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Transmembrane Ferricyanide Reduction by Cells of the Yeast *Saccharomyces cerevisiae*

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Abstract

Both respiratory-competent and respiratory-deficient yeast cells reduce external ferricyanide. The reduction is stimulated by ethanol and inhibited by the alcohol dehydrogenase inhibitor, pyrazole. The reduction of ferricyanide is not inhibited by inhibitors of mitochondrial or microsomal ferricyanide reduction. Cells in exponential-phase growth show a much higher rate of ferricyanide reduction. The reduction of ferricyanide is accompanied by increased release of protons by the yeast cells. We propose that the ferricyanide reduction is carried out by a transmembrane NADH dehydrogenase.

Key Words: Plasmalemma redox; transmembrane dehydrogenase; proton transport.

Introduction

The reduction of external ferricyanide by intact erythrocytes has been demonstrated by Dormandy and Zarday (1965). It has been proposed that the reduction is carried out by a transmembrane dehydrogenase which can transfer electrons from an internal reducing agent to external ferricyanide (Mishra and Passow, 1969). On the other hand, Ottinger and Roer (1979) have proposed that ascorbic acid acts as a redox carrier across the erythrocyte plasma membrane to reduce ferricyanide on the outside of the cell.

The plasmalemma from many types of cells contains NADH ferricyanide reductase activity (Crane *et al.*, 1979; Löw and Crane 1978). Part of this activity may be attributed to the NADH cytochrome b_5 reductase (Strittmatter and Velick, 1956) which has been identified in plasma membrane fractions

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from all tissues studied (Crane *et al.*, 1979; Masudo *et al.*, 1973; Kilberg and Christensen, 1979). Kant and Steck (1972) have shown that in erythrocyte plasma membrane the NADH cytochrome b_5 reductase is exclusively on the cytoplasmic side of the membrane. In liver cells the cytochrome *c* reductase has also been shown to be exclusively on the cytoplasmic side of the endoplasmic reticulum membranes (DePierre and Ernster, 1977). It is therefore unlikely that the NADH cytochrome *c* reductase can act as the transmembrane electron carrier in erythrocytes and presumably in other cells. A second NADH dehydrogenase which has been purified from erythrocyte plasma membranes acts only as a ferricyanide reductase (Wang and Alaupovic, 1978). In erythrocyte plasma membranes which have been prepared so that both surfaces are exposed to the media, it is possible to show that there is more NADH ferricyanide reductase activity than in resealed membrane fragments which exclusively expose the cytoplasmic side. These findings are consistent with a transmembrane NADH ferricyanide reductase which oxidizes internal NADH by reduction of external ferricyanide (Löw *et al.*, 1979; MacKellar *et al.*, 1979).

In this paper we describe ferricyanide reduction by intact respiratory-competent ($\rho+$) and respiratory-deficient yeast cells which would support a broad phylogenetic distribution of transmembrane dehydrogenases. The activity also shows remarkable changes related to the transition from exponential-phase to stationary-phase growth. Stimulation of the transmembrane electron flow by uncouplers suggests that the activity may be coupled to proton transport across the plasma membrane.

Study of the transmembrane redox reaction in yeast is especially favored because of the availability of mutants which are completely deficient in a functional mitochondrial respiratory system although the cells retain 10% of the oxygen consumption of wild-type cells. These mutants can only grow on fermentable substrate. The respiratory-competent and respiratory-deficient cells have been used to demonstrate the presence of the transmembrane NADH dehydrogenase when mitochondrial respiratory function is absent.

Methods

Growth of Yeast Cells

Cells of the wild-type strain of *Saccharomyces cerevisiae,* J69-B (α *ade his* ρ^+), and of the petite strain EJO (α *ade his* ρ^0) derived from it, were grown aerobically at 28°C with 2% glucose as the carbon source. Catabolite-repressed exponential-phase cells harvested before the cultures reached 2.4 mg cell dry wt./mg, or derepressed cells harvested when they had reached 2.4

mg/ml (stationary phase ρ^0 cells) or higher (in the case of ρ^+ cells), were used as indicated in the text, after being washed twice with water. Cell densities were calculated from calibrated hematocrit volumes.

