ISOLATION AND CHARACTERIZATION OF TRICHOMONAS VAGINALIS FERREDOXIN*

by

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the occasion of his 80th birthday

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A ferredoxin was purified from the anaerobic protozoon *Trichomonas vaginalis*. The protein had a molecular weight of 12,000 as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, gel filtration and amino acid analysis. The protein contained seven 1/2-cystine (cystine plus cysteine) residues and one tyrosine, and lacked tryptophan. Chemical analysis and spectral properties of the ferredoxin indicated the presence of a [2Fe-2S] cluster. The complex optical spectrum of the native ferredoxin had peaks at 310 and 450 nm and shoulders near 415 and 550 nm. The molar absorbance of the protein at 450 nm was 8,000 M⁻¹cm⁻¹. The EPR spectrum of reduced ferredoxin had two features at g values of 2.02 and 1.94 and revealed axial symmetry. Results of subcellular fractionation studies indicated the ferredoxin to be a major iron-sulfur protein of the cells and to be located in the hydrogenosome. The ability of the ferredoxin to function as an electron carrier was demonstrated by its reduction by pyruvate:ferredoxin oxidoreductase and hydrogenase as detected by EPR spectroscopy and by its stimulation of metronidazole reduction by these enzymes. These observations implicate ferredoxin as an important electron transport component in hydrogenosomes of *T. vaginalis*.

Abbreviations: P_{1/2} = half saturation parameter; SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

1. INTRODUCTION

Metabolic pathways, in which ferredoxin-like electron transport components participate, are assumed to play a significant role in various anaerobic protozoa which contain no mitochondria (16). Low molecular weight iron-sulfur proteins with characteristic properties of ferredoxin were isolated, however, only from two protozoan species, *Entamoeba histolytica* and *Tritrichomonas foetus*. These ferredoxins accept electrons from homologous pyruvate:ferredoxin oxidoreductases (14, 20). In this paper we report the isolation and partial characterization of a [2Fe-2S] ferredoxin from *Trichomonas vaginalis*. Similarly to other protozoan ferredoxins this protein is likely to be a natural electron transport component in the energy metabolism of *T. vaginalis*. These results were presented in part at the 83rd Annual Meeting of the American Society for Microbiology (New Orleans, LA, March 6-11, 1983) and at the 36th Annual Meeting of the Society of Protozoologists (New York, NY, 20 to 24 June, 1983; T.E. GORRELL, N. YARLETT and M. MULLER, J. Protozool. 30, 4A-5A, 1983).

2. EXPERIMENTAL PROCEDURES

2.1. Organism

Trichomonas vaginalis, ATCC 30001, was grown for one day at 37 °C in a tryptone-yeast extract-maltose medium supplemented with 10 µg ml⁻¹ (170 µM) iron and 10% heat inactivated horse serum (4). Iron was added as ferrous ammonium sulfate dissolved in 5-sulfosalicylic acid. For cell fractionation experiments, cells were collected from 3 l cultures. For purification of ferredoxin, larger amounts of material were collected from three 15 l batches of cultures and then stored at -20 °C until processed, within 3 weeks (14).

2.2. Purification of ferredoxin

Ferredoxin was purified from whole cells of *T. vaginalis* by deoxycholate treatment and subsequent ion exchange chromatography and gel filtration (14). In detail, the cells were resuspended to 30 mg×ml⁻¹ protein in a solution containing a final concentration of 50 mM-Tris

base (adjusted to pH 8.0 with HCl) and 10 mg×ml⁻¹ sodium deoxycholate. The solution was kept at 4 °C for 20 hours, then centrifuged at 50000 rpm for 45 min at 4 °C (Ti70 rotor in a Sorvall OTD 75 ultracentrifuge). After centrifugation the tube contained a clear golden supernatant with a turbid layer on top and a dark pellet at the bottom of the tube. Ferredoxin was purified from the clear supernatant solution by ion exchange chromatography on DEAE cellulose and two cycles of gel filtration on Sephadex G-75. The details of these steps were similar to those used for *T. foetus* ferredoxin (14). Purity and molecular weight of the final product were assessed by gel filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (14).

