Activity of Na^+/K^+-activated Mg^{2+}-dependent ATP-hydrolase in the cell-free extracts of the sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9

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Abstract

The aim of our work was to study Na^+/K^+-activated Mg^{2+}-dependent ATPase activity in cell-free extracts of the sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine, and to carry out the kinetic analysis of the enzyme reaction. The maximum ATPase activity for both bacterial strains at +35 ºC was determined. The highest activities of the studied enzyme in the cell-free extracts of *D. piger* Vib-7 at pH 7.0 and *Desulfomicrobium* sp. Rod-9 at pH 6.5 were measured. Based on experimental data, the analysis of kinetic properties of the ATP-hydrolase reaction by the studied bacteria was carried out. The enzyme activity, initial (instantaneous) reaction rate (V_0) and maximum rate of the ATPase reaction (V_{max}) was significantly higher in *D. piger* Vib-7 cells than in *Desulfomicrobium* sp. Rod-9. Michaelis constants (K_m) of the enzyme reaction for both bacterial strains were determined.

ATPase activity, hydrogen sulfide, intestinal microbiocenosis, bowel diseases, ulcerative colitis

Sulfate-reducing bacteria carry out the dissimilatory sulfate reduction during anaerobic respiration (Barton and Hamilton 2007). The final product of the sulfate reduction in the human intestine is hydrogen sulfide which is carcinogenic to its cells, and can cause inhibition of cytochrome oxidase, colonocytes oxidation of butyrate, destruction of epithelial cells, development of ulcers, and inflammation with subsequent development of colon cancer (Pitcher and Cummings 1996; Gibson et al. 1991; Cummings et al. 2003). The transport of sulfate ions and organic compounds in the cytoplasm of the bacterial cells occurs through active transport using ATP energy (Barton and Hamilton 2007). In this regard, it is very important to study the mechanisms of sulfate ions transport, enzymatic activity and kinetic properties of other ATP-dependent enzymes of sulfate-reducing bacteria from human intestine.

Plentiful data are available on the functions of biological membranes including the integral membrane protein ATP-dependent systems of transport ions (Lodish et al. 2000; Yuan et al. 2005; Tian et al. 2006). However, the ATPase activity of the sulfate-reducing bacteria *Desulfovibrio piger* and *Desulfomicrobium* sp. isolated from the human large intestine has not been studied yet. A comprehensive study of the functioning and role of Na^+/K^+-pump as a system of energy-dependent transport of different ions in the regulation of the dissimilatory sulfate reduction and accumulation of hydrogen sulfide will enable to form a holistic view on the participation of these systems in maintaining ion homeostasis of the sulfate-reducing bacteria cells.

The aim of our work was to study Na^+/K^+-activated Mg^{2+}-dependent ATPase activity in cell-free extracts of the sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine, and to carry out the kinetic analysis of the enzyme reaction.

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Materials and Methods

Bacterial strains

Objects of the study were sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine (Kushkevych 2013; Kushkevych et al. 2014). The strains have been kept in the collection of microorganisms at the Biotechnology Laboratory of the Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic).

Preparation of cell-free extracts

Cell-free extracts were prepared from the exponential phase of growth. The bacteria were grown anaerobically in nutrition-modified Kravtsov-Sorokin's liquid medium (Kushkevych 2013). The cold extraction buffer (50 mM potassium phosphate buffer, pH 7.5, 10^{-5} M EDTA (ethylenediaminetetraacetic acid) was added to centrifuged sedimented cells to bind heavy metal ions. A total of 10^{-5} M PMSF (phenylmethylsulfonyl fluoride) for the inhibition of proteases, which is effective at pH above 7.0, was added. After this procedure, a suspension of cells (150–200 mg/ml, which correspond to optical density 0.569–0.761) was obtained. The cells were homogenized using the ultrasonic disintegrator (Soniprep 150 Plus MSE Limited, United Kingdom) at 22 kHz for 5 min at 0 ºC. The soluble extracts constituted by the supernatant were used as the source of the enzyme. The soluble fraction was displaced into centrifugal tubes and cell-free extracts were separated from the cells fragments by centrifugation for 30 min at 14,000 × *g* and at 4 ºC. Pure supernatant was then used as cell-free extracts. The spinned cells fragments were used as sedimentary fraction. Protein concentration in the cell-free extracts was determined by the Lowry method (Lowry et al. 1951).