Ferricyanide reductase activity by whole yeast cells was measured in an Aminco DW-2A spectrophotometer using the dual wavelength mode to subtract absorbance at 500 nm from 400 nm. With 5 to 25 mg of cells per 3.0 ml there was no significant change caused by turbidity. Various buffers were used with 0.31 mM potassium ferricyanide and 10 μl ethanol as standard conditions. The extinction coefficient of 1.0 mM^{-1} cm^{-1} was used for ferricyanide at 400 nm. Cytochrome *c* reduction was measured in similar fashion using 550–500 nm and an extinction coefficient of 19 mM^{-1} cm^{-1}. Cytochrome *c* (0.2 mg) was added as acceptor and 10^{-3} M KCN was required. The buffer was 0.2 M potassium phosphate buffer, pH 8.0.

Oxygen uptake was determined in 2.0 ml of buffer on the oxygen electrode using 10 to 30 mg of cells (dry wt.). For indigotetrasulfonate reduction the increase in oxygen uptake upon addition of 0.2 mg indigotetrasulfonate was measured. The basal oxygen uptake was subtracted from the total.

Changes in pH were measured with a pH meter recording in the range from 7.0 to 6.0. A 10-μl quantity of 0.2 M potassium phosphate, pH 8.0, and 10 to 30 mg of yeast cells were added in a volume of 7.0 ml water. A 50-μl quantity of ferricyanide solution (0.042 M) was added. Calibration was made with 0.5 M NaOH or HCl. Difference spectra were measured on the DW-2a spectrophotometer using the split beam in 1.0 ml total volume. Cell concentration was 30 to 50 mg/ml with 2 μmol ferricyanide or a few crystals of ammonium persulfate as oxidants. Ethanol (10 μl) was used as reductant or a few crystals of sodium dithionite.

Results

As shown in Fig. 1, both respiratory-competent wild-type (ρ^+) and respiratory-deficient petite (ρ^0) yeast cells reduce ferricyanide utilizing endogenous substrate. The kinetics of reduction by the two cell types may not be the same. The ρ^+ cells can show an initial rapid rate of reduction followed by a slower rate of reduction. The duration of the initial fast rate is modified by the buffer or salts used in the aşsay media. The slower secondary stage can be maintained for more than 10 min if ethanol is present. The ρ^0 cells do not show the initial rapid rate but only a steady rate which may continue for more than 10 min. High ionic strength increases the secondary or ρ^0 cell rate as shown by addition of calcium chloride in Fig. 1.

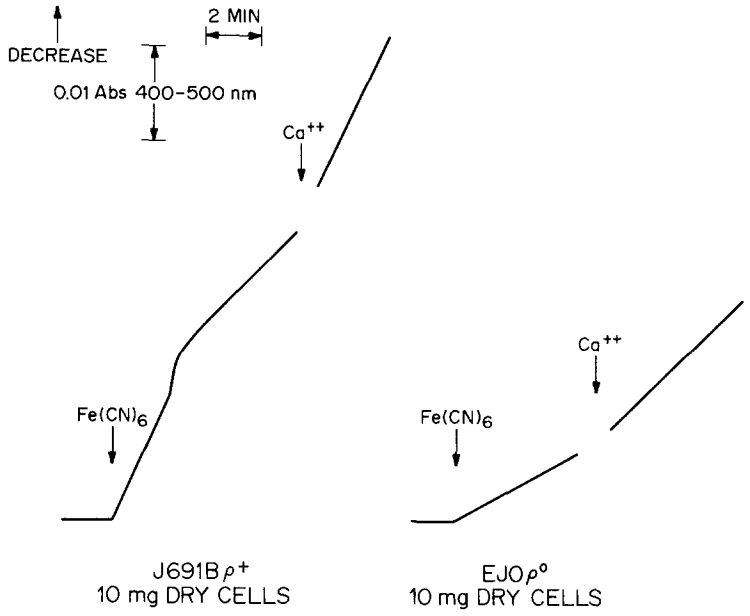

Fig. 1. Ferricyanide reduction by J691B ρ^+ respiratory-competent and EJO ρ^0 petite yeast cells. 10 mg dry wt. cells suspended in 60 mM Tris chloride, pH 8.0, plus 10 μl ethanol. Total volume 2.8 ml. Temperature 21°C, reaction started with potassium ferricyanide at 0.31 mM. Calcium chloride added at second arrow to 3.7×10^{-4} M to show salt-stimulated rate. Absorbance change at 400 nm minus 500 nm. Spurt of absorbance decrease in ρ^+ at 2 min is caused by reduction of cytochromes when cells go anaerobic and occur even without ferricyanide.

