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Effect of alkyl-β,D-glucopyranosides on hypertonic haemolysis of erythrocytes

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Abstract

The effect of temperature (5–20 °C) and treatment with phenylhydrazine on the hypertonic lysis of erythrocytes in the presence of alkyl-β,D-glucopyranosides was studied. The results highlight an important role for the cytoskeleton-membrane complex, which allows the cells to both withstand hypertonic stress within the temperature range studied and facilitate the protective effect of alkylglucopyranosides at low temperatures (5 °C).

Keywords: Erythrocyte; Hypertonic haemolysis; Alkyl-β,D-glucopyranosides; Temperature; Phenylhydrazine

1. Introduction

The effect of highly concentrated salt solutions has been established as the main cause of cryoinjury during erythrocyte preservation [1,2]. Considerable increases in the tonicity of the solution in combination with temperature reduction trigger the processes resulting in defects in the plasma membrane structure and cell haemolysis. Previously, we have demonstrated [3] that derivatives of glucopyranoside, which are referred to as non-ionic amphiphilic compounds, considerably increase the erythrocyte's ability to resist the damaging effects of highly concentrated salts. The protective effect of some amphiphiles is modulated by temperature [2]. We were interested in investigating the temperature dependence of hypertonic haemolysis of erythrocytes in the presence of alkylglucopyranosides. Furthermore, erythrocyte sensitivity to the combined effects of hypertonic medium and low temperature is regulated by the state of cytoskeleton [1], the proteins of which control the process of formation and stabilization of transmembrane pores [4]. Therefore, amphiphilic compounds, directly affecting the lipid bilayer, may change these processes, resulting in the impairment of membrane integrity.

The aim of this work was to study the effect of temperature on the hypertonic haemolysis of erythrocytes and to determine how the antihaemolytic properties of activity of alkyl-β,D-glycopyranosides are manifested.

2. Materials and methods

Hexyl-β,D-glycopyranoside, octyl-β,D-glucopyranoside, decyl-β,D-glucopyranoside and phenylhydrazine were supplied by Sigma. All solutions were prepared with 5 mM phosphate buffer, pH 7.4.

The study was conducted using erythrocytes harvested from donor's blood. The erythrocytes were removed by washing with centrifugation three times in 10 volumes of physiological solution (0.15 M NaCl, pH 7.4). After washing, the cells were stored for no more than 4 h at 4 °C.

The dynamics of erythrocyte haemolysis was measured by the scattering of light ($\lambda = 720$ nm).

Aliquots of cell suspension were transferred into a thermostatic cuvette, containing 4.0 M NaCl, maintained at various temperatures. A final concentration is 3.1×10^6 cells/ml. Red cell haemolysis was evaluated by monitoring the optical density of the suspension, which is proportional to the concentration of intact cells at high dilutions. The rate of red cell haemolysis was determined as the slope of the line, drawn to the kinetic curve of the change of red cell suspension density under 4.0 M NaCl solution. The of

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maximum antihaemolytical activity (AH) of amphiphilic compounds was calculated according the formula:

$$\mathrm{AH} = \frac{k - a}{k} \times 100\%$$

where k = the degree of erythrocyte haemolysis under experimental conditions in the absence of an amphiphilic compound and a = the degree of erythrocyte haemolysis in the presence of the amphiphilic substance.

Modification of red cells cytoskeleton by phenylhydrazine was according to the method of Arduini et al. [5]. The cells were incubated with phenylhydrazine (1 mM) with constant shaking at 37 °C for 10 min. They were then removed from this solution by two washes, used for further experiments.

3. Results

Fig. 1 shows the temperature dependencies of hypertonic haemolysis of erythrocytes, placed into 4.0 M NaCl. These data demonstrate that hypertonic haemolysis of intact cells is not changed over the whole temperature range (10–20 °C), significantly decreasing to 5 °C. After treatment with phenylhydrazine, hypertonic haemolysis of the treated cells exceeds that of the control erythrocytes and does not depend on the temperature of the lysis medium when between 10 and 20 °C. The data presented in Fig. 2 demonstrate that a temperature decrease from 20 to 10 °C causes a reduction in the rate of haemolysis in both control and phenylhydrazine modified cells is significantly higher than that of the control cells. At 5 °C, the lysis rate of the cells, modified with phenylhydrazine, sharply rises when compared to that of the control erythrocytes (Fig. 2).

