Biochimica et Biophysica Acta, 1058 (1991) 261–268

ADONIS 000527289100159O

BBABIO 43427

Chloroquine-sensitive transplasmalemma electron transport in *Tetrahymena pyriformis*: a hypothesis for control of parasite protozoa through transmembrane redox

R. Barr [1], B.A. Branstetter [1], A. Rajnicek [1], F.L. Crane [1] and H. Löw [2]

[1] *Department of Biological Sciences, Purdue University, West Lafayette, IN (U.S.A.)*
and [2] *Endocrinology Department, Karolinska Institute, Stockholm (Sweden)*

(Received 13 February 1991)

Key words: Plasma membrane redox; Iron reduction; Chloroquine; Malaria; Sleeping sickness; Giardiasis; Suramin; (*Tetrahymena*)

Plasma membrane electron transport was studied in a protozoan cell, *Tetrahymena pyriformis*, by assaying transmembrane ferricyanide reduction and the reduction of iron compounds. The rates of ferricyanide reduction varied between 0.5 and 2.5 μmol/g dry wt. per min, with a pH optimum at 7.0–7.5. Other active non-permeable electron acceptors, with redox potentials from +360 to −125 mV, were cytochrome *c*, hexaammine ruthenium chloride, ferric-EDTA, ammonium ferric citrate, and indigo di-, tri- and tetrasulfonates. It was found that *Tetrahymena* cells can reduce external electron acceptors with redox potentials at pH 7.0 down to −125 mV. Ferricyanide stimulates ciliary action. Transmembrane ferricyanide reduction by *Tetrahymena* was not inhibited by such mitochondrial inhibitors as antimycin A, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide, or potassium cyanide, but it responded to inhibitors of glycolysis. Transmembrane ferricyanide reduction by *Tetrahymena* appears to involve a plasma membrane electron transport chain similar to those of other animal cells. As in other cells, the transmembrane electron transport is associated with proton release which may be involved in internal pH control. The transmembrane redox system differs from that of mammalian cells in a 20-fold greater sensitivity to chloroquine and quinacrine. The *Tetrahymena* ferricyanide reduction is also inhibited by chlorpromazine and suramin. Sensitivity to these drugs indicates that the transplasma membrane electron transport and associated proton pumping may be a target for drugs used against malaria, Trypanosomes and other protozoa.

Introduction

Plasma membrane redox systems have been established in many eukaryotic cells [1,2], such as erythrocytes [3], liver [4], heart [5], transformed liver cells [6], HeLa cells [7], neutrophils [8], yeast [9] and other plant cells [2,10,11]. In tumor cells the protonophoric transplasma electron transport has been shown to stimulate growth [1,12–14] and inhibitors of the transmembrane enzyme inhibit growth [6,15,16]. This is, to our knowledge, the first study of the properties of a plasma membrane redox system in a protozoan. Here we provide evidence for basic similarity to plasma membrane oxidoreductases found in both eukaryotic plant and animal cells [1]. A distinguishing characteristic of the plasma membrane redox system in *Tetrahymena* is its sensitivity to the antimalarial drugs chloroquine and quinacrine. The present study, therefore, provides a possible explanation for selective inhibition of growth of parasitic protozoa by these drugs. The exposure of a redox-stimulated proton release system on the surface of protozoa also can provide a target for drugs such as suramin, which have poor permeability into cells.

The plasma membrane electron transport system which acts as a cyanide-insensitive oxidase in other cells [17] also provides a new approach to understanding the cyanide-insensitive respiration of protozoal parasites, especially those without mitochondria.

Materials and Methods

Cell culture. Tetrahymena cells were obtained from the Carolina Biological Supply Co. and grown in a medium consisting of protease peptone (5 g), tryptone (5 g), K_2HPO_4 (0.2 g) and distilled water to 1.0 liter. The pH was adjusted to 7.2. 20-ml test-tubes were filled with 5 ml of growth medium, plugged with cotton,

Correspondence: F.L. Crane, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

autoclaved 15 min at 120 lb pressure/in^2, cooled and inoculated with *Tetrahymena*. Racks containing the inoculated test tubes were stored in a dark at room temperature without shaking for 3–6 days.

Preparation for assays. Cells were harvested by centrifugation in an International table model centrifuge (1500 rpm for 2 min). The supernatant was discarded and cells were rewashed three times with 15 ml of sucrose-salts solution (0.1 M sucrose with 10 mM each NaCl, KCl and $CaCl_2$). After the final wash, the cells were suspended in 5 ml of sucrose-salts. Before removal of cell aliguots, the suspension was bubbled with air for 30 s to aerate and to obtain a uniform cell suspension.

