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Permeability of rat and rabbit erythrocyte membranes for a series of amides

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ABSTRACT

Permeability coefficients of rat and rabbit erythrocyte membranes for a series of amides, as well as for erythrocytes treated with *p*-chloromercuribenzenesulfonic acid monosodium salt (pCMBS) have been determined at 25 and 37 °C. Directly proportional dependence of the pCMBS treated erythrocyte permeability for investigated substances and their partition coefficients between the hydrophobic phase and water as well as the values of activation energy of this process indicate that penetration of small hydrophilic molecules is realized by passive diffusion through the lipid bilayer. The results obtained indicate that penetration of small hydrophilic molecules of formamide through lipids is determined by the existence of a free space between hydrocarbon chains that arises from kink formation. The differences in permeability between rat and rabbit erythrocyte membranes could arise in particular as a result of the differences in lipid composition.

1. Introduction

In the previous studies, we characterised the permeability of human erythrocyte membranes for non-electrolytes such as series of diols [1] and amides [2]. These substances were shown to permeate the erythrocyte membrane by passive diffusion in two different ways: through the lipid bilayer and along protein channels. The membrane permeability of human erythrocytes treated with pCMBS correlates with the partition coefficients of substances between the hydrophobic phase and water. It means that pCMBS treatment of erythrocytes abolishes the hydrophilic protein-mediated pathway for substances to cross the membrane, leaving only their residual penetration through a lipid bilayer. The rate of permeation in this case is limited at the stage of molecule entering into lipid bilayer.

The permeability of a lipid bilayer depends on its phospholipid composition and the content of cholesterol. The study of lipid bilayer permeability showed that factors increasing the order and decreasing the motility of molecules in hydrocarbon regions generally decrease membrane permeability. Higher carbon chain saturation and the increased length correlate with decreased permeability for water and small electrically neutral molecules [3]. Increased temperature leads to fatty acid chain motility and changes in membrane lipid surface density, as well as enhanced bilayer permeability.

Our further investigations are directed to the study of erythrocyte membrane permeability in animal species with varying cell membranes lipid composition. In the present report the membrane permeability of rat and rabbit erythrocytes (native and pCMBS treated) for a series of amides has been characterised.

2. Materials and methods

2.1. Materials

The following non-electrolytes were investigated: formamide, acetamide, dimethyl formamide, dimethyl acetamide. All substances were of chemical pure standards and additionally purified [2].

Erythrocytes were obtained from rat and rabbit blood collected with “Glugitsyr” preservative: from rat (white breedless animals) by paracentesis of the caudal vein, from rabbit (Chinchilla breed) by insection or paracentesis of a lateral auricular vein.

2.2. Methods

Permeability coefficients for non-electrolytes were determined as described in [1,2]. The theoretical background of the method was presented in [2,4].

Aqueous protein channels were inhibited using *p*-cloromercuribenzenesulfonic acid, monosodium salt (pCMBS, Sigma). The erythrocytes were incubated with 2 mM pCMBS for 1 h at 22 °C [5]. Native erythrocytes and erythrocytes that had been incubated with pCMBS were washed in phosphate-buffered saline (pH 7.4).

The geometrical parameters of the molecules were estimated using Stewart models using the Hyper Chem Pro v.5.1 software.

The partition coefficients of the substances between water and the non-polar phase were determined as described in [2].

3. Results and discussion

The permeability coefficients of rat and rabbit erythrocyte membranes for the substances under investigation (concentration

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Table 1
Membrane permeability coefficients for rat erythrocytes (P) and calculated activation energy values (E_A)