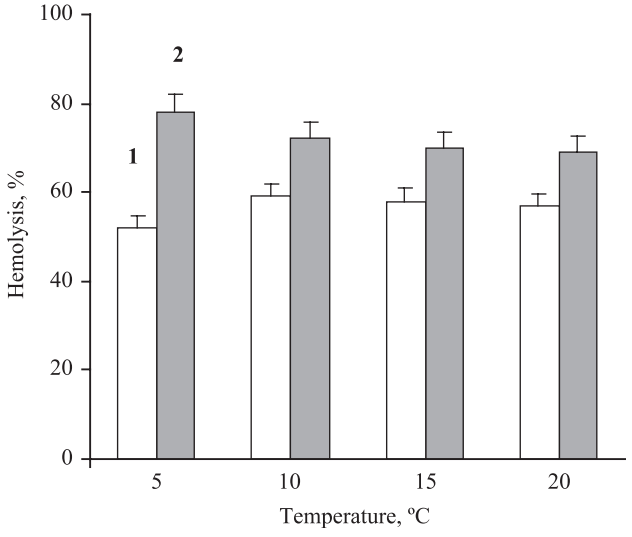

Fig. 1. Effect of phenylhydrazine on temperature dependent haemolysis of erythrocytes under hypertonic conditions (4.0 M NaCl): (1) control cells, (2) phenylhydrazine-modified cells, 1 mM. Values are the mean of six experiments ± standard deviation between experiments.

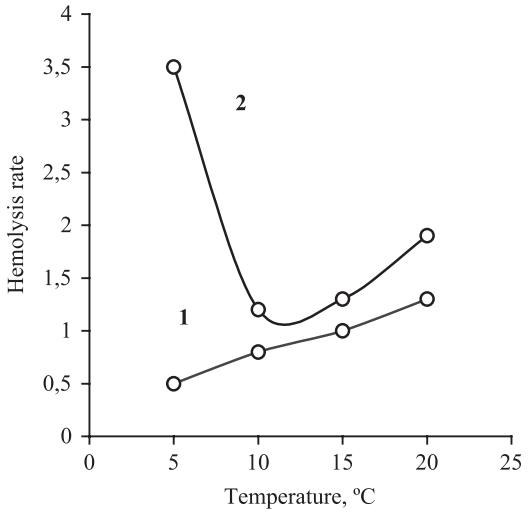

Fig. 2. Effect of temperature on rate of haemolysis under hypertonic conditions (4.0 M NaCl) for erythrocytes with and without phenylhydrazine modification: (1) control cells, (2) phenylhydrazine-modified cells, 1 mM.

Fig. 3 demonstrates how hypertonic haemolysis of erythrocytes in the presence of glucopyranoside derivatives with alkyl chain lengths from 6 to 10 carbonic atoms: hexyl-β,D-glucopyranoside (C6), octyl-β,D-glucopyranoside (C8) and decyl-β,D-glucopyranoside (C10) varies with temperature. Homologues were used at concentrations, which cause minimal hypertonic haemolysis of the cells. The maximum protective effect of alkylglucopyranosides is found when applied simultaneously with highly concentrated salts [3]; therefore, erythrocytes were placed into lysis medium (4.0 M NaCl), which already contained the appropriate amphiphilic compound. From Fig. 3, it can be seen that all of the amphiphilic substances studied reduce the level of hypertonic haemolysis in the temperature range

