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Short communication

Spherocyte shape transformation and release of tubular nanovesicles in human erythrocytes

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Abstract

We have studied dodecylmaltoside-induced echinocyte–spheroechincyte–spherocyte shape transformation and membrane vesiculation using transmission electron microscopy (TEM) on freeze-fracture replicas. It is indicated that spherical erythrocyte shape at higher dodecylmaltoside concentration is formed due to loss of membrane in the process where small, mostly tubular nanovesicles are released predominantly from the top of echinocyte and spheroechinocyte spicules.

Keywords: Dodecylmaltoside; Nanovesicles; Erythrocyte membrane; Erythrocyte shape; Membrane shear energy

1. Introduction

Water-soluble detergents embed readily into the red blood cell membrane thereby causing changes of the red blood cell shape. It has been observed that amphiphiles induce changes of the normal discoid shape into either spiculated echinocytic or invaginated stomatocytic shapes [1–3]. Dodecylmaltoside is a detergent composed of a single tail and a bulky head-group. When incubated with erythrocytes it induces echinocyte (Fig. 1) shape transformation. Spheroechinocyte shapes (Fig. 1c), having narrower spicules than echinocytes, are developed at higher dodecylmaltoside concentrations. When the concentration of dodecylmaltoside is further increased, spheres (spherocytes) are created. Finally, hemolysis occurs. According to the bilayer couple model [2], the echinocyte shape transformation is driven by intercalating the exogeneously added molecules preferentially into the outer membrane layer [4]. The dodecylmaltoside molecules may therefore increase the area difference between the outer and inner membrane lipid layer and thus affect the cell shape. It has been shown that the stability of echinocyte shape is primarily determined by competition of the membrane bilayer bending energy and membrane skeleton shear energy [4]. The echinocyte shape is additionally modulated also by stretching energy of the skeleton [5] and band 3 molecules [3, 6]. Dodecylmaltoside-induced erythrocyte shape transformation is accompanied by membrane shedding in the form of predominantly tubular nanoexovesicles [7]. It is the aim of this short communication to elucidate the possible mechanism leading to the final

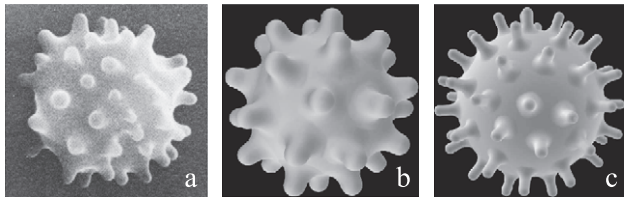

Fig. 1. Micrograph of echinocyte shape (adapted from Ref. [8]) (a) and the calculated echinocyte shapes determined by minimization of membrane elastic energy (bending and shear) for two values of the relative area difference between the areas of the outer and inner membrane lipid layer (Δa): 2.06 (b) and 3.2 (c) at the relative cell volume $v = 0.6$ (adapted from Ref. [4]). The quantities Δa and v are normalized relative to the corresponding values for the spherical cell with the same membrane area [4,9].

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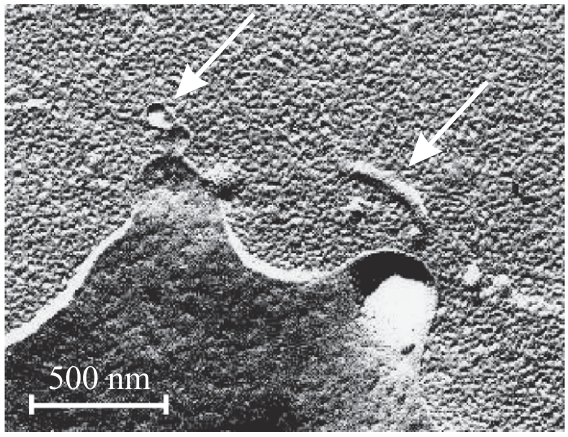

Fig. 2. TEM micrographs of the freeze fracture replica of the buds (arrows) on top of the echinocyte spicules induced by adding 40 mM dodecylmaltoside to the erythrocyte suspension.

spherical erythrocyte shape induced at sublytic concentrations of dodecylmaltoside.