Substance	Permeability coefficients, P ($\times 10^6$) m/s				Activation energy, E_A kJ/mol	
	25 °C		37 °C		Native erythrocytes	pCMBS treated erythrocytes
	Native erythrocytes	PCMBS treated erythrocytes	Native erythrocytes	pCMBS treated erythrocytes		
Formamide	23.97±2.06	6.76±1.73	32.23±4.47	21.84±3.43	19.09	75.62
Acetamide	15.68±3.23	4.38±0.78	21.86±1.71	14.33±1.1	21.42	76.43
Dimethyl formamide	21.70±3.69	16.22±1.97	33.45±4.44	29.11±1.44	27.90	37.71
Dimethyl acetamide	21.08±2.60	17.11±1.12	32.82±4.09	30.09±2.12	28.55	36.40

of 1 M) were measured at 25 °C and 37 °C and after preincubation with pCMBS. Experimental data reporting permeability coefficients and calculated activation energy values for rat and rabbit erythrocyte membranes are presented in Tables 1 and 2. The plots of erythrocyte membrane permeability coefficients (P, m/s) vs. partition coefficients (K_p) of the penetrating substances are shown in the Fig. 1.

The results presented show that, for all substances under investigation, permeability coefficients are reduced after incubation of rat and rabbit erythrocytes with sulfhydryl reagent, in a similar fashion to that of human erythrocytes. This phenomenon confirms that these molecules permeate the erythrocyte membrane by two pathways: through the lipid bilayer and through protein channels. Reduction in permeability is significantly smaller for the more hydrophobic molecules: dimethyl formamide (K_p=0.233) and dimethyl acetamide (K_p=0.291), because the majority of their flow occurs through the lipid bilayer. With the exception of formamide, at both temperatures tested the coefficient of correlation between the permeability coefficient of pCMBS treated erythrocytes and the water-hydrophobic phase partition coefficient is 0.95 for rabbit and 0.98 for rat erythrocytes. That is the membrane permeability of pCMBS treated erythrocytes definitely correlates with solubility increase in lipid. In both species, the monotonic relation between membrane permeability coefficient of pCMBS treated erythrocytes and partition coefficient is disrupted by formamide (Fig. 1). However, activation energy values show that both formamide and other substances under investigation permeate trough lipid bilayer by passive diffusion. There are no reasons to suppose that there are other mechanisms of these substances for permeating like facilitated diffusion. High permeating ability of formamide in pCMBS treated erythrocytes can be explained by the significantly smaller size of formamide molecules (D=2.0 Å, L=3.3 Å, V=10.4 Å^3) when compared with acetamide molecules (D=3.2 Å, L=3.8 Å, V=30.5 Å^3). The size of formamide molecule is close to that of water with an effective radius (R_{ef}) of 1.4 Å [6]. For a molecule of this size, effective penetration through fluctuating pores or lipid bilayer defects (kinks) is possible. The theoretical analysis of fluctuating pore formation process in lipid bilayer showed that it was most likely that pores have the diameter up to 5 Å [7]. The appearance of a kink leads to a hydrocarbon chain shift of approximately 1.5 Å, with simultaneous formation of a free space. The total space occupied by the lipid molecule increases by 25–50 Å^3 [8].

Increasing the temperature from 25 °C to 37 °C results in an increase of the flux mediated by lipid bilayer for all the substances. This increase was most apparent for the smallest molecules under investigation: acetamide and especially formamide (Table 3). This observation is consistent with the notion that a temperature dependent rise in lipid bilayer permeability occurs. It should be noted that the ratio P(pCMBS treated erythrocytes)/P(native erythrocytes) for hydrophilic substances in rabbit erythrocytes at 25 °C is significantly lower than that for the rat cells, while the opposite is true at 37 °C.