Ferricyanide reduction assay. Ferricyanide reduction by *Tetrahymena* cells was measured at 420–500 nm in a dual-beam Aminco DW-2A spectrophotometer equipped with a magnetic stirrer [7]. Temperature was 25 °C. Assay media was 0.15 ml Tris-Mes (0.25 M, pH 7.5), 1.25 ml sucrose-salts solution and 0.1 ml cells (0.0015 g dry wt.) allowed to equilibrate for 3 min. The reaction was initiated by the addition of ferricyanide to give 100 μM final concentration. A millimolar extinction coefficient of 1 $mM^{-1}\,cm^{-1}$ was used to calculate the rate.

Cytochrome c reduction assay. Cytochrome *c* reduction by *Tetrahymena* cells was measured as the difference in absorbance between 550–541 nm at 25 °C using the dual beam. The reaction mixture was the same as ferricyanide reduction, with 0.1 mg cytochrome *c* added in place of ferricyanide. The millimolar extinction coefficient for cytochrome *c* reduction is 19 ($\Delta A_{550-541} = 19\ mM^{-1}\,cm^{-1}$).

Iron reduction assays. Iron reduction by *Tetrahymena* cells was assayed as the formation of a red complex between reduced iron and bathophenanthroline disulfonate, using the difference in absorbance between 535–600 nm at 25 °C. The reaction mixture consisted of 0.1 ml cells (0.0015 g dry wt.), 25 mM Tris-Mes (pH 7.5, 10 μM bathophenanthroline sulfonate (BPS) and one of the following: Fe^{3+}-EDTA, ammonium Fe(III) citrate or $FeCl_3$ in concentrations from 50 to 100 μM. The volume was adjusted to 1.5 ml with sucrose-salts solution. The millimolar extinction coefficient for ferrous BPS is 17.6 ($\Delta A_{535-600} = 17.6\ mM^{-1}\,cm^{-1}$). In all cases a preincubation period of 3 min was allowed for cells, bathophenanthroline sulfonate, buffer and sucrose-salts solution before the addition of the iron source.

Proton release. Proton release associated with ferricyanide reduction was measured by determining the change in external pH when ferricyanide was added to a cell suspension. The pH was measured with a glass electrode in a cuvette continuously bubbled with air to equilibrate CO_2 or with CO_2-free air to remove CO_2. Cells, 0.001–0.0001 g dry wt., were suspended in sucrose-salts solution with low buffering capacity (25 μM Tris-HCl (pH 7.4)) in a total volume of 3 ml at 25 °C. Proton release was calibrated with 50 μmol HCl. The basal rate was determined after the rate of pH change had reached equilibrium before addition of ferricyanide [7].

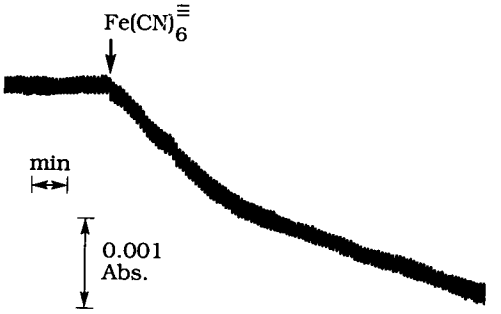

Fig. 1. Tracing of a recording of transmembrane ferricyanide reduction by *Tetrahymena* cells. 0.5 mg dry wt. cells in 1.5 ml sucrose-salts with Tris-HCl and 0.1 mM potassium ferricyanide. Assay at 25 °C.

Results

Ferricyanide concentration

Ferricyanide reduction by *Tetrahymena* cells shows a fast and a slow phase (Fig. 1). The fast phase is observed in the first 2–3 min, while the slow phase continues for as long as 30 min. The effect of ferricyanide concentration on the two rates is shown in Fig. 2. The K_m for ferricyanide calculated from a Lineweaver-Burk plot is 21.5 μM for both the fast and slow rate.

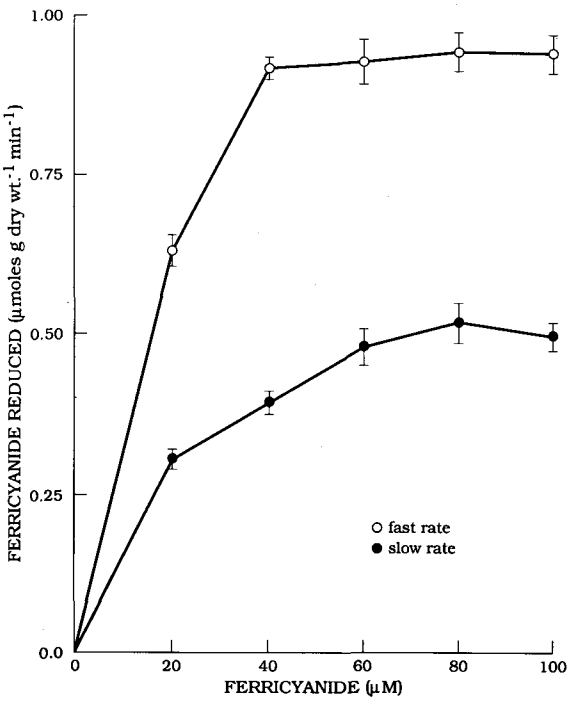

Fig. 2. Effect of ferricyanide concentration on the rate of ferricyanide reduction by *Tetrahymena* cells. Conditions as in Materials and Methods.

