UDC 579.846.2:22

KINETIC PROPERTIES OF ADENOSINE TRIPHOSPHATE SULFURYLASE OF INTESTINAL SULFATE-REDUCING BACTERIA

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The investigation of specific activity of ATP sulfurylase and kinetic properties of the enzyme in cell-free extracts of intestinal bacterial strains Desulfovibrio piger Vib-7 and Desulfomicrobium sp. Rod-9 is presented. The microbiological, biochemical, biophysical and statistical methods were used in the work. The optimal temperature (35ºC) and pH 8.0-8.5 for enzyme reaction were determined. An analysis of kinetic properties of ATP sulfurylase has been carried out. Initial (instantaneous) reaction velocity (V_0), maximum amount of the product of reaction (P_{max}), the reaction time (half saturation period, τ) and maximum velocity of the ATP sulfurylase reaction (V_{max}) have been defined. Michaelis constants ($K_m^{Sulfate}$, K_m^{ATP}, K_m^{APS}, and $K_m^{Pyrophosphate}$) of the enzyme reaction were demonstrated for both D. piger Vib-7 and Desulfomicrobium sp. Rod-9 intestinal bacterial strains.

K e y w o r d s: kinetic analysis, sulfate-reducing bacteria, Desulfovibrio piger, Desulfomicrobium sp., ATP sulfurylase, intestinal microbiocenosis, inflammatory bowel diseases, ulcerative colitis.

Ulcerative colitis is one of the most common acute inflammatory bowel diseases. The development of this disease depends on human nutrition and the qualitative and quantitative composition of intestinal microbiocenosis [3, 12]. The active agents of ulcerative colitis and inflammatory bowel diseases can be sulfate-reducing bacteria which occur in the gut flora of about 50% of healthy persons [7].

The process of the dissimilatory sulfate reduction to sulfide is provided due to the enzyme activity of the bacteria [2]. Sulfate is a stable nonreactive compound that must be activated to participate in subsequent metabolic reactions, such as sulfate reduction and sulfur transfer [11]. The first activation step in assimilation and dissimilation pathways of sulfate reduction is accomplished by transferring and coupling the adenosine 5′-phosphoryl moiety of ATP to sulfate [2, 16]. This reaction catalyzed by ATP sulfurylase (ATP sulfate adenyltransferase, EC 2.7.7.4) is represented by the equation: ATP + + SO_4^{2-} ↔ APS + PP_i. The enzyme plays a crucial role in sulfate activation, the key step for sulfate utilization. It was found in *Desulfovibrio desulfuricans* ATCC 27774 and *Desulfovibrio gigas* [6].

The dissimilation process occurring in these organisms involves adenosine phosphosulfate (APS) as the active intermediate compound [11, 16]. In sulfate-reducing bacteria of the genus *Desulfovibrio* the APS is reduced to AMP and sulfite through the mediation of APS reductase [2, 6]. Sulfite is further reduced to hydrogen sulfide, the final product of the sequential reactions. Hydrogen sulfide accumulated in the human intestine is cytotoxic and carcinogenic to its cells causing inhibition of cytochrome oxidase, oxidation processes of butyrate by colonocytes, and destruction of epithelial cells, ulcers, inflammation with subsequent development of colon cancer [3, 7, 12].

As far as it is aware, ATP sulfurylase from intestinal sulfate-reducing bacteria *D. piger* and *Desulfomicrobium* has never been well-characterized. In literature, there are a lot of data about ATP sulfurylase of the sulfate-reducing bacteria isolated from the environment [2, 6, 10, 16]. However, the data about activity of this enzyme from intestinal sulfate-reducing bacteria *Desulfovibrio piger* and *Desulfomicrobium* sp. has not been yet reported.

The aim of our work was to study specific activity of ATP sulfurylase in cell-free extract of in-

testinal sulfate-reducing bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 and to carry out the kinetic analysis of enzymatic reaction.

Materials and Methods

Objects of the study were sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine and identified [13, 14].

Bacterial growth and cultivation. Bacteria were grown in a nutrition-modified Kravtsov-Sorokin's liquid medium [13]. Before seeding bacteria in the medium, 0.05 ml/l of sterile solution of $Na_2S\times9H_2O$ (1%) was added. A sterile 10N solution of NaOH (0.9 ml/l) in the medium was used to provide the final pH 7.2. The medium was heated in boiling water for 30 min in order to obtain the oxygen-free medium, and then cooled to 30 °C. The bacteria were grown for 72 h at 37 °C under anaerobic conditions. The tubes were brim-filled with the medium and closed to provide anaerobic conditions.

