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Covalent immobilization as a stimulus of cell wall composition changes

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Abstract. Covalent immobilization of yeast cells by an activated diamine spacer is accompanied by increased levels of cell wall proteins, lipids, amino sugars, amino acids and acid phosphatase leakage, and by altered composition of mannoproteins. The observed changes in cell wall composition are attributed to the effect of cell-solid surface contact.
Key words. Covalent immobilization; cell wall composition changes.

Phenotypic changes in soil and pathogenic microorganisms are often associated with cell attachment to a wide variety of solid surfaces[1]. This immobilization of whole, viable cells (surface colonization), determining their spatio-temporal position, may be a process of fundamental importance[2]. An essential question is whether cells use this physical contact – a multipoint linkage – purely for nutritional or protective purposes, or whether these interactions are also perceived as mechano-physiological signals (stimuli), modulating cellular structure or function. To obtain a deep insight, an in vitro simulation of possible types of natural immobilization is necessary.

Previous findings that covalently immobilized growing yeasts have an increased content of cell wall polysaccharides[3] as well as changes in membrane composition[4], encouraged the idea that multipoint covalent attachment of cells, simulating the natural conditions of cellular attachment[5], is sensed and responded to. In this connection, the cell wall chemistry of free and immobilized yeast cells was investigated in more detail. The multipoint, covalent linkage of cells to a physiologically inert support[6] was accomplished via dialdehyde-amino spacers.

Materials and methods

The strain of *Saccharomyces cerevisiae* Hansen 92 used in this study was a wild type supplied by the culture collection of the Prague Institute of Chemical Technology. It was grown aerobically in a rotary shaker at 28 °C in Olson-Johnson[7] synthetic medium (pH 4.7). The washed cells were prepared for immobilization by suspension in 50 mM citrate-phosphate buffer (also pH 4.7). The support for covalent immobilization was prepared by incorporating epichlorohydrin into the hydroxyalkyl methacrylate gel Separon H-1000 according to Jirků and Turková[6]. The epoxide derivative obtained (5 g) was suspended in 50 ml of 1 M tetra-methylene-diamine solution. After 48 h stirring at room temperature the suspension was transferred to the column and the gel was washed with 10 times its volume each of water, ethanol, butanol, and ethanol again. The content of attached diamine was determined from the amount of nitrogen, quantified by Kjeldahl's method. Unreacted epoxide groups were eliminated using overnight hydrolysis with 0.1 M $HClO_4$. The NH_2-Separon obtained was suspended in 10 ml of 10% v/v glutaraldehyde. After stirring the gel for 40 h it was washed with water until the reaction with 2,4-dinitrophenylhydrazine, indicating the presence of free gluteraldehyde, was negative. Afterwards the gel was washed with 300 ml of 50 mM citrate-phosphate buffer (pH 4.7). The suction-dried support (5 g) was placed in a yeast cell suspension (10^9 cells.ml^{-1}). After 5 h stirring at 20 °C the support was harvested and washed with 1000 ml of 50 mM citrate-phosphate buffer (pH 4.7, 28 °C). Immobilization was carried out under sterile conditions. The prepared free and immobilized cells were transferred to Olson-Johnson[7] medium and incubated at 28 °C on a rotary shaker. The total content of proteins, readily extractable and bound lipids, and amino sugars of isolated cell walls was analysed according to the methodology described by Cole et al.[8] Cell wall isolation and purification was carried out according to Jirků[9]. Free and immobilized cells suspended in 50 mM Tris-HCl buffer, pH 7.5, 1 M 2-mercaptoethanol, 1.2 M KCl, and 20 mM $MgSO_4$ were treated with *Arthrobactor luteus* lytic system containing neither mannanase nor protease activity. Cell walls were separated by differential centrifugation and washed to remove the lytic enzymes completely. Acid hydrolysis of freeze-dried walls was performed according to Puerser and Beuchler[10]. Amino acid profiles were determined by chromatography on a Hitachi amino acid autoanalyser. Extraction of covalently linked mannoproteins was carried out according to Kitamura[11], using laminarinase secreted by *Alternaria tenuissima*[12] in combination with separation on a

Table. Time course of the change in the amount of bound cell dry weight and free cell counts.

Incubation (h)	Bound cell dry weight (mg.g^{-1} dry support)[a]	Free cells (variable count.ml^{-1})[b]
0	1.9	–
4	3.1	30
6	3.8	75
8	4.6	115
10	5.7	217

[a]Determined on the basis of the amount of nitrogen, determined by Kjeldahl's method.
[b]Samples of cultivation medium devoid of support were applied to agar plates containing all components of Olson-Johnson[7] medium.