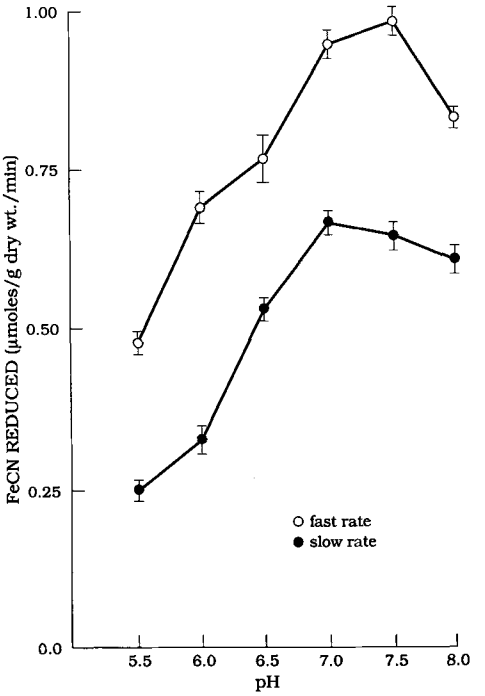

Fig. 3. Effect of pH on the rate of ferricyanide reduction by *Tetrahymena* cells.

pH optimum for assays

Ferricyanide reduction by *Tetrahymena* cells gives optimum rates between pH 7 and 7.5 (Fig. 3). The reaction is much slower at low pH. At high pH (> 8.5) the determination of ferricyanide reduction rates becomes unreliable, i.e., the blank rates without cells are too high as a result of nonphysiological ferricyanide reduction.

Ion effects on ferricyanide reduction

When *Tetrahymena* cells are washed free of growth medium with 0.1 M sucrose instead of the sucrose-salts solution normally used (0.1 M sucrose with 10 mM each of KCl, NaCl and $CaCl_2$), the effect of individual ions on ferricyanide reduction can be studied. 6 mM Mg^{2+} and Ca^{2+} ions stimulate the fast rate of ferricyanide reduction 2-fold. However, very little stimulation by divalent cations is seen on the slow phase of ferricyanide reduction by *Tetrahymena* cells.

TABLE II

Recovery of ferricyanide in the media after removal of Tetrahymena cells

Cells in Eppendorf tubes (0.003 g dry wt.) and 0.1 mM ferricyanide were incubated in 1 ml 25 mM Tris-Mes (pH 7.0) with sucrose-salts at 22°C. At the end of the incubation, cells were immersed in ice and centrifuged in the cold, 4°C, for 2 min. Absorbance of supernatant was measured at 420 nm. Supernatant after 5 min incubation was treated with 5 μl 10 mM sodium persulfate to oxidize the ferrocyanide. Standard deviation based on three assays with the same cell culture.

Treatment	Ferricyanide in supernatant (μmol)
Cells + ferricyanide (zero time)	0.102 ± 0.01
Cells + ferricyanide (5 min incubation)	0.085 ± 0.01
5 min supernatant plus persulfate	0.113 ± 0.003
Persulfate only (blank)	0.005

Reduction of other electron acceptors

Tetrahymena cells can also reduce other non-permeant electron acceptors besides ferricyanide, such as cytochrome *c*, hexaammine ruthenium(III) chloride, ferric EDTA, ferric ammonium citrate and indigo sulfonates. Table I lists the concentrations of these compounds which give maximum reduction rates with *Tetrahymena* cells. The tested compounds that are active have standard redox potentials at pH 7.0 ranging from +360 mV to −125 mV.

Criteria for extracellular ferricyanide reduction

If ferricyanide is reduced on the outside of *Tetrahymena* cells, it should be recovered when the supernatant is reoxidized. The results of such an experiment are

TABLE I

Nonpermeable electron acceptors reduced by Tetrahymena cells

Activities were measured on one cell culture using three aliquots of cells to compute standard deviation. An 8-day-old cell culture was used. Younger cultures can have ferricyanide reduction rates up to 2.5 μmol/g dry wt. per min.

Acceptor	Concn. (μM)	Rate of reduction (μmol/g dry wt. per min)	Redox potential E^0 (pH 7.0) (mV)
Ferricyanide	100	0.85 ± 0.05	+360
Cytochrome *c*	90	0.04 ± 0.009	+225
1,2-Naphthoquinone sulfonate	20	0.013 ± 0.001	+187
Ferric EDTA	100	0.02 ± 0.004	+90
Hexammine ruthenium(III)	200	0.02 ± 0.002	+54
Fe(III) ammonium citrate	3[a]	0.03 ± 0.006	–
Indigo tetrasulfonate	6.6	0.003	−46
Indigo trisulfonate	20	0.003	−70
Indigo disulfonate	40	0.001	−125

[a] mg/ml.

shown in Table II. Sodium persulfate is highly acidic and was dissolved in 0.25 M Tris-Mes (pH 8) before the addition of a few microliters to the supernatant. It also gave a slight blank, which was subtracted from the appropriate absorbance readings.

