Temperature Dependence of H^+ Transport Across Erythrocyte Membrane of *Rana temporaria* Grass Frog in Media Containing Cl^- and SO_4^{2-}

A. A. Mishchenko and L. I. Irzhak

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H^+ transport across the erythrocyte membrane was studied in *Rana temporaria* grass frog. The temperature coefficients and activation energy of H^+ transport were calculated in media containing Cl^- and SO_4^{2-}. Our results show that kinetic characteristics of H^+ transport depend on function of band 3 protein in the erythrocyte membrane.

Key Words: *H^+ transport; band 3 protein; temperature coefficient; activation energy*

Physicochemical processes maintaining vital activity and providing cell function are temperature-dependent. Temperature determines the value of thermodynamic constants and, therefore, modulates the direction and rate of chemical reactions, conformational changes in biological macromolecules, phasic transition in lipids, and other processes [1].

Here we studied the temperature dependence of H^+ transport in nucleated erythrocytes of *Rana temporaria* grass frog. The membrane of nucleated erythrocytes has considerable amount of band 3 protein, which is involved in neutral exchange of Cl^- for HCO_3^- [10]. The exception is *Agnata* erythrocytes not containing this transporter [8]. H^+ transport across the erythrocyte membrane depends on reaction rates in the Jacobs—Stewart cycle [9]. Carboanhydrase, or band 3 protein of the erythrocyte membrane, serves as a target for the influence of exogenous factors [10]. Band 3 protein plays an important physiological role, because 80% carbon dioxide are converted into hydrocarbonate and transported from tissues into the lungs. Hydrocarbonate is formed in erythrocytes and released into the plasma during HCO_3/Cl exchange [5,12]. The study of band 3 protein in frog erythrocytes would provide valuable data for comparative evolutionary analysis.

Syktyvkar State University

MATERIALS AND METHODS

Experiments were performed with blood samples from *Rana temporaria* grass frog (n=15). The animals were captured in the Kirov region in autumn. In winter they were maintained in a refrigerator (water temperature 5-7°C). The study was conducted in winter and spring. The animals were decapitated under light ether anesthesia. The blood was obtained by cardiac puncture and stabilized with heparin.

For erythrocyte isolation the blood was centrifuged at 3000 rpm for 5 min. Erythrocyte pellet was washed twice and resuspended in Ringer's solution for cold-blooded animals (pH 7.2). The rate of H^+ transport was estimated [2]. pH was measured using an EV-74 ion meter. The signal was transferred to an H-306 automatic recorder or V7-267 digital voltmeter.

The rate of H^+ transport was measured at 18 and 28°C to estimate the temperature coefficient. The activation energy was calculated as follows: $E_a=RT_2T_1\times(\ln k_1-\ln k_2)/(T_2-T_1)$, where E_a is the activation energy; T_1 and T_2 are values of temperature; and k_1 and k_2 are the reaction rate constants at T_1 and T_2, respectively.

The results were analyzed by the method of pairwise and non-pairwise comparisons. The significance of differences was evaluated by Student's t test.

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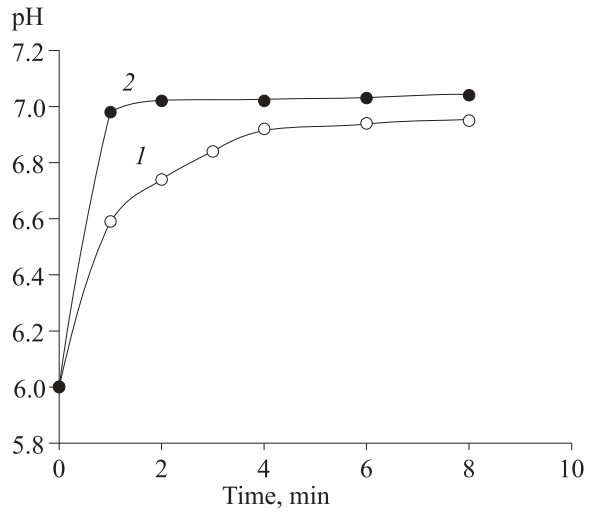

Fig. 1. Changes in pH of a Cl^--containing medium (Ringer's solution for cold-blooded animals) after mixing with erythrocytes from *Rana temporaria* frog (0.02 ml). Here and in Fig. 2: 18 (*1*) and 28°C (*2*).

