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Erythrocyte membrane permeability for a series of diols

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Abstract

Erythrocyte membrane permeability coefficients for a series of diols have been defined by the method developed. The method is based on the physical and mathematical modeling of hypotonic hemolysis process. There have been also determined membrane permeability coefficients for erythrocytes treated with *p*-chloromercuribenzenesulfonic acid monosodium salt (pCMBS), which is known to block aqueous protein channels. Permeating process is shown to be conditioned both by hydrophilic/hydrophobic properties of the molecules and their geometrical parameters. The obtained results propose that, when exceeding the molecules diameter over a value of 4 Å, the permeability coefficient reduces due to decreasing of flow through the aqueous protein pores of a constant size. Permeability coefficients for comparatively hydrophobic molecules are almost directly proportional to the coefficients of partition between hydrophobic and hydrophilic phases, by pointing to a lipid way of permeation of these molecules through erythrocyte membranes.

Keywords: Erythrocyte membrane; Permeability; Diols

1. Introduction

Plasmatic membrane is the key structure, which separates a cell from the environment and interacts with it. Passive permeability of the cell membrane is conditioned by its general structure, the properties of the compounds and the way they interact. Comparison of permeability coefficients for different substances showed that permeability coefficient depends significantly on lipophilic properties of the molecules. For a number of substances, the value of permeability coefficient is almost directly proportional to that of hydrophilic/hydrophobic partition [1,2]. The small neutral hydrophilic molecules, such as urea, ethyleneglycol and especially water, are known to penetrate easily through membranes of certain cells. The permeability models for penetration of hydrophilic molecules through biological membranes were developed based on the analysis of the experimental data. One of the models supposes a fluctuative formation of pores in the lipid bilayer as the result of thermal motion [3]. Another modification of this hypothesis considers permanent presence of hydrophilic pores of a certain diameter formed by protein structures. In an erythrocyte membrane, the anion-exchange protein of band III is an example of such a transmembrane structure [4]. This protein is known to bind to such organic reagent as *p*-chloromercuribenzenesulfonic acid monosodium salt (pCMBS) [5], which inhibits transport of water and small hydrophilic molecules. It was assumed that incubation of erythrocytes with such reagents resulted in blockage of protein water channels due to conformational changes in the protein of band III, while lipid ways for water penetration are still available [4]. In the work presented, we analysed the permeability of erythrocyte membranes to a series of diols. This choice was conditioned by the possibility to compare physical and chemical properties for the molecules in homologous series and structural isomers and to elucidate the influence of these properties on their permeability through biomembranes. From the practical point of view, such a choice was made due to a wide use of certain diols and glycerol in cryobiological routine.

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2. Materials and methods

2.1. Materials

For investigation, we have chosen the following polar non-electrolytes: ethane diol (ethylene glycol), two structural isomers of propane diol (1,2- and 1,3-propane diol), four structural isomers of buthane diol (1,2-, 1,3-, 2,3-, 1,4-buthane diol), di- and triethylene glycol and propane triol (glycerol). Substances were "chemical pure" or "pure for analysis" standards and additionally purified. Diols purification was accomplished by double vacuum distillation (under 2 mm Hg) followed by absorption with activated aluminium oxide. Glycerol was purified by single vacuum distillation after pre-absorption with activated coal.

Erythrocytes were obtained from bank blood supplied by Kharkov State Blood Perfusion Station. Erythrocytes were isolated 2 days after blood collection. This was conditioned by the necessity to test blood for AIDS, hepatitis and other infections. Only uninfected blood was used in the experiments. The state of the erythrocytes was controlled by light microscopy. After 2 days of storage at hypothermic conditions, erythrocytes have normal discoid shape and keep the ability to rouleaux formation.

To block aqueous protein channels, we used pCMBS (Sigma).