In starved cells, or in cells which have reacted with ferricyanide long enough to show a decline in rate, addition of ethanol to both ρ^+ and ρ^0 cells as a source of reducing equivalents for NADH formation will restore maximal ferricyanide reduction rates (Table I). Further evidence that ethanol stimulation of ferricyanide reduction requires ethanol dehydrogenase to generate cytosolic NADH is derived from inhibition of ferricyanide reduction by pyrazole. Since pyrazole is an inhibitor of ethanol dehydrogenase (Lumeng, 1979), it would decrease generation of NADH (Fig. 2).

Table I. Ethanol Stimulation of Ferricyanide Reduction by Whole Yeast Cells[a]

	Ferricyanide reduction (nmole/min/mg dry wt.)	
Strain	− Ethanol	+0.01 ml ethanol
EJO ρ^0	0.30	1.08
J69 1B ρ^+	0.45	0.91

[a]Assay conditions: 0.2 M potassium phosphate, pH 8.0, total volume 2.8 ml, 12 mg dry wt. cells.

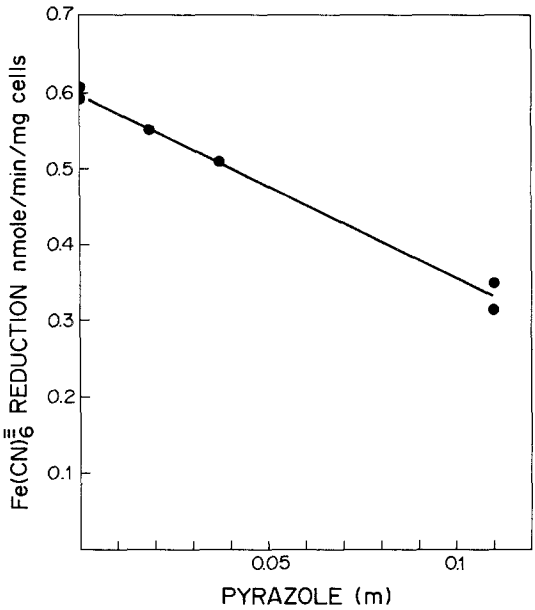

Fig. 2. Pyrazole inhibition of ferricyanide reduction by yeast cells. Assay with 10 mg EJO ρ^0 cells as for Fig. 1.

The rate of reduction of ferricyanide in both the fast and slow phase for the ρ^+ cells and by the ρ^0 cells is proportional to the amount of cells added to 25 mg dry weight of cells per 2.5 ml (data not shown).

The pH of the medium has very little effect on the rate of ferricyanide reduction by the ρ^0 cells over the pH range from 6.0 to 8.7 in 0.2 M phosphate buffer. There is a sharp decline in the rate of reduction as the pH decreased from pH 6.0. It should be noted that the normal growth condition for yeast is below pH 6.0. The affinity of the external reduction site in ρ^0 cells for ferricyanide appears to be quite high. For stationary-phase cells the half maximal rate is reached at 13 μM ferricyanide and for exponential-phase cells the half maximal rate is at 45 μM ferricyanide. The maximal rate of ferricyanide reduction by exponential-phase ρ^0 cells is at least five times greater than the rate of reduction by stationary-phase cells. As shown in Fig. 3, the change in ferricyanide reduction rate is very abrupt as the cells enter the stationary phase.

