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Concanavalin A induced activity change in yeast PM-bound NADH-HCF(III) oxidoreductase

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Abstract

The activity of plasma membrane bound redox enzyme, NADH-HCF(III) oxidoreductase, in wild and mutant strains of the yeast *Saccharomyces cerevisiae* is modulated by Con A in a dose-dependent manner. The solubilized activity is enhanced at lower concentration and inhibited at higher concentration of Con A. The enzyme in mutant strain is more sensitive to inhibition. The activation of enzyme by Con A is suppressed in the presence of either α-methyl-D-mannoside or 2-deoxy-D-glucose, indicating the glycoproteic nature of enzyme as well as the resulting conformational change due to interaction with Con A as the factor for modulated activities. This was supported by recording the decrease in K_m value of enzyme with respect to substrate NADH in the presence of lower concentration of Con A. The purified enzyme was more sensitive to lectin stimulation and, on the basis of comparative stimulatory effects of Con A and PSA on activity, it is likely that mannosyl moiety in enzyme is involved in binding the lectins to cause enzymic activation.

Keywords: Plasma membrane; *Saccharomyces cerevisiae*; Redox activity; Con A; PSA

1. Introduction

Lectins have been proved to be useful tools in the study of cell surface glycoproteins involved in initiating various cellular responses [1–4]. Con A is the most widely used plant lectin that demonstrated its influence on mitochondrial respiration [5], PM potential [6], ATP turnover [7], endocytosis of adrenergic receptors [8] and apoptosis [9]. In addition, Con A has also been implicated to modulate the activity of enzymes and proteins [10–12]. Alteration of temperature dependency of rat plasma membrane bound Mg^{2+}-ATPase has been found to be due to lectin-induced activation of enzyme [11]. Also, in vivo activation of enzymes/proteins by Con A is attributed to be due to induced cell signaling resulting in covalent modification of proteins [13]. In thymocytes, ROS are identified to be modulators of signaling cascade initiated by Con A [14]. Regulation of protein-C activity has been attributed to be due to interaction between Con A and oligosaccharide moieties of protein-C [12]. High affinity of Con A for terminal α-D-glucosyl and α-D-mannosyl residues is known [15]. However, the precise mechanism by which lectins function to regulate cell activities is far from clear.

In this paper, we show that the interaction of Con A with *Saccharomyces cerevisiae* (wild and mutant strains) PM redox enzyme, NADH-HCF(III) oxidoreductase, modulated its activity in a dose-dependent manner. The studies with membrane solubilized enzyme indicated that Con A interaction with enzyme changed its affinity for substrate. The findings with Con A and PSA effects indicated that mannosyl moiety of the enzyme plays an important role in determining its conformation in the presence of lectin.

2. Materials and methods

2.1. Yeast strains and growth conditions

The mutant-strain of *S. cerevisiae* (MG 21290 pma 1-1 having reduced PM H^+-ATPase, a kind gift from Prof. Andre Goffeau, University Catholique de Louvain, Louvain-la-Neuve, Belgium) was cultivated for 24 h at 30 °C after Ulaszewski et al. [16] in a medium consisting bacto-

Abbreviations: BSA, bovine serum albumin; Chaps, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate; Con A, concanavalin A; HCF(III), ferricyanide; MWCO, molecular weight cut off; PM, plasma membrane; PSA, *Pisum sativum* agglutinin; ROS, reactive oxygen species

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peptone (2.0%), glucose (2.0%) and yeast extract (1.0%). The wild-type cells of *S. cerevisiae* (NCIM 3078, obtained from 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.

2.2. Isolation of PM vesicles and solubilization of proteins

The isolation of PM vesicles from *S. cerevisiae* cells was as per procedure described by us earlier [17]. Solubilization of PM bound proteins was carried out using Chaps (1.5%, w/v) in the membrane suspension by stirring for 30 min at 0–4 °C [18]. The solubilized proteins were separated by centrifugation at 50,000 × *g* for 15 min, and the supernatant containing the desired protein was passed through a Millipore filter (Millex-GV, pore size 0.22 μm) at 4 °C. The solubilized enzyme was partially purified and enriched by microcentrifugation at 4 °C using Amicon's (MWCO-100) microconcentrator as per 'operating procedure' provided by the manufacturer. The retentate of the Microcone-100 had bulk of redox activity.