Excretion of reducing agents from cells, such as phenols or thiols, could be a basis for external ferricyanide reduction. The supernatant removed from cells after 15 to 30 min incubation produces no significant ferricyanide reduction, showing that transmembrane electron transport by whole cells is necessary for ferricyanide reduction.

The mitochondrial inhibitor antimycin A shows no effect on transplasma membrane electron transport in *Tetrahymena*. HOQNO stimulates the rate 62% (Table III), KCN 36%. Inhibitors of glycolysis such as iodoacetamide in millimolar concentrations inhibit transmembrane ferricyanide reduction up to 40% after a 3 min incubation period. Superoxide dismutase, used to check for the production of superoxide radicals in transplasma membrane electron transport, showed only a slight stimulation (27%) of ferricyanide reduction.

Stimulation of motility by ferricyanide

Cells suspended in assay media (sucrose-salts with 25 mM Tris-Mes (pH 7.0)) with and without 100 μm potassium ferricyanide were observed at 430 × magnification. In absence of ferricyanide only slight movement is observed and cilia are not readily apparent. With ferricyanide present, the cells move rapidly and the ciliary movement produces a halo around each cell. The fast movement lasted for more than 15 min. Ferrocyanide does not produce the effect.

TABLE III

The effect of mitochondrial and metabolic inhibitors on plasma membrane electron transport in Tetrahymena

Inhibitor	Concn.	Electron transport rate (μmol/g dry wt. per min)	Stimulation or inhibition (%) [a,b]
None	–	0.8 ±0.14	–
Antimycin	1 μM	0.8 ±0.03	0
HOQNO	5 μM	1.3 ±0.21	+62
KCN	1 mM	1.10±0.32	+36
PCMBS	100 μM	0.7 ±0.09	−12
Sodium arsenite	20 mM	0.85±0.05	+6
Sodium arsenate	20 mM	0.5 ±0	−31
Iodoacetamide	3 mM	0.49±0.11	−40
Maleic hydrazide	6 mM	0.51±0.16	−37
Superoxide dismutase	60 units/ml	1.03±0.07	+27

[a] +indicates stimulation, −inhibition of rate in comparison to control.

[b] After a 3 min incubation.

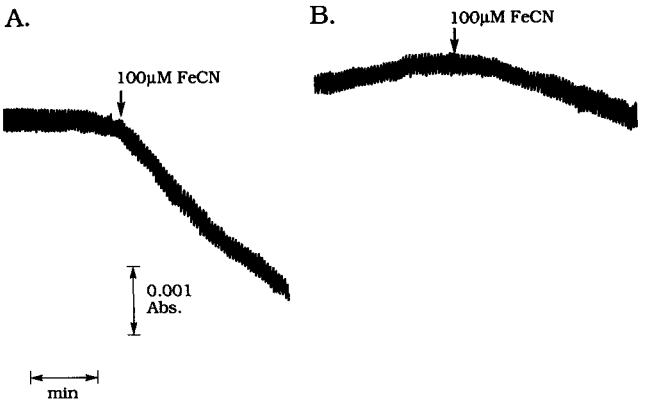

Fig. 4. Tracings of ferricyanide reduction by *Tetrahymena* cells (A) in the absence of chloroquine and (B) with 100 μM chloroquine. Cells incubated without and with chloroquine for 3 min before addition of ferricyanide. The control rate (A) is 0.68 μmol $Fe(CN)_6^{3-}$ reduced/g dry wt. per min, with chloroquine (B) 0.21 μmol/g dry wt. per min.

Specific inhibitors

The best specific inhibitor of transmembrane ferricyanide reduction by *Tetrahymena* cells is chloroquine (Fig. 4). It gives 50% inhibition at 50 μM. A spectrophotometric tracing showing the effect of chloroquine on plasma membrane electron transport in *Tetrahymena* is shown in Fig. 4. While the control rate is 0.67 μmol FeCN reduced/g dry wt. per min, 100 μM chloroquine reduces this rate to 0.25 μmol/g dry wt. per min, which represents a 63% inhibition. The effect of chloroquine concentration on ferricyanide reduction by *Tetrahymena* is shown in Fig. 5.

In addition to chloroquine, the following show inhibition of the *Tetrahymena* transmembrane electron transport: quinacrine gives 50% inhibition at 120 μM (Fig. 6); suramin gives 40% inhibition at 200 μM (Fig. 7); and chlorpromazine gives 65% inhibition at 150 μM (Table IV). All of these compounds have been used to inhibit growth in various parasitic protozoa [18–22]. Chloroquine and quinacrine also inhibit electron transport in erythrocyte and liver plasma membrane, but the concentrations required are much higher [23]. With HeLa cells, 100 μM chloroquine gives only 40% inhibition of ferricyanide reduction (Table V).

