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Molecular Organization of the *Saccharomyces cerevisiae* Cell Wall

Molekularno ustrojstvo stanične stijenke *Saccharomyces cerevisiae*

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Summary

Saccharomyces cerevisiae *cell wall is composed of a glucan network in which different mannoproteins are interspersed. 2–3 % of the wall consists of chitin which plays a role in cell septation during the budding process. Interactions among wall components and the organization of these components in the cell wall are to a great extent unexplained. Recently, it has been observed that most proteins interact in vitro with glucan by hydrogen bonding at acidic pH in a rather nonspecific manner. Similar experiments have, therefore, been performed with other cell wall components and it has been found that proteins interact with chitin in a similar fashion, although not as strongly as with glucan. Glucan-glucan and glucan-chitin interactions have also been observed, but no binding of either proteins, glucan, or chitin to mannan chains of mannoproteins have been detected. Cell walls of intact cells bind proteins added to the yeast suspension very poorly. Similar results have also been obtained with isolated cell walls which still retained the shape of the cell. If cells are broken in a mechanical homogenizer at acidic pH, released intracellular proteins bind to the inner side of the wall efficiently. Results suggest a layered structure of the* ***S. cerevisiae*** *cell wall with mannan chains forming the outer, and glucan building the inner layer.*

Sažetak

Stanična stijenka kvasca ***Saccharomyces cerevisiae*** *izgrađena je od glukanske strukture u koju su ugrađeni različiti manoproteini. Hitin čini 2–3 % stijenke. Sudjeluje u odvajanju stanica tijekom procesa pupanja. Nije objašnjeno međudjelovanje staničnih komponenata i njihova organizacija u staničnoj stijenci. Nedavno je primijećeno da većina proteina nespecifično,* ***in vitro****, reagira s glukanom stvarajući vodikove veze u kiselom pH. Stoga su slični pokusi provedeni s drugim sastojcima stanične stijenke, te je utvrđeno da proteini na sličan način reagiraju i s hitinom, iako ne tako čvrsto kao s glukanom. Međudjelovanja glukan-glukan i glukan-hitin također su opažena, ali nije primijećeno vezanje bilo proteina, glukana ili hitina za mananske lance manoproteina. Stanične stijenke cijelih stanica vrlo slabo vežu proteine dodane u suspenziju kvasca. Slični rezultati dobiveni su i s izoliranim staničnim stijenkama koje su još zadržale izvorni oblik. Kada su stanice mehanički razbijene u kiselom pH, oslobođeni intracelularni proteini vrlo su se dobro vezali za unutrašnju stranu stijenke. Rezultati pokazuju slojevitu strukturu stanične stijenke kvasca* ***S. cerevisiae*** *s mananskim lancima koji tvore vanjski i glukanom koji izgrađuje unutrašnji sloj.*

Introduction

The characteristic morphology of yeast cell, its clearly defined shape, as well as morphological changes occurring during particular cell cycle events such as mating or sporulation, result from properties of the yeast cell wall. Therefore, this peripheral cellular structure has been a subject of extensive studies in many laboratories *(1-3)*. Particular attention has been paid to the structure and function(s) of the cell wall of *Saccharomyces cerevisiae* because of the importance of this yeast in both fundamental and applied research in practically all life sciences.

Cell wall of *S. cerevisiae* is composed of about 10–20 % protein and 80–90 % carbohydrates, glucan and mannan each contributing about half of this amount *(4,5)*. Chitin, the third cell wall polysaccharide, is located only in bud scars and constitutes not more than 2–3 % of wall carbohydrates *(6)*. The structure of individual cell wall com-

ponents has been studied in detail in several laboratories in the world and has therefore been well elucidated *(7-9)*. Their molecular organization, however, is still to a great extent unexplained. Except for the covalent attachment of mannan chains to proteins in the well established process of protein glycosylation *(10)*, which has already become a part of most biochemistry textbooks, little is known about intermolecular interactions of other cell wall components. The aim of this work was to elucidate potential interactions among these components and to gain insight into their molecular organization in the *S. cerevisiae* cell wall.

