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Triton X-100 inhibition of yeast plasma membrane associated NADH-dependent redox activities

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Abstract

Plasma membrane (PM) vesicles isolated from the yeast *Saccharomyces cerevisiae* (wild-type NCIM 3078, and a MG 21290 mutant pma 1-1) were used to monitor the effect of the detergents, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (Chaps) and Triton X-100, on H^+-ATPase (E.C. 3.6.1.35), NADH oxidase and NADH- hexacynoferrate (III)[HCF (III)] oxidoreductase (E.C. 1.6.99.3) activities. The results obtained show that Triton X-100 inhibited both membrane bound and solubilized NADH-dependent redox activities. The nature of this inhibition as determined for NADH–HCF(III) oxidoreductase was non-competitive and the Ki values for wild and mutant enzymes were 1.2×10^{-5} M and 8.0×10^{-6} M, respectively. The findings are interpreted, in view of the established reports, that the active site architecture of PM bound NADH-dependent oxidoreductase in yeast is likely to be different than in other eukaryotes.

Keywords: *Saccharomyces cerevisiae*, plasma membrane, H^+-ATPase, NADH-HCF(III) oxidoreductase, triton X-100 inhibition, chaps

Introduction

The presence of plasma membrane bound redox activities in eukaryotic cells has been demonstrated either as transplasma membrane electron transport with tissue segments or cell preparations using membrane impermeable exogenous electron acceptors [1–3] or as NAD(P)H oxidase or NAD(P)H-acceptor oxidoreductase with isolated plasma membrane vesicles [3–6]. Presence of such redox activity in the yeast *Saccharomyces cerevisiae* has been reported [7–9]. The transplasma membrane electron transport induced by membrane impermeable exogenous electron acceptors is associated with H^+-extrusion [3,6]. Whether this H^+-extrusion is tightly coupled to induced transplasma membrane electron transport or is a consequence of plasma membrane H^+-ATPase activation resulting from depolarization of the membrane potential associated with such an electron transport is still debatable [10–12] nevertheless the involvement of such a redox system in many diverse cellular functions has been shown [3,5,6]. In a recent communication [13] we have shown that the activity of purified PM bound redox enzyme is modulated by concanavalin A (Con A), indicating the possibility of involvement of such a redox enzyme in cell signal transduction.

In our attempts to get a better understanding of the relationship between cellular functions and PM bound redox enzymes, we isolated plasma membrane vesicles from the cells of wild and H^+-ATPase deficient mutant of *Saccharomyces cerevisiae*. In the study presented in this paper, the activities of H^+-ATPase and NADH-dependent oxidoreductases in the isolated plasma membrane vesicles were found to be modulated in a different fashion in the presence and absence of a zwitterionic detergent 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (Chaps) and a non-ionic detergent Triton X-100. The comparison of activities indicated that contrary to stimulation of all the activities by Chaps, the NADH-dependent redox activities were inhibited by Triton

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X-100. The findings indicated that, according to our knowledge, this is the first report on Triton X-100 inhibition of PM bound NADH-linked redox activities in eukaryotic cells. It is interpreted, *vis-à-vis* to Triton X-100 activation of plant plasma membrane bound redox activities, that the active site architecture of yeast redox enzyme is likely to be different.

Materials and methods

Yeast strain and growth conditions

The mutant-strain of *Saccharomyces cerevisiae* (MG 21290 pma 1-1 having reduced H^+-ATPase, a kind gift from Prof. Andre Goffeau, University of Catholique de Louvain, Louvain-la-Neuve, Belgium) was cultivated for 24 h at 30°C after Ulaszewski *et al.* [14] in a medium consisting of bactopeptone (2.0%), glucose (2.0%) and yeast extract (1.0%). The wild-type cells of *Saccharomyces cerevisiae* (NCIM 3078, obtained from the National Chemical Laboratory, Pune, India) were cultivated in a medium containing malt extract (0.3%), bactopeptone (0.5%), glucose (1.0%) and yeast extract (0.3%) under similar conditions.