2.3. Purification of enzyme

The purification of solubilized and partially enriched enzyme preparation was carried out by affinity chromatography the procedure of which was broadly based after Parez-Campos et al. [19]. The enzyme was applied directly to a Con A–Sepharose 4B column at a flow rate of 0.5 ml/min. The affinity column had been previously calibrated with a buffer (100 mM Tris–HCl pH 7.5) containing 1% Chaps, 0.1 mM EDTA, 0.1 mM PMSF, 0.1 mM DTT, 1.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM $MnCl_2$, 0.5 M NaCl and 30% (v/v) glycerol. The enzyme loaded column was washed with 10-column volumes of the same buffer and 0.5-ml fractions were collected in tubes containing 200 μl of 30% (v/v) glycerol. The protein bound to the column was eluted by the addition of 0.2 M methyl-mannopyranoside solution and fractions were collected as above. The fractions were immediately tested for the presence of reductase activity. The fraction having maximum specific activity was taken for further studies with Con A and PSA bindings.

2.4. Enzyme assay

The procedure for measurement of NADH-HCF(III) oxidoreductase activity was after Morre et al. [20]. The assay was carried out both in presence and absence of detergent. The decrease in optical density with time at 28 °C was recorded at 340 nm. Millimolar extinction value of 6.22 for NADH was used in calculation. The values reported are after appropriate corrections for the rates of controls.

2.5. Estimation of protein

The protein was measured by Bradford method [21]. BSA was used as standard run.

3. Results and discussion

3.1. Effect of Con A on NADH-HCF(III) oxidoreductase activity associated with PM vesicles

The presence of NADH-acceptor oxidoreductase activities in the PM of eukaryotic cells is well known [22,23]. The PM of yeast *S. cerevisiae* has also been shown to possess such redox activity [17]. Presence of this redox activity at the external surface of protoplasts of *Cuscuta reflexa* was

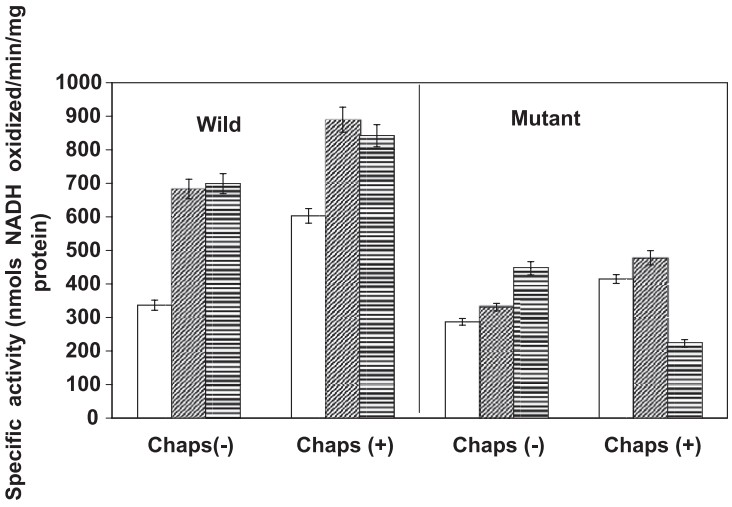

Fig. 1. Effect of concanavalin A on PM vesicle-bound NADH-HCF(III) oxidoreductase activity. The 3.0-ml assay mixture contained 25 mM Tris–HCl buffer (pH 7.0), sucrose (100 mM)–salt (10 mM each of KCl and NaCl) solution, 0.1-ml membrane preparation (30–40 μg protein) and 0.5 mM HCF(III) in the presence and absence of Chaps (0.2%). The reaction was initiated by NADH (100 μM) addition. (▭) Control, (▨) Con A (10 μg/3 ml), (▤) Con A (50 μg/3.0 ml).

reported earlier from this laboratory by demonstrating its sensitivity to exogenous Con A [3]. This also indicated the glycoproteic nature of this activity. Similarly, in present studies, we attempted to test whether such a redox activity in *S. cerevisiae* was also sensitive to lectin. The findings of these studies are presented in Fig. 1.