Obtaining of cell-free extracts. Cells were harvested at the beginning of the stationary phase, suspended in 10 mM Tris-HCl buffer in a 1/1 ratio (w/v) at pH 7.6, and disrupted using a Manton-Gaulin press at 9000 psi. The extract was centrifuged at 15,000g for 1 h; the pellet was then used as sedimentary fraction, and the supernatant obtained was the termed soluble fraction [6]. This extract was subjected to further centrifugation at 180,000g for 1 h to eliminate the membrane fraction. A pure supernatant, containing the soluble fraction, was then used as cell-free extract. Protein concentration in the cell-free extracts was determined by the Lowry method [15].

Assays for ATP sulfurylase activity. The enzyme was assayed as described in paper [18]. Initial reaction velocities were determined by observing the rate of change in absorbance of pyridine nucleotide at 340 nm in 500 µl systems at 25 °C using a Beckman spectrophotometer. For providing the forward APS synthesis reaction the assay system was used which consisted of 50 mM Tris (pH 8.0), 15 mM $MgCl_2$, 100 mM NaCl, 0.4 mM phosphoenolpyruvate, 0.2 mM NADH, 0.05 units of APS kinase, 20 units of pyruvate kinase and 30 units of lactate dehydrogenase. The specific activity of APS kinase (1.5 $\mu mol\times min^{-1}\times mg^{-1}$) was determined spectrophotometrically, as described in [17]. For the reverse ATP synthesis reaction we used an assay system of 50 mM Tris (pH 8.0), 5 mM $MgCl_2$, 1 mM $NADP^+$, 1 mM glucose, 2 units of hexokinase and 1 unit of glucose-6-phosphate dehydrogenase [4]. All reactions were initiated by addition of enzyme and were corrected for non-enzymatic rates. One unit of enzyme activity is defined as the amount of enzyme that produces 1 µmol of primary product per minute. Specific enzyme activity was expressed as $U\times mg^{-1}$ protein. The activity of the studied enzyme in the cell-free extracts of both bacterial strains under the effect of different temperature (from 20 to 45 °C) and pH (in the range from 4.0 to 10.0) in the incubation medium was measured.

Kinetic analysis. Kinetic analysis of the enzyme reaction was performed in the standard incubation medium (as it was described above) with modified physical and chemical characteristics of the respective parameters (the incubation time, substrate concentration, temperature and pH). The determined kinetic parameters characterizing the ATP sulfurylase reaction are as follows: the initial (instantaneous) reaction velocity (V_0), maximum velocity of the reaction (V_{max}), maximum amount of the reaction product (P_{max}) and characteristic reaction time (time of half saturation) [9]. The amount of the reaction product was calculated stoichiometrically. The kinetic parameters characterizing ATP sulfurylase reactions such as Michaelis constant (K_m) and maximum reaction velocity of substrate decomposition were determined by Lineweaver-Burk plot [8]. To analyze the substrate kinetic mechanism of ATP sulfurylase, initial velocities were measured under standard assay conditions with different substrate concentrations. In the forward direction, assays used varied Na_2SO_4 and ATP concentrations. For the reverse reaction, initial velocities were determined using varied APS and sodium PP_i concentrations. The resulting data were also analyzed by global curve fitting in SigmaPlot (Systat Software, Inc.) to model the kinetic data for rapid equilibrium rate equations describing ordered sequential, $V = (V_{max}[A][B])/(K_AK_B + K_B[A] + + [A][B])$, and random sequential, $V = (V_{max}[A][B])/(\alpha K_A K_B + K_B[A] + K_A[B] + [A][B])$, kinetic mechanisms, where V is the initial velocity, V_{max} is the maximum velocity, K_A and K_B are the K_m values for substrates A and B, respectively, and α is the interaction factor if the binding of one substrate changes the dissociation constant for the other [19].

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 processed by

the methods of variation statistics using Student *t*-test. The equation of the straight line that the best approximates the experimental data was calculated by the method of least squares. The absolute value of the correlation coefficient r was from 0.90 to 0.98. The significance of the calculated parameters of the line was tested by the Fisher's F-test. The accurate approximation was observed when $P \leq 0.05$ [1].

Results and Discussion

Specific activity of ATP sulfurylase, an important enzyme in the process of sulfate transport in sulfate-reducing bacteria, in different fractions of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was determined (Table 1). Results of our study showed that the specific activity of the enzyme in cell-free extracts (2.26 ± 0.231 and 0.98 ± 0.0082 U×mg^{-1} protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively) was measured. Slightly higher values of activity of ATP sulfurylase was determined in the soluble fraction compared to cell-free extracts. Its values designated 2.94 ± 0.272 U×mg^{-1} protein for *D. piger* Vib-7 and 1.12 ± 0.121 U×mg^{-1} protein for *Desulfomicrobium* sp. Rod-9. The enzyme activity was not observed in sedimentary fraction.