Oxidant-induced proton release

Addition of ferricyanide to the *Tetrahymena* cells induces increased proton release (Table VI). Addition of ferrocyanide does not cause proton release, so the *Tetrahymena* resemble other cells in releasing protons when ferricyanide reduction occurs. The presence of calcium ions increases the redox induced proton release.

Inhibition of oxygen uptake by ferricyanide

Transfer of electrons across the plasma membrane is related to a cyanide insensitive NADH oxidase in plant and animal cells [17]. When impermeable ferricyanide is

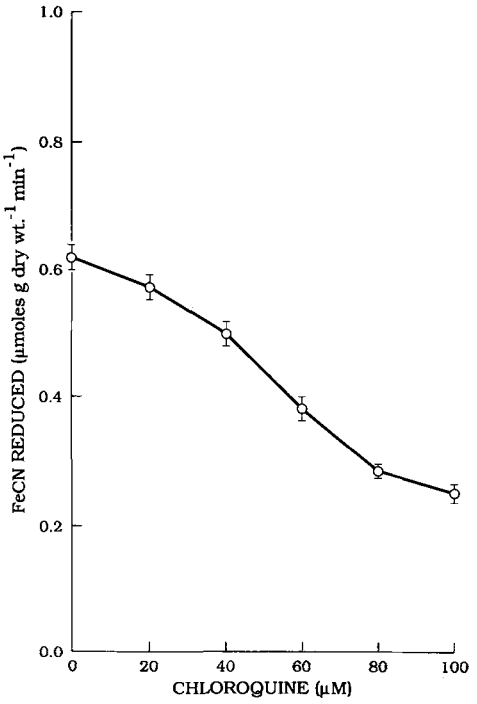

Fig. 5. Effect of chloroquine concentration on the rate of transplasma membrane ferricyanide reduction by *Tetrahymena*. Cells were incubated in assay mixture with chloroquine as indicated for 3 min before addition of ferricyanide. Chloroquine alone does not reduce ferricyanide.

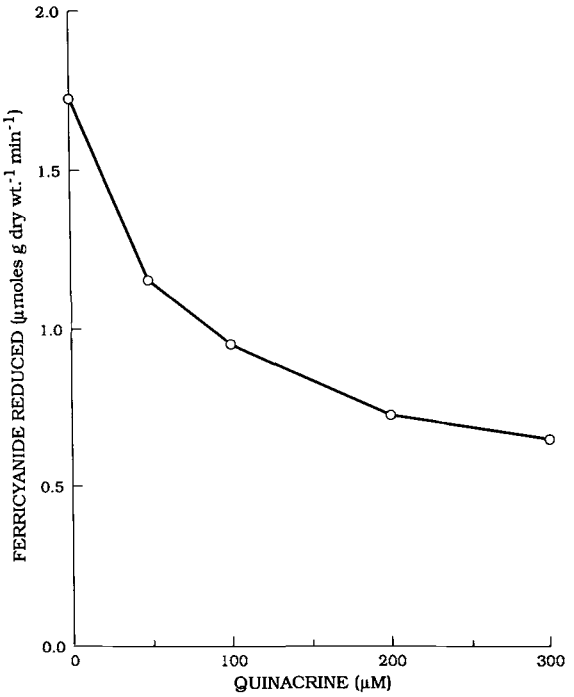

Fig. 6. Effect of quinacrine concentration on the rate of ferricyanide reduction by *Tetrahymena* cells. Cells preincubated in the assay media with quinacrine at the concentrations indicated for 3 min before starting the assay with ferricyanide.

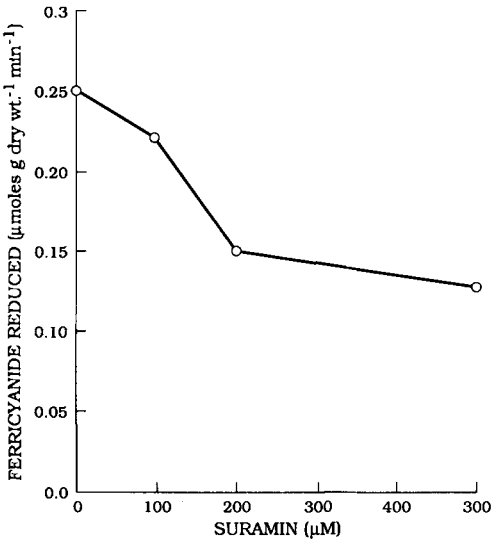

Fig. 7. Effect of suramin concentration on the rate of ferricyanide reduction by *Tetrahymena*. Assay as in Materials and Methods after 3 min incubation with suramin.

reduced by the plasma membrane electron transport the oxygen uptake at the plasma membrane may be decreased by competition of ferricyanide for electrons [24,25]. Ferricyanide inhibition of oxygen uptake by *Tetrahymena* cells consistent with the presence of an oxidase in the membrane is shown in Table VII.