2.3. Chemical analysis

For amino acid analysis, apoferredoxin was prepared by precipitation of the native protein with 920 mM-trichloroacetic acid (23). A sample of the apoferredoxin was oxidized with performic acid (15) for determination of total cysteine plus cystine. Samples were hydrolysed for 20 hours in 6 N HCl and 1 µl×ml⁻¹ phenol. Amino acid composition was determined in a Dionex D-500 amino acid analyzer (Dionex Inc., Sunnyvale, CA). Protein iron, and labile sulfur were determined as described (14).

2.4. Spectroscopy

Ultraviolet-visible spectra of *T. vaginalis* ferredoxin were recorded in a Cary 210 spectrophotometer. Samples in quartz cuvettes (1 cm light path) sealed with rubber stoppers were made anaerobic by repeated evacuation and filling with O₂-free N₂. Dithionite was added with a syringe through the stopper (14). Samples for EPR spectroscopy were prepared in a titration vessel (5) at 30 °C. The solution was flushed with air or O₂-free N₂. After 5 min, samples were transferred with a syringe into quartz tubes and rapidly frozen by immersion into an isopentane-methylcyclohexane (5:1, v/v) freezing mixture chilled with liquid N₂. The tubes were stored in liquid N₂ until spectra were recorded on an E 109 spectrometer (Varian Associates, Palo

Alto, CA). The conditions used were: microwave frequency 9.3 GHz; modulation frequency 100 kHz; modulation amplitude 12.5 G; scanning rate 250 G min⁻¹; time constant 0.25 s. Spectra were recorded at various temperatures (10 to 30 K) and at a wide range of microwave power (0.01 to 200 mW). The half saturation parameter ($P_{1/2}$) of EPR signals was determined by the method of BLUM and OHNISHI (2).

2.5. Subcellular fractionation

Subcellular localization of ferredoxin was determined by differential centrifugation of a cell free homogenate of *T. vaginalis* under anaerobic conditions. The homogenization medium was 225 mM-sucrose supplemented with 100 mM-potassium phosphate pH 8.0 and 20 mM- β -mercaptoethanol. The following fractions were separated: nuclear, large cytoplasmic granule, small cytoplasmic granule and non-sedimentable cytoplasmic fraction. Conditions of centrifugation were as described (10), except that the large granule fraction was obtained by centrifugation at a higher centrifugal force (7750 rpm for 10 min). Malate dehydrogenase (decarboxylating), β -N-acetylglucosaminidase, acid phosphatase, and NADH oxidase were assayed spectrophotometrically in all fractions (9). EPR spectra of fractions reduced with 2 mM-sodium dithionite were also recorded.

2.6. Hydrogenosomal extracts

Extracts were prepared under anaerobic conditions in an anaerobic glove chamber (Coy Laboratory Products, Ann Arbor, MI) as described (14). Fractions enriched in large cytoplasmic granules, including hydrogenosomes, were solubilized by deoxycholate treatment and subjected to ion exchange chromatography. In detail, a 10 ml fraction was diluted with the same volume of a solution containing 100 mM-potassium phosphate pH 8.0, 20 mg $\times$ ml⁻¹ sodium deoxycholate, 20 mM- β -mercaptoethanol, and 20 mM-sodium pyruvate. This material was centrifuged at 50,000 rpm for 45 min at 4 °C and the clear golden solution obtained was applied to a jacketed column at 4 °C of DEAE Sephacel (1.5 $\times$ 5 cm). Excluded material con-

tained the hydrogenosomal enzymes pyruvate:ferredoxin oxidoreductase and hydrogenase. Reduction of metronidazole by these enzymes was determined by the procedure described earlier (14). Aliquots of the excluded material were stored under H₂ atmosphere at 4 °C.

3. RESULTS

3.1. Purification and chemical composition

Our experience with purification of *T. foetus* ferredoxin (14) indicated that extraction of

Figure 1. SDS-PAGE of *T. vaginalis* ferredoxin on a 12 to 18% polyacrylamide gradient gel. Molecular weight markers: (A) bovine serum albumin (M_r = 68,000), ovalbumin (45,000), trypsinogen (24,000), soy bean trypsin inhibitor (21,000), lactalbumin (18,000), lysozyme (14,000) and aprotinin (6,500) (B) myoglobin (17,000) and cytochrome c (12,000). *T. vaginalis* ferredoxin: (C) 5 μ g; (D) 25 μ g.