The effect of temperature and pH of the reaction mixture on ATP sulfurylase activity in the cell-free extracts of the sulfate-reducing bacteria was studied (Fig. 1). The maximum specific activity for both bacterial strains was determined at 35 ºC. An increase or decrease in temperature led to a decrease of the activity of studied enzyme in the cell-free bacterial extracts. The highest enzyme activity of ATP sulfurylase was determined in the cell-free extracts of the *D. piger* Vib-7 and the *Desulfomicrobium* sp. Rod-9 at pH 8.0-8.5.

Thus, temperature and pH optimum of this enzyme was 35 ºC and pH 8.0-8.5, respectively. The enzyme activity exhibited typical bell-shaped curves as a function of temperature and pH.

To study the characteristics and mechanism of ATP sulfurylase reaction, the initial (instantaneous) reaction velocity (V_0), maximum velocity of the reaction (V_{max}), maximum amount of reaction product (P_{max}) and reaction time (τ) were determined. Dynamics of APS accumulation in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was studied for investigation of the kinetic parameters of ATP sulfurylase (Fig. 2).

Experimental data showed that the kinetic curves of ATP sulfurylase activity have a tendency to saturation (Fig. 2, *A*). The analysis of the results allows one to make the conclusion that the kinetics of ATP sulfurylase activity in cell-free extracts of the sulfate-reducing bacteria was consistent to the zero-order reaction in the range of 0-10 min (the graph of the dependence of product formation on the incubation time was almost linear in this interval of time). Therefore the duration of the incubation of bacterial cells extracts was 10 min in subsequent experiments.

The amount of ATP sulfurylase reaction product in the *D. piger* Vib-7 was higher (97.13 ± 8.91 μmol×mg^{-1} protein) compared to the *Desulfomicrobium* sp. Rod-9 (36.24 ± 3.43 μmol×mg^{-1} protein) in the entire range of time factor. The basic kinetic properties of the reaction in the cell-free extracts of the sulfate-reducing bacteria were calculated by linearization of the data in the {P/t; P} coordinates (Fig. 2, *B*, Table 2).

The kinetic parameters of ATP sulfurylase in cell-free extracts of both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were significantly different. The values of initial (instantaneous) reaction velocity (V_0) for ATP sulfurylase activity was calculated by the maximal amount of the product reaction (P_{max}). As shown in Table 2, V_0 for ATP sulfurylase reac-

Table 1. ATP sulfurylase activity in different fractions obtained from cells of Desulfovibrio piger Vib-7 and Desulfomicrobium sp. Rod-9

Sulfate-reducing bacteria	Specific activity of ATP sulfurylase (U×mg^{-1} protein)		
	Cell-free extract	Individual fractions	
		Soluble	Sedimentary
Desulfovibrio piger Vib-7	2.26 ± 0.231	2.94 ± 0.272	0
Desulfomicrobium sp. Rod-9	0.98 ± 0.0082**	1.12 ± 0.121***	0

Comment: The assays were carried out at a protein concentration of 44.93 mg/ml (for *D. piger* Vib-7) and 38.14 mg/ml (for *Desulfomicrobium* sp. Rod-9). Enzyme activity has been determined after 20 min incubation. Significance of the values $M \pm m$, $n = 3$; ** $P < 0.01$, *** $P < 0.001$, compared to *D. piger* Vib-7 strain.