Such behavior might reflect the different lipid composition of the erythrocyte membrane in these animals. It is known that the cholesterol content in erythrocyte membranes of different animal species vary slightly [9]. At the same time it was shown, that only considerable alteration of cholesterol relative content results in visible change of lipid bilayer permeability [10,11]. Chernitskiy and Vorobey reported [12] that the relative content of sphingomyelin, almost entirely lacking double bonds in its hydrocarbon tails, constitutes 19% of the lipid in rabbit erythrocyte membranes, whereas this number is 12% in rat cells. At the same time, however, the acidic phospholipid content (phosphatidyletanolamine and phosphatidylserine) in rabbit erythrocyte membranes is greater than that found in rat. These phospholipids have larger hydrocarbon chains with up to 6 double bonds. Increasing the temperature the probability of gauche–isomers formation increases. The energy of gauche-conformation exceeds best relatively slightly the energy of *trans*-conformation (2–3 kJ/mol higher), but these states are separated by energetic barrier of ~12–17 kJ/mol. Moreover, it is more likely that larger chains will exhibit 120° rotations around C – C bonds, resulting in formation of a hydrocarbon chain kink. Double (cys-) bonds in non-saturated fatty acid chains of membrane lipids can serve as "nucleation centers" for kink formation in neighboring saturated chains. In this case, the kink formation in the non-saturated chain requires only one gauche-conformation with a chain incurvation of 80° and the steric restrictions for the location of a non-saturated chain in the membrane hydrocarbon zone are eliminated. This situation is consistent with the experimental observation of sharp decrease in temperature of phase transition in membranes comprised of saturated lipids on addition of small quantities of non-saturated fatty acid chains. Estimation of gauche-conformation

Table 2
Membrane permeability coefficients for rabbit erythrocytes (P) and calculated activation energies (E_A)

Substance	Permeability coefficients, P($\times 10^6$) m/s				Activation energy, E_A kJ/mol	
	25 °C		37 °C		Native erythrocytes	pCMBS treated erythrocytes
	Native erythrocytes	pCMBS treated erythrocytes	Native erythrocytes	pCMBS treated erythrocytes		
Formamide	21.61±3.04	3.63±0.21	29.10±4.64	23.54±4.79	19.19	120.54
Acetamide	17.59±0.91	2.32±0.38	24.39±3.26	14.85±1.91	21.07	119.72
Dimethyl formamide	18.03±2.23	12.97±1.72	27.35±4.05	25.28±2.25	26.87	43.03
Dimethyl acetamide	16.71±1.95	12.01±1.95	25.62±1.63	23.66±1.64	27.56	43.72

