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Kinetic Properties of Pyruvate Ferredoxin Oxidoreductase of Intestinal Sulfate-Reducing Bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9

IVAN V. KUSHKEVYCH*

Institute of Animal Biology of NAAS of Ukraine, Lviv, Ukraine

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Abstract

Intestinal sulfate-reducing bacteria reduce sulfate ions to hydrogen sulfide causing inflammatory bowel diseases of humans and animals. The bacteria consume lactate as electron donor which is oxidized to acetate *via* pyruvate in process of the dissimilatory sulfate reduction. Pyruvate-ferredoxin oxidoreductase activity and the kinetic properties of the enzyme from intestinal sulfate-reducing bacteria *Desulfovibrio piger* and *Desulfomicrobium* sp. have never been well-characterized and have not been yet studied. In this paper we present for the first time the specific activity of pyruvate-ferredoxin oxidoreductase and the kinetic properties of the enzyme in cell-free extracts of both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 intestinal bacterial strains. Microbiological, biochemical, biophysical and statistical methods were used in this work. The optimal temperature (+35°C) and pH 8.5 for enzyme reaction were determined. The spectral analysis of the purified pyruvate-ferredoxin oxidoreductase from the cell-free extracts was demonstrated. Analysis of the kinetic properties of the studied enzyme was carried out. Initial (instantaneous) reaction velocity (V_0), maximum amount of the product of reaction (P_{max}), the reaction time (half saturation period) and maximum velocity of the pyruvate-ferredoxin oxidoreductase reaction (V_{max}) were defined. Michaelis constants (K_m) of the enzyme reaction were calculated for both intestinal bacterial strains. The studies of the kinetic enzyme properties in the intestinal sulfate-reducing bacteria strains in detail can be prospects for clarifying the etiological role of these bacteria in the development of inflammatory bowel diseases.

Key words: kinetic analysis, inflammatory bowel diseases, pyruvate ferredoxin oxidoreductase, sulfate-reducing bacteria

Introduction

Intestinal sulfate-reducing bacteria are often isolated from the gut of healthy humans and persons with ulcerative colitis and inflammatory bowel diseases (Gibson *et al.*, 1991; Barton and Hamilton, 2010). A greater number of these bacteria is found mainly in sick people (Cummings *et al.*, 2003; Gibson *et al.*, 1991). In the presence of sulfate, lactate in human intestine contributes to the intensive bacteria growth and the accumulation of their final metabolism product, hydrogen sulfide, which is toxic, mutagenic and cancerogenic to epithelial intestinal cells (Pitcher and Cummings, 2003; Gibson *et al.*, 1991; Kushkevych, 2012a). The increased number of sulfate-reducing bacteria and the intensity of dissimilatory sulfate reduction in the gut can cause inflammatory bowel diseases of humans and animals (Cummings *et al.*, 2003; Gibson *et al.*, 1991; Kushkevych, 2012b).

Lactate is the most common substrate used by the species belonging to the sulfate-reducing bacteria (Kushkevych, 2012a). This compound is oxidized to acetate *via* pyruvate. The type of enzyme present in these microorganisms appears to be a pyruvate-ferredoxin oxidoreductase, as can be deduced from the low potential electron carriers, ferredoxin and flavodoxin, which serve as electron acceptors for the enzyme (Akagi, 1967; Hatchikian *et al.*, 1979; Guerlesquin *et al.*, 1980). In strict anaerobes microorganisms, pyruvate is oxidatively decarboxylated by pyruvate oxidoreductase (EC 1.2.7.1). Pyruvate ferredoxin oxidoreductase catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA and CO_2 (Akagi, 1967; Barton and Hamilton, 2010; Kushkevych, 2012a).

The reaction of this enzyme has been most extensively studied in the forward (oxidative decarboxylation) direction beginning with a series of seminal studies published in 1971 by Raeburn and Rabinowitz which have isolated and characterized pyruvate-ferredoxin oxidoreductase. They have also demonstrated that low potential electron donors, like reduced ferredoxin, can drive the reductive carboxylation of acetyl-CoA (Raeburn and Rabinowitz, 1971).

