Activation energy of sulfate ion transport across methylated human erythrocyte membranes

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Activation energy E_A of the sulfate ions transport process across human erythrocyte membranes modified by reductive methylation has been measured. It has been found that exhaustive reductive methylation (3 times) with formaldehyde and borohydride inhibits the sulfate-equilibrium exchange, by a maximum of about 40%. However, methylation has no measurable effect on activation energy, since the evaluated E_A values for control and methylated cells remain the same within the experimental error range.

1. INTRODUCTION

Inorganic anion transport across the red blood cell membrane is mediated by an integral membrane protein with a molecular mass of about 95 kDa, the so-called Band 3 protein [1]. Chemical and enzymatic modifications have been widely used for the study of structure and function of the Band 3 protein (for review see [2,3]). The results obtained indicate that the transport centre is a heterogenous assembly of charged groups such as lysine residues, arginyl residues, carboxyl groups, aliphatic or aromatic amino acid residues and SH groups.

This paper reports the effect of reductive methylation of amino groups in the lysine residues of Band 3 protein on divalent anion exchange as measured with $^{35}SO_4^{2-}$. In order to find out whether such a modification affects the energy

barrier for sulfate ion transport across the membrane, the activation energy has been measured.

2. MATERIALS AND METHODS

Experiments were performed with erythrocytes from fresh heparinized human blood. Rate constants for SO_4^{2-}/SO_4^{2-} equilibrium exchange were measured as reported by Schnell et al. [4]. $^{35}SO_4^{2-}$ efflux was measured in a medium containing 110 mM K_2SO_4 and 2 mM K_2HPO_4 at pH 7.2. Reductive methylation of erythrocyte membranes was performed using the method of Jennings [5]. Cells were washed in a medium of 165 mM KCl buffered with 2 mM K_2HPO_4 at pH 7.2, resuspended in this medium at a hematocrit of 5% and cooled to 0°C. The cells were then methylated with formaldehyde (final concentration 16 mM) and borohydride (final concentration 5 mM). Reductive methylation was repeated 3 times.

The activation energy of sulfate ion transport across control and methylated cells was determined from the Arrhenius plot in the temperature range from 12°C to 37°C.

3. RESULTS AND DISCUSSION

Repeated reductive methylation with formaldehyde inactivates sulfate transport across the erythrocyte membrane, as follows from our flux measurements in this paper. After exhaustive (3 times, 16 mM HCHO) reductive methylation of the membranes, we obtained a maximum degree of sulfate inhibition of about 40%. This result was

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confirmed by our measurements for every temperature studied in the range 12–37°C. The rate constant for $^{35}SO_4^{2-}$ efflux at 37°C was 0.069 ± 0.008 min^{-1} for control cells and 0.041 ± 0.01 min^{-1} for 3× methylated cells.

In the case of Cl-Cl exchange and Cl-Br exchange the maximum inhibition produced by reductive methylation was 75% and 80%, as observed by Jennings [5]. It is known [3,5] that reductive methylation takes place at two lysine residues called Lys a and Lys b, respectively in the 60 kDa and the 35 kDa chymotryptic fragments of Band 3 protein. The results presented in this paper and Jennings' [5] observations probably point to different roles of methylated Lys a and Lys b groups in mono- and divalent anion translocation across red blood cell membranes.

In order to elucidate the effect of methylation of lysine residues in Band 3 on the energy barrier for sulfate ion transport, we have measured the activation energy of this process. The temperature dependence of sulfate equilibrium exchange has been studied in the range from 12°C to 37°C. Typical relationships for control and methylated cells are presented in fig.1. In both cases the Arrhenius plots are linear and with similar slopes. The activation energy of sulfate efflux was calculated from these plots. The mean values obtained (± SD) were 131 ± 16 kJ/mol and 129 ± 14 kJ/mol for control and methylated cells, respectively. Thus the methylation of lysine residues in the Band 3 protein has no measurable effect on the activation energy of sulfate transport. The activation energy value determined by us for control cells is in agreement with the values obtained by others [3]. According to the Arrhenius formula: $k = k_0 \exp(-E_A/RT)$ (where k, k_0 are rate constants; E_A, activation energy; R, gas constant; T, temperature), a small increase in E_A can result in a relatively large decrease in k. Therefore we can expect an increase in E_A to be observed. However, this increase should be less than the experimental error (14 kJ/mol).

Inorganic anion exchange mediated by the Band 3 protein proceeds via three steps [2,3]: (i) binding of the transported anion to the active centre; (ii) spontaneous alternation of inward- and outward-facing conformations permitted by the bound
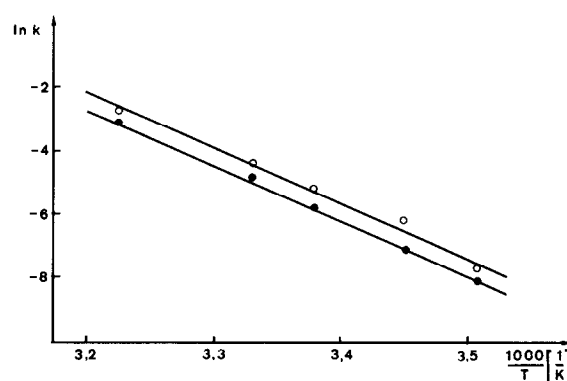

Fig.1. Temperature dependence of sulfate equilibrium exchange in control (○) and methylated (●) cells. The fluxes were measured after repeated reductive methylation. Ordinate: logarithm of rate constant. Abscissa: reciprocal value of absolute temperature.

anion; (iii) dissociation of the transported anion from the centre on the opposite side of the membrane. It can be expected that all of these steps make some contribution to the energy barrier for anion translocation across the membrane. According to Passow [3] the temperature dependence of the anion penetration rate is essentially due to the conformational transition of the loaded forms of the transfer site from facing one membrane surface to facing the other (step 2). Therefore this conformational transition mainly creates the energy barrier for anion transport in erythrocyte membranes. It seems that methylation of lysine residues diminishes the ability of the transfer site to undergo conformational transition.

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