Table I.
Amino Acid Composition of *Trichomonas vaginalis* Ferredoxin

Amino Acid	Amount Detected ^{a)}		mol mol M _r 12,000 ^{b)}
	Not oxidized	Oxidized	
Cys	-	5.44	7
Asx	9.53	12.23	13
Thr	5.10	5.97	7
Ser	2.53	2.85	3
Glx	10.65	12.62	15
Pro	1.32	1.51	2
Gly	7.76	9.03	11
Ala	9.96	11.29	14
Cys	2.45	-	-
Val	5.10	6.05	7
Met	1.06	-	1
Ile	2.92	3.47	4
Leu	7.25	8.11	10
Tyr	0.74	-	1
Phe	3.69	3.78	5
His	0.98	-	1
Lys	7.71	9.00	11
Arg	1.05	1.25	1
Trp	-	-	0 ^{c)}

^{a)} Amounts expressed as mol recovered from 1.25 nmol native protein that was treated with 920 mM-trichloroacetic acid and then oxidized by performic acid or not oxidized.

^{b)} Calculation based on the amount of tyrosine detected.

^{c)} Indicated by low absorbance at 280 nm of the native protein.

whole cell suspensions under aerobic conditions gives good recovery thus subcellular fractionation and strict anaerobic techniques are not necessary. After an initial bulk DEAE-cellulose treatment of deoxycholate-solubilized cell suspensions, the protein could be purified to apparent homogeneity by two cycles of Sephadex G75 gel filtration. The affinity of *T. vaginalis* ferredoxin to DEAE-cellulose was slightly greater than that of *T. foetus* ferredoxin. Consequently, the ferredoxin had to be eluted with 300 mM, instead of 200 mM, NaCl.

The *T. vaginalis* ferredoxin preparations obtained were essentially pure since they gave only a single protein peak in the second cycle of gel filtration and a single protein band by SDS-PAGE (Figure 1). From 13 g of starting protein 6.5 mg of purified ferredoxin was obtained which indicates it represented at least 0.05% of total cell protein.

The protein isolated had a low molecular

weight as indicated by its comigration with cytochrome c during gel filtration on Sephadex G-75 (not shown) and SDS-PAGE (Figure 1). Both methods gave an apparent M_r of 12,000. Anomalous migration of ferredoxins during gel filtration and SDS-PAGE has been reported, however, which casts some uncertainty on molecular weight values obtained by these methods alone (7, 12, 14).

Results of amino acid analysis are presented in Table I. Assuming the presence of one tyrosine residue per molecule, again an approximate M_r of 12,000 could be calculated. The seven 1/2-cystine (cystine plus cysteine) residues detected could accommodate either a [2Fe-2S] or a [4Fe-4S] cluster characteristic of ferredoxins or one iron atom characteristic of rubredoxin (18). Spectral properties of the native protein suggested that the protein lacked tryptophan (see below).

The purified protein had approximately equal

Table II.
Composition of *Trichomonas vaginalis* and *Spinacea oleracea* ferredoxins

Ferredoxin	A_{458}/A_{280}	Protein $\text{mg} \times \text{ml}^{-1}$	Protein	Iron $\text{nmol} \times \text{ml}^{-1}$	Labile Sulfur	Iron $\text{mol} \times \text{mol}^{-1}$	Labile Sulfur ferredoxin
<i>T. vaginalis</i> (M_r 12,000)							
Sample A	0.69	$1.0 \pm 0.12^{\text{a}}$	82.5	145 ± 10	172 ± 4	1.76	2.09
Sample B	0.80	1.9 ± 0.11	158	317 ± 13	350 ± 19	2.01	2.21
<i>S. oleracea</i> (M_r 12,000)	-	1.45 ± 0.15 [2.00]	120.9 166.8 ^{b)}	318.7 ± 6.2	308.7 ± 8.7	2.64 [1.90]	2.55 1.85 ^{b)}

^{a)} Amounts per ml represent means + standard deviations obtained in two determinations.

^{b)} Values within brackets were based on estimation of protein content by absorbance measurements at 420 nm.

amounts of iron and labile sulfur (Table II). In view of numerous difficulties (18) that can be encountered in determination of protein, iron and labile sulfur we also analysed samples of *Spinacea oleracea* ferredoxin. The amounts of iron and labile sulfur detected in *T. vaginalis* ferredoxin were similar to those detected in *S. oleracea* ferredoxin indicating the presence of one [2Fe-2S] cluster if a M_r of 12,000 is assumed.