Inhibitors of mitochondrial electron transport do not inhibit the ferricyanide reduction by ρ^0 cells (Table II). On the other hand, the initial rate of ferricyanide reduction by the ρ^+ cells appears to have a component which is inhibited by mitochondrial electron transport inhibitors. Cyanide, azide, and 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) all show an inhibition. This effect suggests that the early rapid absorption changes at 400 nm in the ρ^+

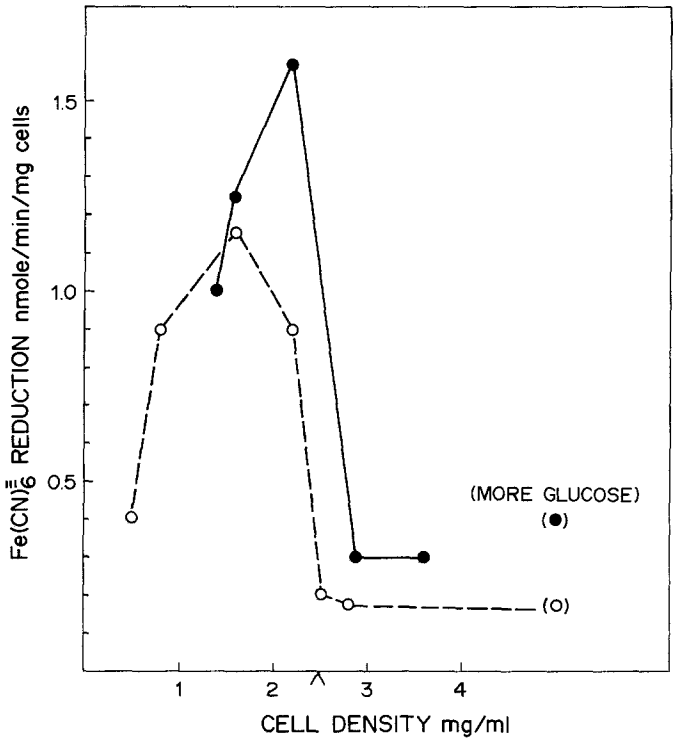

Fig. 3. Ferricyanide reduction by J691B ρ^+ and EJO ρ^0 yeast cells harvested at different growth stages. Cells grown aerobically on 2% glucose with glucose exhaustion at cell density of 2.5. Ferricyanide reduction assay as for Fig. 1 except that the buffer was 0.2 M potassium phosphate, pH 8.0. ●, ρ^+ cells; ○, ρ^0 cells. The mark at 2.5 mg/ml indicates where glucose would be exhausted except for cells shown as (more glucose) grown at 5% glucose.

cells are in some manner partly related to mitochondrial function. Because of this apparent mitochondrial involvement, we have concentrated the studies on the ρ^0 cells.

p-Hydroxymercuribenzoate is an inhibitor of the NADH ferricyanide reductase activity of microsomes. At 5×10^{-5} M there is only a slight inhibition of ρ^0 cell ferricyanide reduction, whereas this concentration of *p*-hydroxymercuribenzoate would completely inhibit microsomal activity (Huang *et al.*, 1979).

Ferricyanide reduction by yeast cells is favored in a medium of high ionic strength. The best activity we have observed is in the presence of sodium salts (Table III). On the other hand, potassium salts tend to give lower activity as compared to Tris or sodium. The divalent cations magnesium and calcium appear to give similar stimulation of the activity. The maximal stimulation by Mg^{2+} and Ca^{2+} occurred at 7×10^{-4} M.

Table II. Effect of Mitochondrial and Microsomal Electron Transport Inhibitors on the Initial Rapid and Slow Ferricyanide Reduction Rate by Respiratory-Component ρ^+ Yeast Cells and ρ^0 Cells[a]

Inhibitor added		Ferricyanide reduction (nmol/min/mg dry wt.)		
		ρ^+ initial	ρ^+ after 2 min	ρ^0 π^0
None	Tris Cl, pH 8.0	2.06	0.81	0.43
NaN_3, 3.7×10^{-3} M		1.37	1.37	0.53
None	KPO_4, pH 7.0	3.3	1.08	
HOQNO, 3.7×10^{-5} M		1.21	1.21	
None	Tris Cl, pH 8.0	1.12	0.89	
KCN, 1×10^{-3} M		0.72	0.72	
None	KPO_4, pH 8.0			0.86
KCN, 1×10^{-3} M	KPO_4, pH 8.0			0.93
None	KPO_4, pH 8.0	3.3	1.1	0.92
PCMB, 3.7×10^{-3} M		3.3	1.2	0.77
None	0.2 M KPO_4, pH 8.0			0.14
HOQNO, 3.6×10^{-5} M				0.22
KCN, 1.7×10^{-3} M				0.15

[a] Different control rates represent different cell preparations or different assay conditions. KPO_4 indicates 0.2 M potassium phosphate buffer. HOQNO is 2-heptyl-4-hydroxyquinoline-*N*-oxide.