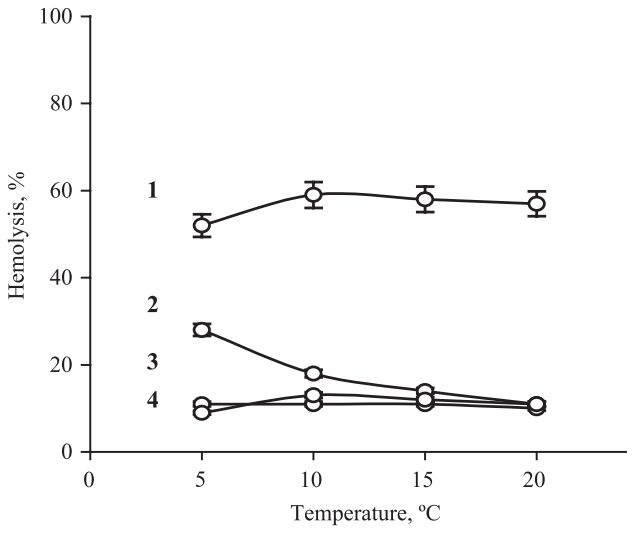

Fig. 3. Temperature dependent hypertonic haemolysis (4.0 M NaCl) of erythrocytes in the presence of alkylglucopyranosides: (1) control cells, (2) C6 (2.8 mM), (3) C8 (0.45 mM), (4) C10 (0.03 mM). Values are the mean of six experiments ± standard deviation between experiments.

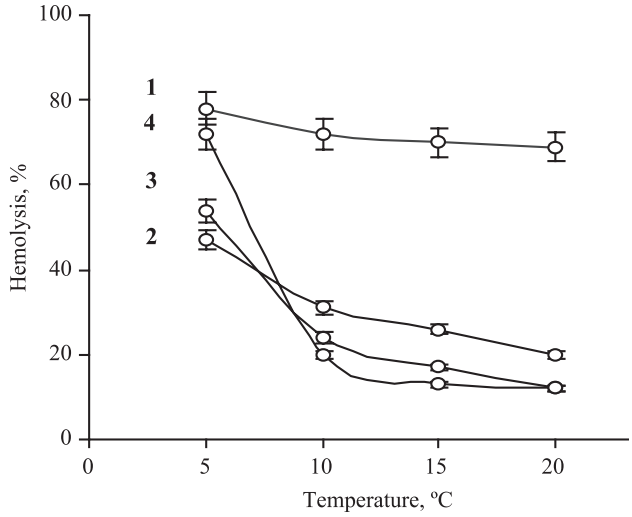

Fig. 4. Temperature dependent hypertonic haemolysis (4.0 M NaCl) of phenylhydrazine-modified erythrocytes in the presence of alkylglucopyranosides: (1) control cells, (2) C6 (2.8 mM), (3) C8 (0.45 mM), (4) C10 (0.03 mM). Values are the mean of six experiments ± standard deviation between experiments.

(5–20 °C). The glucopyranoside derivatives with longer carbon chains (C8 and C10) significantly decrease the level of hypertonic haemolysis irrespective of temperature. However, the antihaemolytic properties of the shorter C6 glucopyranoside is temperature-dependent. The protective effect of this homologue of alkylglucopyranoside is greater at higher temperatures.

Thus, a temperature-dependent effect on hypertonic haemolysis of erythrocytes is only found with short chain homologues of alkylglucopyranoside.

Introducing glucopyranoside derivatives to the lysis medium also reduces the haemolysis of phenylhydrazine-modified erythrocytes (Fig. 4). In this case, the effect of all glucopyranoside homologues is temperature-dependent. C6 glucopyranoside decreases the damage to phenylhydrazine-modified erythrocytes over the whole temperature range (curve 2). However, as the temperature of the lysis medium reduces, the protective effect of this compound is diminished. When comparing curve 2 in Figs. 3 and 4, it becomes apparent that the level of damage of phenylhydrazine-modified cells in the presence of C6 glucopyranoside is greater at all temperatures investigated.

The curve describing the effect of C8 glucopyranoside on phenylhydrazine-modified cells is on the whole similar to the temperature-dependent haemolysis in the presence of C6 glucopyranoside. However, as the temperature decreases down to 5 °C, the level of hypertonic haemolysis of phenylhydrazine-modified erythrocytes in the presence of C8 glucopyranoside increases when compared with that of the C6 glucopyranoside (Fig. 4, curve 3). Of all the homologous tested, C10 glucopyranoside has the greatest temperature dependence effects. At low temperatures, the capacity of this homologous to reduce the hypertonic haemolysis of phenylhydrazine-modified cells is greater than the other glucopyranosides tested. In contrast to high temperature (20 °C) at 5 °C, the haemolysis of modified erythrocytes reduces with the decrease of the length of a substance alkyl chain. Thus, the response of phenylhydrazine-modified erythrocytes to hyperconcentrated salt solutions containing amphiphilic compounds is dependent on temperature and is determined by the hydrophobicity of the alkylglucopyranoside used.