Discussion

Transmembrane electron transport depends on the production of an internal reducing agent, such as NADH

TABLE IV

Effect of phenothiazines on transplasmalemma electron transport in Tetrahymena

14-day-old cells in 25 mM Tris-Mes (pH 7.0).

Addition	Ferricyanide reduction (μmol/g dry wt. per min)
None	0.52 ± 0.03
150 μM chlorpromazine	0.18
150 μM trifluoperazine	0.25 ± 0.03

TABLE V

Comparison of chloroquine inhibition of ferricyanide reduction by Tetrahymena and HeLa cells

Chloroquine added (μM)	Rate of ferricyanide reduction (μmol/g dry wt. per min)	
	Tetrahymena	HeLa
None (control)	0.61 ± 0.01	0.35
40	0.50 ± 0.01	–
80	0.26 ± 0.005	–
100	0.24 ± 0.007	0.21
200	–	0.05

TABLE VI

Oxidant-induced proton release from Tetrahymena cells

All assays were conducted in 3 ml buffer as indicated. Assay in sucrose-salts with 0.1 mg dry wt. of cells and in calcium sulfate plus potassium phosphate 0.2 mg dry wt. of cells. Proton release was titrated with 50 μmol standard HCl. pH changed from 7.56 to 7.51 during the assay.

Suspension medium	Proton release (μequiv./g dry wt. per min)	
	basal	increase with ferricyanide
Sucrose-salts + 25 μM Tris	3.5	0.7
1 mM $CaSO_4$ + 25 μM phosphate	2.0	3.6

or NADPH [9,26,27], the oxidation of which liberates electrons which travel to the outside of the cell via a transplasma membrane electron transport chain. Various non-permeable artificial electron acceptors can therefore be reduced on the outer surface of the cell. In this study, we have used such non-permeable electron acceptors as ferricyanide, ruthenium hexaammine chloride, indigo sulfonates and various iron complexes to accept electrons at the cell surface.

Transmembrane electron transport is well established in many eukaryotic cells of both plant and animal origin [1,2], but this is, to our knowledge, the first demonstration with a protozoan cell, *Tetrahymena*. We find that plasma membrane electron transport of *Tetrahymena*, assayed as transmembrane ferricyanide reduction, is similar to that in other eukaryotic cells. Ferricyanide reduction rates of 0.5–2.5 μmol/g dry wt. per min are similar to those of liver [4], HeLa [7] or carrot [10] cells. *Tetrahymena* cells show a high affinity for ferricyanide, with a K_m of 21.5 μM. This is similar to the K_m found for rat heart (22 μM) [28] but much higher than that found with HeLa (240 μM) or rat liver (130 μM) cells [4,7]. The broad pH optimum at pH 7.0–7.5 is similar to other cells [7,9] but there is much less activity at pH 6.0. *Tetrahymena* cells are also able to reduce iron-containing compounds, such as ferric-EDTA, ammonium ferric citrate, or ferric tartrate, as do mammalian cells. The transmembrane enzyme can reduce compounds with a negative redox potential down to −125 mV, as with HeLa cells [7,13] and plant cells [29]. Excretion of reducing compounds does not account for the transmembrane ferricyanide reduction rates observed with *Tetrahymena* because the supernatant, which has been in contact with cells for 30 min, does not appreciably reduce ferricyanide. *Tetrahymena* has been reported to excrete dopamine at a rate of 0.3 pmol/10^6 cell per min^{-1}. This would represent approx. 5 nmol/g dry wt. per min or excretion of reducing agent at a 100-times lower rate than the rate of ferricyanide reduction [30]. If thiols were excreted, the reduction should be inhibited by *p*-phenylchloromercuri sulfonate. The recovery of 95–100% of added ferricyanide after reoxidation of the reduced ferricyanide is consistent with an extracellular site of ferricyanide reduction, as with other cells [4,7]. Stimulation of ciliary action by ferricyanide clearly indicates that the cells are not dead. Ferricyanide has previously been shown to have inotropic effects in perfused rat heart [28] and to increase membrane potential in HeLa cells [31], so the ciliary response may be related to changes in cytosolic calcium or membrane potential.

TABLE VII

Effect of ferricyanide on oxygen uptake by Tetrahymena cells

Assay was done in 3 ml sucrose salts buffer (pH 7.0) with an oxygen electrode.

Addition	Oxygen uptake (μmol O_2/g dry wt. per min)
None	5.08 ± 0.51 (3)
0.1 mM ferricyanide	0.0

Extracellular ferricyanide reduction by *Tetrahymena* or other cells is not inhibited by such mitochondrial inhibitors as antimycin A, HOQNO or cyanide, which shows the reaction is not based on mitochondria from broken cells. Inhibitors of glycolysis, on the other hand, can inhibit transmembrane ferricyanide reduction from 30–40% after 3 min incubation with cells. A decrease in the rate of formation of internal NADH would limit substrate availability for the transmembrane electron transport. Increased ferricyanide reduction with HOQNO or cyanide could be a response to increased NADH in the cytoplasm because of inhibition of mitochondria.