It was indicated that this redox activity associated with yeast PM vesicles was stimulated by relatively lower concentration (10 μg/3.0 ml) of Con A in assay medium added immediately before the initiation of reaction both in the absence and presence of detergent, Chaps, in wild and mutant strains. The percent stimulation of activity was more than 100 in the absence of detergent in wild strain, but in mutant strain this stimulation was marginally higher and nearly the same both in the absence and presence of detergent. On increasing the Con A concentration in assay medium to 50 μg/3.0 ml, there was no significant change in activities compared to the values seen in the presence of lower concentration of Con A in wild cells, but in the case of mutant cells, there was an opposing effect in the absence and presence of Chaps; whereas in the absence of Chaps there was stimulation of activity by Con A but in the presence of Chaps there was significant inhibition (~ 50%). These observations indicated that binding of lectin to glycoproteins in the yeast PM, in all probability, was responsible for the observed changes in activities. It may be that either the redox enzyme itself is a glycoprotein or the other glycoproteins in the vicinity of the redox enzyme influenced its activity in presence of Con A. In order to ascertain any of these possibilities, it was important to study the effect of Con A on solubilized enzyme.

3.2. Effect of Con A on solubilized and partially purified NADH-HCF(III) oxidoreductase activity

Table 1 indicated the distribution of the solubilized redox activity in two fractions obtained on filtration through MWCO-100 filter. It showed that the enzyme has a molecular size greater than 100 kDa because the bulk of the solubilized activity was present in retentate both in wild and mutant strains. The findings of Table 2 indicated that at relatively lower concentration of Con A (10 μg/3.0 ml) in assay medium, there was activation of redox activity in solubilized and partially purified state. However, at higher concentration (50 μg/3.0 ml) there was marginal inhibition of activity with partially purified enzyme in case of wild strain but a significant inhibition (>75%) of activity in case the mutant strain was recorded. These findings are quite in line with the observations reported in presence of detergent for PM vesicles (Fig. 1). It was also to be noticed that the mutant enzyme was more sensitive to inhibition by higher concentration of Con A in the presence of detergent.

In a subsequent study, the effect of Con A on partially purified enzyme was further evaluated by incorporating varying amounts of Con A (1.0 to 50 μg) in assay system. The findings are presented in Fig. 2. It is indicated that stimulation of enzyme activity in wild strain occurred up to 10 μg Con A/3.0 ml of assay system, but in the case of mutant enzyme, this activation ceased beyond 7.5 μg of Con A. The inhibition of the activity at higher concentrations of Con A indicated that mutant enzyme was more susceptible than wild enzyme, thus confirming the findings of earlier experiments (Fig. 1 and Table 2).

Table 1
Microconcentrator filtration enrichment of NADH-HCF(III) oxidoreductase activity solubilized by Chaps from the PM vesicles of the yeast *S. cerevisiae*

Sample	Specific activity (nmol NADH oxidized/min/mg protein)	
	Wild	Mutant
Solubilized	747.78	207.06
MWCO-100 retentate	1270.44	962.22
MWCO-100 filtrate	187.31	173.70

The 3.0-ml assay mixture included 2.5 mM Tris–HCl buffer (pH 7.0), sucrose (100 mM)–salt (10 mM each of KCl and NaCl) solution, 0.1-ml solubilized and partially purified membrane preparation (30–40 μg protein) and 0.5 mM HCF(III). The reaction was initiated by NADH (100 μM) addition.

Table 2
Effect of concanavalin-A on plasma membrane solubilized and enriched NADH-HCF(III) oxidoreductase activity in the yeast *S. cerevisiae*[a]

Sample	Specific activity (nmol NADH oxidized/min/mg protein)	
	Wild	Mutant
Solubilized preparation	747.78	207.00
+10 μg Con A	955.49	258.7
+50 μg Con A	872.41	258.72
MWCO-100 retentate	1270.44	962.22
+10 μg Con A	1724.17	1202.78
+50 μg Con A	1225.10	240.56
MWCO-100 filtrate	187.31	173.70
+10 μg Con A	231.60	187.31
+50 μg Con A	173.70	93.65

The details of enzyme assay are same as described in Table 1.

[a] The values presented are representative of one such typical run from among many such experiments where the absolute values differed probably because of different extent of solubilization in each case but the pattern remained the same.