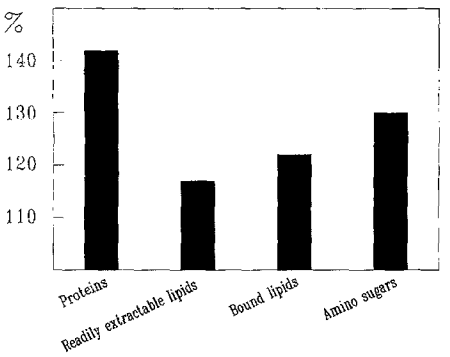

Figure 2. Protein, lipid, and amino sugar content in cell walls of immobilized *Saccharomyces cerevisiae* expressed as a percentage of the content of walls of free cells. Based on the values determined (as % of wall dwt) in the middle (4 h) of the cultivation period – see text. Values shown are the means of three independent determinations; ± SEs did not exceed 7.8%.

ConA-Sepharose column[13]. To remove non-covalently linked mannoproteins, cell walls were extracted in sodium dodecylsulphate[14]. The desalted mannoprotein fractions were fractionated on a Bio-Gel P-150 column. Acid phosphatase was assayed by the method described by Torriani[15]. The dry weight of free and bound cells was calculated from their nitrogen content, measured using Kjeldahl's procedure. Specimens of immobilized cell preparations were coated with gold and observed with a JEOL scanning electron microscope.

Results and discussion

A key element in covalent immobilization is the reaction of properly situated aldehyde groups of the support with reactive amino acid groups in the accessible cell surface proteins. Counts of free cells in the medium and the time course of changes in dry weight of bound cells (table) show that yeast cells immobilized in this manner grow and divide practically without leakage of progeny into the medium. The immediate immobilization of daughter cells during their longitudinal growth (fig. 1a) is assured by the content of homogeneously coupled diamine arms (778 µmol.g^{-1} dry support), extended with monomeric glutaraldehyde. The extent and homogeneity of this modification is determined by the basic composition[16] and previous epoxidation[6] of the support. The formation of chain-like filaments (fig. 1b) indicates a polarization of budding. The conversion of the ellipsoid shape of free *Saccharomyces cerevisiae* cells into rod-shaped forms of immobilized cells is probably caused by a cytomechanical effect[17] of the immobilization which could be similar to the cytomechanics of surface-associate growth of adhered cells[18], causing the same morphological changes. A comparison of total content of cell wall proteins, lipids, and amino sugars in free and immobilized cells shows (fig. 2) that the cell walls of immobilized yeasts contain increased amounts of each compound. The illustrated increase (fig. 2) is virtually stable over a 7 h interval of vegetative reproduction. In addition, except for serine and tyrosine, the

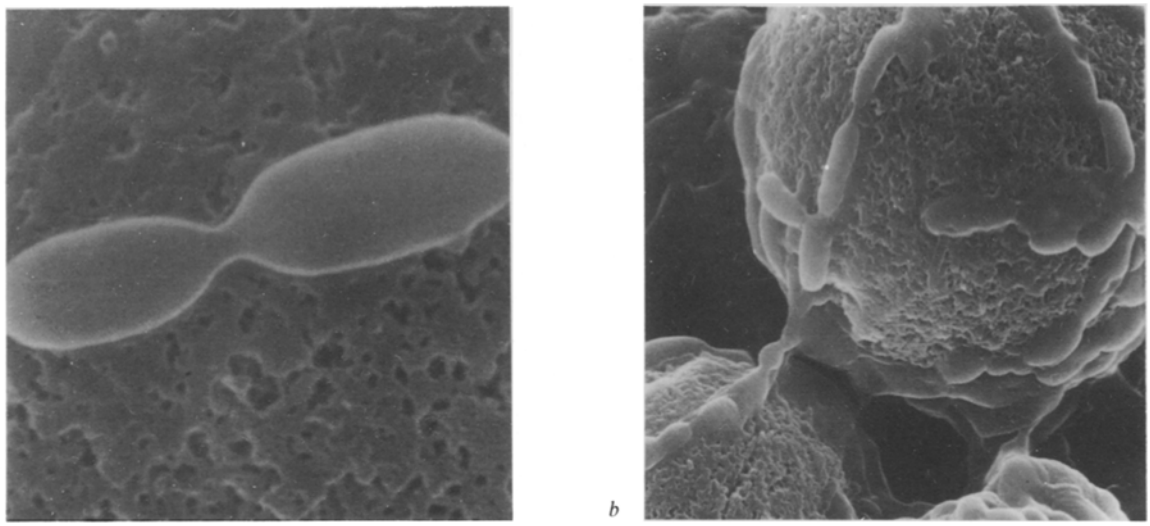

Figure 1. Scanning electron micrographs of immobilized *Saccharomyces cerevisiae* cells grown at 28 °C (*a* × 6000; *b* × 3000).