Isolation of plasma membrane vesicles and solubilization of proteins

Yeast cells harvested in mid-exponential growth phase were used for isolation of plasma membrane vesicles by the procedure given by us earlier [15]. Solubilization of plasma membrane bound proteins was carried out using Chaps (1.5%, w/v) in the membrane suspension by stirring for 30 min at 0–4°C [16]. The solubilized proteins were separated by centrifugation at 50,000 × *g* for 15 min, and the supernatant containing the desired proteins was passed through a Millipore filter (Millex-GV, pore size 0.22 μm) at 4°C. The solubilized enzyme preparation was partially purified and enriched by microcentrifugation at 4°C using Amicon's (MWCO-100) microconcentrator according to the 'operating procedure' provided by the manufacturer. The retentate of microcon-100 had the bulk of the redox activity.

Enzyme assay

Adenosine triphosphatase. The microassay of ATPase (EC 1.6.1.35) was performed at 28°C for 15 min after Delhez *et al.* [17] in a final volume of 0.33 ml containing 6 mM each of ATP and $MgCl_2$, 25 mM Tris–HCl buffer (pH 6.0 or 9.0) and 0.1 ml of membrane preparation containing 30–40 μg of protein in presence and absence of detergents, Chaps (0.02%, w/v) and Triton X-100 (0.01%, w/v). The reaction was stopped by the addition of 0.33 ml of 10% (w/v) TCA. The release of inorganic phosphate in the supernatant was measured by the method of Fiske and Subbarow [18].

Plasma membrane bound reductase. The procedure for measurement of enzyme activities was after Morre *et al.* [19] The assay was carried out both in presence and absence of detergent. The 3.0 ml assay volume contained 25 mM Tris–HCl buffer (pH 7.0), 100 mM sucrose, 10 mM each of NaCl and KCl and 30–40 μg membrane protein. The reaction was initiated by the addition of 100 μM NADH. In the case of NADH-HCF(III) oxidoreductase, incorporation of 0.5 mM HCF(III) was made prior to NADH addition. The decrease in optical density with time at 28°C was recorded at 340 nm.
Millimolar extinction values of 1.0 for HCF(III) and 6.22 for NADH were used in the calculation. The values reported are after appropriate corrections for the rates of controls.

Protein estimation. The protein was measured by Bradford's method [20]. BSA was used as standard run.

Results and discussion

The data in Table I showed the stimulation of plasma membrane ATPase in presence of detergents both in wild and mutant strains of the yeast. The stimulation of this activity was relatively higher in the presence of Triton X-100. Stimulation of PM ATPases by detergents is well known [21,22] and the findings of Table I are also in agreement with the established reports. However, the effect of these detergents on the plasma membrane bound redox activities, NADH oxidase and NADH-HCF(III) oxidoreductase (Table II), was opposite in nature. Chaps stimulated both the redox activities albeit the extent of stimulation was widely different. The NADH oxidase, which was generally reported to be very low [19,23] was not detected in the absence of Chaps in the plasma membrane vesicles of the mutant-strain, but NADH-HCF(III) oxidoreductase was measurable with these preparations in the absence of detergents. Contrary to the stimulatory effect of Chaps on these redox activities, Triton X-100

Table I. Effect of detergents on membrane bound H^+-ATPase activity.

	Specific activity (nmoles Pi released $min^{-1} mg^{-1}$ protein)	
Sample	Wild	Mutant
Control	53.6 ± 2.35	38.0 ± 2.0
+ Chaps	97.8 ± 5.49	72.0 ± 0.00
+ Triton X-100	131.4 ± 6.0	76.2 ± 12.9

The concentration of Chaps was 0.02% (w/v). The concentration of Triton X-100 was 0.01% (w/v). The values are mean ± S.D. of 3 independent experiments.

Table II. Effect of detergents on redox activities associated with plasma membrane vesicles isolated from the yeast *Saccharomyces cerevisiae*.