The *Tetrahymena* plasma membrane electron transport resembles that of higher animal cells. We have found significant differences, however, in the response of the protozoa to chloroquine and quinacrine. Quinacrine (atebrin) was the first specific inhibitor found for the NADH dehydrogenase in plasma membranes from rat liver and adipocytes [32] and ascites tumor cells [33]. It was a rather poor inhibitor because $3 \cdot 10^{-3}$ M was required to achieve 50% inhibition [32]. Much lower concentrations inhibit ferricyanide reduction by *Tetrahymena*. Chloroquine, likewise, inhibited plasma membrane dehydrogenase and required 5 mM to give 50% inhibition with human erythrocyte membranes [23] and rat liver plasma membranes [34]. 60 μM chloroquine inhibits transplasma membrane electron transport 50% in *Tetrahymena*. A 2-times greater chloroquine concentration only inhibits HeLa cell ferricyanide reduction by only 40% [35]. If increased sensi-

tivity occurs in parasitic protozoa, then quinacrine or chloroquine could inhibit growth of the protozoa by inhibition of the plasma membrane redox system, while leaving the host cells unaffected.

Chlorpromazine and trifluoperazine are antagonists of calmodulin. The plasma membrane electron transport may be another site of action, since the concentration of chlorpromazine which inhibits electron transport (150 μM) is similar to concentrations which are lethal to *Tetrahymena* (160 μM) [36]. Alternatively, calmodulin may contribute to control of electron transport [37]. A much higher chlorpromazine concentration (500 μM) is required to inhibit NADH-ferricyanide reductase of human erythrocyte membranes [23].

As with other eukaryotic cells, the transmembrane electron transport is associated with increased release of protons to the medium. In animal cells this proton release has been attributed to activation of the Na^+/H^+ antiport [7,31,38]. In plant cells the activation of a proton pumping ATPase in response to the plasma membrane electron transport has been proposed [1,11]. The basis for the ferricyanide stimulated proton release by *Tetrahymena* remains to be established.

The transplasma membrane electron transport system in liver cell membranes has been shown to act as a cyanide insensitive NADH oxidase [17]. If the redox system in protozoal plasma membranes can also act as an oxidase, it may contribute to the total oxygen uptake of these cells [18]. The inhibition of oxygen uptake by *Tetrahymena* cells by ferricyanide is consistent with the presence of a transmembrane oxidase, since ferricyanide can compete for electrons only at the outer surface. Similar inhibition of part of the oxygen uptake by liver and carrot cells with ferricyanide has been observed [24,25]. In some parasitic protozoa which lack mitochondria [18], such as *Giardia* or *Entamoeba*, the plasma membrane electron transport may play an essential role as an oxidase. If this is so, then inhibitors of the system, such as chloroquine or atebrin, may be especially effective in inhibition of the growth of these cells.

Fry [39] has recently demonstrated that when malaria parasites incorporate transferrin receptors into erythrocyte membranes, transferrin reductase activity is induced in the membranes. Since electron transfer to transferrin iron appears to depend on the transmembrane electron transport system [40], and this activity is associated with NADH oxidase activity [38], the effects of chloroquine and atebrin on malaria and other protozoa [41,42] may also be based on inhibition which these compounds have on plasma membrane electron transport [34,35].

Acknowledgements

Supported under NSF grant PCM8314603 and NIH Career Award GM-K6-21839 (F.L.C.).