Measuring ATPase activity

The total Na^+/K^+-ATPase enzymatic activity of bacterial cell-free extracts was measured in the incubation medium (volume 1 ml) of the following composition: 120 mM NaCl, 30 mM KCl, 5 mM $MgCl_2$, 1.5 mM ATP, 1 mM EGTA, 20 mM Hepes-Tris-buffer (pH 7.4), 0.1 μM thapsigargin (selective inhibitor of Ca^{2+}/Mg^{2+}-ATPase E (C)) (Flynn et al. 2001). Calcium chelators EGTA were added for the binding of endogenous Ca^{2+} ions in the incubation medium. The enzymatic reaction was initiated by adding bacterial cell-free extracts (100 μl) in the incubation medium (the amount of the protein in the sample did not exceed 50–150 mg/ml). The incubation period was 1–20 min. The enzymatic reaction was stopped by adding 1 ml of cooled stop solution of the following composition: 1.5 M sodium acetate, 3.7% formaldehyde, 14% ethanol, 5% trichloroacetic acid (pH 4.3). The suspension was centrifuged (1,600 × *g*, 10 min) and the concentration of inorganic phosphorus (P_i) was measured spectrophotometrically (Biospectrophotometer, Eppendorf, USA) in the obtained supernatant without protein. The amount of reaction products were determined by Rathbun and Betlach (1969) and expressed as μmol P_i/min·mg^{-1} protein. Magnesium-ATPase activity of the bacterial cell-free extracts was tested in the same incubation medium, in the presence of 1 mM ouabain (selective inhibitor of Na^+/K^+-ATPase). The Na^+/K^+-ATPase activity was calculated by the difference between the value of total ATPase and Mg^{2+}-ATPase activity. The standard incubation medium (without the adding of cell-free extract) of non-enzymatic hydrolysis of ATP was used in the experiments as a control. A mixture of bacterial cells in saline for measuring the amount of endogenous P_i in the bacterial suspension was also used as a control.

Kinetic analysis

The study of kinetic properties of enzymatic reactions Na^+/K^+-activated Mg^{2+}-dependent ATP-hydrolase reaction was performed in a standard incubation medium, which was modified by physical and chemical characteristics or composition of the respective components (incubation time, protein bacterial mixture in the sample, the concentration of ATP, $Na^+(K^+)$, Mg^{2+}, ouabain). All experiments to study the properties of the Na^+/K^+-ATPase reaction were performed using the initial rate V_0 (linear accumulation of product (P_i) in time). The kinetic indicators characterizing the reaction release P_i during Na^+/K^+-activated Mg^{2+}-dependent of ATP hydrolysis were the initial (instantaneous) reaction rate (V_0), maximum rate of the Na^+/K^+-ATPase reaction (V_{max}), maximum amount (plateau) of the reaction product (P_{max}) and the characteristic reaction time (time half saturation) τ were determined. The kinetic indicators that characterize the Na^+/K^+-activated Mg^{2+}-dependent ATP-hydrolase reaction (the constant activation of ions Mg^{2+}, K^+ (K_{Mg}^{2+}, K_K^+), Michaelis constant (K_{mATP}) and maximal rate of ATP hydrolysis by the Mg^{2+}, K^+ (V_{Mg}^{2+}, V_K^+) and ATP (V_{ATP}) were determined by the Lineweaver-Burk plot (Keleti 1988). The obtained concentration dependence of the rate of enzymatic reaction on the studied reagents (ATP and Mg^{2+}, Na^+ (K^+)) was constructed in the coordinates {1/V on 1/S}, where S is the concentration of the reagent (ATP, Mg^{2+} or Na^+ (K^+)), and V is the rate of enzymatic hydrolysis of ATP at a concentration of ATP, Mg^{2+} or Na^+ (K^+), respectively. In determining the effectiveness of the ouabain on ATPase activity (the constant of inhibition ($I_{0,5}$) and Hill coefficient (n_H)), the linearized curves of the concentration dependence were constructed in Hill coordinates {lg[(A_0–A)/A]; lg[I]} according to the empirical Hill's equation: $\lg[(A_0-A)/A] = -n_H \lg I_{0,5} + n_H \lg [I]$, where A_0 and A are the specific activity of the enzyme in the absence and in the presence of ouabain in a concentration I in medium.

