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Effect of Culture Medium Iron Content on the Biochemical Composition and Metabolism of *Trichomonas vaginalis*

THOMAS E. GORRELL†

The Rockefeller University, New York, New York 10021

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Trichomonas vaginalis **grown in iron-enriched medium contained increased concentrations of iron-sulfur proteins, including ferredoxin and pyruvate-ferredoxin oxidoreductase. The increases in hydrogenosomal constituents correlated with increased in vivo hydrogenosomal metabolism.**

Trichomonad flagellates are eucaryotic microorganisms with a fermentative metabolism in which iron-sulfur proteins play an important role. Two iron-two sulfur ferredoxins have been isolated from *Tritrichomonas foetus* (6) and *Trichomonas vaginalis* (3), and electron paramagnetic resonance (EPR) studies have indicated the presence of other iron-sulfur proteins in these species (9). Pyruvate-ferredoxin oxidoreductase and hydrogenase, present in both species (7), are likely to correspond to some of the centers detected. Enzymes of fermentative metabolism have characteristic subcellular localization in trichomonad flagellates. The cytosol contains most of the activities (16), but enzymes that oxidize pyruvate to acetate and H_2 are located in a membrane-bounded organelle, the hydrogenosome (7).

Ferredoxin concentrations in bacteria (11, 14) and possibly in plants (17) are known to be correlated with the iron content of the growth medium. I decided to test whether a similar effect of iron could be detected in a protozoon, prompted also by earlier studies that showed increased anaerobic gas production by *T. vaginalis* grown in a medium containing iron-rich liver extract (12). I report here that high concentrations of iron in the medium are required to maintain maximal levels of ferredoxin and pyruvate-ferredoxin oxidoreductase activity in *T. vaginalis*.

(Some of these results were presented at the 30th Annual Meeting of the Society of Protozoologists, Washington, D.C. [T. E. Gorrell, J. Protozool. 27:17A, 1980].)

T. vaginalis ATCC 30001 was grown for 1 day at 37°C under an N_2 atmosphere in tryptose-yeast extract-maltose medium supplemented with 10% heat-inactivated horse serum (2). This medium contains 20 μM iron, as shown by analysis of several batches. The source of additional iron was 25 mM ferrous ammonium sulfate in 5 mM 5-sulfosalicylic acid (15). Cells were collected by centrifugation, suspended in phosphate-buffered salt solution (pH 6.4), and counted in a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.) (4). Determinations of fermentation products, enzyme activities, protein (8), iron (5), and labile sulfur (1) and methods of EPR spectroscopy (9) were as described previously.

The growth rate (generation time, 5.1 h) and maximum cell number attained (5×10^6 cells ml^{-1}) were not affected by a 10-fold difference in iron concentration. At the stationary phase, both cultures (iron enriched and control) contained about 500 μg of cell protein per ml. The cell pellet from cultures enriched with iron was a darker brown than the cell pellet from control cultures.

† Present address: Pfizer Central Research, Groton, CT 06340.

Cells grown in iron-enriched medium (iron-enriched cells) had increased levels of iron but not of labile sulfur (Table 1). Low-temperature EPR spectroscopy showed that all features attributable to paramagnetic iron-sulfur clusters were enhanced in spectra of iron-enriched cells as compared with spectra of control cells, indicating that the overall concentration of such clusters was markedly dependent on the iron content of the medium (Fig. 1). Spectra of cell homogenates reduced with dithionite (data not shown) were identical to those of intact cells, demonstrating that the observed differences were not a result of a different state of oxidation of the cells. Spectra recorded at 10 K consisted of overlapping signals caused by several paramagnetic components and did not permit quantitation of individual proteins. At a higher temperature (25 K) and a lower microwave power, however, ferredoxin was the major constituent detected (6). Measurement of the g = 1.94 signal amplitude revealed a threefold increase in ferredoxin concentration in iron-enriched cells (Table 1).

Iron-enriched cells also exhibited increased activities of certain but not all hydrogenosomal enzymes (Table 1). Pyruvate-ferredoxin oxidoreductase activity showed the greatest increase, and a smaller increase in hydrogenase activity was also observed, as determined with methyl

TABLE 1. Composition of *T. vaginalis* grown in media containing different iron concentrations

Constituent or parameter	Amt or activity (no. of expt) at indicated iron concn[a]		Ratio
	20 μM	200 μM	
Iron	1.5 ± 0.5 (7)	5.1 ± 0.4 (8)	3.4
Labile sulfur	4.2 ± 1.3 (6)	4.9 ± 1.6 (7)	1.2
EPR signal amplitude (g = 1.94, 25 K)	1.1 ± 0.2 (3)	3.2 ± 0.9 (3)	2.9
Pyruvate-ferredoxin oxidoreductase	350 ± 190 (13)	2,600 ± 590 (12)	7.4
Hydrogenase	2,100 ± 890 (4)	3,100 ± 350 (4)	1.4
Malate dehydrogenase (decarboxylating)	280 ± 80 (6)	310 ± 90 (6)	1.1
Lactate dehydrogenase	2,000 ± 740 (5)	1,000 ± 100 (5)	0.5
NADH oxidase	19 ± 1 (2)	33 ± 7 (3)	1.8

[a] Values are the mean ± standard deviation of results obtained with the indicated concentrations of iron in the growth medium. Iron and labile sulfur are expressed as nanomoles per milligram of protein. The EPR signal amplitude is given as the peak height (in arbitrary units) per milligram of cell protein. Enzyme activities are expressed as nanomoles per minute per milligram of protein.