	Redox activity (nmoles NADH oxidized min^{-1} mg^{-1} protein)	
Enzyme	Wild	Mutant
NADH oxidase		
Control	7.81 ± 4.51	0.00
+Chaps	19.25 ± 5.44	6.82 ± 4.8
+Triton X-100	5.24 ± 3.24	0.00 ± 0.00
NADH-HCF (III) oxidoreductase		
Control	18.29 ± 4.38	12.48 ± 4.42
+Chaps	27.3 ± 4.82	18.73 ± 6.00
+Triton X-100	13.04 ± 4.3	0.00 ± 0.00

a Values are average ± standard deviation of 3 independent incubation experiments. The concentration(w/v) of Chaps was 0.02% and that of Triton X-100 was 0.01%.

inhibited both NADH oxidase and NADH-HCF (III) oxidoreductase. The extent of inhibition was about 30 percent in both the activities associated with vesicles of the wild strain. In the case of NADH oxidase associated with membrane vesicles of the mutant strain, Triton X-100, unlike Chaps, failed to elicit any stimulatory response but at the same time this detergent completely inhibited the membrane associated NADH-HCF(III) oxidoreductase activity. It was, therefore, inferred that Triton X-100 caused inhibition of plasma membrane bound NADH-dependent oxidoreductase in yeast *Saccharomyces cerevisiae*. In order to evaluate this effect of Triton X-100 on solubilized and partially purified enzyme preparations, we treated the membrane vesicles with higher concentration of Chaps (1.5%, w/v) and the resulting solubilized preparation was subjected to microcentrifugation using Microcon concentrators (as described under Materials and Methods) to partially purify the solubilized preparation. The effect of Triton X-100 was checked on the redox activities present in these preparations and the results are presented in Table III. In the case of the wild strain the Triton X-100 inhibition of solubilized NADH oxidase was about 70% but with relatively purified preparation 100 percent inhibition of this activity was found, however in the case of the mutant strain there was 100 percent inhibition in both cases. Similarly, the extent of inhibition of solubilized and purified NADH-HCF(III) oxidoreductase in the wild strain was 80% and 95%, respectively. These values were 100% both in solubilized and purified preparations in the mutant strain. These findings are therefore clearly in support of the results presented in Table II, establishing that Triton X-100 was inhibiting the NADH-dependent redox activities whether membrane bound or present in solution. Besides, these activities were more sensitive to Triton X-100 inhibition in soluble and relatively purer preparations.

The stimulatory effect of detergents on particulate bound enzyme activities is well known [24]. The stimulating effect of Chaps and Triton X-100 on a variety of membrane bound enzymes has been reported [5,25]. The findings were explained on the presumption that detergents open up the vesicle membranes to give the enzyme free access to its substrate [26]. However, some reports [21,22] suggested that detergents can directly activate membrane bound enzymes besides altering vesicle permeability to substrate. Stimulation of plant plasma membrane bound NADH-dependent redox activities by Triton X-100 has been reported by different workers [27,28]. On the other hand, Triton X-100 inhibition of NADH-dependent redox activities associated with the mammalian mitochondrial membrane has been reported [29–32]. According to our knowledge, inhibition of NADH-dependent PM redox activity by Triton X-100 has not been reported so far. In view of the reported [33] non-specific inhibition of enzymes by sub-micrometer aggregates, it is important to mention that the amount of Triton X-100 used in

Table III. Effect of Triton X-100 on Chaps solubilized and partially purified redox activities from the plasma membrane vesicles of yeast *Saccharomyces cerevisiae*.

