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Influence of magnesium sulfate on HCO_3/Cl transmembrane exchange rate in human erythrocytes

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HIGHLIGHTS

- A novel molecular-kinetic model of Band 3 activation by Mg^{2+} is introduced.
- The model is verified experimentally with the use of a scanning flow cytometer.
- The association constant of Mg^{2+} with Band 3 is evaluated as 0.07 mM.
- The method developed allows quantitative control and optimization of $MgSO_4$ treatment.
- Results of the work are useful particularly for early detection of risk of hypoxia.

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ABSTRACT

Magnesium sulfate ($MgSO_4$) is widely used in medicine but molecular mechanisms of its protection through influence on erythrocytes are not fully understood and are considerably controversial. Using scanning flow cytometry, in this work for the first time we observed experimentally (both *in situ* and *in vitro*) a significant increase of HCO_3^-/Cl^- transmembrane exchange rate of human erythrocytes in the presence of $MgSO_4$ in blood. For a quantitative analysis of the obtained experimental data, we introduced and verified a molecular kinetic model, which describes activation of major anion exchanger Band 3 (or AE1) by its complexation with free intracellular Mg^{2+} (taking into account Mg^{2+} membrane transport and intracellular buffering). Fitting the model to our *in vitro* experimental data, we observed a good correspondence between theoretical and experimental kinetic curves that allowed us to evaluate the model parameters and to estimate for the first time the association constant of Mg^{2+} with Band 3 as $K_B \sim 0.07$ mM, which is in agreement with known values of the apparent Mg^{2+} dissociation constant (from 0.01 to 0.1 mM) that reflects experiments on enrichment of Mg^{2+} at the inner erythrocyte membrane (Gunther, 2007). Results of this work partly clarify the molecular mechanisms of $MgSO_4$ action in human erythrocytes. The method developed allows one to estimate quantitatively a perspective of $MgSO_4$ treatment for a patient. It should be particularly helpful in prenatal medicine for early detection of pathologies associated with the risk of fetal hypoxia.

1. Introduction

Magnesium sulfate ($MgSO_4$) is a pharmaceutical preparation of magnesium, widely used in medicine (Fawcett et al., 1999; McLean, 1994). It is effective in numerous diseases associated with

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magnesium deficiency (Swaminathan, 2003; Ulger et al., 2010). Magnesium sulfate is known as an antiarrhythmic agent to prevent cardiac arrest (Sugiyama et al., 1996), and as a bronchodilator to reduce the symptoms of acute asthma (Blitz et al., 2005). It is also effective as a tocolytic agent in decreasing the risk of eclampsia and preterm birth (Duley and Neilson, 1999; Euser and Cipolla, 2009). Intravenous administration of magnesium sulfate before birth could reduce the risk of cerebral palsy and motor dysfunction in preterm infants (Doyle et al., 2009). However, the effect of the treatment is dose sensitive. A high dosage of magnesium sulfate for attempted tocolysis in preterm labor was shown to cause more harm than do good (Mittendorf et al., 2004). It was found that "aggressive tocolysis" increases a risk of intracranial hemorrhage as well as deaths of fetus and very low body weight neonates (Mittendorf et al., 2002; Murata et al., 2005). In addition, the prolonged exposure to magnesium sulfate was associated with an increased risk of clinical chorioamnionitis (Elimian et al., 2002). By contrast, low doses of magnesium sulfate, when used as prophylaxis in cases of preterm labor, appeared to be neuroprotective for a number of children (Deering et al., 2005). So far, there is no method for determining the optimal dosage of magnesium sulfate for a particular patient before the injection. This is largely due to the lack of understanding of molecular kinetic mechanisms of $MgSO_4$ action. In particular, the role of the influence of magnesium on erythrocytes in blood is known (Abad et al., 2005, 2010; Ariza et al., 2005; Gulczynska et al., 2006), but far from completely understood. In some cases, the anti-hypoxic mechanism of the protection through the activation of erythrocytes physiological properties by magnesium is supposed (Szemraj et al., 2005), but not explained quantitatively.

Magnesium is the second most abundant cation in erythrocytes. The total concentration of Mg^{2+} in human erythrocytes is around 2 mM, but only a small fraction ($\sim$0.2 mM) is free (uncomplexed) (Grubbs, 2002; Gunther, 2007). Many enzymes require the presence of Mg^{2+} for their catalytic action (Beydemir et al., 2000). Magnesium influences ionic traffic through Ca^{2+}, K^+, and Na^+ channels (Antonov and Johnson, 1999; Bara et al., 1993; Michelet-Habchi et al., 2003) and reduces lipids peroxidation of erythrocytes membranes (Abad et al., 2005). It is known that the elevation of intracellular magnesium modifies the main anion exchanger Band 3 (or AE1) in human erythrocytes (Barbul et al., 1999; Gulczynska et al., 2006). In particular, the rate of SO_4^- anions influx (which is going through Band 3) is higher (Teti et al., 2002) in the erythrocytes with increased concentration of intracellular Mg^{2+}.

