents is *in vitro* assessed by their impact on the growth of various microorganisms including yeasts [8], protozoa [9] and most frequently on the hemolysis of human erythrocytes they induce at room temperature [10] or at 37 °C [1, 11].

The organic solvents exert only a weak type of interactions with the proteins of erythrocyte membrane changing their conformation and, consequently, function. These solvents could have, however, different impact on the structure of various membrane proteins that could be important for their cytotoxicity. Measuring only the final hemolysis at 37 °C does not allow discrimination of the conformation changes these solvents produce on various portions of erythrocyte membrane proteins, peripheral and intrinsic. On the other hand, the change in conformation of membrane proteins could be assessed by the relevant change in their thermal stability and denaturation temperatures T_m, respectively.

In order to better elucidate the cytotoxic effect of some frequently used organic solvents, the aim of the present report was to estimate their potency to destabilize or stabilize the conformation of peripheral and integral portions of erythrocyte membrane proteins by the respective changes in T_m.

2. Investigations, results and discussion

The potency P of the solvents to affect the conformation stability of both peripheral and intrinsic groups of membrane proteins displayed strongly species variations (Table). For each solvent the value of P was compared to the solvent coefficients of polarity (δ_P) and capability to form hydrogen bonds (δ_H). Generally, P was inversely affected by both polarity and ability of the solvent to form hydrogen bonds although there were exceptions possibly related to the molecular structure of the solvent.

Table: Impact of organic solvents on thermal stability of erythrocyte membrane proteins

Solvent	Potency to affect the conformation of spectrin P (°C/mol · l)	Potency to affect the conformation of integral protein P (°C/mol · l)	Polarity of the solvent (δ_P)	Ability of the solvent to form hydrogen bonds (δ_H)
H_2O (control)	0	0	15.3	16.7
Erythritol – i	+2.3	+1.0	–	–
Glycerol	+1.60	+0.51	5.9	14.3
Ethylene glycol	−0.25	−1.20	5.4	12.7
Methanol	−1.3	−3.42	6.3	10.9
Ethanol	−2.8	−9.8	4.3	9.5
Pyridine	−13.5	−35	4.3	2.9
Acetone	−6.5	−12.5	5.1	3.4
Dimethyl sulfoxide	−1.04	−1.15	8.0	5.0
Formamide	−8.5	−2.5	12.8	9.3
Dimethyl formamide	−5.10	−5.65	6.7	5.5
Ethyl acetate	−7.2	−71	2.6	4.5
Diethyl maleate	−30.0	−243	≅1.5	≅2.0

The effect is represented by the $\Delta T_m/C_m$ ratio (potency to affect the conformation stability of protein). ΔT_m is the change in the temperature T_m of protein denaturation by heat and C_{ex} is the molar concentration of solvent in suspension. The coefficients corresponding to the polarity of the solvent (δ_P) and to its capability to form hydrogen bonds (δ_H) were taken from the literature [17]

The more hydrophobic and cytotoxic solvents produced labilization on membrane proteins and the less polar was the solvent, the stronger the thermal destabilization was. The conformation stability of membrane proteins was also affected by the capability of the solvent to form hydrogen bonds. The greater this capability was the less destabilization was produced. Compared to pyridine and acetone, ethanol has almost the same polarity but it produced smaller destabilization possibly due to its greater capability to form hydrogen bonds (Table). Owing to their exclusive capability to form hydrogen bonds glycerol and, especially, erythritol increased the thermal stability of mem-