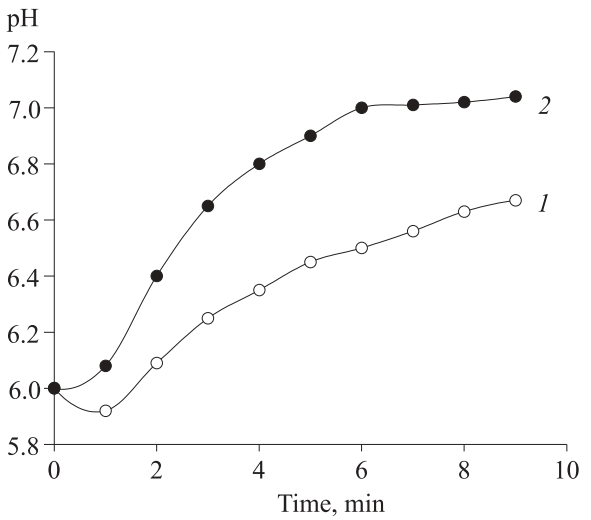

Fig. 2. Changes in pH of a SO_4^{2-}-containing medium (0.6% Na_2SO_4, 8 ml) after mixing with erythrocytes from *Rana temporaria* frog (0.02 ml).

RESULTS

Fig. 1 illustrates the recovery of pH in an acidified erythrocyte suspension (Fig. 1). The rate of pH changes decreased due to decrease in proton gradient during their transport in erythrocytes (reactions of the Jacobs—Stewart cycle) and binding to the hemoglobin buffer system.

Heating increased the rate of pH changes. The estimated values of Q_{10} and activation energy were 1.8 and 9.3 kcal/mol, respectively. The estimated Q_{10} was similar to that observed in trout acclimatized at 5°C [7]. Our results show that H^+ transport across the erythrocyte membrane in these animals has the same thermodynamic characteristics (despite various ecological conditions). Probably, these animals do not differ in the dependence of H^+ transport on the rate of extracellular noncatalyzed reactions of the Jacobs—Stewart cycle [9].

In a SO_4^{2-}-containing medium pH initially decreased (Fig. 2), which was probably related to Cl^- efflux from erythrocytes along the concentration gradient (exchange for SO_4^{2-}) and membrane hyperpolarization [3]. The duration of this stage at 18°C was 2.5-4 min. Heating to 28°C was followed by shortening of this stage by 2.5 times. In the follow-up period pH progressively increased due to H^+ transport, which prevailed over SO_4^{2-}/Cl^- exchange.

During the increase in pH, Q_{10} and activation energy were 2.2 and 14 kcal/mol, respectively. The estimated values exceeded those observed in a Cl^--containing medium. The rate of H^+ transport in a SO_4^{2-}-containing medium depends on function of band 3 protein, which is associated with slow transfer of SO_4^{2-} (as distinct from H^+ transport in a Cl^--containing medium). The results of experiments with a SO_4^{2-}-containing medium reflect thermodynamic characteristics of band 3 protein.

We found no published data on kinetic characteristics of HCO_3^-/SO_4^{2-} exchange. The activation energy for SO_4^{2-}/Cl^- exchange in human erythrocytes is 21-24 kcal/ml [11], which exceeds the estimated value for SO_4^{2-}/HCO_3^- exchange.

Our results suggest that the observed differences are associated with various reactions of band 3 protein in human and frog erythrocytes to a rise in temperature.

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