Experimental

*Preparation of **Saccharomyces cerevisiae** cell wall constituents*

Yeast glucan was isolated by the method of Peat et al. *(11)*, and the purity of the preparation was tested by thin-layer chromatography of glucan hydrolysis products as described *(12)*.

Chitin was prepared by the acetylation of chitosan according to the method of Molano et al. *(13)*.

Mannan was obtained by autoclaving yeast cells in citrate buffer, pH = 7.0 as described by Nakajima and Ballou *(14)*.

Crude extract of yeast intracellular proteins was obtained by disrupting cells in a Braun MSK homogenizer and the subsequent centrifugation of cell debris, 30 minutes at 15000 rpm.

Cell walls were prepared as described *(15)*.

Binding reactions

Protein-glucan and protein-chitin interactions were analyzed as described *(12)*. The effect of mannan on the protein-glucan or protein-chitin binding was examined by the addition of 100 μg mannan to the reaction mixture composed of 20 μg proteins and 100 μg of either glucan or chitin.

Laminarin-glucan and laminarin-chitin interactions were determined in a binding test containing 100 000 min^{-1} (100 μg) ^{14}C-laminarin and 60 μg of the insoluble polysaccharide.

Interactions of proteins with whole cells or purified cell walls were performed as described for protein-glucan interactions. Binding of intracellular proteins to the inner surface of the cell wall was analyzed by disrupting cells in a Braun MSK disintegrator in the 0.1 M Na-acetate buffer, pH = 3.8 and subsequent washing of cell walls with the same buffer, or with 0.1 M Tris-HCl buffer, pH = 8.5. Released proteins were analyzed by SDS-electrophoresis.

Other methods

SDS-electrophoresis was performed by the method of Laemmli *(16)*.

Proteins were determined as described by Lowry et al. *(17)*.

Neutral carbohydrates were assayed by the orcinol-H_2SO_4 method *(18)*.

Results and Discussion

To check for abilities of different cell wall constituents to react and bind to each other, a simple *in vitro* test has been established in our laboratory, by which it was possible to follow the binding of different soluble wall components to insoluble constituents *(12)*. First results already pointed out that *S. cerevisiae* cell wall proteins react with glucan, the major structural polysaccharide of the wall. However, most of the other proteins tested, among which most yeast intracellular proteins (Fig. 1, lanes 1 and 2) and also proteins from other sources, reacted with glucan chains as well, suggesting that the binding is unspecific. The nature of the interaction was shown to be predominantly hydrogen bonding, since reagents interfering with other types of chemical linkages, like high salt concentrations, EDTA, nonionic detergents, β-mercaptoethanol, or group-specific reagents, had little or no effect on the observed protein-glucan interactions. The reaction could only be prevented by the addition of urea. It was also noticed that proteins interacted with glucan only at pH lower than about 7, while at higher pH no interactions could be recorded. The interesting observation was that yeast periplasmic proteins, invertase and acid phosphatase failed to react with glucan *(12)*. Such result could explain why these proteins, albeit secreted to the periplasmic space, where they come in contact with glucan, still remain soluble periplasmic proteins *(19,20)*. The reason why periplasmic proteins differ from most other proteins in this property is not clear and probably resides in their particular tertiary structure, since no peculiarities in the amino acid composition or sequence of these proteins were recorded.