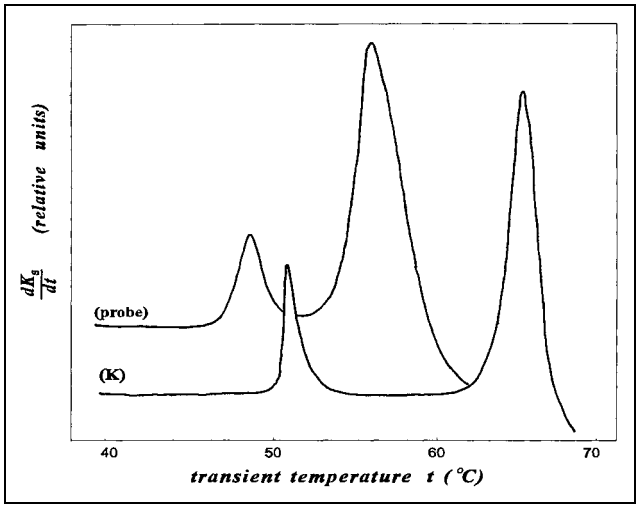

Fig.: Temperature profile of suspension conductivity derivative. Human erythrocytes were suspended in isotonic low-salt media, hematocrit 0.10 and heated at 2.0 °C/min rate. The 52,2 °C peak detects change in the beta-dispersion of cell membranes that involves the heat denaturation of spectrin. The 65,5 °C peak indicates egress of conductive material from the cells following formation of pores during denaturation of integral proteins. The suspension media contained 60 mM NaCl/sucrose (the curve K) plus organic solvent (the curve probe)

brane proteins. These results are consistent with the reports that polyols enhance the thermal stability of water-soluble proteins [15] while alcohols decrease it [16].

Formamide, a very polar solvent, was specific in destabilizing spectrin while strong hydrophobic solvents (ethyl acetate and diethyl maleate) destabilized largely the integral proteins. By contrast, dimethyl sulfoxide and dimethyl formamide were equipotent in destabilizing the structure of peripheral and intrinsic proteins as well, the latter being about 5 times more aggressive.

These findings demonstrate that the ability of organic solvents to destabilize membrane proteins correlate to their cytotoxicity and should be considered in biomedical and pharmaceutical applications. Where it is possible, more polar solvents with greater capability to form hydrogen bonds should be preferred inasmuch as they produce less membrane destabilization and, hence, minor cytotoxic effects.

3. Experimental

The solvents under study (glycerol, erythritol, ethylene glycol, methanol, ethanol, acetone, pyridine, formamide, dimethyl formamide, dimethyl sulfoxide, ethyl acetate and diethyl maleate) were purchased from Sigma Chemical Co, St. Louis, MO, USA. For each solvent, the coefficients corresponding to its polarity (δ_P) and its capability to form hydrogen bonds (δ_H) were taken from the literature [17].

The method for determining denaturation temperatures of both groups membrane proteins, peripheral and intrinsic, in erythrocyte membrane was explained earlier [12]. Briefly, erythrocytes isolated from human blood and their resealed ghosts were suspended in isotonic 60 mM NaCl/sucrose media with or without the solvent, hematocrit 0.10. The suspension was heated at 2.0 °C/min and the first derivative of suspension impedance, measured at 10 kHz, was recorded on a chart. In this way, two separate thermally-induced alterations could be detected in the membrane of intact cells and their isolated membranes (ghosts) as shown in the Fig. The first one centered at 52.2 °C and corresponding to the change in capacity of membranes [13] involves the heat denaturation of peripheral protein spectrin. During the second one the ion concentration gradient dissipates in a narrow temperature interval that is detected as a sharp peak centered at 65.5 °C. This peak corresponds to the increase in the thermohemolysis – related ion permeability [12, 14] and presumably involves a second order phase transition in the hydrophobic domain of integral proteins. With cells and ghosts the reproducibility for determination of T_m was within +/−0.2 °C.

Before the onset of heating sufficient time was provided for the solvent to attain equilibrium on both side of membranes. In the presence of organic solvents, the denaturation temperatures T_m of both membrane alterations linearly changed by ΔT_m indicating labilization (negative ΔT_m) or stabilization (positive ΔT_m) of the structure of respective membrane protein, spectrin and the integral proteins (Fig.). For each solvent with molar concentration C_{ex} the $\Delta T_m/C_{ex}$ ratio was determined and later referred to as potency P of the solvent to affect the membrane protein conformation stability.

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