ACTA UNIVERSITATIS LODZIENSIS

FOLIA BIOCHIMICA ET BIOPHYSICA 10, 1994

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POLY-(ETHYLENE GLYCOL) PROTECTS ERYTHROCYTES AGAINST THERMAL HEMOLYSIS

In this paper we study the effect of poly-(ethylene glycol) on thermostability of erythrocytes. The addition of PEG_{4000} protects the cells from thermal hemolysis suggesting that the plasma membrane is the critical target for hyperthermia action.

INTRODUCTION

The interaction of poly-(ethylene glycol) – PEG with model and cell membranes has received particular attention for its well-known fusogenic effect [1, 2]. PEG may destabilize the lipid bilayer indirectly by forming phase-separated domains or directly by binding to the membrane surface [3]. Freeze-fracture electron microscopy studies have indicated that PEG treatment leads to formation of regions free of intramembrane particles in the plasma membrane [3, 4].

PEG with molecular weights ranging between 400–1000 may also protects mice against irradiation [5] and red blood cells from hemolysis [6]. Recently, our laboratory has shown that poly- (ethylene glycol) with molecular weight of 4000 (PEG_{4000}) can protects erythrocytes against thermal damage.

The aim of the present paper was to investigate a possible role of the PEG in maintaining of thermal stability of pig erythrocytes. Using PEG solutions of different molecular weight we investigate the interaction of PEG with erythrocytes at various temperatures.

MATERIALS AND METHODS

Pig blood was obtained from the slaughterhouse in acid citrate dextrose. Erythrocytes, separated from plasma and buffy coat by centrifugation at 3000 rpm were washed four times in 5 mM sodium phosphate buffer, containing

150 mM NaCl, pH 7.4. The assessment of hemolysis of erythrocyte suspensions was determined by the method of Minetti et al [7].

Erythrocytes suspended in saline buffer were incubated with and without PEG up to 12 h. For the temperature treatments, samples containing 10% suspensions of erythrocytes in 8 dilutions (from 0.56 to 0.76) of NaCl were immersed in a water bath controlled with a precision of ± 0.1°C. The percent lysis was calculated by comparision of absorbance in supernatants of control cells totally lysed in 5 mM sodium phosphate. NaCl concentration at which 50% of the cells were lysed ($NaCl_{50\%}$) was used to express the results. All experiments were repeated at least four times using erythrocytes from a different animal blood.

RESULTS AND DISCUSSION

Table 1 illustrates the effect of temperature and time of incubation on the $C_{50\%}$ parameter. After single heating cells are more fragile at 20°C than at 37°C or 44°C. The cells indicaté an almost stable osmotic resistance up to 24 h incubation at 37°C [8]. If the cells are exposed to step-down heating they indicate increased sensitivity to heat both at 20°C and 37°C after preincubation at 44°C for 10 min. By contrast, cells treated at 44°C for 10 min, kept at 37°C for 3 h and then exposed to subsequent heating at 44°C are more resistant than cells subjected to single or step-down heating (Fig. 1).

Table 1

Effect of poly-(ethylene glycol) on the osmotic fragility of pig erythrocytes. Cells were incubated with or without 10 mM PEG_{200} or 6 mM PEG_{4000}. After the indicated times of incubation hemolysis was measured at 540 nm. (means ± SD of four experiments)

Treatment	NaCl concentration of the medium for 50% hemolysis ($NaCl_{50\%}$)		
	3 h	5 h	8 h
20°C	0.640 ± 0.015	0.642 ± 0.017	0.658 ± 0.019
20°C + PEG_{4000}	0.628 ± 0.007	0.635 ± 0.010	0.643 ± 0.014
37°C	0.626 ± 0.004	0.627 ± 0.016	0.624 ± 0.016
37°C + PEG_{200}	0.660 ± 0.007	0.692 ± 0.025	0.716 ± 0.018
44°C	0.615 ± 0.023	0.617 ± 0.018	0.619 ± 0.016

The effect of PEG on thermal stability of erythrocytes was studied in the following experimental set-up. PEG was added to the cell suspensions immediately before heating and was present during heat treatment. Immediately after the treatment with heat cells were analyzed for hemoglobin content.