* Corresponding author: I.V. Kushevych, Institute of Animal Biology of NAAS of Ukraine, Lviv, Ukrain; **e-mail: ivan.kushkevych@gmail.com**

As far as we are aware, pyruvate-ferredoxin oxidoreductase from intestinal sulfate-reducing bacteria *D. piger* and *Desulfomicrobium* sp. has never been well-characterized. In the literature there are a lot of data on pyruvate-ferredoxin oxidoreductase in various organisms as well as in sulfate-reducing bacteria isolated from environment (Akagi, 1967; Barton and Hamilton, 2010; Hatchikian *et al.*, 1979; Furdui *et al.*, 2000; Garczarek *et al.*, 2007; Guerlesquin *et al.*, 1980; Zeikus *et al.*, 1977; Raeburn and Rabinowitz, 1971; Uyeda and Rabinowitz, 1971; Ma *et al.*, 1997; Meinecke, *et al.*, 1989; Pieulle *et al.*, 1995). However, data on the activity of this enzyme from intestinal sulfate-reducing bacteria *D. piger* and *Desulfomicrobium* sp. have not yet been reported.

The aim of this work was to study pyruvate-ferredoxin oxidoreductase activity in cell-free extracts of intestinal sulfate-reducing bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 and to carry out the kinetic analysis of enzymatic reaction.

The aim was accomplished using microbiological, biochemical, biophysical methods, and statistical processing of the results; the obtained data were compared with those from the literature.

Experimental

Materials and Methods

The objects of the study were sulfate-reducing bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine and identified by sequence analysis of the 16S rRNA gene (Kushkevych, 2013; Kushkevych *et al.*, 2014).

Bacterial growth and cultivation. Bacteria were grown in a nutrition-modified Kravtsov-Sorokin's liquid medium (Kushkevych, 2013). Before seeding bacteria in the medium, 0.05 ml/l of sterile solution of $Na_2S \times 9H_2O$ (1%) was added. A sterile 10 N 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 an oxygen-free medium, and then cooled to +30°C. The bacteria were grown for 72 hours at +37°C under anaerobic conditions. The tubes were brim-filled with medium and closed to provide anaerobic conditions.

Obtaining 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 15,000 *g* for 1 h; the pellet was then used as sedimentary fraction, and the supernatant obtained was termed the soluble fraction (Gavel *et al.*, 1998). The soluble extract constituted by the supernatant was used as the source of the enzyme. This extract was subjected to further centrifugation at 180,000 *g* 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 (Lowry *et al.*, 1951).

Assays for pyruvate-ferredoxin oxidoreductase activity. The pyruvate-ferredoxin oxidoreductase was assayed and purified as described in paper (Pieulle *et al.*, 1995). The enzyme activity was routinely determined spectrophotometrically by following the reduction of methyl viologen as previously described (Zeikus *et al.*, 1977). All enzyme assays were performed under anaerobic conditions at +35°C using serum-stoppered cuvettes. Samples of enzyme were made anaerobic by flushing the solution with argon as previously reported (Fernandez *et al.*, 1985). The reaction mixture containing 50 μmol Tris-HCl (pH 8.5), 10 μmol sodium pyruvate, 0.1 μmol sodium coenzyme A, 2 μmol methyl viologen and 16 μmol dithioerythritol, in a final volume of 1.0 ml, was bubbled with argon for 20 min and the cell was then incubated at +30°C. The reaction was started by injection of pyruvate-ferredoxin oxidoreductase into the assay cuvette using a gastight syringe and the absorbance at 604 nm was followed. Rates of methyl viologen reduction were calculated using an absorption coefficient of 13.6 $mM^{-1} \times cm^{-1}$. A regenerating system was used to determine the K_m for coenzyme A as previously described (Meinecke *et al.*, 1989). One unit of enzyme activity was defined as the amount of enzyme, which catalyzes the oxidation of 1 μmol of pyruvate or the reduction of 2 μmol of methyl viologen per min under the specified conditions. Specific enzyme activity was expressed as $U \times mg^{-1}$ protein. Michaelis constant (K_m) for pyruvate-ferredoxin oxidoreductase reaction has been determined by substrate (pyruvate and coenzyme A). In order to maintain the concentration of oxidized ferredoxin, a recycling system consisting of spinach ferredoxin-NADP reductase (5 μg/assay) (Sigma) and $NADP^+$ (5 mM) was used. The overall rate was measured by the appearance of NADPH. The activity of the studied enzyme in the cell-free extracts of both bacterial strains at different temperature (from +20°C to +45°C) and pH (in the range from 5.0 to 10.0) in the incubation medium was measured. Spectral analysis of the purified enzyme was carried out as previously described (Pieulle *et al.*, 1995).