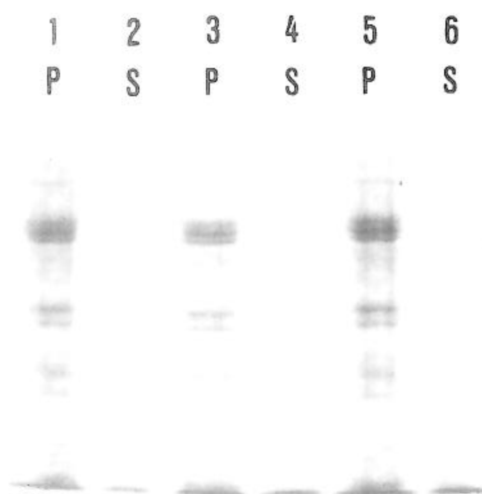

Fig. 1. Binding of yeast intracellular proteins to glucan and chitin. The binding was performed as described in Experimental, and proteins found in supernatants (S), or released from pellets (P) of reaction mixtures were subjected to SDS-electrophoresis. Lanes: 1 and 2, binding to glucan; 3 and 4, binding to chitin; 5 and 6, binding to glucan in the presence of mannan.

Slika 1. Vezanje unutarstaničnih proteina kvasca za glukan i hitin. Vezanje je provedeno kako je opisano u poglavlju Experimental, a proteini nađeni u supernatantu (S), odnosno otopljeni iz taloga (P) reakcijske smjese podvrgnuti su elektroforezi. Pruge: 1 i 2, vezanje za glukan; 3 i 4, vezanje za hitin; 5 i 6, vezanje za glukan uz prisutnost manana.

Although present only as a minor component in the cell wall, chitin plays a very important role in cell budding as the supporting material of the bud neck and also in the formation of the primary septum between the daughter and the mother cell *(21)*. Therefore, it was of interest to investigate if chitin also has binding properties observed for glucan. When the described binding test was applied to protein-chitin interactions, results were very similar to those obtained for the protein-glucan binding (Fig. 1, lanes 3 and 4). The binding was again unspecific, it occurred only at acidic pH and it was shown that the predominant type of interactions was the hydrogen-bonding. The binding of proteins to chitin was somewhat weaker than the reaction with glucan and the optimal pH for the reaction was shifted to the acidic pH (not shown). Interestingly, periplasmic proteins, invertase and acid phosphatase, again failed to interact with the polysaccharide, showing once more that for some reason these proteins have a smaller hydrogen-bonding potential at their surfaces than most other proteins.

Besides glucan, mannan is the most abundant *S. cerevisiae* cell wall component *(5)*. The term mannan actually corresponds to oligomannose chains of cell wall mannoproteins, obtained by the alkaline extraction of walls. Mannan chains are formed during the passage of proteins following the secretory pathway through the endoplasmic reticulum and Golgi *(10)*. Interactions between protein moieties of cell wall proteins and their mannan chains could account for additional interconnections among wall proteins. However, the standard *in vitro* binding test could not be used to study such interactions, since both proteins and mannan are water soluble. Therefore, an indirect approach was chosen in which mannan was added to the protein-glucan or protein-chitin reaction mixtures as a potential binding inhibitor. Results showed, however, that the addition of mannan could not inhibit any of the protein-polysaccharide interactions (Fig. 1, lanes 5 and 6), thus indicating that proteins do not bind to this cell wall constituent. Such a result is not surprising since in case of significant protein-mannan interactions, aggregation of mannoproteins with other proteins in cell extracts or other complex protein mixtures would have been expected, but has never been observed or reported.

Another interesting question was whether cell wall polysaccharides interact with one another. To establish if mannan can bind to glucan or chitin, the standard binding test was used, and the amount of mannan remaining in the supernatant, or bound to the insoluble polysaccharide was assayed by the orcinol method *(18)*. Results showed that neither glucan nor chitin interacted with mannan, since the whole amount of mannan was detected in the supernatant after the binding reaction.