Uncoupler Effects

A transmembrane NADH dehydrogenase could involve the movement of protons into or through the membrane if the final electron acceptor, such as ferricyanide, does not become protonated when it is reduced. If proton migration through the membrane is required, it may serve to limit the rate of electron transport. Several compounds, referred to as uncouplers, have been developed from study of proton gradient formation in mitochondria which facilitate proton movement through membranes and thereby stimulate electron transport. We have tested the effect of one of these protonophoric

Table III. Effect of Salts on Ferricyanide Reduction by ρ^0 Yeast Cells

Salt added	Concentration	Percent stimulation from 60 mM Tris Cl, pH 8.0
Potassium phosphate, pH 8.0	0.2 M	56%
Sodium phosphate, pH 8.0	0.2 M	79%
Sodium chloride	0.2 M	200%
Sodium sulfate	0.2 M	330%
Sodium acetate	7×10^{-3} M	30%
Potassium chloride	7×10^{-3} M	−18%
Magnesium chloride	7×10^{-4} M	53%
Calcium chloride	7×10^{-4} M	53%

uncouplers, carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) on the rate of ferricyanide reduction. The stimulation observed with low concentrations of CCCP is consistent with the idea that proton movement through the membrane is necessary for the maximum rate of electron transport (Fig. 4) (Bakker *et al.*, 1975).

Ferricyanide-Stimulated Proton Release

In order to test directly for movement of protons across the plasmalemma associated with ferricyanide reduction, we have measured the changes in pH of a suspension of yeast cells when ferricyanide is added. A low concentration of buffer is used in the medium so that changes in pH can be followed with a pH meter.

When yeast cells are suspended in a buffer above pH 5.6, they will

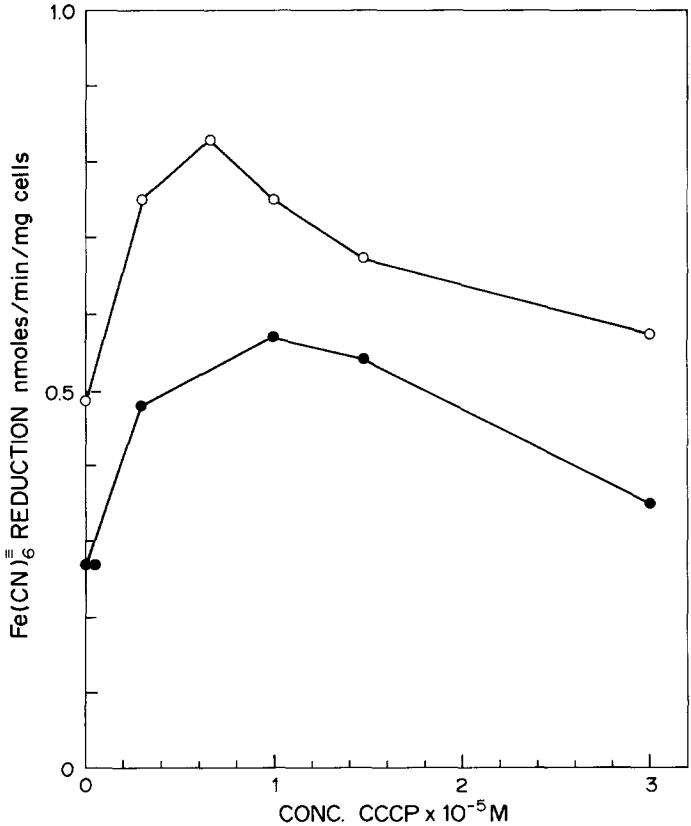

Fig. 4. Effect of carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) on ferricyanide reduction by ρ^0 yeast cells. Assay as for Fig. 1 in (●) 60 mM Tris chloride, pH 8.0, and (○) with 6.6×10^{-4} M magnesium chloride.