Kinetic analysis. Kinetic analysis of the enzyme reaction was performed in a standard incubation medium (as it was described above) with modified physical and chemical characteristics of the respective parameters (incubation time, substrate concentration, temperature and pH). The kinetic parameters characterizing the pyruvate-ferredoxin oxidoreductase reaction are 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 half saturation) were determined. The amount of the reaction product was calculated stoichiometrically. The kinetic parameters characterizing pyruvate-ferredoxin oxidoreductase reactions such as Michaelis constant (K_m) and maximum reaction velocity of substrate decomposition were determined by Lineweaver-Burk plot (Keleti, 1988). For analysis of the substrate kinetic mechanism of pyruvate-ferredoxin oxidoreductase, initial velocities were measured under standard assay conditions with different substrate 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, $V=(V_{max}\ [A]\ [B])/(K_A\ K_B+K_B\ [A]+[A]\ [B])$, 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 (Segal, 1975).

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

Results and Discussion

Specific activity of pyruvate-ferredoxin oxidoreductase, an important enzyme in the process of organic compounds oxidation in sulfate-reducing bacteria, was measured in different fractions obtained from *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 cells (Table I).

Results of our study showed that the highest specific activity of the enzyme was detected in cell-free extracts (1.24 ± 0.127 and 0.48 ± 0.051 $U \times mg^{-1}$ protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively). The slightly lower values of activity of pyruvate-ferredoxin oxidoreductase were determined in the soluble fraction compared to cell-free extracts. Its values designated 1.11 ± 0.114 $U \times mg^{-1}$ protein for *D. piger* Vib-7 and 0.37 ± 0.033 $U \times mg^{-1}$ protein for *Desulfomicrobium* sp. Rod-9. The enzyme activity in sedimentary fraction was not observed.

The effect of temperature and pH of the reaction mixture on pyruvate-ferredoxin oxidoreductase 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°C. The highest enzyme activity of pyruvate-ferredoxin oxidoreductase for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was measured at pH 8.5.

Thus, temperature and pH optimum of this enzyme was +35°C and pH 8.5, respectively. An increase or decrease in temperature and pH led to a decrease of the activity of studied enzyme in the cell-free bacterial extracts of the sulfate-reducing bacteria. The enzyme activity exhibited typical bell-shaped curves as a function of temperature and pH.

Next task of this study was to carry out a spectral analysis of the purified pyruvate-ferredoxin oxidoreductase from the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9. The absorption maxima were 317 and 423, 316 and 425 nm for pyruvate-ferredoxin oxidoreductase from *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively (Fig. 2). Ten-minute incubation of the enzyme with 0.75 mM sodium pyruvate led to a slight decrease in absorption maxima. The same peaks of absorption as without addition of sodium pyruvate were observed. However, the significant decrease in absorption spectra after the addition of 0.75 mM sodium pyruvate and 0.1 mM coenzyme A

Table I
Pyruvate-ferredoxin oxidoreductase activity in different fractions obtained from the bacterial cells

Sulfate-reducing bacteria	Specific activity of the enzyme ($U \times mg^{-1}$ protein)		
	Cell-free extract	Individual fractions	
		Soluble	Sedimentary
Desulfovibrio piger Vib-7	1.24 ± 0.127	1.11 ± 0.114	0
Desulfomicrobium sp. Rod-9	0.48 ± 0.051**	0.37 ± 0.033***	0