Statistical analysis

Kinetic and statistical calculations of the results were carried out using the software MS Office and Origin computer programs. The research results were treated by methods of variation statistics using Student's *t*-test.

The significance of the calculated indicators of line was tested by Fisher's *F*-test. The accurate approximation was when $P \leq 0.05$ (Bailey 1995).

Results

ATPase activity and the effect of temperature and pH

The activity of Na^+/K^+-ATPase was studied in different fractions (Table 1). Our results revealed a high enzyme activity in all fractions (16.11 ± 1.87, 15.89 ± 1.72, 28.27 ± 2.53 $U \cdot mg^{-1}$ protein for cell-free extracts, soluble and sedimentary, respectively), which were obtained from *D. piger* Vib-7 cells. The activity was significantly lower ($P < 0.01$) in the fractions obtained from *Desulfomicrobium* sp. Rod-9.

Table 1. Na^+/K^+-ATPase activity in different fractions.

Fractions	Na^+/K^+-ATPase activity ($U \cdot mg^{-1}$ protein)	
	Desulfovibrio piger Vib-7	*Desulfomicrobium* sp. Rod-9
Cell-free extract	16.11 ± 1.87	7.31 ± 0.98
Individual fractions:		
soluble	15.89 ± 1.72	6.92 ± 0.86
sedimentary	28.27 ± 2.53	15.25 ± 1.62

The activity of the enzyme in cell-free extracts under the effect of temperatures and pH in the medium incubation was determined (Fig. 1). The Na^+/K^+-ATPase activity exhibited bell-shaped curves as a function of temperature and pH. A peak of the activity for both bacterial strains was determined at +35 ºC. An increase or decrease in temperature of the incubation led to a decrease of the activity of the enzyme. Similar peaks of the activity were also determined in extracts of *D. piger* Vib-7 at pH 7.0 and *Desulfomicrobium* sp. Rod-9 at pH 6.5.

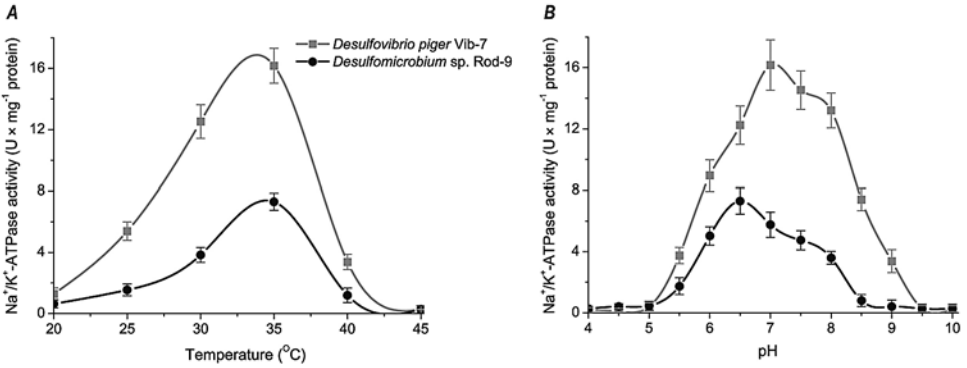

Fig. 1. Effect of temperature (A) and pH (B) on the Na^+/K^+-ATPase activity in the cell-free extracts

Kinetic analysis of the release of inorganic phosphorus in the ATP hydrolysis

To study the characteristics and mechanism of Na^+/K^+-ATPase, the initial (instantaneous) reaction rate (V_0), maximum (plateau) amount formation of product reaction (P_{max}) and reaction time (τ) was defined. To calculate the kinetic indicators of ATP hydrolysis which is

catalyzed by Na^+/K^+-ATPase, the dynamics of the product accumulation of ATP hydrolysis reaction was studied. For that, bacterial suspensions were incubated in a standard incubation medium for different periods of time (1–20 min). Our experimental data imply that the kinetic curves of ATP hydrolysis in both bacterial strains have tendency to saturation (Fig. 2).