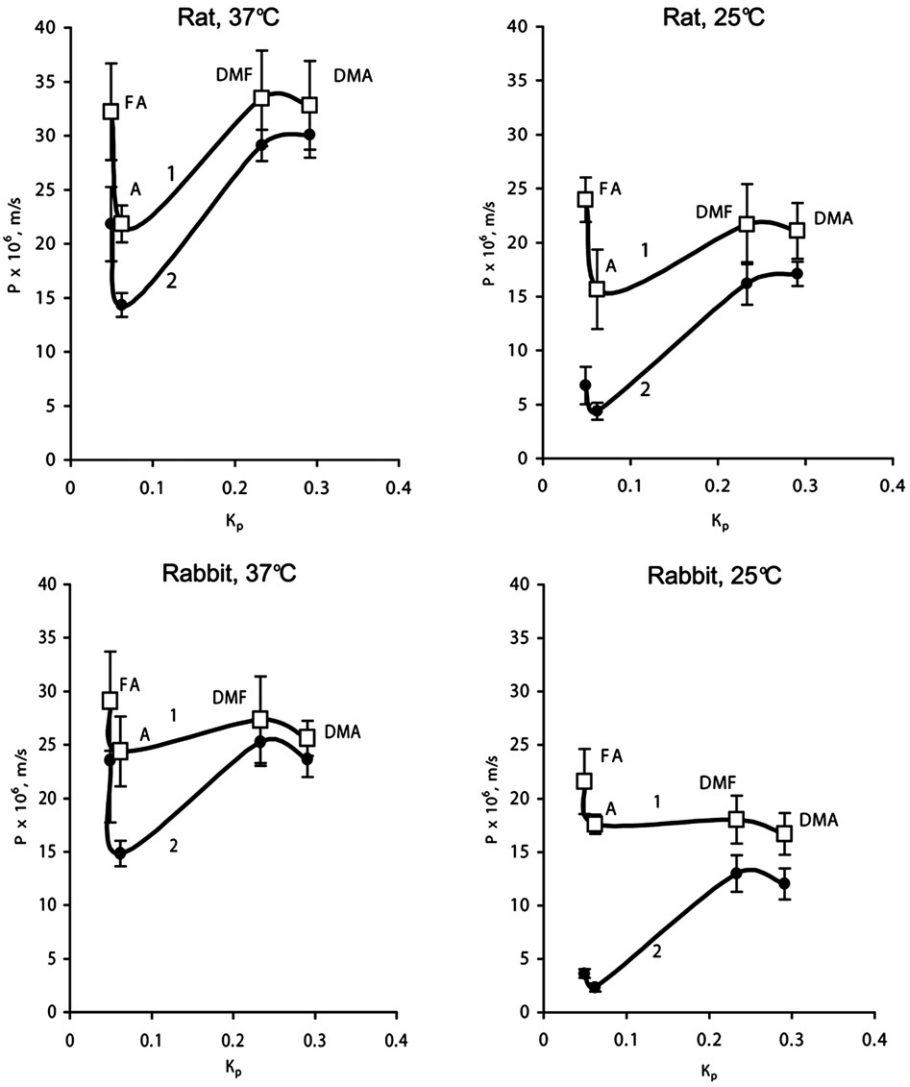

Fig. 1. The relation between erythrocyte membrane permeability coefficients (P, m/s) and partition coefficient (K_p) for penetrating substances in the '*n*-octanol water' system. 1 — native erythrocytes; 2 — pCMBS treated erythrocytes; FA — formamide; A — acetamide; DMF — dimethyl formamide; DMA — dimethyl acetamide.

occurrence frequency at 37 °C and 12 kJ/mol barrier gives the value of $\sim 10^{10}$ s^{-1} [8].

Besides the stated differences between neutral and acidic phospholipids, it should be noted that various phospholipid molecules exhibit spontaneous curvature, e.g. phosphatidylcholine, sphyngomyelin and phosphatidylserine have a cylinder shape, and phosphatidylethanolamine is conical. Analysis of fluctuating hydrophilic pore formation in a lipid bilayer shows that the energy of pore formation strongly depends on the linear tension of the pore periphery. The minimal values of this parameter are characteristic for the membranes comprising lysolecithin, which exhibits positive spontaneous curvature (inverse cone). Membranes of the phosphatidylcholine demonstrate intermediate values for linear tension. Maximum linear tension values are characteristic of membranes consisting of phospholipids of phophatidylethanolamine type, the molecules of which exhibit a negative spontaneous curvature. The probability of local disturbances in bilayer by pore formation therefore depends on the geometry of lipid molecules present. Molecules with positive spontaneous curvature (e.g. lysolecithin) facilitate aqueous pore formation in the bilayer, in contrast to molecules with zero or negative spontaneous curvature [8]. The phosphatidylethanolamine content of rabbit erythrocyte membrane (31.8% of total lipids) significantly exceeds that for rat (21.5%) [12]. The more pronounced temperature dependent increase of the ratio P (pCMBS treated erythrocytes)/P(native erythrocytes) in rabbit erythrocytes for formamide as compared with that in rat cells and accordingly greater activation energy of formamide molecule permeating through lipid bilayer indicates that the comparatively higher permeability of lipid bilayer for small molecules such as formamide arises from a kink translocation along hydrocarbon chains, rather than as a result of fluctuating hydrophilic pores.

Table 3
Ratio of permeability coefficients for pCMBS treated and native erythrocytes

Substance	P(pCMBS treated erythrocytes)/P(native erythrocytes)			
	Rat erythrocytes		Rabbit erythrocytes	
	25 °C	37 °C	25 °C	37 °C
Formamide	0.28	0.68	0.17	0.81
Acetamide	0.28	0.66	0.13	0.61
Dimethyl formamide	0.75	0.87	0.72	0.92
Dimethyl acetamide	0.87	0.92	0.72	0.92

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