References

1 Crane, F.L., Morré, D.J. and Low, H. (eds.) (1989) Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth, Plenum, New York.
2 Crane, F.L., Sun, I.L., Clark, M.G., Grebing, C. and Löw, H. (1985) Biochim. Biophys. Acta 811, 233–264.
3 Mishra R.K. and Passow, H. (1969) J. Membr. Biol. 1, 214–224.
4 Clark, M.G., Partick, E.J., Patten, G.S., Crane, F.L., Löw, H. and Grebing, C. (1981) Biochem. J. 200, 567–572.
5 Löw, H., Crane, F.L., Partick, E.J. and Clark, M.G. (1985) Biochim. Biophys. Acta 844, 142–148.
6 Sun, I.L., Crane, F.L., Chou, J.Y., Löw, H. and Grebing, C. (1983) Biochem. Biophys. Res. Commun. 116, 210–216.
7 Sun, I.L., Crane, F.L., Grebing, C. and Löw, H. (1984) J. Bioenerg. Biomembr. 16, 583–595.
8 McLoughlin, P.M., Sun, I.L. and Crane, F.L. (1984) Biochim. Biophys. Acta 802, 71–76.
9 Crane, F.L., Roberts, H., Linnane, A.W. and Löw, H. (1982) J. Bioenerg. Biomembr. 14, 191–205.
10 Barr, R., Craig, T.A. and Crane, F.L. (1985) Biochim. Biophys. Acta 812, 49–54.
11 Sijmons, P.C., Lanfermeijer, F.C., DeBoer, A.H., Prins, H.B.A. and Bienfait, H.F. (1984) Plant Physiol. 76, 943–946.
12 Ellem, K.A.O. and Kay G.F. (1983) Biochem. Biophys. Res. Commun. 112, 183–190.
13 Sun, I.L., Crane, F.L., Löw, H. and Grebing, C. (1984) Biochem. Biophys. Res. Commun. 125, 649–654.
14 Sun, I.L., Crane, F.L., Grebing, C. and Löw, H. (1985) Exp. Cell Res. 156, 528–536.
15 Sun, I.L. and Crane, F.L. (1984) Biochem. Int. 9, 299–306.
16 Sun, I.L. and Crane, F.L. (1990) in Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport, Vol. I, Animals (Crane, F.L., Morré, D.J. and Löw, H., eds.), pp. 257–280, CRC Press, Boca Raton.
17 Morré, D.J. and Crane, F.L. (1990) in Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport, Vol. I, Animals (Crane, F.L., Morré, D.J. and Löw, H., eds.), pp. 67–84, CRC Press, Boca Raton.
18 Weinbach, E.C. (1981) Trends Biochem. Sci. 6, 254–257.
19 Lindmark, D.G. (1980) Mol. Biochem. Parasitol. 1, 1–12.
20 Fairlamb, A. (1982) Trends Biochem. Sci. 7, 249–253.
21 Ruben, L., Egwuagu, C. and Patton, C.L. (1983) Biochim. Biophys. Acta 758, 104–113.
22 Pearson, R.D., Manian, A.A., Harcus, J.L., Hall, D. and Hewlett, E.L. (1982) Science 217, 369–371.
23 Grebing, C., Crane, F.L., Löw, H. and Hall, K. (1984) J. Bioenerg. Biomembr. 16, 517–534.
24 Crane, F.L., Sun, I.L., Barr, R. and Löw, H. (1991) J. Bioenerg. Biomembr. in press.
25 Crane, F.L., Barr, R., Morré, D.J., Brightman, A.O. and Craig, T.A. (1988) in Current Topics in Plant Biochemistry and Physiology, Vol. 7 (Randall, D.D., Blevins, D.G. and Campbell, W.H., eds.), p. 224, University of Missouri Press, Columbia.
26 Navas, P., Sun, I.L., Morré, D.J. and Crane, F.L. (1986) Biochem. Biophys. Res. Commun. 135, 110–115.
27 Sijmons, P.C., Van den Briel, W. and Bienfait, H.F. (1984) Plant Physiol. 75, 219–221.
28 Löw, H., Crane, F.L., Partick, E.J., Patten, G.S. and Clark, M.G. (1984) Biochim. Biophys. Acta 804, 253–260.
29 Ullrich, C.I., Köhler, K., Baier, M., Förster, B. and Hartung, W. (1990) Bot. Acta 103, 214–221.
30 Gunderson, R.E. and Thompson, G.A., Jr. (1983) Biochim. Biophys. Acta 755, 186–194.
31 Toole-Simms, W. (1988) Regulation of Proton Release from HeLa Cells by Ferric Reductase. p. 161, Ph.D. Thesis, Purdue University, W. Lafayette.

32 Crane, F.L. and Löw, H. (1976) FEBS Lett. 68, 153–156.
33 Kilberg, M.S. and Christensen, H.N. (1979) Biochemistry 18, 1525–1530.
34 Löw, H., Crane, F.L., Morré, D.J. and Sun, I.L. (1990) in Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport, Vol. 1, Animals (Crane, F.L., Morré, D.J. and Löw, H., eds.), p. 29–66, CRC Press, Boca Raton.
35 Toole-Simms, W., Sun, I.L., Morré, D.J. and Crane, F.L. (1990) Biochem. Int. 21, 761–769.
36 Forrest, I.S., Quesada, F. and Deitchman, G.L. (1964) Proc. West. Pharmacol. Soc. 7, 42.
37 Barr, R., Stone, B. and Crane, F.L. (1985) Biochem. Biophys. Res. Commun. 126, 262–267.
38 Crane, F.L., Löw, H., Sun, I.L. and Morré, D.J. (1990) in Growth Factors: From Genes to Clinical Applications (Sara, V., Hall, K. and Löw, H., eds.), pp. 288–297, Raven, New York.
39 Fry, M. (1989) Biochem. Biophys. Res. Commun. 158, 469–473.
40 Sun, I.L., Navas, P., Crane, F.L., Morré, D.J. and Löw, H. (1987) J. Biol. Chem. 262, 15915–15921.
41 Weinbach, E.C., Claggett, C.E., Keister, D.E., Diamond, L.S. and Kon, H. (1980) J. Parasitol. 66, 347–350.
42 Lindmark, D.G. (1980) Mol. Biochem. Parasitol. 1, 1–12.