2.2. Methods

Permeability coefficients were determined with elaborated by us method, which is based on physical and mathematical model of hemolysis in aqueous solutions of permeating substance. For the determination of permeability coefficients by measuring the time required for 50% hemolysis in hypotonic medium, the erythrocyte volume change in time is usually calculated using the Kedem–Katchalsky equations at different values of permeability coefficients. Such a permeability coefficient at which the calculated time required for the cell to reach a critical volume coincides with the experimentally determined time of 50% hemolysis is supposed to be the unknown value. Hemolysis is assumed to occur immediately when the cell reaches a certain critical size [6]. However, it has been shown the assumption that hemolysis occurs immediately when an erythrocyte reaches a certain fixed critical volume is not valid [7]. The time t_h, during which hypotonic hemolysis in erythrocyte suspension reaches 50%, was shown to consist of two items: $t_h = t_k + t_s$, where t_k represents the mentioned time required for an erythrocyte to reach a critical volume. The existence of the spherical time period t_s is connected with the fact that the pore formation in membrane is an activated process and requires some time to occur. To this aim, a theoretical model was developed for measuring the time required for 50% hemolysis [8–11]. It introduces into consideration the concept of the so-called "spherical period" and dependence of critical erythrocyte volume on membrane transport characteristics. We obtained the analytical solution of the equation system, which describes the cell relative volume change from the time when the cells come into contact with a hypertonic aqueous solution of permeable substance until the cell becomes spherical [9,11] and the average time of a pore formation [10,11]. The developed physical and mathematical model explains the process of hypotonic hemolysis as a whole, and in particular connects the time required for 50% release of hemoglobin with the cell membrane permeability coefficient for non-electrolyte. This theory, therefore, can be used for the experimental determination of cell membrane permeability coefficients for non-electrolytes.

Permeability coefficients for non-electrolytes were determined as follows. At the initial moment, 3 μl of erythrocyte suspension were added to 3 ml of aqueous solution of the penetrating substance in the chamber of the device for measuring of the intensity of the scattered light. The time dependence of the light scattered by the cell suspension was registered at 9° towards the reference beam ($\lambda = 1000$ nm). The intensity of the light scattered can be determined theoretically based on the physical and mathematical model of hypotonic hemolysis developed in our laboratory and using Mie scattering theory well known in theoretical optics. Therefore, we obtain theoretical dependence of the light scattered by the cell suspension under the certain angle upon the concentration of hemoglobin in the cell during hemolysis. The value of permeability coefficient could be obtained by the coincidence of the theoretical and experimental time curves of the intensity of the light scattered by erythrocyte suspension at the convenient calculated value of this coefficient.

Geometrical parameters of molecules were estimated basing on Stewart models using the Hyper Chem Pro v.5.1 software.

To block the protein channels, erythrocytes were incubated with 2 mM pCMBS during 1 h at 22 °C [12]. After incubation, erythrocytes were washed with phosphate buffer pH 7.4.

3. Results and discussion

Following our method, the permeability coefficients of human erythrocyte membranes for the substances under investigation were determined in native conditions and after pre-incubation with pCMBS. Measurements were carried out at 20 °C and the concentrations used were 1 M.

Experimental results and calculated geometrical parameters of the molecules are presented in the Table 1 as well as previously obtained [13] octanol/aqua partition coefficients (K_p), which describe hydrophobic properties of the molecules.

These data give evidence that butanediol penetrates into the cells through the lipid phase. Permeability coefficients for butanediol isomers depend greatly on the coefficient of hydrophobic/hydrophilic partition with correlation coeffi-

Table 1
Erythrocyte membrane permeability coefficients (P) at 20 °C and partition coefficients (K_p) and geometrical parameters of penetrating molecules (D—diameter, L—length, V—volume)