release protons causing a lowering of the pH of the medium. In normal ρ^+ cells the release of protons is very rapid (Rothstein and Enns, 1946; Riemersma and Alsbach, 1974). Evidence has been presented to show that this rapid proton extrusion depends on an ATPase in the plasma membrane (Misra and Höfer, 1975; Serrano, 1980). When cyanide is added to the ρ^+ cells to inhibit mitochondrial function, the rate of proton release is decreased to about 2% of the uninhibited rate (Table IV). The ρ^0 cells show only a slow rate of proton extrusion similar to that of the ρ^+ cells in presence of cyanide. The addition of ferricyanide to either ρ^+ cells, treated with cyanide, or ρ^0 cells stimulates a proton release into the media. The quantitative increase in proton release is the same order of magnitude as the rate of ferricyanide reduction by the same cells (Table IV). If the protonophoric uncouplers CCCP or 2,4-dinitrophenol are added, the ferricyanide-induced increase in proton release is eliminated. The ferricyanide reduction rate is stimulated by CCCP. The effect of 2,4-dinitrophenol on ferricyanide reduction rate cannot be measured because of interference with absorbance at 400 nm.

Proton permeability of the plasmalemmae can also be increased by heating the cells to 68°C for 15 min (Misra and Höfer, 1975). After such a treatment proton release is greatly reduced and the stimulation by ferricyanide is eliminated.

Transmembrane Redox Acceptors

The nature of the natural electron acceptor for the transmembrane dehydrogenase is not clear. The most obvious possibility would be oxygen through part of the cyanide-insensitive oxidase which is present in both ρ^+ and ρ^0 cells (DeTroostemberg and Nyms, 1978; Downie and Garland, 1973). At present it is not possible to test whether the cyanide-insensitive oxidase is transmembranous. The different electron acceptors which have been used are shown in Table V. We have used indigotetrasulfonate as an impermeable alternate to ferricyanide. It gives a similar rate of transmembrane electron transport to that with ferricyanide.

Cytochrome *c* added to the intact cells shows only a very slow rate of reduction, so it appears to be a very poor acceptor for the transmembrane electron transport. There is a tenfold increase of cytochrome *c* reduction compared to ferricyanide reduction when cells are broken by shaking with glass beads.

Spheroplasts of yeast cells were prepared to increase accessibility of sites on the cell membrane surface for ferricyanide reduction. However, spheroplasts were too fragile since cytochrome *c* oxidase becomes more apparent in preparations from ρ^+ cells and therefore cyanide must be present to observe cytochrome *c* reduction. It should be noted that cytochrome *c* reduction by whole cells is inhibited by 10^{-3} M cyanide which indicates a nonmitochondrial type of reductase. The lack of inhibition by superoxide dismutase (Table V)

Table IV. Effect of Ferricyanide on the Rate of Proton Release by Yeast Cells[a]

Cell preparation	Additions	Rate of ferricyanide reduction (nmol/min/mg)	Rate of proton release (nmol H^+ min/mg)	Increase of proton release in presence of ferricyanide (Δ nmol H^+/min/mg)
J69 ρ^+	—	0.26	105	—
J69 ρ^+	KCN 10^{-3} M		1.7	—
J69 ρ^+	KCN + $Fe(CN)_6^{3-}$	0.20	1.9	0.20
ρ^0	—	—	0.12	
ρ^0	$Fe(CN)_6^{3-}$	0.13	0.31	0.19
ρ^0	—	—	0.75	
ρ^0	$Fe(CN)_6^{3-}$	0.27	0.98	0.23
ρ^0	$Fe(CN)_6^{3-}$ + CCCP	0.375	0.75	0.0
ρ^0	—	—	1.1	
ρ^0	$Fe(CN)_6$	0.87	1.75	0.65
ρ^0	$Fe(CN)_6^{3-1}$ + 2,4-DNP	—	0.97	−0.13
ρ^0	heated 68°	—	0.65	
ρ^0	heated + $Fe(CN)_6^{3-}$		0.60	−0.05

[a]The rate of ferricyanide reduction was determined separately by the spectrophotometric procedure. Each series represents a different batch of cells at different growth stages. The initial pH was adjusted to 7.0 to 7.8 with 0.01 ml 0.1 M KPO_4, pH 8.0, plus a few microliters of 0.05 M NaOH to start proton release. Concentration of CCCP is 0.6×10^{-5} M and that of 2,4-dinitrophenol is 1.4×10^{-4} M.