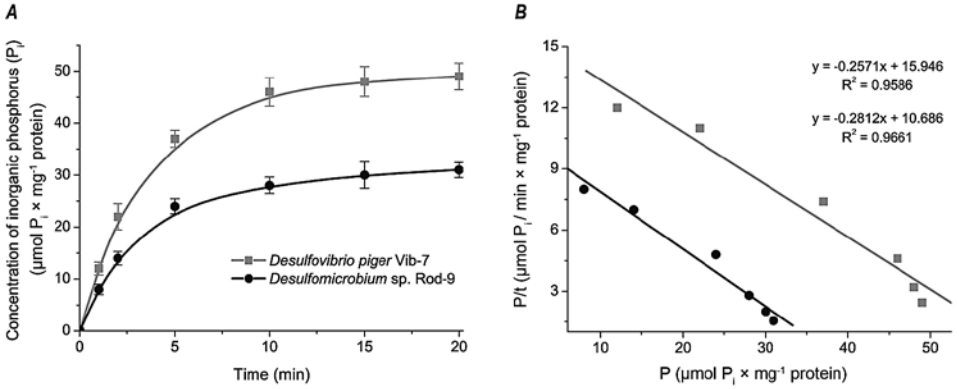

Fig. 2. Dynamics of inorganic phosphorus (P_i) release in the process of ATP hydrolysis (A) and linearization curves of inorganic phosphorus (P_i) accumulation in {P/t; P} coordinates (B) (M ± m, n = 5, $R^2 > 0.95$; $P < 0.02$).

The kinetic of ATP hydrolysis catalyzed in the bacterial cells was consistent with the zero-order reaction in the range 0–5 min (the graph of dependence of P on the incubation time is almost linear in this time interval). Therefore, the duration of incubation of bacterial cell extracts and accordingly the ATP hydrolysis was 5 min in subsequent experiments. The amount of P_i released by the ATPase from *D. piger* Vib-7 was higher compared to *Desulfomicrobium* sp. Rod-9 in the entire range of time factor. The main kinetic properties of the ATP hydrolysis reaction were calculated by the linearization of the data in the {P/t; P} coordinates (Fig. 2B).

Kinetic indicators of ATP hydrolysis in the cell-free extracts were significantly different ($P > 0.05$). Values of initial reaction rate for the enzyme reaction by the maximum amount of the reaction product (P_{max}) were calculated. The V_0 (15.95 ± 1.58 μmol P_i/min·mg^{-1} protein) for *D. piger* Vib-7 was 1.6 greater compared to *Desulfomicrobium* sp. Rod-9 (10.69 ± 0.93 μmol P_i/min·mg^{-1} protein). P_{max} was 62.02 ± 4.21 and 38.01 ± 2.81 μmol P_i·mg^{-1} protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively. Based on these data, we assume that the transport of Na^+ and K^+ ions through the membrane is slower and less active in *Desulfomicrobium* sp. Rod-9 cells compared to *D. piger* Vib-7. However, they are characterized by almost the same reaction time, which was 3.89 ± 0.34 and 3.56 ± 2.28 min for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively.

Initial rate of ATP hydrolysis depending on protein concentration in incubation mixture

The dependence of the Na^+/K^+-ATPase activity on the protein concentration was studied in a range of concentrations from 15 to 150 mg protein/ml. A gradual increase in the concentration of protein in the bacterial cell-free extracts of the incubation medium increased the initial (instantaneous) rate of enzyme reaction; the V_0 reached its a maximum value at 115 μg/ml for extract of *D. piger* Vib-7 and 75 μg/ml for *Desulfomicrobium* sp. Rod-9 (Fig. 3).

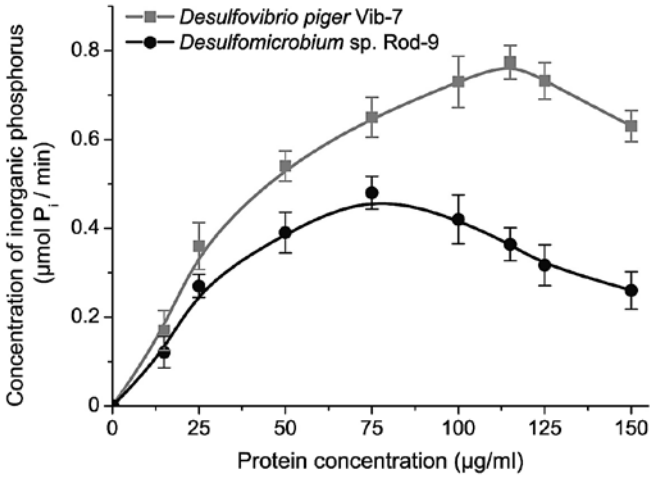

Fig. 3. Initial rate of ATP hydrolysis depending on protein concentration (M ± m, n = 5)

Similarly, the dependency of P_i accumulation depending on the protein content of bacterial cells in the incubation medium had the same character for both strains. However, the data imply that the initial reaction rate was significantly different ($P > 0.05$).