3.2. Spectral properties

Solutions of the native protein were red-orange. The complex optical spectrum of *T. vaginalis* ferredoxin had peaks at 310 nm and 450 nm and shoulders near 415 nm and 550 nm (Figure 2). In contrast to most [2Fe-2S] ferredoxins (18) which show two peaks in the 415-460 nm region, only one peak was observed in this region of the spectrum of *T. vaginalis* ferredoxin similarly to *T. foetus* ferredoxin (14). Assuming an M_r of 12,000, fresh preparations of *T. vaginalis* ferredoxin had a molar absorbance at 450 nm of $8000 \text{ M}^{-1} \times \text{cm}^{-1}$ which is close to that of other [2Fe-2S] ferredoxins (18).

The absorbance of the native ferredoxin at 280 nm was $10,000 \text{ M}^{-1} \times \text{cm}^{-1}$. As noted by KERESZTES-NAGY (6), a significant part of the absorbance of ferredoxins at 280 nm is due to iron, because of the low content in aromatic amino acid residues. The molar absorbance at 280 nm of *T. vaginalis* and *T. foetus* (14)

Figure 2. Absorption of native (A) and reduced (B) *T. vaginalis* ferredoxin. The ferredoxin solution ($31 \mu\text{M}$ protein) was rendered anaerobic in a sealed quartz cuvette (10 mm optical path). After recording the spectrum of the native protein, the solution was reduced with $150 \mu\text{M}$ -sodium dithionite.

ferredoxins is similar to that of bovine adrenodoxin which contains a single tyrosine residue and no tryptophan (22, 24), indicating that trichomonad ferredoxins lack tryptophan. The A_{458}/A_{280} ratio which is regarded to reflect the purity of holoferreredoxins, was 0.8 in a fresh preparation, a value similar to those reported for bovine adrenodoxin (14) and *T. foetus* ferredoxin (22), and larger than a value of 0.4 to 0.5 found for other [2Fe-2S] ferredoxins with

Figure 3. EPR spectrum at 25 K of *T. vaginalis* ferredoxin (31 μM) reduced with 150 μM -sodium dithionite. Microwave power - 0.5 mW; receiver gain - 5×10^3 .

larger numbers of tyrosine and tryptophan residues (6, 7, 8, 24). In older samples of *T. vaginalis* ferredoxin lower ratios were observed, presumably corresponding to a loss of iron-sulfur clusters (6).

T. vaginalis ferredoxin could be reduced with dithionite (Figures 2 and 3). Upon reduction, A_{450} decreased by 60% and the shoulder at 550 nm became a broad peak. When air was admitted to the reduced sample, the red-orange color reappeared and the spectrum was identical to that of the native protein. The EPR spectrum of the native ferredoxin was featureless (not shown) whereas the spectrum of the protein reduced with dithionite had a complex shape of an axial symmetry with $g_{\parallel} = 2.02$ and $g_{\perp} = 1.94$ (Figure 3). No spectral distortion appeared over a broad range of temperature and microwave power. At 25 K, the value of $P_{1/2}$ of the $g = 1.94$ signal was approximately 1.5 mW whereas this feature was already saturated at 0.01 mW in spectra recorded at 10 K. These results are consistent with the presence of a [2Fe-2S] cluster (21) with virtually axial symmetry as seen for [2Fe-2S] clusters in hydroxylase-type ferredoxins (18).

3.3. Subcellular distribution

EPR spectroscopy of dithionite reduced subcellular fractions indicated that the ferredoxin was enriched in hydrogenosomes (Figures 4 and 5). Spectra of the hydrogenosome enriched frac-

Figure 4. Distribution of the component giving the $g = 1.94$ EPR signal and of marker enzymes after differential centrifugation of a homogenate of *T. vaginalis*. Relative specific activity was plotted against cumulative percentage of protein recovered in each fraction. In the graph the direction from left to right corresponds to increasing centrifugal field. The far right-hand block represents the final supernatant. Percentage recoveries were 104% for EPR signal height at $g = 1.94$, 100% for malate dehydrogenase (decarboxylating), 86% for NADH dehydrogenase, 100% for acid phosphatase, 95% for β -N-acetylglucosaminidase and 106% for protein. Samples for EPR studies were reduced by 2 mM-sodium dithionite and spectra were recorded at 25 K 0.5 mW.