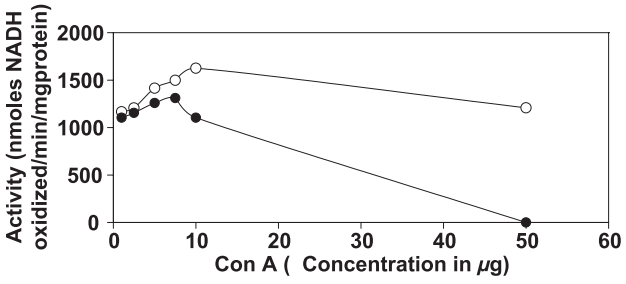

Fig. 2. Concentration-dependent effect of Con A on solubilized-enriched NADH-HCF(III) oxidoreductase activity. The experimental details are same as in Table 1. (○) Wild, (●) mutant.

Table 3
Effect of lectins on plasma membrane solubilized and affinity column purified NADH – HCF(III) oxidoreductase activity

NADH-HCF(III) oxidoreductase activity	Specific activity (nmol NADH oxidized/min/mg protein)	
	Wild	Mutant
Solubilized	422.24 ± 39.16	290.82 ± 23.53
Purified	1033.79 ± 80.20	355.29 ± 32.35
+Con A[a]		
0.05 μg	1162.95 ± 96.19	444.12 ± 42.23
0.1 μg	1472.64 ± 99.23	503.34 ± 41.88
0.3 μg	1588.12 ± 119.42	236.86 ± 31.87
0.5 μg	1227.23 ± 95.11	148.04 ± 21.88
+PSA[b]		
0.05 μg	1414.83 ± 85.72	503.33 ± 41.87
0.1 μg	1632.36 ± 110.47	710.59 ± 72.53
0.3 μg	1201.27 ± 93.54	651.38 ± 41.88

The details of enzyme assay are described under Table 1.
[a] Specific for the presence of mannosyl and glucosyl moiety in enzyme.
[b] Specific for the presence of mannosyl moiety in enzyme.

3.3. Effect of lectin on purified enzyme

For the characterization of the enzyme, NADH-HCF(III) oxidoreductase, the activity was carried out with Sepharose-4B column-purified enzyme in the presence of Con A (α-D-glucosyl and α-D-mannosyl specific) and PSA (α-D-mannosyl specific). The results (Table 3) indicated that Con A still activated the enzyme but at much lower concentrations while it showed inhibition of activity at marginal higher concentrations. At the same time, the other lectin, PSA (α-D-mannosyl-specific), showed more stimulation of NADH-HCF(III) oxidoreductase activity at concentrations comparative to that of Con A. This was true for enzymes both in wild and mutant strains.

The dose-dependent effect of Con A on redox activity, as observed in present studies, was quite in agreement with the reported observations of Con A effect on anticoagulant activity of protein-C [12]. The observed modulation of activity by Con A led authors to explain that an oligomannosidic portion of protein-C participates in the regulation of its catalytic activity. Molecular events mediating cellular response to lectins are still not very well understood. The studies on Con A activation of rat thymocytes showed production of ROS which modulated signaling cascade [14], stimulation of cell cycle [24], and expression of actin sequestering peptide, thymosin beta 4 [25]. In order to explain the observed activation of pyruvate dehydrogenase by Con A in pig lymphocytes [10], it was suggested that Con A treatment enhanced cytoplasmic free [Ca^{2+}] which activated pyruvate dehydrogenase phosphatase. The action of this enzyme on dephosphorylation activation of pyruvate dehydrogenase is well known. Almost all these studies investigating the effect of Con A were carried out with cells where intracellular machinery was involved in modulating the end results ascribed to be caused by Con A. However, in present studies the activity of the purified redox enzyme was altered by lectins; therefore, it is a case of direct effect of Con A and PSA on enzyme protein. The observed changes in enzyme activity may be due to altered conformation in protein structure initiated by lectin binding to the carbohydrate chain of the protein. To evaluate the effect of lectin on redox activity supposedly due to binding to oligosaccharide chain of the enzyme, the effect of inhibitor sugars, sugars inhibiting Con A–polysaccharide interaction [26], on the efficacy of Con A activation of redox activity was tested. In this study, Con A was pretreated with these sugars prior to its incorporation to the assay media of the redox enzyme.