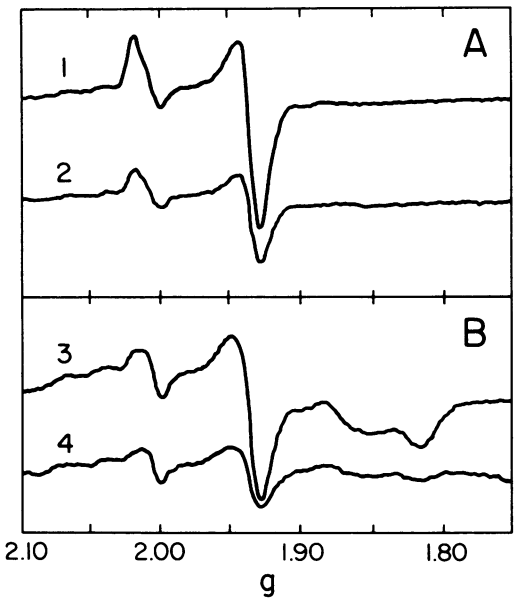

FIG. 1. EPR spectra of *T. vaginalis* grown in media containing 20 μM (lines 2 and 4) or 200 μM (lines 1 and 3) iron. Cells (70 mg of protein ml^{-1}) were incubated anaerobically at 30°C for 5 min in a buffered salt solution containing 50 mM glucose before being frozen. Spectra were recorded at 25 K and 0.5 mW (A) and at 10 K and 2 mW (B). The gain was 5×10^3, the microwave frequency was 9.3 GHz, the scan time was 2 min, the time constant was 0.128 s, and the modulation amplitude was 12.5.

viologen as the electron acceptor. Malate dehydrogenase (decarboxylating) activity, however, was the same in both cell populations.

Two cytosolic activities, NADH oxidase and lactate dehydrogenase, also showed differences (Table 1). Subcellular fractionation studies revealed that the location of all enzymes and EPR-detectable constituents remained unchanged (data not shown).

The changes in the composition of the organisms and especially of their hydrogenosomes were accompanied by increased in vivo metabolic activity of the organelles (Table 2). Hydrogenosomal metabolic flux, as reflected by hydrogen and acetate production, showed a dependence on the concentration of iron in the medium similar to that of pyruvate-ferredoxin oxidoreductase (Fig. 2). Lactate production by iron-enriched cells considerably decreased. After a single subculture of the iron-enriched cells in nonsupplemented medium, enzyme activities and the rate of hydrogenosomal metabolism returned to those observed in cells grown continuously without additional iron (data not shown).

TABLE 2. Anaerobic fermentative metabolism of *T. vaginalis* grown in media containing different iron concentrations

Fermentation product	Rate of production (no. of expt) at indicated iron concn[a]		Ratio
	20 μM	200 μM	
Hydrogen	19 ± 9 (6)	83 ± 9 (5)	4.4
Acetate	20 ± 5 (3)	79 ± 9 (4)	4.3
Lactate	42 ± 19 (3)	8 ± 3 (4)	0.2

[a] Values are the mean ± standard deviation of results obtained with the indicated concentrations of iron in the growth medium and are expressed as nanomoles of product formed per minute per milligram of protein. Cells were incubated at 37°C in a buffered salt solution containing 50 mM glucose.

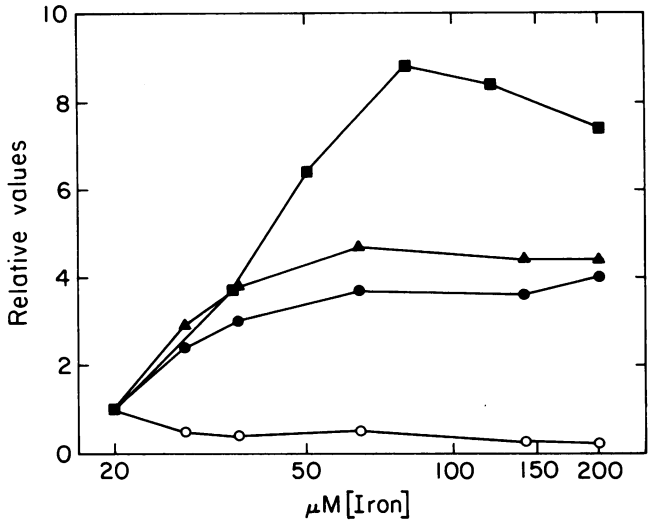

FIG. 2. Effect of the iron concentration in the growth medium on pyruvate-ferredoxin oxidoreductase activity (■) and on hydrogen (▲), acetate (●), and lactate (○) production by *T. vaginalis*. The ordinate represents the ratio of experimental and control (cells grown in medium containing 20 μM iron) values.

My results demonstrate that in *T. vaginalis*, hydrogenosomal activities are limited by the iron content of the routine growth medium, even though it is relatively high. A recent paper described similar effects of iron supplementation on pyruvate-ferredoxin oxidoreductase activity in *T. vaginalis* (10). The concentration of iron that *T. vaginalis* needs to maintain maximal levels of pyruvate-ferredoxin oxidoreductase and ferredoxin is similar to that required by other organisms for maintaining maximal ferredoxin concentrations (11, 13, 14, 17). In clostridia, pyruvate-ferredoxin oxidoreductase is not as dependent on the iron concentration of the medium as it is in *T. vaginalis* (14). Although changes in the kinetic properties of the enzyme could explain the increase in pyruvate-ferredoxin oxidoreductase activity, the dependence on iron concentration of all iron-sulfur clusters supports the idea that the observed changes in the activity of this enzyme reflect the concentration of the enzyme. The in vivo studies showed that levels of iron-sulfur components limit the rate of hydrogenosomal metabolism.

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