Figure 5. EPR spectra of a dithionite reduced large granule fraction (21 $\text{mg} \times \text{ml}^{-1}$ protein). Conditions for spectroscopy: (A) 25 K, 0.5 mW, and 4×10^3 gain; (B) 25 K, 50 mW, and 2×10^3 gain; and (C) 10 K, 2 mW, and 4×10^3 gain.

Table III.**Effect of ferredoxin on enzyme activities of hydrogenosomal extracts of *Trichomonas vaginalis***

Enzyme	Ferredoxin (μM)	Electron Acceptor ^{a)}	
		Metronidazole	Methyl Viologen
Pyruvate:ferredoxin oxidoreductase	none	46	5840
	4	171	5530
	8	394	-
Hydrogenase	none	< 10	7680
	4	105	8050
	8	175	-

^{a)} Rates are expressed as nmol metronidazole or methyl viologen reduced $\text{min}^{-1} \times \text{mg}^{-1}$ protein (hydrogenosomal extract). For metronidazole reduction by pyruvate:ferredoxin oxidoreductase and hydrogenase the assay mixtures (625 μl) contained 28.4 μg and 56.8 μg protein, respectively; and for methyl viologen reduction the assay mixtures (1.0 ml) contained 1.42 μg protein.

tion recorded at 25 K and 0.5 mW had two dominant features at g values of 1.94 and 2.02 characteristic of the ferredoxin (Figure 5). Moreover, the relaxation behavior of the feature at $g = 1.94$ was similar to that of the ferredoxin. At 25 K, the value of $P_{1/2}$ of the signal at $g = 1.94$ in spectra of the large granule fraction was approximately 1.5 mW and this feature was already saturated at 0.01 mW when spectra were recorded at 10 K. Spectra of the large granule fraction, however, became more complex upon changes in power or temperature. The most complex spectra were seen at 10 K when they contained a feature with an unusual g value of 1.82. These complex spectra presumably reflected the overlapping signals of several components as reported for the analogous spectra of *T. foetus* (17). Nevertheless, the ferredoxin could be selectively detected by recording at 25 K and 0.5 mW.

The distribution profile of the EPR signals obtained at 25 K and 0.5 mW, and at 10 K and 2 mW (data not shown) matched closely the profile of the hydrogenosomal marker enzyme, malate dehydrogenase (decarboxylating) (Figure 4), and was quite distinct from the marker enzymes for other subcellular components. The distribution patterns observed for various marker enzymes are similar to those reported earlier (10) but the hydrogenosomal marker enzyme, malate dehydrogenase (decar-

boxylating), showed a higher relative specific activity which can be attributed to the use of increased centrifugal force in preparing the large granule fraction.

It has been estimated that hydrogenosomes represent about 5-10% of the protein in *T. vaginalis* cells (10). Based on this value and the amount of ferredoxin recovered from cells, the ferredoxin represents at least 0.5% of the protein in the organelle. Assuming the hydrogenosomes have 20% protein on a weight per volume basis, the concentration of ferredoxin in the organelle is at least 80 μM .

3.4. Enzymatic reduction

Purified *T. vaginalis* ferredoxin served as an electron acceptor for homologous hydrogenosomal enzymes, pyruvate:ferredoxin oxidoreductase and hydrogenase. Reduction of ferredoxin could be detected by EPR spectroscopy of DEAE treated hydrogenosomal extract. EPR spectra of the hydrogenosomal extract incubated with dithionite (spectra not shown) or pyruvate and cofactors necessary for pyruvate:ferredoxin oxidoreductase activity had a small feature at $g = 2.00$ and lacked a feature at $g = 1.94$ indicating efficient removal of the ferredoxin by the DEAE treatment (Figure 6). Supplementation of the protein extract with purified ferredoxin gave more complex spectra

Figure 6. EPR spectra at 25 K of a DEAE treated hydrogenosomal extract *T. vaginalis* ($2.1 \text{ mg} \times \text{ml}^{-1}$ protein), supplemented with pyruvate, coenzyme A and β -mercaptoethanol, and $4.0 \mu\text{M}$ -ferredoxin (A) or no ferredoxin (B). Microwave power - 0.5 mW ; receiver gain - 16×10^3 .

with dominant features at $g = 2.02$ and 1.94 characteristic of ferredoxin.