Substance	Structural formulae	Permeability coefficients, P x 10_6, m/s		Partition coefficient, Kp	Molecules' geometrical parameters		
		Native erythrocytes	pCMBS treated erythrocytes		D,Å	L,Å	V,Å^3
Ethylene glycol (ethane diol)	CH_2–CH_2 OH OH	1.98±0.48	0.526±0.092	0.04	2.6	5.2	27.6
Diethylene glycol	CH_2–CH_2–O–CH_2–CH_2 OH OH	0.421±0.045	0.271±0.037	-	4.1	7.2	95.0
Triethylene glycol	CH_2–CH_2–(O–CH_2–CH_2)$_2$–OH OH	0.162±0.044	0.14±0.002	-	4.4	10.4	158
1,2-Propane diol	CH_3–CH–CH_2 OH OH	1.6±0.24	0.664±0.18	0.076	3.7	5.0	53.7
1,3-Propane diol	CH_2–CH_2–CH_2 OH OH	0.897±0.3	0.572±0.057	0.064	4.1	5.7	75.2
1,4-Buthane diol	CH_2–CH_2–CH_2–CH_2 OH OH	0.979±0.292	0.646±0.141	0.137	4.0	7.4	92.9
1,3-Buthane diol	CH_3–CH–CH_2–CH_2 OH OH	1.89±0.18	1.01±0.27	0.182	3.6	6.0	61.0
2,3-Buthane diol	CH_3–CH–CH–CH_3 OH OH	2.64±0.22	1.69±0.22	0.227	3.9	5.8	69.2
1,2-Buthane diol	CH_3–CH_2–CH–CH_2 OH OH	2.89±0.11	2.67±0.3	0.308	4.3	6.1	88.5
Glycerol	CH_2–CH–CH_2 OH OH OH	0.038±0.003	0.038±0.008	0.005	4.7	5.2	90.2

cient of 0.927 (Fig. 1, curve 1, right branch). Moreover, taking into account that the correlation coefficient has the value of 0.996 (Fig. 1, curve 2) for the erythrocytes pre-incubated with pCMBS, the suggestion of butanediol penetration through lipid bilayer becomes very probable. On the other hand, erythrocytes pre-incubation with pCMBS leads to a quite considerable inhibition of butanediol penetration that can provide the evidence for the protein aqueous pores to be available for butanediol as well.

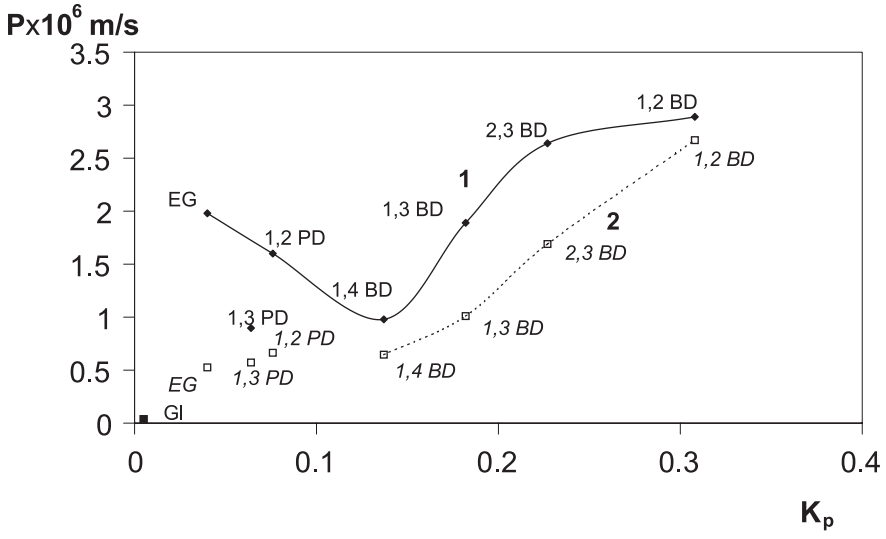

Fig. 1. Dependence of erythrocyte membrane permeability coefficients (P, m/s) at 20 °C vs. partition coefficient of the penetrating substances in the "n-octanol–water" system (K_p). ◆—native erythrocytes, □—pCMBS-treated erythrocytes.