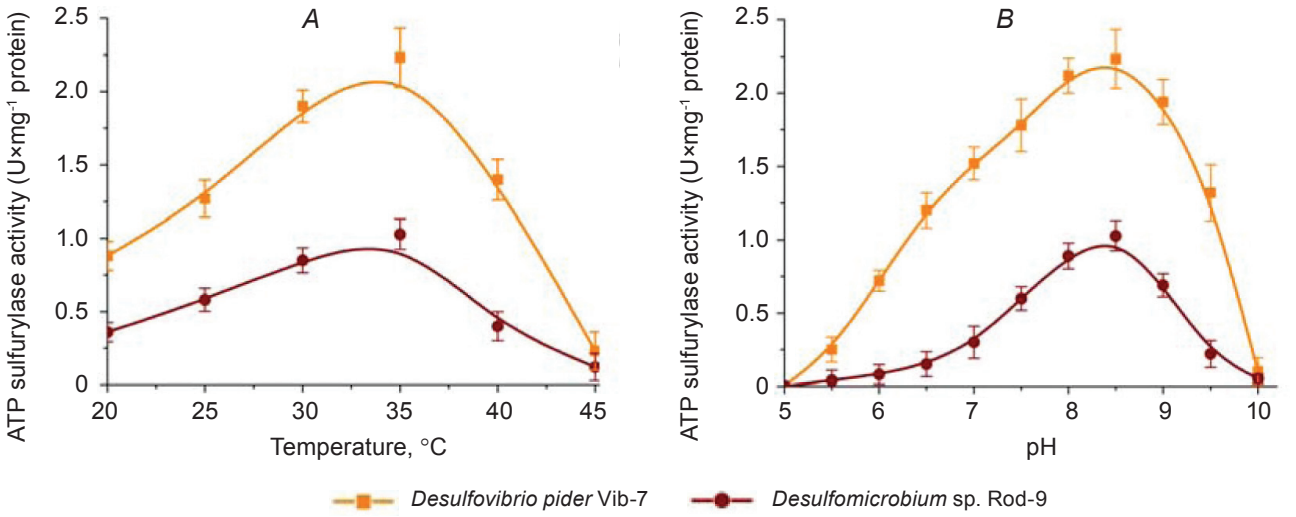


Fig. 1. The effect of temperature (A) and pH (B) on the dissimilatory ATP sulfurylase activity in the cell-free extracts of D. piger Vib-7 and Desulfomicrobium sp. Rod-9

Table 2. Kinetic parameters of the ATP sulfurylase in the cell-free extracts of Desulfovibrio piger Vib-7 and Desulfomicrobium sp. Rod-9

Kinetic parameters	Sulfate-reducing bacteria	
	Desulfovibrio piger Vib-7	*Desulfomicrobium* sp. Rod-9
V_0 (μmol×min^{-1}×mg^{-1} protein)	5.48 ± 0.57	4.12 ± 0.38
P_{max} (μmol×mg protein)	97.13 ± 8.91	36.24 ± 3.43***
τ (min)	17.73 ± 1.62	8.80 ± 0.76**

Comment: V_0 is initial (instantaneous) reaction velocity; P_{max} is maximum amount (plateau) of the product of reaction; is the reaction time (half saturation period). Significance of the values $M \pm m$, $n = 5$; $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the *D. piger* Vib-7 strain.

tion was slightly higher in the cell-free extracts of *D. piger* Vib-7 (5.48 ± 0.57 μmol×min^{-1}×mg^{-1} protein) compared to *Desulfomicrobium* sp. Rod-9 (4.12 ± 0.38 μmol×min^{-1}×mg^{-1} protein). In this case, the value of the reaction time (τ) was also significantly higher for *D. piger* Vib-7 than for *Desulfomicrobium* sp. Rod-9 strain. Based on these data, there is an assumption that *D. piger* Vib-7 can consume sulfate ion much faster in their cells than *Desulfomicrobium* sp. Rod-9. Moreover, this hypothetical assumption can be also confirmed by the data obtained with maximal velocities of accumulation of the final reaction products, where V_{max} for SO_4^{2-}, ATP, APS, and PPi in the cell-free extracts of *D. piger* Vib-7 were also more intensively increased compared to *Desulfomicrobium* sp. Rod-9 (Table 3).

The kinetic analysis of ATP sulfurylase activity dependence on concentration of substrates (SO_4^{2-} and ATP) was performed. According to the obtained results, the increase of substráte concentrations from 0.5 to 5.0 mM (for sulfate) and from 0.25 to 3.5 mM (for ATP) causes a monotonic rise of the studied enzyme activity and the activity was maintained on unchanged level (plateau) under substrate concentrations over 5.0 and 3.5 mM, respectively (Fig. 2, *C, E*). Curves of the dependence {1/*V*; 1/[S]} were distinguished by the tangent slope and intersect the vertical axis in one point (Fig. 2, *D, F*). Additionally, the activity of ATP sulfurylase in cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 under the effect of different concentrations of APS and pyrophosphate was studied by the reverse reac-