Comment: The assays were carried out at a protein concentration of 43.57 μg/ml (for *D. piger* Vib-7) and 41.94 μg/ml (for *Desulfomicrobium* sp. Rod-9). Enzyme activity was determined after 20 min incubation. Statistical significance of the values $M \pm m$, $n = 5$; **$P < 0.01$, ***$P < 0.001$, compared to *D. piger* Vib-7 strain

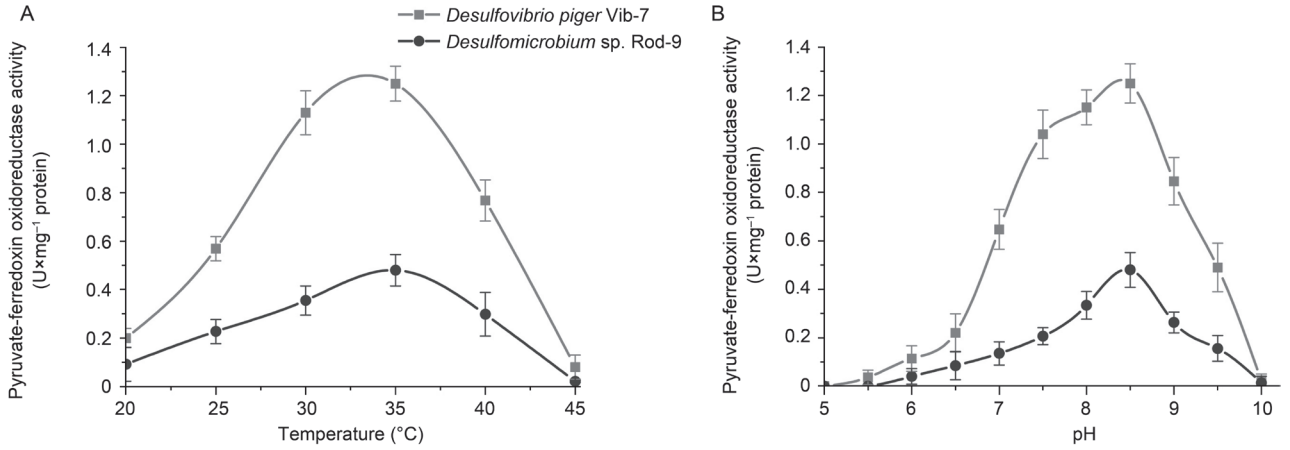


Fig. 1. The effect of temperature (A) and pH (B) on the pyruvate-ferredoxin oxidoreductase activity in the cell-free extracts of the sulfate-reducing bacteria