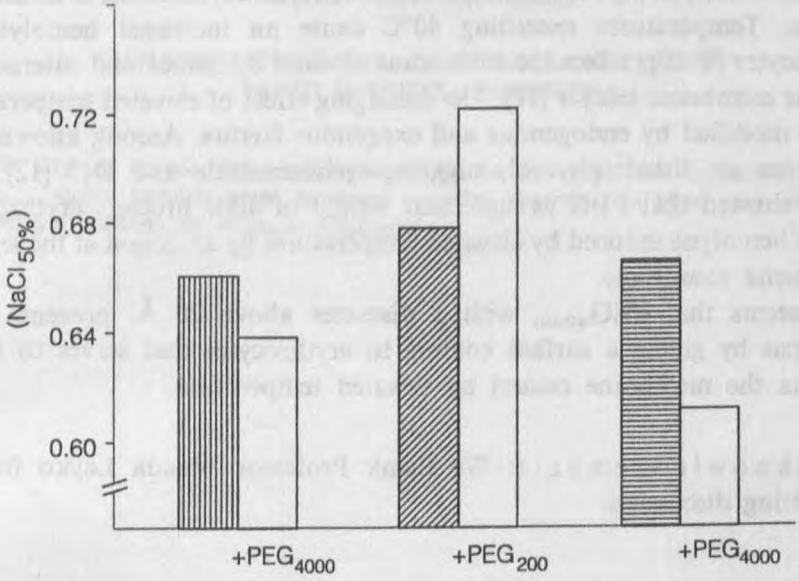

Fig. 1. Effect of poly-(ethylene glycol) on the thermostability of erythrocytes. Cells were exposed in the absence or presence of PEG as indicated to 44°C for 10 min followed by incubation for 12 h at 20°C (▥) or 44°C for 10 min followed by incubation at 37°C for 8 h (▨) or 44°C for 10 min, kept at 37°C for 3 h and then subjected to a second heat shock at 44°C for 20 min (▤). The data represent the average of five different experiments. The concentration of PEG_{200} and PEG_{4000} was 10 mM and 6 mM, respectively

The effects of the presence of PEG on thermal sensitivity of erythrocytes are shown in Fig. 1 and Tab. 1. It can be seen that thermal sensitivity of cells depends on the molecular weight of PEG and conditions of incubation. When cells are incubated with PEG_{4000} for periods up to 12 h at 20°C the increased osmotic resistance is observed (Tab. 1). Also erythrocytes exposed to step-down heating or subjected to a double heat shock in the presence of PEG_{4000} indicate the decreased sensitivity to heat treatment (Fig. 1). On the other hand, the cells at almost constant osmotic resistance followed by 8 h incubation with PEG_{200} at 37°C have indicated nearly completly hemolysis (Tab. 1). PEG as a water-soluble polymer may perturbe the organization of membrane domains. It may bind to phospholipids as well as aggregate membrane proteins [3]. The mechanism by which PEG causes reorganization of the membrane components is still unknown. For ethanol-treated human red blood cells it has been shown that this molecule induces the formation of membrane pores [6]. Such membrane damage can lead to the lysis of cells. PEG_{1000} with a diameter of 20 Å fully protects red blood cells from ethanol

-induced hemolysis [6]. Also heat treatment of erythrocytes result in membrane damage. Temperatures exceeding 40°C cause an increased hemolysis of erythrocytes [8–10], affect the membrane skeletal dynamics and interactions with the membrane bilayer [11]. The damaging effect of elevated temperatures can be modified by endogenous and exogenous factors. Among known heat protectors are listed: glycerol, suggars, cyclohexamide and D_20 [12]. Our results showed that PEG at molecular weight of 4000 protects erythrocytes against hemolysis induced by elevated temperatures by an action at the level of the plasma membrane.

It seems that PEG_{4000}, with a diameter above 20 Å, prevents from hemolysis by giving a surface coating to erythrocytes that serves to repair pores in the membrane caused by elevated temperature.

Acknowledgements: We thank Professor Wanda Leyko for the stimulating discussion.

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Wpłynęło do Redakcji Folii
1.07.1992 r.

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POLI-(ETYLENOGLIKOL) ZABEZPIECZA ERYTROCYTY PRZED HEMOLIZĄ TERMICZNĄ

W artykule badano wpływ polietylenoglikolu na termostabilność erytrocytów. Dodany PEG_{4000} chroni komórki przed termiczną hemolizą, sugerując, że błona plazmatyczna jest krytycznym miejscem dla działania hipertermii.