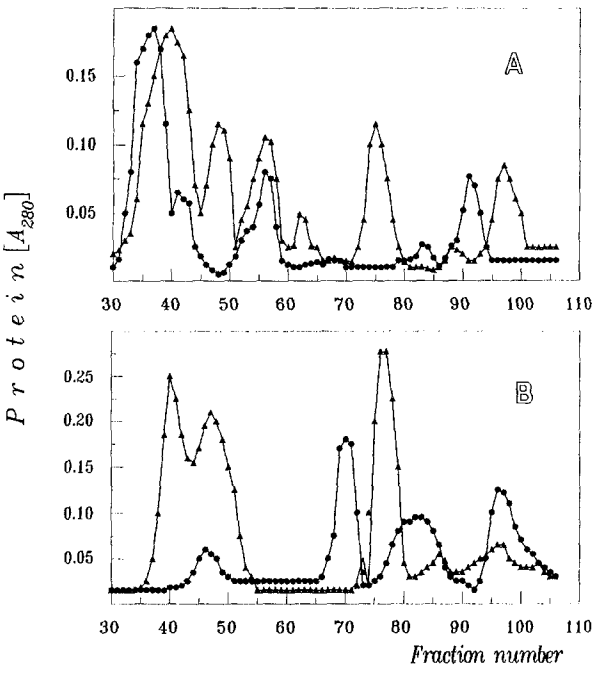

Figure 3. Gel chromatography profile of covalently (*A*) and non-covalently (*B*) linked mannoproteins of free (●) and immobilized (▲) *Saccharomyces cerevisiae* cells.

amino acid content of immobilized *Saccharomyces cerevisiae* walls were consistently higher (5–25%) than that of free cells (not shown). The 30% and 50% increase in serine and tyrosine could enhance the O-glycosidic linkage of short mannosyl chains via these amino acids residues[13]. It has been shown previously that the walls of covalently immobilized yeasts also have increased levels of β-1,3, β-1,6 glucans and mannan[3]. Considering the role of all components investigated we assume that the changes detected must primarily affect the rigid wall framework, as well as the anchorage and biochemical functions of mannoproteins which are an integral part of the cell wall matrix[19]. Therefore, the difference in the elution patterns of covalently or non-covalently bound mannoproteins, between fractions extracted from walls of free and immobilized cells, is not unexpected (fig. 3). Moreover, the strong leakage of acid phosphatase (a suspected mannoprotein[20]) into the culture medium of immobilized yeasts (fig. 4) also indicates changes in the cell wall. It is probably physical contact of the cell surface with the support that induces a change in total cell wall composition. Such a 'contact stimulation' of changes in cellular structure must be connected with the transfer of an informational signal (stimulus) from the cell microenvironment to the cell. However, the long-term stablity of the cell wall alterations observed suggests that their development is not stimulated by transient (physiological) stimuli in the cell surface microenvironment. On the other hand, if we assume that a single cell organism is capable of recognizing a physical contact, then at least some of the

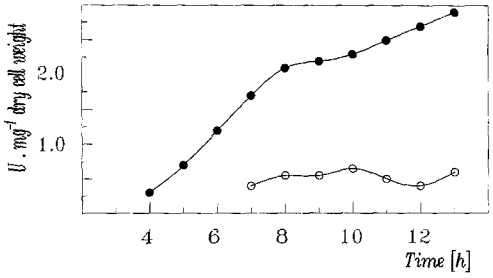

Figure 4. Profile of acid phosphatase leakage into the culture medium from free (○) and immobilized (●) *Saccharomyces cerevisiae*.

property changes of immobilized cells[21] could be direct or indirect consequences of two processes: 1) mechanical signal transduction across surface structures, 2) signal translation into activation of intracellular pathways, initiating events that result in a final property change. The stable alteration of plasma membrane composition, stimulated by the same covalent immobilization of yeast cells[4,22], suggests a membrane involvement in these processes.

Further studies will be required to clarify the way that microbial cell-solid surface contact is sensed as a mechano-physiological signal, stimulating a programmed response that may be important for sustaining growth, expression of a differentiated state, or a development of a resistance.

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