Kinetic analysis of ATP hydrolytic activity depending on ATP concentration

According to the results obtained for the extracts by ATP at concentrations ranging from 0.25 to 2.5 mM (at a constant concentration of 5 mM of Mg^{2+}), there was observed a monotonic increase in enzymatic activity of Na^+/K^+-ATPase to maximum values under substrate concentrations over 1.75 mM and after that activity was maintained on an unchanged (plateau) level (Fig. 4).

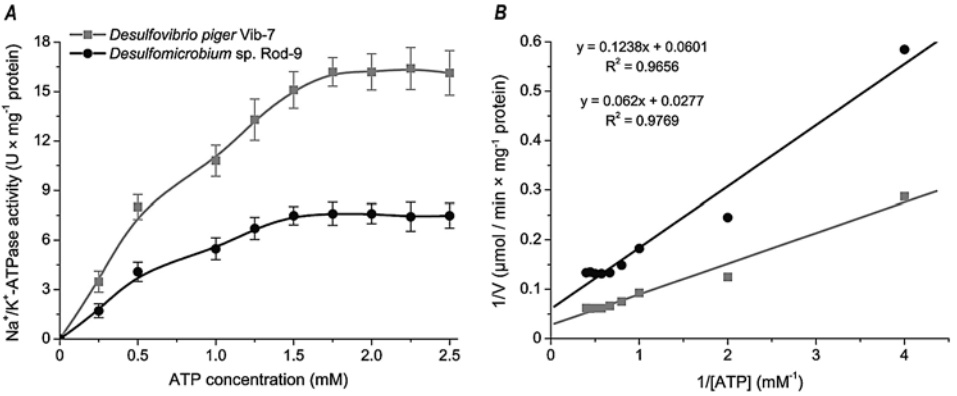

Fig. 4. ATP effect on the Na^+/K^+-ATPase activity (A) and the linearization of the concentration curves (B), which are shown in Fig. 4A, in the Lineweaver-Burk plot, where V is the rate of enzyme reaction (M ± m, n = 5; $R^2 > 0.95$; $F < 0.005$).

The enzyme activity in the *D. piger* Vib-7 was higher compared to the *Desulfomicrobium* sp. Rod-9 within the range of ATP concentrations. To elucidate the possible mechanism of changes of the enzymatic ATPase activity, the kinetic indicators of ATP hydrolysis were defined. The maximum rate of ATP hydrolysis was 36.10 ± 2.87 and 16.64 ± 1.73 μmol P_i/min·mg^{-1} protein for *D. piger* Vib-7 and *Desulfomicrobium* sp., respectively. The value for *D. piger* Vib-7 was × 2 greater than for *Desulfomicrobium* sp. ($P < 0.001$). However, the Michaelis affinity constant (K_m) of the ATPase to ATP in the extract from *D. piger* Vib-7 (2.24 ± 0.21 mM) was not significantly different from *Desulfomicrobium* sp. Rod-9 (2.06 ± 0.18 mM). Thus, according to the obtained kinetic indicators of the ATP hydrolysis for both bacterial strains, we concluded that the activity of Na^+/K^+-ATPase, V_0 and V_{max} was significantly higher ($P < 0.001$) in *D. piger* Vib-7 cells than *Desulfomicrobium* sp. Rod-9.

Kinetic analysis of ATP hydrolytic activity depending on Mg^{2+} ion concentration

Experiments focusing on the effect of Mg^{2+} ions on the ATPase activity were performed in a range of $MgCl_2$ concentrations from 0 to 7 mM (at constant ATP concentration of 1.5 mM) (Fig. 5).