To compare the efficiency of alkylglucopyranosides, we have studied the temperature dependencies of antihaemolytic activity of the substances on native or phenylhydrazine-treated cells in hypertonic media. The data presented in Fig. 5 show that, in native cells, the antihaemolytic activity of C8 glucopyranoside and C10 glucopyranoside is not temperature-dependent. In contrast, the efficiency of C6 glucopyranoside is reduced at 5 °C. At high temperatures, the homologues with longer chain (C8 and C10) (Fig. 5B,C) are more efficient when compared with that of C6 glucopyranoside (Fig. 5A).

In the case of phenylhydrazine-modified cells (Fig. 5, curve 2), the antihaemolytic activity of each homologue showed a marked dependence on temperature. The antihae-

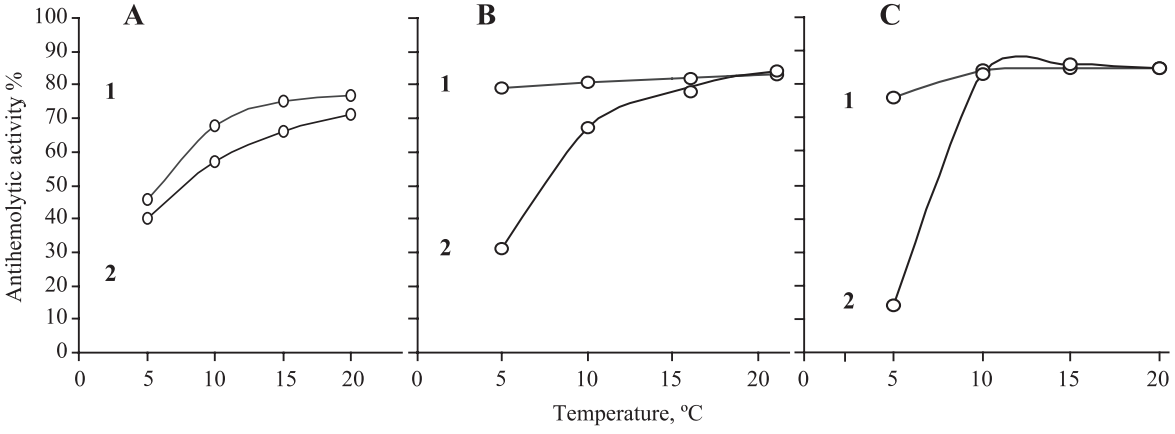

Fig. 5. Temperature dependent antihaemolytic activity of alkylglucopyranosides when (1) native erythrocytes and (2) phenylhydrazine-modified erythrocytes are transferred into 4.0 M NaCl: (A) C6 (2.8 mM), (B) C8 (0.45 mM), (C) C10 (0.03 mM).

molytic ability of C8 glucopyranoside and C10 glucopyranoside at 20 °C was greater than for C6 glucopyranoside. At low temperature, the protective effects of the homologues of glucopyranoside can be put in the following order, according to their degree of antihaemolytic activity: C6>C8>C10.

The data presented in Fig. 5 demonstrate that the antihaemolytic activity of amphiphilic substances under hypertonic haemolysis depends on the initial state of the cells and the temperature of the lysis medium. For C6 glucopyranoside, the decrease in the efficiency of phenylhydrazine-treated cells is approximately the same over the temperature range. However, for homologues with a longer chain length, there is a different picture. In this case, under high temperatures, there are practically no differences in antihaemolytic activity, but at 5 °C, C8 glucopyranoside and C10 glucopyranoside achieve maximum effects.

4. Discussion

The processes responsible for the formation of the haemolytic pore are a common factor in the haemolysis of erythrocytes, irrespective of the stressor causing the haemolysis. This macroscopic pore is a dynamic lipid–protein ensemble, the state of which is determined by physical and chemical factors of environment [6].