Table V. Reduction Rates for Various Acceptors with ρ^+ and ρ^0 Yeast Cells

Preparation	Acceptor (nmol/min/mg dry wt.)				
	Oxygen (O) 2 e^-	Indigo tetra-sulfonate	Ferricyanide	Cytochrome *c*	$\frac{Fe(CN)_6}{Cyt.\ c}$
1. J69 ρ^+	7.8	—	1.08	0.0045	240
J69 ρ^+ + KCN	1.1	1.0	0.54	0	—
2. EJO ρ^0	0.67	0.2	0.39	0.0035	111
EJO ρ^0 + KCN			0.47	0	—
J69 spheroplasts[a]	4.0	0	9.2	1.4	6.6
EJO ρ^0 spheroplasts	0	0	0.97	0.39	2.5
3. EJO ρ^0 broken cells			12.2	1.35	9
EJO ρ^0			1.22	0.012	102
4. EJO ρ^0	0.56				
EJO ρ^0 + 10^{-3} M KCN	0.40				
5. EJO ρ^0			1.08		
EJO ρ^0 + 7500 units SOD[b]			1.13		

[a]Spheroplasts prepared by zymolyase digestion according to Cobon *et al.* (1974). Broken cells prepared by shaking 3.5 ml of cells at 60 mg dry wt./ml with 3 g glass beads for 2 min. The ferricyanide rate for broken cells is initial rate only. In contrast to whole cells the rate decreases rapidly over 5 min. Cytochrome *c* reduction with spheroplasts of J69 required cyanide to show activity because of cytochrome *c* oxidase.
[b]SOD, superoxide dismutase.

indicates that the donor of electrons is not superoxide ions secreted by the cells.

Oxidation of Cytochromes

If ferricyanide accepts electrons at sites on the interior of the cell by direct interaction with redox chains of mitochondria and microsomes, it would be expected to show a strong oxidation of the cytochromes on these membranes. If ferricyanide is acting on the exterior surface through a relatively slow electron carrier system, then it would be expected to cause only a minor shift in redox state of the internal cytochromes.

The oxidation of only a small part of the total cytochrome in the cell is observed when ferricyanide is added. If ethanol-reduced cells are oxidized with persulfate, there is a rapid oxidation of all cytochromes. On the other hand, the addition of ferricyanide to oxidize the cytochromes gives only a 5 to 25% oxidation of cytochromes compared to complete oxidation by persulfate (Table VI).

Discussion

The results of these studies are consistent with the concept of a transmembrane electron transport system across the plasma membrane of

Table VI. Changes in Redox State of Cytochromes *b* and *c* by Adding Ferricyanide to Whole Yeast Cells[a]

Cells	Treatment	Absorbancy change	
		Δ552–540 nm cyt. *c*	Δ562–540 nm cyt. *c*
J69 ρ^+ + KCN	$Fe(CN)_6^{3-}$	−0.0005	−0.0002
J69 ρ^+ + KCN	Pesulfate	−0.0020	−0.0040
ρ^0	$Fe(CN)_6^{3-}$	−0.0003	−0.0003
ρ^0	Pesulfate	−0.0010	−0.0013

[a] 10 μl of ethanol added to reduce cytochromes in all cells prior to addition of $Fe(CN_6^{3-}$ or persulfate.

yeast cells. The enzyme can react with the artificial nonpermeable electron acceptors, ferricyanide and indigotetrasulfonate, but shows very little activity with cytochrome *c*. If the transmembrane reduction were accomplished by movement of a reducing agent such as glutathione or a high-potential reductant through the membrane, then cytochrome *c* reduction would be expected. The low potential of indigotetrasulfonate (−0.07 V) would exclude reduction by high-potential carriers. The lack of inhibition by parahydroxymercuribenzoate, which has poor permeability in membranes, indicates that a sulfhydryl compound cannot be the transmembrane carrier.