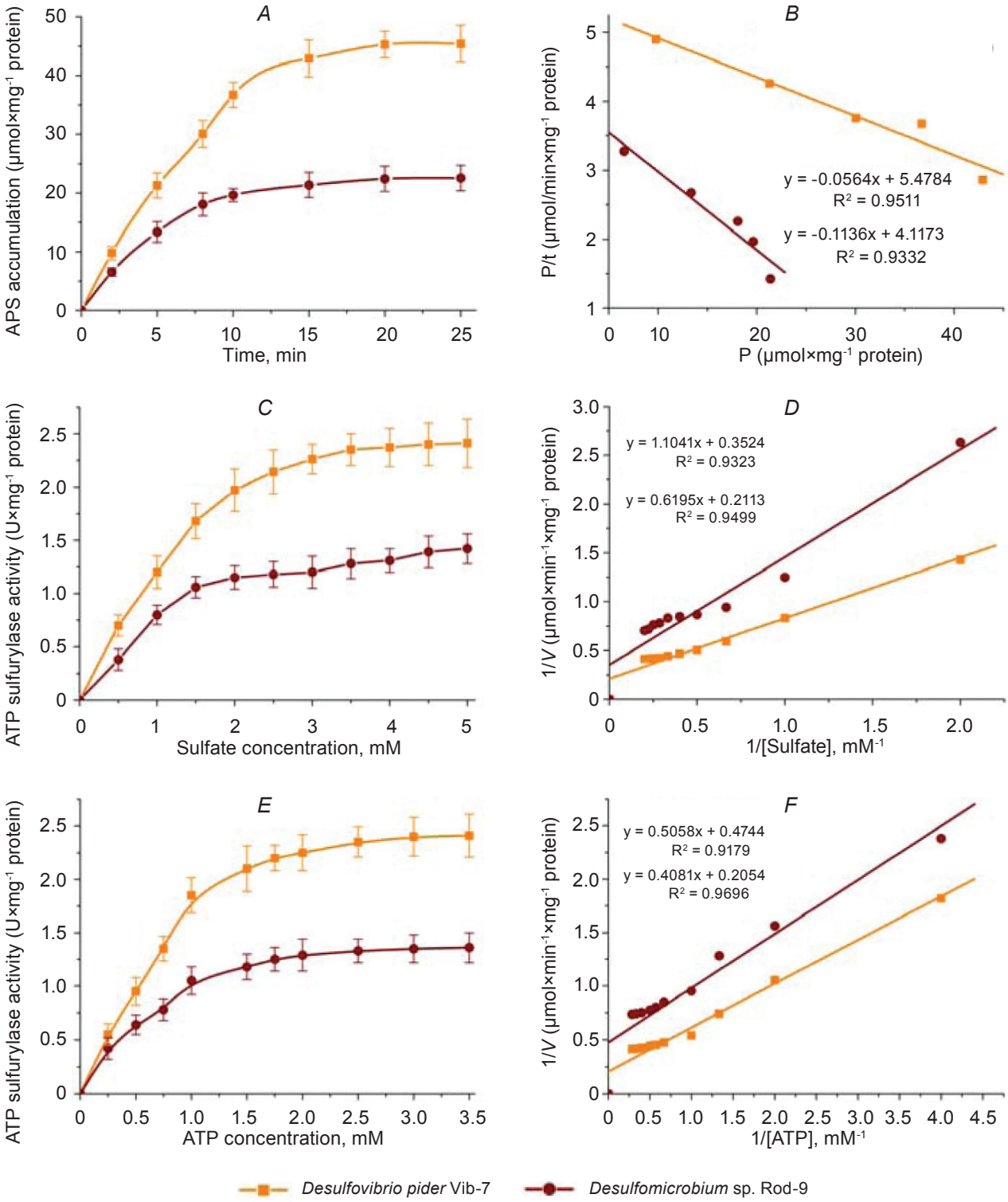


Fig. 2. Kinetic parameters of ATP sulfurylase activity in cell-free extracts of D. piger Vib-7 and Desulfomicrobium sp. Rod-9: A – dynamics of product accumulation (M ± m, n = 3); B – linearization of curves of product accumulation in {P/t; P} coordinates (n = 3; $R^2 > 0.95$; $F < 0.02$); C, E – the effect of different concentrations of substrate on ATP sulfurylase activity (M ± m, n = 5); D, F – linearization of concentration curves, which are shown in fig. 3C, E, in the Lineweaver-Burk plot, where V is velocity of ATP sulfurylase reaction and [Sulfate] or [ATP] is substrate concentration (n = 5; $R^2 > 0.95$; $F < 0.005$)