Temperature and osmolarity play a vital role in the onset of damage to erythrocytes. In the absence of a temperature change, increasing the in tonicity of the solution results in an increase in erythrocyte damage [7]. However, under constant conditions of hypertonic osmolarity (4.0 M NaCl), reducing the temperature to 5 °C lowers the level of erythrocyte haemolysis (Fig. 1) in the accordance with Arrhenius' law.

The changes in the structure of the erythrocyte membrane in the presence of phenylhydrazine result in a greater degree of damage across the whole temperature range, when in compared with the control cells (Fig. 1). One can suppose that the effect of phenylhydrazine results in the formation of an unstable pre-lytic fraction of cells, which rapidly haemolyse in a hypertonic solution. The high rate of hypertonic lysis observed in of phenylhydrazine-modified erythrocytes is most prominent at low temperatures (Fig. 2), reflecting highly synchronous damage in the unstable fraction of erythrocytes.

Phenylhydrazine is a well-known modifier of the erythrocyte skeleton [5] and when used at a concentration of 0.2 mg/ml (as in these experimental conditions) causes 35–40% degradation of spectrin [8]. Phenylhydrazine destroys α- and β-chains of spectrin without the formation of high molecular weight products [5]; it causes a considerable reduction in free sulfhydrylic groups of spectrin and the majority of other polypeptides [9]. However, at this time, little experimental data is available to support the role of phenylhydrazine not only at the level of membrane protein component. It has been shown [10] that the degradation of cytoskeletal proteins, caused by phenylhydrazine, is accompanied by an increase in the transbilayer movement of membrane phospholipids. Furthermore, although phosphatidylserine exposure on the membrane surface has not been observed, but a disorder of aminophospholipidtranslocase activity has been found in cells treated with phenylhydrazine [11].

Thus, data available in the literature [5,10,11] suggest that phenylhydrazine not only modifies the cytoskeleton, but also cytoskeletal-membrane complex as a whole. Incubating erythrocyte with such a highly reactive substance results in changes in the plasma membrane, which substantially reduce a cell's ability to withstand an unfavourable environment. This is the most clearly observed under conditions where high salt concentration and low temperature are combined. Phenylhydrazine synchronises the cells responses and reduces the variation in the ability of the erythrocyte population to respond to highly salt concentrates.

Alkyl-β,D-glucopyranosides, which are non-ionic amphiphilic compounds, are characterised by high antihaemolytic activity in the presence of various stressors [3,12]. They protect the cells from hypotonic and hypertonic lysis [3,12].

During their incorporation into the erythrocyte membrane, alkylglucopyranosides can modify its structure up to vesiculation, depending on the concentration of compound used. At the molecular level, these processes are mediated by the transbilayer movement of lipids, as well as by the formation of non-bilayer lipid structures [13]. There is evidence that non-ionic amphiphilic compounds make easier transbilayer movement of lipid molecules [13,14]. The ability of amphiphiles to cause the reorganization of membrane is probably an important factor when cells encounter hypertonic solutions. Under hypertonic conditions, processes are initiated in the cell membrane, which result in both the appearance of membrane defects and the activation of pre-existing membrane defects. The addition of amphiphiles to lysis medium results in the reorganization of the membrane, stabilizing and preventing further growth of membrane defects.

For many amphiphilic compounds, it is known that temperatures close to 0 °C cause a sharp reduction or even the loss of their ability for many amphiphilic compounds to protect the cells in an unfavourable environment [2,7]. Alkylglucopyranosides are peculiar in that only the short chain, C6 demonstrated a decrease in antihaemolytic activity as the temperature of the lysis solution was reduced. Stronger homologues (C8 and C10) are characterised by a lack of sensitivity to temperature-dependent changes in membrane. The observed reduction in antihaemolytic activity of C8 and C10 alkylglucopyranosides under hypertonic conditions in phenylhydrazine-modified erythrocytes at 5 °C is probably brought about by substantial changes in the membrane structure caused by the combined effect of the

three environmental factors (phenylhydrazine, hypertonic solution and low temperature; Figs. 4 and 5). ESR spectroscopy has shown a reduction in membrane fluidity in the presence of high concentrations of phenylhydrazine [8], which affected the hydrophobic portion of the membrane. Furthermore, decreasing temperature also reduces membrane fluidity [15].