In the attempt to test potential glucan-chitin interactions which may have particular physiological significance in the formation of the secondary septum which finally divides the daughter from the mother cell in the budding process, and which is composed of both chitin and glucan *(22)*, the fact that both carbohydrates are water-insoluble presented a problem. Therefore, laminarin, a soluble glucan analog which differs from glucan only in the length of carbohydrate chains, was used in the binding reaction. ^{14}C-labelled laminarin provided the

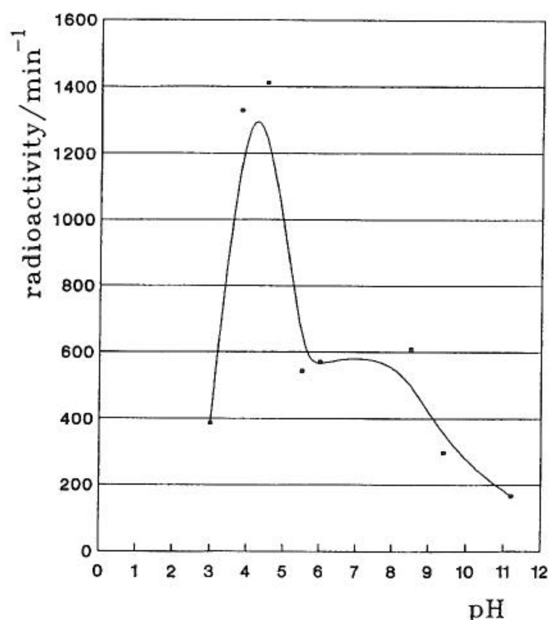

Fig. 2. Interactions of laminarin with chitin at different pH. ^{14}C-laminarin was bound to chitin at different pH, as described in Experimental, and the bound radioactivity was determined by scintillation counting

Slika 2. Međudjelovanja laminarina s hitinom pri različitim pH-vrijednostima. ^{14}C-laminarin je vezan za hitin pri različitim pH-vrijednostima kako je opisano u poglavlju Experimental, a vezana radioaktivnost je određivana scintilacijskim brojanjem

possibility to detect the bound oligosaccharide by counting the radioactivity. Results showed that laminarin indeed bound to chitin and that the reaction occurred in a broad pH-range from 3 to 9, but had an optimal pH at 4–5 (Fig. 2). Similar results were obtained for laminarin-glucan binding (not shown) which may reflect interactions between glucan chains in the cell wall, perhaps determining the density of the wall structural network.

To find out if interactions among purified cell wall constituents described so far could be extrapolated to intact cell walls as well, the binding of proteins to whole cells, or purified cell walls has been tested. Results revealed no binding of proteins to intact yeast cells, and a very weak binding to purified cell walls (not shown). Such results could be explained by a layered composition of the wall, first proposed by Zlotnik et al. *(23)*. According to this model, the outer layer of the *S. cerevisiae* cell wall is composed of mannan, and the inner layer is predominantly formed by glucan chains. As shown by our experiments, mannan does not interact with proteins, thus whole cells or purified cell walls, most of which still possess the original shape of the cell, are not able to bind externally added proteins. To support this idea, yeast cells were disrupted in a mechanical homogenizer under the binding conditions (i.e. at pH = 3.8) and the binding of released intracellular proteins to the inner surface of the cell wall was checked by solubilizing bound proteins at basic pH and subjecting them to SDS-electrophoresis. In that case pronounced interactions of proteins with the cell wall were detected (not shown) confirming a marked asymmetry in the *S. cerevisiae* cell wall com-

posed by an inner, protein-binding layer and an outerly localized material which does not interact with proteins. Such a composition may have a particular physiological significance for the yeast cell enabling the interactions of proteins secreted into the periplasmic space with the inner surface of the cell wall as the first step of their inclusion into the wall, but, at the same time, protecting the cell from potentially hazardous proteins reaching the cell from its surrounding.

The results presented in this paper indicate that the *Saccharomyces cerevisiae* cell wall is a complex cellular structure with a sophisticated molecular organization. Except for mannan chains of wall glycoproteins, which do not interact with any other cell wall component, all other wall constituents can react with each other. Although data strongly suggest that the result of these interactions is a defined molecular organization, the mechanism(s) for achieving this organization are so far completely unexplained.

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