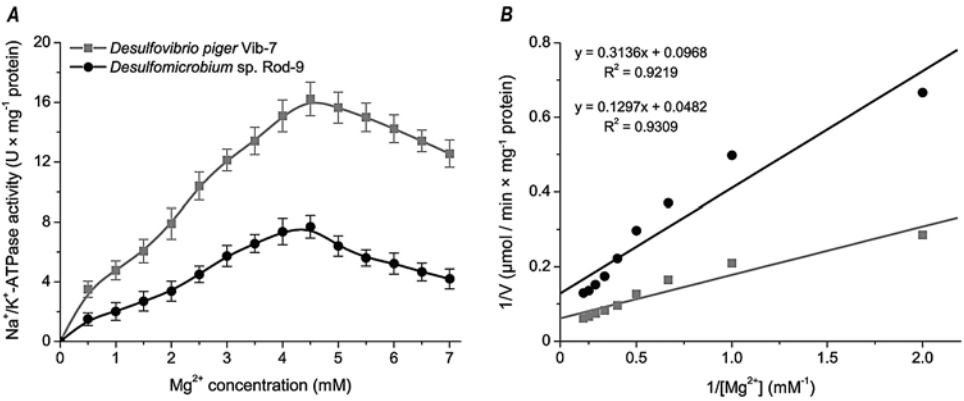

Fig. 5. Mg^{2+} ions effect on the Na^+/K^+-ATPase activity (A) and linearization of concentration curves (B), which are shown in Fig. 5A, in the Lineweaver-Burk plot, where V is the rate of the enzyme reaction (M ± m, n = 5; $R^2 > 0.9$; $P < 0.001$).

Enzyme activity depended on the concentration of Mg^{2+} ions in the incubation medium, and the activity curves had a typical bell-shape. Results presented in Fig. 5A indicate that the maximum value of activity was observed at 4.5 mM $MgCl_2$ for both bacterial strains. As shown in Fig. 5B, the curves of dose-dependent of Mg^{2+} ions effect on the ATPase activity in {1/V, 1/[Mg^{2+}]} coordinates differed by the tangent slope. The V_{max} (20.75 ± 2.3 and 10.33 ± 1.22 μmol P_i/min·mg^{-1} protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively) were significantly different from each other ($P < 0.001$). The apparent activation constant of Mg^{2+} ions was 2.65 ± 0.21 mM for *D. piger* Vib-7 and 3.04 ± 0.29 mM for *Desulfomicrobium* sp. Rod-9.

Kinetic analysis of ATP hydrolytic activity depending on Na^+ and K^+ ion concentration

To study the effect of different concentrations of Na^+ and K^+ ions on the specific enzymatic activity of the Na^+/K^+-ATPase, NaCl was changed by isotonic KCl in the incubation medium

(the isotonic conditions of $Na^+ + K^+$ ions equal 150 mM). ATPase activity depended on concentration of Na^+ and K^+ ions and had a typical dome shape (Fig. 6).

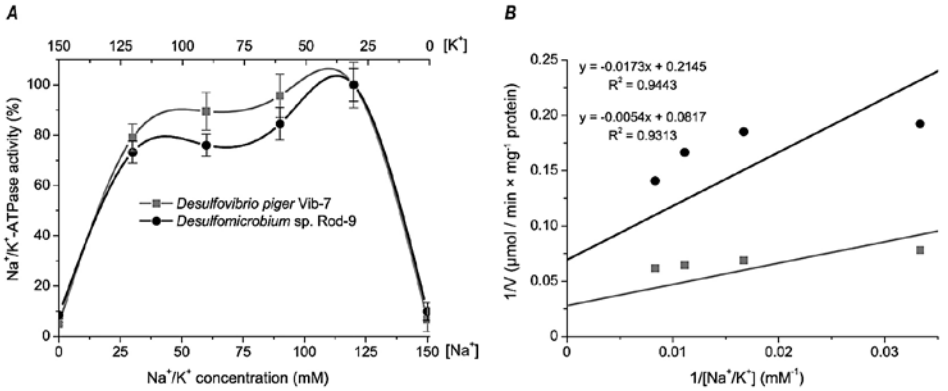

Fig. 6. Effect of Na^+ and K^+ ions (isotonic conditions of $Na^+ + K^+$ is 150 mM) on the Na^+/K^+-ATPase activity (A) and linearization of concentration curves (B), which are shown in Fig. 6A, in the Lineweaver-Burk plot, where V is the rate of the enzyme reaction (M ± m, n = 5; $R^2 > 0.9$; $P < 0.001$).