	Redox Activity (nmoles NADH oxidized min^{-1} mg^{-1} protein)			
	Wild		Mutant	
Enzyme	Solubilized	Purified*	Solubilized	Purified*
NADH-oxidase				
Control	25.71 ± 4.48	196.62 ± 42.72	10.24 ± 0.00	118.79 ± 0.00
+Triton X-100	7.80 ± 4.51	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NADH-HCF(III) oxidoreductase				
Control	38.5 ± 4.1	967.96 ± 21.39	20.48 ± 0.00	673.18 ± 56.00
+Triton X-100	7.80 ± 4.25	45.37 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*The preparation was partially purified using Microcon microconcentrators, as per the procedure described in the text.
The concentration of Triton X-100 was 0.01% (w/v). Values are mean ± standard deviation of 3 independent experiments.

the present studies was much below the critical miceller concentration value of 1.3 mM, as reported for this detergent [34]. Further, it may be added that physical association of enzyme with sub-micrometer aggregates is prevented by Triton X-100 [33]. It was therefore logical to infer that the inhibitory effect of Triton X-100 in the present studies was not due to physical aggregation of protein, lipid and the detergent. Further, the solubilized and partially purified enzyme preparations from wild and mutant strains were used to measure Ki values for Triton X-100 inhibition of NADH-HCF (III) oxidoreductases by the Dixon method [35]. The data from these studies are presented in Figure 1(A and B). It is evident that Triton X-100 inhibition with both the enzyme preparations was of the non-competitive type and the Ki values obtained for activities in wild and mutant strains were 1.2×10^{-5} M and 8.0×10^{-6} M, respectively. This indicated that Triton X-100 as an inhibitor has relatively more affinity for the enzyme in the mutant strain and the observations presented in Table III also support this view.

Ushakova et al. [31] have claimed that Triton X-100 is a specific inhibitor of mammalian mitochondrial NADH-ubiquinone oxidoreductase. In a recent report [36], Triton X-100 at high concentration (>1%) was shown to inhibit the activity of the enzyme undecaprenyl pyrophosphate synthase (UPPs) from *Escherichia coli*. The inhibitory effect has been presumed to be due to occupancy of the active site by the detergent. It is therefore to be emphasized that the amount of Triton X-100 used in our studies was comparable to that where it caused stimulation of plant plasma membrane bound NADH-acceptor oxidoreductase activities [27,28]. Therefore there is ample scope to believe that the active site architecture of PM bound NADH-dependent oxidoreductases in yeast may be different from that of the plant enzyme.

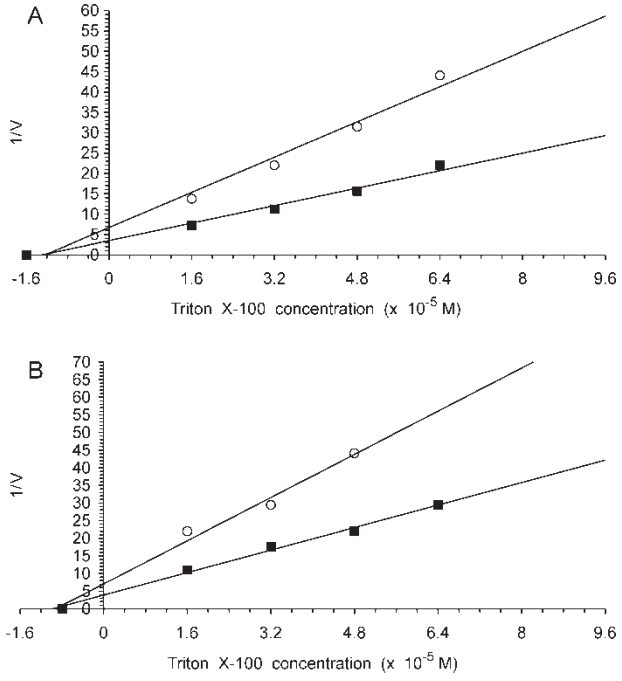

Figure 1. Kinetics of NADH-HCF (III) oxidoreductase activity of partially purified preparations in presence of Triton X-100 as inhibitor after the method of Dixon [35]. The experimental details are described in the text. Two NADH concentrations were used; 0.5 mM (O) and 1.0 mM (■) with both the preparations, A (wild) and B (mutant).

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