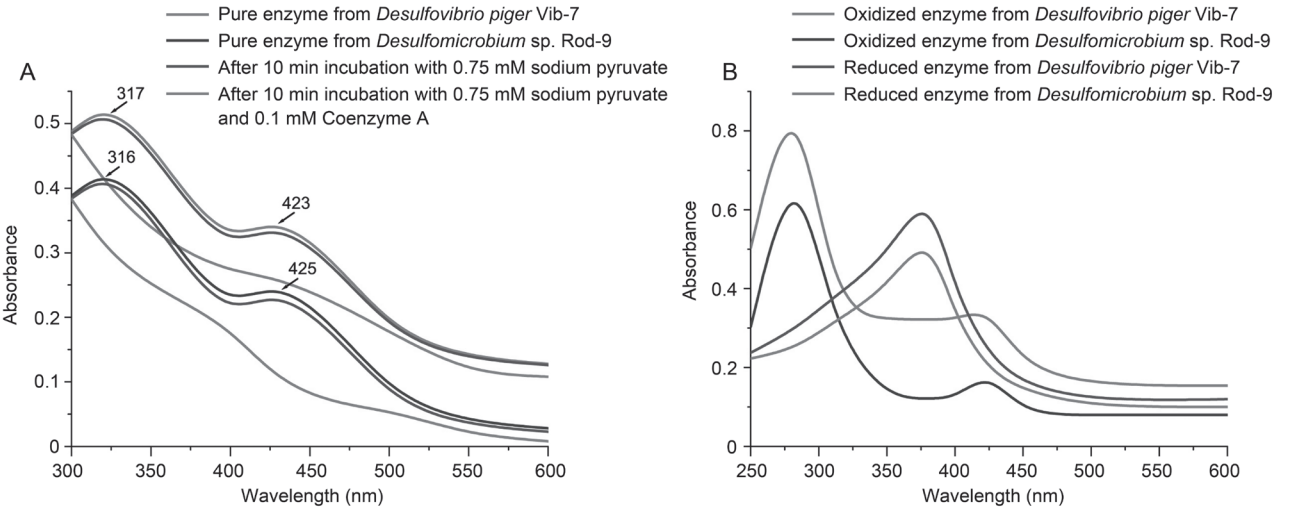


Fig. 2. Absorption spectra of pyruvate-ferredoxin oxidoreductase from *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9. The serum-stoppered cuvette contains 3 μM of pure enzyme in 50 mM Tris-HC1 (pH 8.5) under argon at +35°C, final volume, 1 ml (A). The spectra were recorded in a final volume of 1 ml in a serum-stoppered cuvette of path length 1 cm under argon. Spectrum of the oxidized enzyme and spectrum of the reduced enzyme after injection of 2 μl dithionite (150 mM) (B)

in the incubation medium was registered. The absorption peaks was no observed (Fig. 2A). The spectroscopic analyses of oxidized and reduced pyruvate-ferredoxin oxidoreductase from *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 strains were also carried out (Fig. 2B).

Similar data on the absorption spectra of pyruvate-ferredoxin oxidoreductase from *Desulfovibrio africanus* were obtained by Pieulle *et al.* (1995). The authors described the ultraviolet-visible spectrum of studied enzyme which was typical of an iron-sulfur protein with a broad absorbance band around 400 nm and a shoulder in the 315 nm region (Pieulle *et al.*, 1995). Iron and acid-labile sulfide content, as well as the absorption coefficient at 400 nm suggest the presence of six [4Fe-4S] clusters per molecule of enzyme. The absorption band at 400 nm was partially bleached after addition of dithionite; this indicates only partial reduction of the protein, if one considers that full reduction of iron-sulfur clusters should lead to about 50% decrease of the absorption band. Pyruvate reduced the enzyme slightly, whereas pyruvate and CoASH produced a more pronounced reduction of the protein than that obtained with dithionite (Pieulle *et al.*, 1995).

To study the characteristics and mechanism of pyruvate-ferredoxin oxidoreductase 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 defined. Dynamics of reaction product accumulation was studied for investigation of the kinetic parameters of pyruvate-ferredoxin oxidoreductase (Fig. 3).

Experimental data showed that the kinetic curves of pyruvate-ferredoxin oxidoreductase activity have a saturation tendency (Fig. 3A). Analysis of the results allows to reach the conclusion that the kinetics of pyruvate-ferredoxin oxidoreductase activity in the