Amphiphilic molecules are usually located in the liquid region of the erythrocyte membrane, the proportion of which can vary significantly in membranes modified by phenylhydrazine and low temperature. We believe that antihaemolytic effects may be determined by the saturation peculiarities of the membrane after modification by amphiphilic compounds. Their local concentration within the membrane may exceed the optimal concentration and lysis may occur, depending on physical and chemical properties of amphiphile molecules used and the state of the erythrocyte membrane.

5. Conclusions

These observations suggest an important role for cytoskeletal-membrane complex in conferring a cell's capability to resist the hypertonic stress over a range of temperatures. Furthermore, the cytoskeletal-membrane complex plays a significant role in mode of action of alkylglucopyranosides, which protect cells from lysis at low temperatures.

References

[1] A.M. Belous, The role of regulatory systems modulating the state of cytoskeletal proteins under the temperature and osmotic effects, Probl. Cryobiol. 4 (1992) 3–14.
[2] N.M. Shpakova, E.R. Pantaler, V.A. Bondarenko, Antihaemolytic effect of chlorpromazine on hyperosmotic and cold shock of erythrocytes, Biokhimiya 10 (1995) 1624–1631.
[3] O.P. Synchykova, Glucopyranosides effect on human erythrocyte resistance to hyperosmotic stress, Probl. Cryobiol. 3 (1999) 77–79.
[4] F.A. Green, C.Y. Jung, J. Cuppoletti, N. Owens, Hypertonic cryohemolysis and the cytoskeletal system, Biochim. Biophys. Acta 648 (1981) 225–230.
[5] A. Arduini, S. Storto, M. Belfigilo, G. Mancinelli, G. Federici, Mechanism of spectrin degradation induced by phenylhydrazine in intact human erythrocytes, Biochim. Biophys. Acta 979 (1989) 1–6.
[6] M.R. Lieber, T.L. Steck, Dynamics of the holes in human erythrocyte membrane ghosts, J. Biol. Chem. 257 (1982) 11660–11679.
[7] O.N. Dunayevskaya, N.M. Shpakova, V.A. Bondarenko, Hyperosmotic stress of red cells and the antihaemolytic activity of trifluoperazine, Probl. Cryobiol. 4 (1997) 28–33.
[8] T. Ogiso, Y. Ito, M. Iwaki, K. Nakanishi, H. Siato, Effect of phenylhydrazine-induced structural alterations of human erythrocytes on basic drug penetration, Chem. Pharm. Bull. (Tokyo) 37 (1989) 430–434.
[9] A.N. Hashmi, M. Saleemuddin, Phenylhydrazine causes sulfhydyl oxidation and protein aggregation in hemoglobin-free human erythrocyte membranes, Biochem. Mol. Biol. Int. 40 (1996) 543–550.
[10] T.D. Choudhury, N. Das, A. Chattopadhyay, A.G. Datta, Effect of oxidative stress and erythropoietin on cytoskeletal protein and lipid organization in human erythrocytes, Pol. J. Pharmacol. 51 (1999) 341–350.
[11] K. de Jong, D. Geldwerth, F.A. Kuypers, Oxidative damage does not alter membrane phospholipid asymmetry in human erythrocytes, Biochemistry 36 (1997) 6768–6776.
[12] H. Hagerstrand, B. Isomaa, Amphiphile-induced antihaemolysis is not causally related to shape changes and vesiculation, Chem.-Biol. Interact. 79 (1991) 335–347.
[13] H. Hagerstrand, T.H. Holmstron, M. Bobrovska-Hagerstrand, J.E. Eriksson, B. Isomaa, Amphiphile-induced phosphatidylserine exposure in human erythrocytes, Mol. Membr. Biol. 15 (1998) 89–95.
[14] S. Schreier, S.V.P. Malheiros, E. Paula, Surface active drugs: self-association and interaction with membranes and surfactants. Physicochemical and biological aspects, Biochim. Biophys. Acta 1508 (2000) 210–234.
[15] H.U. Weltzien, H.G. Kalkoff, Quantitative studies on lysolecithin mediated hemolysis. Bensylated lysolecithin as a probe to study effects of temperature and red cell species on the hemolytic reaction, Biochim. Biophys. Acta 455 (1976) 56–65.