The optimal ion concentration ratio for the functioning of the enzyme is 125 Na^+ : 30 K^+ in incubation medium for both strains. In the absence of one of the ion species in the incubation medium, the Na^+/K^+-ATPase activity was not observed. The maximum rate of ATP hydrolysis (12.24 ± 1.29 and 4.66 ± 0.38 µmol P_i/min·mg^{-1} protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively) was determined by Na^+ ions. The V_{max} in both strains was significantly different ($P < 0.001$). However, the apparent constants of activation of Na^+ and K^+ ions were close (15.12 ± 1.4 and 12.39 ± 1.25 mM) in both strains.

Thus, based on calculation of the data received from the kinetic indicators, we concluded that the enzyme activity is lower in the *Desulfomicrobium* sp. Rod-9 than *D. piger* Vib-7 strain. Perhaps, it is through reduced indicators of the enzyme rate. It can be assumed that the decrease in values of V_{max} in the *Desulfomicrobium* sp. Rod-9 can be a species-specific trait, which could be contributed to a lower Na^+/K^+ electrochemical gradient of the cytoplasmic membrane of the *Desulfomicrobium* sp. Rod-9 and a decrease in the number of the transported units (a decrease of their expression in the membrane) or a decrease in the enzyme rate. Perhaps, the decreased value of the apparent activation constants K_{Na}^+ in the *Desulfomicrobium* sp. Rod- 9 indicated the affinity increase of the Na^+/K^+-ATPase to sodium ions.

Analysis of the inhibition effect of ouabain on the ATP hydrolytic activity

As shown in Fig. 7, inhibition by ouabain is the same for both cell-free extracts. To clarify the indicators of the inhibition of Na^+/K^+-ATPase by ouabain, the linearization of the concentration curves in Hill's coordinates was implemented. Values of apparent inhibition constants ($I_{0.5}$) and Hill coefficient (n_H) did not differ significantly. The $I_{0.5}$ was 22.9 ± 4.6 µM and 15.8 ± 2.6 µM as well as n_H was 1.1 ± 0.12 and 0.99 ± 0.08, for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively. However, it should be noted that inhibition coefficient does not match to the apparent inhibition constant, which is determined when achieving a stationary level of inhibition of the enzyme.

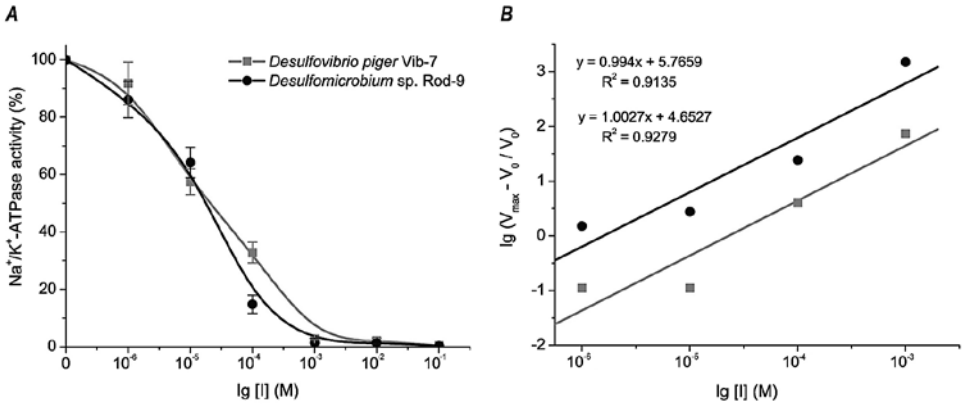

Fig. 7. Ouabain inhibited effect on the Na^+/K^+-ATPase activity (A) and linearization of concentration curves (B) in Hill's coordinates (M ± m, n = 5; $R^2 > 0.9$; $P < 0.001$).

Discussion

Except for ATP, magnesium ions are necessary for the functioning of Na^+/K^+-ATPase, where ions act as cofactors. The Mg^{2+} ions form Mg-ATP chelate complex, which is a substrate of enzymatic reaction. The Mg^{2+} ions interact with the phosphate group of ATP and polarize them, and thus facilitate the nucleophilic attack on the terminal γ-phosphate. The Mg^{2+} ions can also bind to the regulatory center of Na^+/K^+-ATPase (Yuan et al. 2005; Tian et al. 2006).