2. Materials and methods

Dodecylmaltoside was dissolved in the buffer. Human blood was drawn from authors by vein puncture into heparinized tubes. The red blood cells were washed three times in a buffer (pH 7.4). The red blood cells were then suspended in the buffer and kept at 4 °C until used. The aliquots of a prewarmed (37 °C) red blood cell stock suspension were pipetted into polystyrene tubes containing prewarmed (37 °C) buffer with dodecylmaltoside. The final cell density was 1.65×10^8 cells/ml (about 1.5% haematocrit) and the incubations were carried out in a shaking thermostat bath at 37 °C. Dodecylmaltoside was used at sublytic concentrations.

The cells were then fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3, for 2 h at 4 °C. Fixed cells were soaked in 30% glycerol in 0.1 M cacodylate buffer for at least 2 h before being frozen in liquid freon 22. Freeze-fracture replicas were produced in a Balzers freeze-fracture unit and examined in JEOL 100 CX transmission electron microscope (TEM).

3. Results and conclusions

Dodecylmaltoside induces shape transformation of the erythrocyte from an initial discocyte shape to the final shape composed of a spherical mother cell and the released tubular nanoexovesicles. In this process the erythrocyte first undergoes a discocyte–echinocyte transformation and subsequently echinocyte–spheroechinocyte transformation (Fig. 1). The budding occurs predominantly at the top of the echinocyte or spheroechinocyte spicules. Fig. 2 shows buds on the top of echinocyte spicules as observed by TEM on the freeze fracture replica.

It was previously assumed that the spherical erythrocyte shape (spherocyte) at sublytic concentrations of echinocytogenic detergents could arise due to progressive narrowing of echinocyte spicules with increasing detergent concentration (Fig. 1b and c) and final release of the spheroechinocyte spicules from the cell surface as a whole in the form of tubular nanovesicles. It was also proposed that the released tubular nanovesicles may subsequently disintegrate into smaller vesicles [10]. In accordance with previous suggestions [4], we have shown in this work that the spherical erythrocyte shape (spherocyte) at higher echinocytogenic detergent concentrations is not the consequence of the release of the spicules from the spheroechinocyte surface.

Based on the results presented in Fig. 2, it can be concluded that the loss of membrane material in the form of nanovesicles created predominantly on the top of the echinocyte and spheroechinocyte spicules (Fig. 2) may be the principal cause of the spherical erythrocyte shape at sublytic concentrations of dodecylmaltoside.

Protein analysis showed that spherical and tubular micro and nano vesicles of erythrocyte membrane are highly depleted in the membrane skeleton [11]. It has been shown recently that the shear energy of the membrane skeleton in the budding region of the membrane may strongly increase during the budding process [11]. Therefore, it can be expected that the budding is most probable on the top of echinocyte spicules (Fig. 2) since in this region the skeleton may be detached from the membrane bilayer already before the budding starts [12]. If it is not detached, the skeleton at the tip of the echinocyte spicule is maximally expanded, therefore the budding between the cytoskeleton anchors would be expected [5]. However, the budding process can take place also in other regions of the erythrocyte membrane (except on the spicule tips) and it is also not necessarily connected with the gross changes of erythrocyte shape [3]. In this case, the gap in the skeleton network [13], the local disruption in the skeleton network [10] or the local detachment of the skeleton [11] seems to be a necessary condition for the formation of the skeleton depleted daughter vesicles [3]. The partial detachment of the skeleton in the budding region of the erythrocyte membrane is energetically favourable, since in this way, the accumulated skeleton shear energy in the membrane protrusions may be relaxed [11].

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