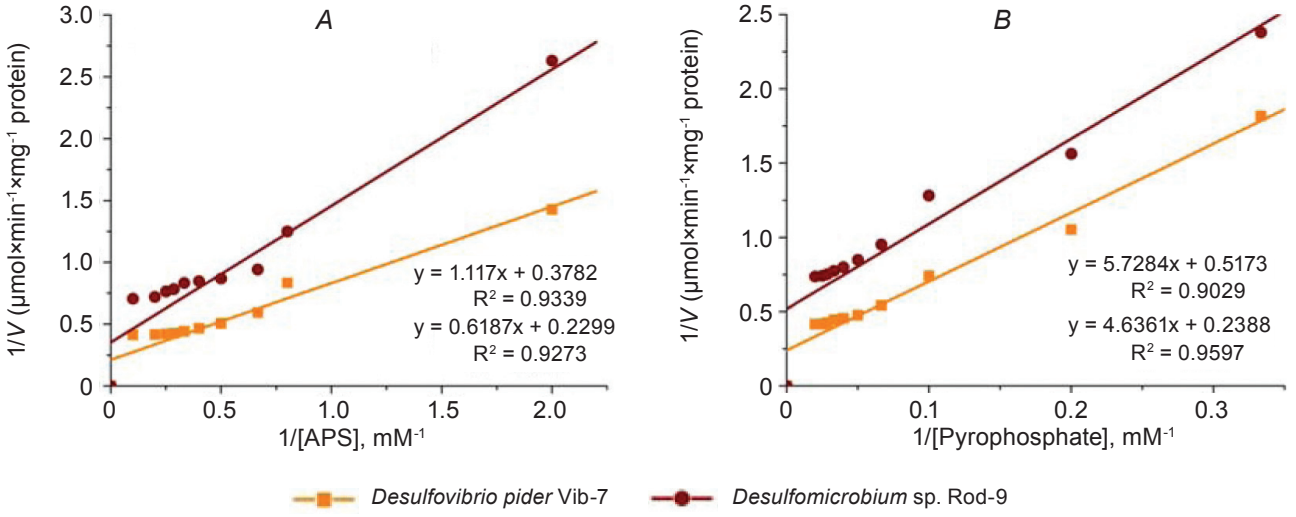


Fig. 3. Linearization of the effect of different concentrations of APS (A) and pyrophosphate (B) on ATP sulfurylase activity in cell-free extracts of D. piger Vib-7 and Desulfomicrobium sp. Rod-9, in Lineweaver-Burk plot, where V is velocity of ATP sulfurylase reaction and [APS] or [pyrophosphate] is substrate concentration ($n = 3$; $R^2 > 0.9$; $F < 0.005$)

tion (figures not shown). There was similar tendency as described for sulfate and ATP substrates. The linearization of the effect of different concentrations of APS and pyrophosphate on ATP sulfurylase activity was presented in Fig. 3.

The basic kinetic parameters of ATP sulfurylase activity in *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 cell-free extracts were identified by linearization of the data in the Lineweaver-Burk plot (Table 3).

Calculation of the kinetic parameters of ATP sulfurylase activity indicates that the maximum velocities (V_{max}) of SO_4^{2-}, ATP, APS, and PP_i in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were significantly different from each other. However, it was observed a correlative

Table 3. Kinetic parameters of ATP sulfurylase activity

Kinetic parameters	Sulfate-reducing bacteria	
	Desulfovibrio piger Vib-7	*Desulfomicrobium* sp. Rod-9
Forward reaction		
V_{max} for SO_4^{2-} (μmol×min^{-1}×mg^{-1} protein)	4.73 ± 0.44	2.84 ± 0.29*
$K_m^{Sulfate}$ (mM)	2.93 ± 0.26	3.13 ± 0.27
V_{max} for ATP (μmol×min^{-1}×mg^{-1} protein)	4.87 ± 0.55	2.11 ± 0.22**
K_m^{ATP} (mM)	1.98 ± 0.21	1.07 ± 0.12*
Reverse reaction		
V_{max} for APS (μmol×min^{-1}×mg^{-1} protein)	4.34 ± 0.42	2.64 ± 0.25*
K_m^{APS} (mM)	2.69 ± 0.23	2.95 ± 0.31
V_{max} for PP_i (μmol×min^{-1}×mg^{-1} protein)	4.19 ± 0.39	1.93 ± 0.18**
$K_m^{Pyrophosphate}$ (mM)	19.41 ± 1.85	11.07 ± 1.11*

Comment: V_{max} is maximum velocity of the enzyme reaction; K_m is Michaelis constant which was determined by substrate. Significance of the values $M \pm m$, $n = 5$; *$P < 0.05$, **$P < 0.01$, compared to the *D. piger* Vib-7 strain.

relationship between $V_{max}^{Sulfate}$ and V_{max}^{APS} as well as V_{max}^{ATP} and $V_{max}^{Pyrophosphate}$ in both intestinal bacterial strains.

Michaelis constants (K_m) of ATP sulfurylase reaction was identified for SO_4^{2-}, ATP, APS, and PP_i in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 strains. The values of K_m were similar for sulfate (2.93 ± 0.26, 3.13 ± 0.27 mM) and APS (2.69 ± 0.23, 2.95 ± 0.31 mM) in extracts of both strains, respectively. However, K_m for ATP (1.98 ± 0.21, 1.07 ± 0.12 mM) and PP_i (19.41 ± 1.85, 11.07 ± 1.11 mM) were significantly different from each other.