Na^+/K^+-activated Mg^{2+}-dependent ATPase combines transport, hydrolytic and receptor functions and specifically interacts with exogenous inhibitors in particular with glycosides or their endogenous counterparts (Lodish et al. 2000; Yuan et al. 2005; Tian et al. 2006). Ouabain is a steroid compound, which is a highly selective inhibitor of Na^+/K^+-ATPase. Ouabain enzyme binds to the outside of the cytoplasmic membrane. It is supposed that ouabain blocks an enzyme in the conformation of the $P\text{-}E_2$, which, in turn, prevents the further course of the catalytic cycle of the enzyme. In experimental conditions (insufficient time for binding ouabain in unsteady mode, antagonism of K^+ ions at its high concentration in samples) the coefficient inhibition does not match the apparent inhibition constant, determined in achieving the stationary level of inhibition the enzyme. Inhibition indicators characterize the highly sensitive phenotype of the Na^+/K^+-ATPase to ouabain under these experimental conditions of inhibition. Ouabain is defined by the similarity of patterns of the receptor sites, which is typical for all isoenzymes Na^+/K^+-ATPase (Yuan et al. 2005; Tian et al. 2006). Perhaps, Na^+/K^+-ATPase activity of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 can have the same receptors, which are characterized by sensitivity to inhibition.

The reduction of sulfate ions to hydrogen sulfide occurs as a result of the formation of many intermediate compounds. The sulfate reduction enzymes are located in the cytoplasm and peripheral plasma. The initial stages of the sulfate reduction include uptake of sulfate ions in the bacterial cells. The sulfate ions can be transported into the cells simultaneously with protons and some sulfate-reducing bacteria can absorb sulfate from the flow of sodium ions (Barton and Hamilton 2007). It is known that the center of the ATP hydrolysis is located on the cytoplasmic surface of the membrane (Yuan et al. 2005; Tian et al. 2006).

Guarraia and Peck (1971) determined the ATPase activity in both the soluble and

particulate fractions of the anaerobic sulfate-reducing bacterium, *Desulfovibrio gigas*. As the soluble ATPase was labile to storage, only the particulate enzyme was studied in detail. ATPase was stimulated by both Ca^{2+} and Mg^{2+}, but the magnitude of stimulation depended on pH. In the presence of Ca^{2+} the optimum pH was 6.5, whereas, in the presence of Mg^{2+} the pH optimum was 8.0. However, under optimum conditions, the activity was the same with both Mg^{2+} and Ca^{2+}.

Taking into consideration all of the obtained results, according to change of the hydrolase activity, the transport of Na^+ and K^+ ions by Na^+/K^+-ATPase in *Desulfomicrobium* sp. Rod-9 is slower and less intense compared to the *D. piger* Vib-7 strain. However, it is characterized by almost the same capacity. The Na^+/K^+-ATPase activity, V_0 and V_{max} were significantly higher in the *D. piger* Vib- 7 cells than in *Desulfomicrobium* sp. Rod-9.

The affinity of Na^+/K^+-ATPase to ATP is also the lowest in the *Desulfomicrobium* sp. Rod-9 compared to *D. piger* Vib-7. At the same time, the Mg^{2+}-binding site of Na^+/K^+-ATPase of the studied bacterial strains is native. Increasing adenosine triphosphate in the concentration range from 0.25 to 2.5 mM caused a monotonic increase in enzymatic activity to maximum values and after that activity was maintained on an unchanged (plateau) level. The increase in the affinity of enzyme activity to Na^+ ions in the cell-free bacterial extracts and maintaining the receptor properties of Na^+/K^+-ATPase to ouabain was observed.

The above described experimental data can be used for further clarification of the mechanisms of ion exchange membrane of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 as well as to study the transport mechanisms. According to these data, we can assume that the transports of sulfate ions into *D. piger* Vib-7 cells are much more intensive, and can lead, in turn, to an increase of the intensity of sulfate reduction and the accumulation of hydrogen sulfide, which is toxic for epithelial cells of the intestine and can cause bowel diseases, in particular ulcerative colitis both in humans and animals.

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