The nature of the internal donor of electrons is not known, but NADH is likely to be the internal reductant. The requirement for ethanol to maintain activity in starved cells, especially in ρ^0 cells which cannot completely metabolize ethanol, indicates that NADH is the most likely electron donor. The NADH would be generated by alcohol dehydrogenase. This is further supported by inhibition of transmembrane reductase activity by pyrazole, an alcohol dehydrogenase inhibitor, when ethanol is the substrate (Lumeng, 1979). The ferricyanide reductase activity appears to be low when expressed on a dry weight of cells basis. If the plasma membrane protein makes up 1/100 to 1/200 of the total dry weight of the cells, then one can predict that the plasma membrane should have NADH ferricyanide reductase activity up to 200 nmol/min/mg protein. This would be in accord with values seen in other plasma membranes (Crane *et al.*, 1979).

The increased rate of acidification of the medium by yeast cells when ferricyanide is added indicates that the transmembrane ferricyanide reduction is accompanied by an export of protons. The proton transport appears to be stoichiometric to the ferricyanide reduction. The proton export seen in absence of ferricyanide may represent a similar transport when oxygen is the electron acceptor. Inhibition of the proton movement by the protonophoric uncouplers CCCP and dinitrophenol would be expected of a redox-linked proton transfer (Mitchell, 1976; Riemersma and Alsbach, 1974).

For ρ^0 cells the ferricyanide-induced proton release has been observed to represent up to one-half of the total proton release. It is possible that this activity could be associated with a special transport function as in selected amino acid transport (Kilberg and Christensen, 1979). The 100-fold greater proton export by ρ^+ cells is undoubtedly energized by mitochondrial ATP.

Except for the slower rate with potassium salts, the stimulation by salts is relatively nonspecific and can be accounted for as a screening effect against the negative surface charge of the yeast cell which would tend to repel ferricyanide ions (Borst-Pauwels, 1981). The parallel effects of magnesium and calcium ions appears to preclude a relation to the calcium-stimulated proton transport system (Hinnen and Racker, 1979; Stroobant and Scarborough, 1979). The effect of sulfate should be examined further in relation to sulfate transport (Roomans *et al.*, 1979). Phosphate transport has also been related to proton movement (Cockburn *et al.*, 1975).

The similarities between the ferricyanide reduction system of yeast plasma membrane and the transmembrane NADH ferricyanide reductase of erythrocytes is striking. In both cells the reduction of ferricyanide is accompanied by the transport of protons. The erythrocyte transmembrane dehydrogenase also shows ionic stimulation which can be related to surface charge screening. Ionophoric uncouplers stimulate the NADH ferricyanide reductase activity of the erythrocyte membrane at concentrations similar to those which stimulate yeast cell ferricyanide reduction (Löw *et al.*, 1979). Since the NADH dehydrogenase in erythrocyte and other animal cell membranes is responsive to hormones, it has been suggested that it may be involved in control of cell function (Löw and Crane, 1978; Löw and Werner 1976).

The components of the redox system in yeast plasmalemma are mostly unknown. The presence of a *b*-type cytochrome in plasma membranes from glucose-grown *Saccharomyces* and *Candida* yeast has been reported (Schneider *et al.*, 1979). Wiseman *et al.* (1978) have reported the presence of cytochrome P450 in glucose-grown yeast. This would correlate with the activity of the transmembrane dehydrogenase and might provide a link from the dehydrogenase to oxygen. Cytochromes *b* and P450 and P420 have been found in mammalian plasma membrane (Jarasch *et al.*, 1979) and in anaerobic yeast (Cartledge and Lloyd, 1972b). Nurminen *et al.* (1970) have made a careful study of previous reports of NADH oxidase activity in the cell wall fraction from *S. cerevisiae* and conclude that NADH oxidase is not present in the wall. The 2% of total activity they find in the wall fraction corresponds to 0.14 nmol/min/mg cells which would be appropriate to the oxidase activity found in the ρ^0 yeast. Data by Serrano (1978) after correction for 3.6% mitochondrial contamination in the plasma membrane fraction would allow NADH oxidase activity in plasma membrane equal to 4 nmol/min/mg protein. Cartledge and Lloyd (1972a, b) have studied the distribution of

NADH cytochrome *c* reductase in yeast membrane fractions. They find activity in membranes which have high and low oligomycin-sensitive ATPase activity. In glucose-repressed, anaerobically grown yeast they find that the major peak of NADH cytochrome *c* reductase accompanies the ATPase. The distribution of NADH ferricyanide reductase activity has not been studied in yeast membranes.

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