The main anion exchanger Band 3 is a major membrane glycoprotein in human erythrocytes presented in approximately one million copies per cell and making up to 25% of the cell membrane (Poole, 2000). It mediates a rapid transmembrane exchange of Cl^- for HCO_3^-, thereby enhancing the CO_2/O_2 physiological exchange (since more than 90% of dissolved CO_2 are in the form of HCO_3^- at physiological conditions (Kummerow et al., 2000; Swietach et al., 2010) of pH between 7.0 and 8.0). Also, erythrocytes glycolysis was reported to be modulated by the interaction of Band 3 with glycolytic enzymes (von Ruckmann and Schubert, 2002) and hemoglobin (Weber et al., 2004). While the structure and properties of Band 3 have been extensively studied (Yeagle, 2005; Zhang et al., 2000), much less is known about molecular mechanisms of regulation of anion-exchange function of Band 3. The cytoplasmic domain of Band 3 can be phosphorylated at tyrosine residues (Ferru et al., 2011), and its phosphorylation state is critical for glycolysis, cell shape and anion transport (Merciris et al., 1998; Minetti et al., 2004, 1998; Puceat et al., 1998). The phosphorylation state of Band 3 is determined by the balance between the activities of protein tyrosine kinases p72syk (Harrison et al., 1994) and p53/56lyn (Brunati et al., 1996) (PTKs) and of phosphotyrosine phosphatase PTP1B (Zipser and Kosower, 1996). From *in vitro* studies, employing purified proteins and RBC ghosts, it was shown that Band 3 PTKs are activated by Mg^{2+} ions (Vasseur et al., 1987). The band 3 phosphorylation by PTKs involves the Mg/ATP as a substrate; therefore, a general point of view is that the elevation (Abad et al., 2005) of intracellular Mg^{2+} during magnesium sulfate treatment increases Band 3 phosphorylation state leading to the activation of its anion exchange functionality. However, detailed mechanism of Band 3 activation has not been modeled quantitatively. The situation is complicated by the reports that Mg^{2+} enhances also Band 3 dephosphorylation (Zipser and Kosower, 1996) mediated by PTP1B, which may lead to an opposite (*i.e.* Band 3 deactivation) effect. By contrast, Mg^{2+} may bind directly with Band 3 that affects the charge and functionality of Band 3 (Weber and Voelter, 2004; Weber et al., 2004). Until now, it is not established whether solely the molecular kinetics mechanism of direct complexation of Band 3 with intracellular free Mg^{2+} can quantitatively explain the influence of intracellular free Mg^{2+} on the anion exchange activity of Band 3. Our current research is devoted to the study of this question.

In this work, we carried out experiments to observe the influence of magnesium sulfate treatment (both *in vitro* and *in situ*) on the anion exchange activity of Band 3 in human erythrocytes. We determined the anion exchange activity in conventional units of the "number of active Band 3" per erythrocyte applying a scanning flow cytometry (Maltsev, 2000; Shvalov et al., 1999) method of measuring single erythrocytes during their hemolysis in isotonic solution of ammonium chloride (Chernyshev et al., 2008). For the quantitative investigation of the influence of $MgSO_4$ on Band 3, we introduced a molecular kinetic model, which consists of three parts: 1) influx of Mg^{2+} into erythrocytes obeying Michaelis–Menten kinetics (Chanson et al., 2005); 2) intracellular magnesium buffering (Bock et al., 1991; Gunther, 2007; Raftos et al., 1999); and 3) Band 3 activation by its complexation (Weber and Voelter, 2004; Weber et al., 2004) with free intracellular Mg^{2+}.

Fitting the developed model to the obtained experimental data, we observed a good correspondence between theoretical and experimental curves that allowed us to estimate the model parameters with good confidence. The values of these parameters are in agreement with known literature data on the apparent Mg^{2+} dissociation constant (from 0.01 to 0.1 mM) that reflects experiments on enrichment of Mg^{2+} at the inner erythrocyte membrane (Gunther, 2007). The estimated value of magnesium influx is close to the known range from 35 to 500 $\mu M\ h^{-1}$ (depending on a donor) in human erythrocytes (Flatman, 1991). This work contributes to the molecular kinetic study of the anti-hypoxic effect of magnesium, since the transmembrane anion exchange of Cl^- for HCO_3^- through Band 3 in erythrocytes is associated with CO_2/O_2 physiological exchange. Results of this work extend the opportunities for control and adjustment of the magnesium sulfate treatment for a particular patient.

2. Materials and methods

2.1. Instrumentation

We used a scanning flow cytometer (SFC) (Maltsev, 2000; Strokotov et al., 2011) in order to obtain the number of active Band 3 channels per erythrocyte, as described in detail elsewhere (Chernyshev et al., 2008). SFC allows the measurement of the light-scattering pattern (LSP) from single cells for their morphological characterization (*i.e.* determination of cell's size, refractive index, shape parameters, *etc.*). Accurate LSP measurements were performed with the rate of up to 300 cells per second.