Analysis for the hydrophilic substances (ethyleneglycol, 1,2-propanediol, 1,3-propanediol) shows that penetration of ethyleneglycol and propanediols is conditioned by both their hydrophilic properties and sizes. The correlation coefficient between permeability of these substances and their diameter is -0.9 and that, between permeability and the volume, is -0.97. Since these non-electrolytes are highly hydrophilic, they are supposed to penetrate through aqueous pores, and therefore a strong dependence on sizes is clear. It is interesting that, while hydrophilic/hydrophobic partition coefficients for 1,2-propanediol and 1,3-propanediol are very close, the permeability coefficient for 1,2-propanediol is more than twice that for 1,3-propanediol. Pre-incubation of erythrocytes with pCMBS inhibits the penetration of 1,2-propanediol by 58%, but only by 36.6% of that of 1,3-propanediol. The difference in size of these isomers leads to a significant decrease in the penetration of 1,3-propanediol through aqueous pores. Therefore, a change in diameter from 3.7 Å for 1,2-propanediol to 4.1 Å for 1,3-propanediole is a critical for passing through the pore. The data for butanediols confirms this conclusion. The percentage of inhibition in penetration after pre-incubation with pCMBS for 1,2-butanediol with the molecular diameter of 4.3 Å decreases only by 7.6%.

Comparison of permeability coefficients in the series: ethyleneglycol–diethyleneglycol–triethyleneglycol leads to the same conclusions. For example, permeability coefficient significantly decreases from ethyleneglycol (with the molecular diameter $D=2.6$ Å) to diethyleneglycol ($D=4.1$ Å). Pre-incubation of erythrocytes with pCMBS highly inhibits penetration of ethyleneglycol (by 77%). Hence, the major passway for ethyleneglycol is thought to be through aqueous pores. For diethyleneglycol with the molecular diameter of 4.1 Å, that coincides with that for 1,3-propanediol, inhibition of penetration is only by 36.6% and agrees with that of 1,3-propanediol (36.3%). As for triethyleneglycol, its permeability coefficient is more than one order less than that for ethyleneglycol and preincubation with pCMBS does not statistically change it. So, molecules of triethyleneglycol can barely penetrate aqueous pores.

As we can see from the data the permeability coefficient for glycerol, it does not change after pre-incubation with pCMBS and also is one order less than that for ethyleneglycol after pre-incubation. The molecular diameter of glycerol is 4.7 Å, so it can be considered not to penetrate through pores due to sterical limits. While penetration through lipid bilayer is also non-efficient because of low hydrophobic/hydrophilic partition coefficient. It is quite interesting that in contrary to human erythrocytes glycerol almost do not penetrate bovine erythrocytes and its permeability rate for rat erythrocytes is in 242 times higher than that for sheep erythrocytes [14]. It is known also that passive permeability of lipid bilayers is tightly associated with their viscosity, and therefore with content of non-saturated fatty acids. At the same time, the double bonds index for lipids of sheep, bovine, human and rat erythrocytes makes 0.7, 0.8, 1.4 and 1.7 correspondingly [15]. Taking into account these data, we suppose that glycerol for all that penetrate to human erythrocytes through the lipid phase.

As the result of our research, we can postulate that small hydrophilic molecules with the diameter up to 4 Å can easily penetrate through aqueous pores formed by protein structures. But to some extent, they can also penetrate through lipid phase. The more lipophilic the molecule is, the higher is the probability of its penetration through the lipid phase. Therefore, the permeability of 1,2-butanediol, which cannot easily pass through protein pore, is even higher than permeability of ethyleneglycol. It is possible that the penetration mechanism for small hydrophilic molecules through lipid bilayer differs from that for lipophilic molecules, which can be dissolved in lipid phase. However, in both cases, the size of the molecule affects permeability coefficient.

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