Reduction of ferredoxin by pyruvate:ferredoxin oxidoreductase and hydrogenase was also demonstrated by its effects on metronidazole reduction by hydrogenosomal extracts (Table III). Metronidazole is a nitroheterocyclic drug that chemically oxidizes reduced ferredoxin (11). In the absence of ferredoxin, metronidazole was reduced at low rates. Added ferredoxin stimulated the rate of reduction. The extent of stimulation by ferredoxin was proportional to the amount of ferredoxin added. Saturation was not observed even when $20 \mu\text{M}$ -ferredoxin was added. The limited amount of purified protein available precluded the use of concentrations comparable to the assumed concentration of the ferredoxin in the hydrogenosome. Stimulation of metronidazole reduction by *T. vaginalis* ferredoxin was comparable to that by *S. oleracea* or *Clostridium pasteurianum* ferredoxin (data not shown). The extent of the stimulation of metronidazole reduction by pyruvate:ferredoxin oxidoreductase in hydrogenosomal extracts was less than reported earlier for the analogous activity in *T. foetus* (14), possibly due to the higher rate of metronidazole reduction by ferredoxin depleted hydrogenosomal extracts of *T. vaginalis*. Although ferredoxin stimulated metronidazole reduction, no stimulation of methyl viologen reduction was observed indicating that this compound accepts electrons directly from the enzyme.

4. DISCUSSION

Our results show that *T. vaginalis* contains a $[2\text{Fe-2S}]$ ferredoxin. The nature of the iron-sulfur cluster was deduced from the results of chemical analysis and the spectral properties of the protein. The optical spectrum of the native ferredoxin had most features characteristic of $[2\text{Fe-2S}]$ clusters but no peak near 425 nm . As also observed for *T. foetus* ferredoxin and adrenodoxin (22), the A_{458}/A_{280} ratio of *T. vaginalis* ferredoxin was greater than reported for most other $[2\text{Fe-2S}]$ ferredoxins. The higher ratio (purity index) is probably due to the low number of aromatic residues in trichomonad ferredoxins and bovine adrenodoxin (6, 8, 14, 24). The spectrum of reduced *T. vaginalis* ferredoxin had the broad peak at 550 nm present in spectra of several hydroxylase-type ferredoxins (18), which in contrast to these proteins persisted as a shoulder in the spectrum of native *T. vaginalis* ferredoxin. EPR spectroscopy of the reduced protein indicated that the iron-sulfur cluster had a virtually axial symmetry similar to hydroxylase-type ferredoxins and lacked the rhombic distortion of chloroplast-type ferredoxins (18). Trichomonad ferredoxins differ in their spectral properties from *E. histolytica* ferredoxin which is reported to contain 8 atoms of iron and labile sulfur per molecule suggesting the presence of two $[4\text{Fe-4S}]$ clusters (20).

The association of the ferredoxin with hydrogenosomes and its ability to react with hydrogenosomal oxidoreductases, pyruvate:ferredoxin oxidoreductase and hydrogenase, indicates its possible role in hydrogen production in *T. vaginalis* (13, 19). The hydrogenosomal electron transport system of *T. vaginalis* appears quite similar to that of *T. foetus*. Hydrogenosomes of both organisms have the same type of ferredoxin. EPR spectra of hydrogenosomal fractions of both organisms (17) are similar but in *T. vaginalis* an additional feature was seen at a g value of 1.82 . In view of the reports that pyruvate:ferredoxin oxidoreductase and hydrogenase from bacteria have $[4\text{Fe-4S}]$ clusters which are paramagnetic in the reduced state (1, 3), it is probable that iron-sulfur clusters associated with these enzymes contributed to the EPR spectra of the hydrogenosomes.

The results support the idea that hydrogen

production by trichomonad hydrogenosomes is similar to the process in anaerobic fermentative bacteria such as clostridial species (16). In these bacteria a 2 [4Fe-4S] ferredoxin serves as electron carrier between pyruvate:ferredoxin oxidoreductase and hydrogenase (25). Although a similar type of ferredoxin was purified from another anaerobic protozoon (20), this organism lacks hydrogenase and hydrogenosomes. In contrast, trichomonad hydrogenosomes have a ferredoxin with a [2Fe-2S] cluster showing that the protozoan electron transport system involved in H_2 production differs in this respect from the bacterial system.

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