The findings of these studies presented in Fig. 3 indicated that pretreatment of Con A with either α-methyl-D-mannoside or 2-deoxy-D-glucose failed to elicit the same response as was noticed earlier with only Con A, thus confirming the interpretation of the findings presented in Fig. 1 and Table 2. It was thus logical to conclude that the conformational change in enzyme following lectin binding was responsible for the observed activation of enzyme activity. The comparative activation of enzyme by Con A and PSA (Table 3) suggested the involvement of mannosyl moiety in enzyme protein for the observed effect.

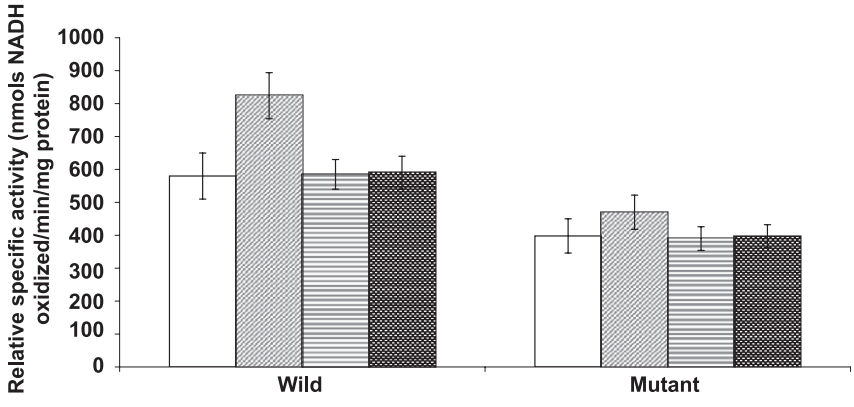

Fig. 3. Effect of sugars on Con A activated PM vesicle-bound NADH-HCF(III) oxidoreductase activity in the presence of Chaps (0.2%). The experimental details are same as in Fig. 1. (▭) Control, (▩) Con A (10 μg/3 ml), (▤) Con A + α-methyl-D-mannoside (1.0 mM), (▩) Con A + 2-deoxy-D-glucose (1.0 mM).

The binding of Con A and other lectins to carbohydrate moiety of glycoproteins resulting alterations in cellular activities is known [11,13,15]. Whether there occurred a change in the affinity of the redox enzyme for a substrate in the presence of lectins, in the present study, it was tested by measuring the Michaelis constant (K_m) of the enzyme for NADH in the presence of low concentration of Con A (six replicates each with enzyme preparation from wild and mutant strains) and comparison was made with the control values. The difference in K_m values in wild (control, $54.80 \pm 7.08 \times 10^{-5}$ M and experimental, $27.64 \pm 4.40 \times 10^{-5}$ M) and mutant (control, $64.23 \pm 12.70 \times 10^{-5}$ M and experimental, $30.43 \pm 6.57 \times 10^{-5}$ M) was statistically ($P < 0.05$) found to be significant. In addition, the findings also indicated that activation of purified enzyme was largely due to interaction between lectin and mannosyl unit of the enzyme. Activation of rat liver plasma membrane-bound Mg^{2+}-ATPase is known [11]. The temperature sensitivity of this activity was found to be counteracted by the lectins. The lectins were also reported to increase V_{max} rather than decrease the K_m. This was interpreted to be due to a change in enzyme's environment rather than in molecule itself. However, in the present studies the significant decrease in K_m value in the presence of low concentration of lectin and solubilized enzyme is suggestive of a change in enzymic conformation. Further, there are no qualitative changes between wild and mutant enzymic activities as a result of lectin effect. The observed quantitative changes between the two types of enzymes suggested that there could be marginal difference in either carbohydrate-chain length or/and active site architecture of the enzyme.

In brief, the findings have led to conclude that the carbohydrate moiety (α-D-mannosyl) on NADH-HCF(III) oxidoreductase protein in the PM of *S. cerevisiae* both in wild and mutant strains plays an important role in determining the conformation of the redox protein. It is likely that interaction of enzyme with Con A and/or other mitogens leads to a cascade of signal transduction thereby influencing the cellular activities. Involvement of MAP kinases in signal transduction is well established [27,28]. Further, according to our knowledge, this is the first report of direct activation of a purified enzyme activity by a lectin.

Acknowledgements

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