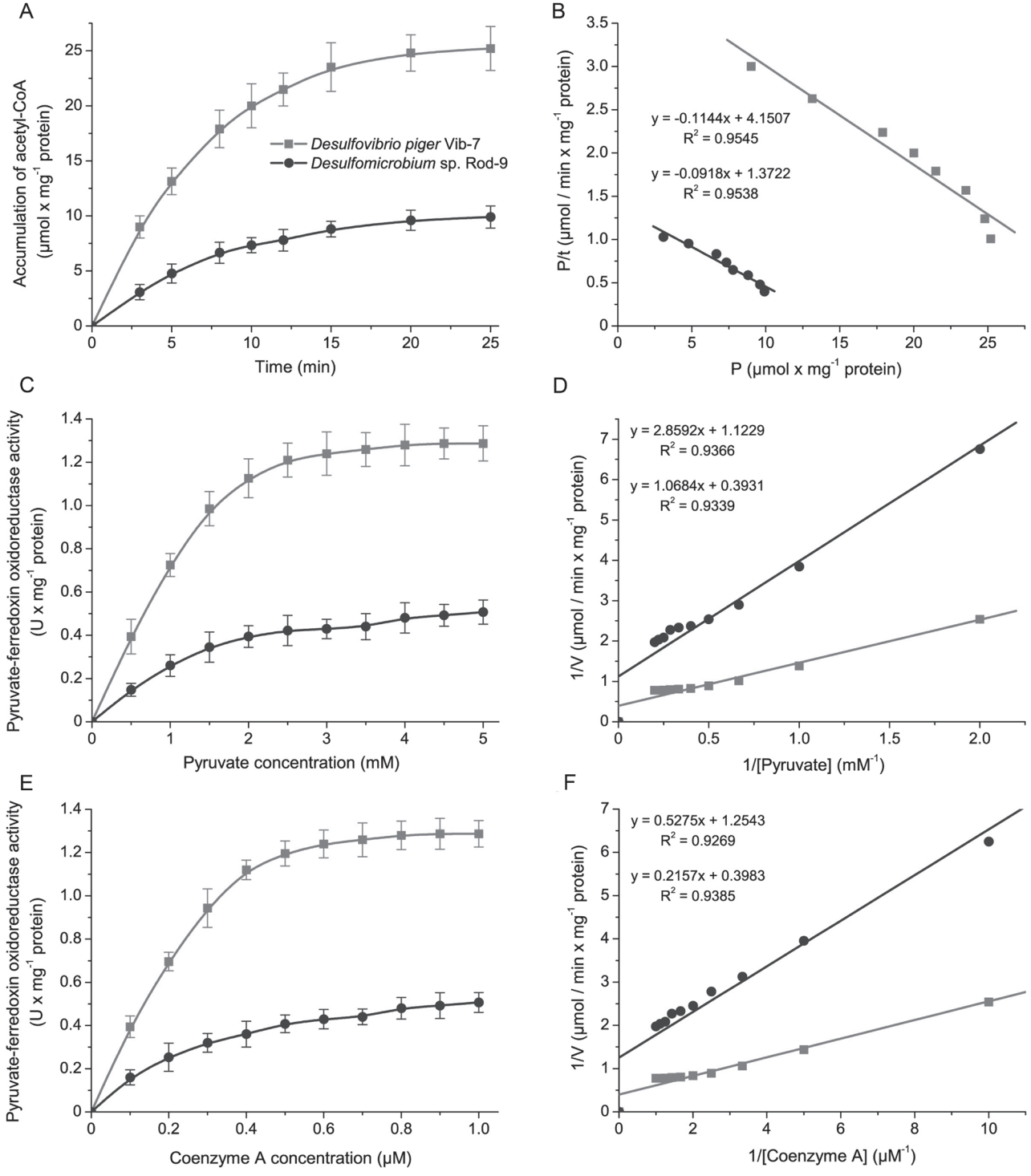


Fig. 3. Kinetic parameters of pyruvate-ferredoxin oxidoreductase activity in *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9: A – dynamics of product accumulation (M ± m, n = 5); B – linearization of curves of product accumulation in {P/t; P} coordinates (n = 5; $R^2 > 0.95$; $F < 0.02$); C, E – the effect of different concentrations of substrate (pyruvate and coenzyme A) on the enzyme 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 the enzyme reaction and [Pyruvate] or [Coenzyme A] is substrate concentration (n = 5; $R^2 > 0.9$; $F < 0.005$)

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 the product of pyruvate-ferredoxin oxidoreductase reaction in the *D. piger* Vib-7 was the higher (36.28 ± 3.59 μmol × mg^{-1} protein) compared to the *Desulfomicrobium* sp. Rod-9 (14.95 ± 1.48 μmol × mg^{-1} protein) in the entire range of time factor. The basic kinetic properties of the reaction in the