The obtained parameters of ATP sulfurylase reaction in the cell-free extracts of *D. piger* Vib-7 are consistent with previously described data by Gavel O. Yu. et al. for ATP sulfurylase activity in extracts of *Desulfovibrio desulfuricans* ATCC 27774 and *Desulfovibrio gigas*. Adenosine triphosphate sulfurylase catalyzes the formation of adenosine 5′-phosphosulfate from adenosine triphosphate and sulfate. The enzyme plays a crucial role in sulfate activation, the key step for sulfate utilization. ATP sulfurylase from *D. desulfuricans* and *D. gigas* was homotrimer and identified, for the first time, as metalloproteins containing cobalt and zinc. Furthermore, it was revealed that either cobalt or zinc binds endogenously to presumably equivalent metal binding sites and is tetrahedrally coordinated to one nitrogen and three sulfur atoms [6]. Perhaps, the described ATP sulfurylase from *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 can contain similar ions because activity of this enzyme in cell-free extracts of studied intestinal bacteria is very similar with activity previously described by Gavel O. Yu. et al. [6].

ATP sulfurylase (MgATP:sulfate adenylyltransferase, EC 2.7.7.4) catalyzes the first reaction in the activation of inorganic sulfate [18]:

APS is the substrate of the second "sulfate activating" enzyme (APS kinase; MgATP:APS 3′-phosphotransferase, EC 2.7.1.25) which forms PAPS [6]:

$$ATP + APS \xrightleftharpoons{\text{APS kinase, } Mg^{2+}} ADP + PAPS$$

The action of inorganic pyrophosphatase helps drive the overall synthesis of PAPS [10, 16]:

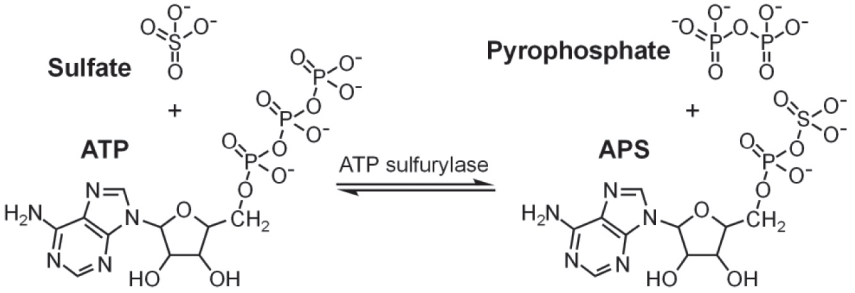
$$PP_i + H_2O \xrightleftharpoons{PP_i\text{tase, } Mg^{2+}} 2P_i$$

PAPS serves as the sulfuryl donor for the biosynthesis of sulfate esters. In bacteria, PAPS also serves as the substrate for a reductive sulfate assimilation pathway leading to cysteine [2].

Kramer M. and Cypionka H. have demonstrated the ATP sulfurylase activity in *Desulfovibrio sulfodismutans* and *D. desulfuricans* CSN strains grown with thiosulfate, sulfite and H_2 + sulfate. Enzyme activity of ATP sulfurylase required for sulfate reduction was detected in sufficient activities to account for the growth rates observed. It is proposed that during thiosulfate and sulfite disproportionation sulfate is formed via APS reductase and ATP sulfurylase [10].

Dissimilatory ATP sulfurylase from the hyperthermophilic sulfate reducer *Archaeoglobus fulgidus* belonging to the group of homo-oligomeric ATP sulfurylases was described by Sperling D. et al. [20]. The kinetic characteristics of Mg^{2+}-dependent ATP hydrolysis (basal Mg^{2+}-ATPase) in the cell membrane of bacteria *Bacillus* sp. was also demonstred by Danylovych G. V. et al. [5].