Table II
Kinetic parameters of the pyruvate-ferredoxin oxidoreductase from intestinal sulfate-reducing bacteria

Kinetic parameters	Sulfate-reducing bacteria	
	Desulfovibrio piger Vib-7	*Desulfomicrobium* sp. Rod-9
V_0 (μmol × min^{-1} × mg^{-1} protein)	4.15 ± 0.43	1.37 ± 0.12***
P_{max} (μmol × mg^{-1} protein)	36.28 ± 3.59	14.95 ± 1.48**
τ (min)	8.74 ± 0.88	10.89 ± 1.11

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

Table III
Kinetic parameters of pyruvate-ferredoxin oxidoreductase reaction

Kinetic parameters	Sulfate-reducing bacteria	
	Desulfovibrio piger Vib-7	*Desulfomicrobium* sp. Rod-9
$V_{max}^{Pyruvate}$ μmol × min^{-1} × mg^{-1} protein)	2.54 ± 0.261	0.89 ± 0.092***
$K_m^{Pyruvate}$ (mM)	2.72 ± 0.283	2.55 ± 0.245
V_{max}^{CoA} (μmol×min^{-1}×mg^{-1} protein)	2.51 ± 0.248	0.81 ± 0.076***
K_m^{CoA} (μM)	0.54 ± 0.052	0.42 ± 0.044

Comment: V_{max} is maximum velocity of the enzyme reaction; K_m is Michaelis constant which was determined by substrate (pyruvate and coenzyme A). Statistical significance of the values M ± m, n = 5; ***P < 0.001, compared to the *D. piger* Vib-7 strain.

sulfate-reducing bacteria were calculated by linearization of the data in the {P/t; P} coordinates (Fig. 3B, Table II).

The kinetic parameters of pyruvate-ferredoxin oxidoreductase from both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were significantly different. Values of initial (instantaneous) reaction velocity (V_0) for the enzyme was calculated by the maximal amount of the product reaction (P_{max}). As shown in Table II, V_0 for pyruvate-ferredoxin oxidoreductase reaction was slightly higher (4.15 ± 0.43 μmol × min^{-1} × mg^{-1} protein) in *D. piger* Vib-7 compared to *Desulfomicrobium* sp. Rod-9 (1.37 ± 0.12 μmol × min^{-1} × mg^{-1} protein). In this case, the values of the reaction time (τ) were more similar for the studied enzyme in both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 strains. Based on these data, it may be assumed that the *D. piger* Vib-7 can consume lactate ion much faster in their cells than a *Desulfomicrobium* sp. Rod-9. Moreover, this hypothetical assumption can be also confirmed by obtained data on maximal velocities of accumulation of the final reaction products, where V_{max} for enzyme reaction in *D. piger* Vib-7 were also more intensively compared to *Desulfomicrobium* sp. Rod-9 (Table III).

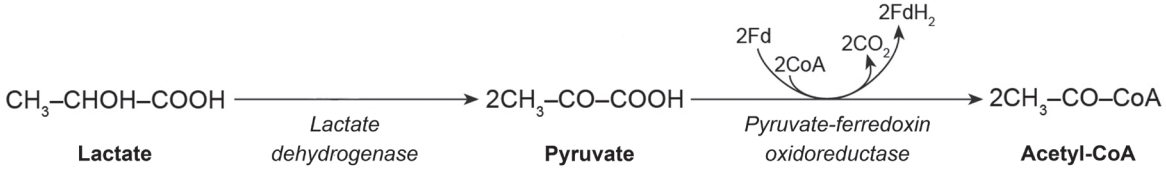
The kinetic analysis of pyruvate-ferredoxin oxidoreductase reaction depending on concentration of substrate (pyruvate and coenzyme A) was carried out. The increasing pyruvate concentrations from 0.5 to 5.0 mM and coenzyme A concentrations from 0.1 to 1.0 μM caused a monotonic rise of the studied enzyme activity and the activity was maintained on unchanged level (plateau) under substrate concentrations over 5.0 mM and 1.0 μM, respectively. (Fig. 3C, E). Curves of the dependence {1/V; 1/[S]} were distinguished by the tangent slope and intersect the vertical axis in one point (Fig. 3D, F). The basic kinetic parameters of pyruvate-ferredoxin oxidoreductase activity in *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were identified by linearization of the data in the Lineweaver-Burk plot (Table III).

Calculation of the kinetic parameters of enzyme activity indicates that the maximum velocities (V_{max}) of pyruvate and coenzyme A in the *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were significantly different from each other. However, it was observed a correlative relationship between $V_{max}^{Pyruvate}$ and V_{max}^{CoA} in both intestinal bacterial strains. Michaelis constants (K_m) of pyruvate-ferredoxin oxidoreductase reaction were identified for pyruvate and coenzyme A. The values of K_m were quite similar for pyruvate (2.72 ± 0.283, 2.55 ± 0.245 mM) and coenzyme A (0.54 ± 0.052, 0.42 ± 0.044 μM) in both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 strains, respectively.

The obtained parameters of pyruvate-ferredoxin oxidoreductase reaction in *D. piger* Vib-7 are consistent with previously described data by Pieulle *et al.* for the activity of pyruvate-ferredoxin oxidoreductase from *D. africanus.* The apparent K_m for pyruvate and coenzyme A were also 2.5 mM and 0.5 μM, respectively and the V_{max} values were 10240 min^{-1} and 5890 min^{-1}, respectively. The apparent K_m for methyl viologen was found to be 0.5 mM in the presence of 10 mM and 0.1 mM of pyruvate and CoASH, respectively. Kinetics studies done with the enzyme and a slight decrease in the affinity for pyruvate and in the catalytic activity (K_m of 5.5 mM and V_{max} of 4810 min^{-1}) were reported (Pieulle *et al.*, 1995).

Furdui and Ragsdale (2000) have described the pyruvate-ferredoxin oxidoreductase from the *Clostridium thermoaceticum.* The Michaelis-Menten parameters for pyruvate synthesis by the enzyme were: V_{max} 1.6 unit/mg, $K_m^{Acetyl\text{-}CoA}$ 9 μM. The intracellular concentrations of acetyl-CoA, CoASH, and pyruvate were also measured (Furdui and Ragsdale, 2000).

Pyruvate-ferredoxin oxidoreductatse, an important enzyme in process of dissimilatory sulfate reduction and organic compounds oxidation in sulfate-reducing bacteria, carries out the central step in oxidative decarboxylation of pyruvate to acetyl-CoA (Kushkevych, 2012a):



Garczarek *et al.* (2007) have purified this enzyme from *Desulfovibrio vulgaris* Hildenborough as part of a systematic characterization of as many multiprotein complexes as possible for this organism (Garczarek *et al*., 2007).

Thus, based on the obtained studies results and according to the kinetic parameters of pyruvate-ferredoxin oxidoreductatse reaction for both bacterial strains, we have concluded that the enzyme activity, V_0 and V_{max} were significantly higher in the *D. piger* Vib-7 cells than *Desulfomicrobium* sp. Rod-9. However, Michaelis constants were quite similar for pyruvate (2.72 ± 0.283, 2.55 ± 0.245 mM) and coenzyme A (0.54 ± 0.052, 0.42 ± 0.044 μM) in both bacterial strains. The maximum enzyme activity for both strains was determined at +35°C and at pH 8.5. These data correspond to conditions which are present in the human large intestine from where the bacterial strains were isolated. Perhaps such conditions favor intensive development of the *D. piger* and *Desulfomicrobium* sp. bacterial strains in the gut. The kinetic parameters of enzyme reaction are depended on the substrate concentration. The studies of the pyruvate-ferredoxin oxidoreductatse in the process of dissimilatory sulfate reduction and kinetic properties of this enzyme in the *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 intestinal strains, their production of acetate in detail can be a perspective for clarification of their etiological role in the development of the humans and animals bowel diseases. 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 the clinical diagnosis of these disease types.

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