Based on the obtained studies results and according to the kinetic parameters of ATP sulfurylase reaction for both bacterial strains, we have concluded that the activity of ATP sulfurylase, V_0 and V_{max} were significantly higher in the *D. piger* Vib-7 cells than in *Desulfomicrobium* sp. Rod-9. However, Michaelis



constants $K_m^{Sulfate}$, K_m^{ATP}, K_m^{APS}, and $K_m^{Pyrophosphate}$ of the ATP sulfurylase reaction were similar for sulfate and ATP as well as significantly different for ATP (1.98 ± 0.21, 1.07 ± 0.12 mM) and PP_i (19.41 ± 1.85, 11.07 ± 1.11 mM), for both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively. The maximum ATP sulfurylase activity for both strains was determined at 35 ºC and at pH 8.0-8.5. These data correspond to conditions which are present in the human large intestine from which the bacterial strains were isolated. Perhaps, such conditions provide their intensive development in the gut. The kinetic parameters of enzyme reaction depend on the substrate concentration. According to these data, the increase of sulfate concentration in the gut can lead to its active assimilation and, accordingly, to the accumulation of hydrogen sulfide in the lumen of large intestine. *D. piger* Vib-7 strain can be more dangerous and have some pathogenic significance in development of inflammatory bowel diseases, consuming sulfate and producing hydrogen sulfide at a higher velocity. The extensive studies of physiological and biochemical properties of the intestinal sulfate-reducing bacteria, the process of the dissimilatory sulfate reduction, in particular participation of ATP sulfurylase in this process, the activity and kinetic properties of this enzyme in the *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 bacterial strains, production of hydrogen sulfide by them can be perspective for revealing the etiological role of these bacteria in the development of various bowel diseases. The data on the concentration of hydrogen sulfide produced by the isolates is supposed to help in establishing and assessing the toxic effect of these compounds on the epithelial cells of the human and animal intestine. These studies might help in predicting the development of diseases of the gastrointestinal tract, by providing further details on the etiology of bowel diseases which are very important for clinical diagnostics of such diseases.

КІНЕТИЧНІ ВЛАСТИВОСТІ АДЕНОЗИНТРИФОСФАТ СУЛЬФУРИЛАЗИ КИШКОВИХ СУЛЬФАТВІДНОВЛЮВАЛЬНИХ БАКТЕРІЙ

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Досліджено питому активність та кінетичні властивості АТР-сульфурилази в безклітинних екстрактах кишкових бактеріальних штамів *Desulfovibrio piger* Vib-7 і *Desulfomicrobium* sp. Rod-9. У роботі використано мікробіологічні, біохімічні, біофізичні та статистичні методи досліджень. Визначено оптимальну температуру (35 °С) і pH 8,0–8,5 для ензиматичної реакції. Проведено кінетичний аналіз властивостей АТР-сульфурилази. Визначено початкову (миттєву) швидкість реакції (V_0), максимальну кількість продукту реакції (P_{max}), час реакції (період напіврозпаду – насиченість, τ) і максимальну швидкість АТР-сульфурилазної реакції (V_{max}). Обчислено константи Міхаеліса ($K_m^{Сульфату}$, $K_m^{АТР}$, $K_m^{АПС}$ і $K_m^{Пірофосфату}$) для ензиматичної реакції обох кишкових бактеріальних штамів *D. piger* Vib-7 і *Desulfomicrobium* sp. Rod-9.

Ключові слова: кінетичний аналіз, сульфатвідновлювальні бактерії, *Desulfovibrio piger*, *Desulfomicrobium sp.*, АТР-сульфурилаза, мікробіоценоз кишечника, запальні кишкові захворювання, виразкові коліти.

КИНЕТИЧЕСКИЕ СВОЙСТВА АДЕНОЗИНТРИФОСФАТ СУЛЬФУРИЛАЗЫ КИШЕЧНЫХ СУЛЬФАТВОССТАНАВЛИВАЮЩИХ БАКТЕРИЙ

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Исследована удельная активность и кинетические свойства АТР-сульфурилазы в бесклеточных экстрактах кишечных бактериальных штаммов *Desulfovibrio piger* Vib-7 и *Desulfomicrobium* sp. Rod-9. В работе использованы микробиологические, биохимические, биофизические и статистические методы исследований. Определена оптимальная температура (35 °С) и pH 8,0–8,5 для энзиматической реакции. Проведен кинетический анализ свойств АТР-сульфурилазы. Установлена начальная (мгновенная) скорость реакции (V_0), максимальное количество продукта реакции (P_{max}), время реакции (период полураспада – насыщенность, τ) и максимальная скорость АТР-сульфурилазной реакции (V_{max}). Рассчитаны константы Михаэлиса ($K_m^{Сульфата}$, $K_m^{АТР}$, $K_m^{АПС}$ и $K_m^{Пирофосфата}$) для энзиматической реакции обоих кишечных бактериальных штаммов *D. piger* Vib-7 и *Desulfomicrobium* sp. Rod-9.

Ключевые слова: кинетический анализ, сульфатвосстанавливающие бактерии, *Desulfovibrio piger*, *Desulfomicrobium sp.*, АТР-сульфурилаза, микробиоценоз кишечника, воспалительные кишечные заболевания, язвенные колиты.

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Received 20.05.2014