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Yeast-assisted synthesis of polypyrrole: Quantification and influence on the mechanical properties of the cell wall

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ABSTRACT

In this study, the metabolism of yeast cells (*Saccharomyces cerevisiae*) was utilized for the synthesis of the conducting polymer – polypyrrole (Ppy).Yeast cells were modified *in situ* by synthesized Ppy. The Ppy was formed in the cell wall by redox-cycling of $[Fe(CN)_6]^{3-/4-}$, performed by the yeast cells. Fluorescence microscopy, enzymatic digestions, atomic force microscopy and isotope ratio mass spectroscopy were applied to determine both the polymerization reaction itself and the polymer location in yeast cells. Ppy formation resulted in enhanced resistance to lytic enzymes, significant increase of elasticity and alteration of other mechanical cell wall properties evaluated by atomic force microscopy (AFM). The suggested method of polymer synthesis allows the introduction of polypyrrole structures within the cell wall, which is build up from polymers consisting of carbohydrates. This cell wall modification strategy could increase the usefulness of yeast as an alternative energy source in biofuel cells, and in cell based biosensors.

1. Introduction

In order to increase practical applicability of cells and microorganisms in some cases it is reasonable to modify the cells and/or at least outer structures of the cells. Such modifications may allow, enhancement or at least maintenance of stability and survivability of cells [1,2], improved efficiency of biosynthesis [3], or application of modified cells as whole cell sensors [4]. Typical strategies used for cell modification with polymers include: (i) chemical modification, (ii) electrostatic interaction, (iii) adsorption and even (iv) genetic engineering [3]. Chemical bonding of synthetic polymers such as polyethylene glycol through N-hydroxysuccinimidyl to the cell surface has been reported [5]. Electrostatic interaction of poly-ionic polymers, such as cationic poly(ethylene imine), anionic carboxyl methyl poly(vinyl alcohol) and polycationic polymers, such as: protamine, poly-L-lysine, diethylaminoethyl-dextran with cell membranes have all been shown to occur [6]. More advanced cell modification techniques are based on layer-by-layer deposition of self-assembling nanomaterials. This type of adaptation of cell surface function is usually achieved *via* electrostatic interaction between the cell membrane/wall and the materials which are applied for the modification [7,8]. A number of layer-by-layer (LbL) coatings have been manufactured on various types of cells and microorganisms [2]. Additionally, many 'hybrid' approaches have been developed adapting cell surface function; for example, performing phospholipid copolymer adhesion to the membrane followed by sequential LbL deposition [5,9]. Using LbL-based deposition it has even been possible to form calcium phosphate mineral shells around yeast cells [10]. Nevertheless, most of these techniques are enhancing cell 'barriers' towards some chemical compounds. In the cell modifications mentioned above pre- synthesized, defined size polymer nanoparticles are mainly used.

In vivo encapsulating polymerization is rarely based on the application of simple low molecular weight monomers; it is how-

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ever a very promising technique due to the ability to specifically distribute monomers in a polymer molecule in a manner which can compliment the existing components of the living cells surface. This may facilitate development of new techniques for characterization of cell surface structure or new diagnostics methods [11].

Currently it is difficult to localize *in situ* synthesized polymers without sophisticated fluorescent labelling, thus more efficient and simple alternative methods to determine and characterize polymers formed within cell structures are desirable. Detection of the functional groups of polymers is very important in their identification. For molecules without characteristic functional groups, such as amino-, keto-, hydroxyl-, carboxyl- etc, it is almost impossible to identify them in complex matrixes, without sophisticated labelling based techniques. One indirect way to detect such polymeric structures is through the use of isotopic labelling based methods where either radioactive [12] and/or stable isotopes [13] are applied. Isotopically labelled molecules provide an ability to detect molecules of interest even after their introduction into complex matrices. Labelled substances, coupled with the use of isotope ratio mass spectrometry (IRMS), can provide information on the dynamics of chemical reactions and rough estimation of reaction yield [14,15]. IRMS has even been used to localize nanoparticles and estimate their diffusion process [16].

In this study we take advantage of a cell wall modification method where polypyrrole (Ppy) is synthesized in yeast *Saccharomyces cerevisiae* cell walls [17]. The reported synthesis is based on application of redox-cycling of $[Fe(CN)_6]^{3-/4-}$, performed by the yeast cells themselves. Here, we provide a more advanced evaluation of the Ppy synthesis and estimates on the formed polymer localization within the cell structures using tandem-application of multiple methods including fluorescence microscopy, enzymatic digestions, atomic force microscopy and isotope ratio mass spectroscopy. We suggest a potential mechanism of how the formed polypyrrole structures affect the mechanical properties of yeast cells.

2. Experimental section

2.1. Materials

Potassium ferrocyanide trihydrate ($K_4[Fe(CN)_6]\cdot 3H_20$), D-(+)-glucose (Glu) and sorbitol were purchased from Carl Roth GmbH&Co (Karlsruhe, Germany). Pyrrole (Py) (98%), FITS labelled concanavalin A, lyticase from Arthrobacter luteus and poly–L–lysine (0,01 w/v) (PLL) were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Nitrogen isotope (^{15}N) labelled pyrrole (98% +) (Py*) was purchased from Cambridge Isotope Laboratories Inc. (USA). Sodium phosphate was purchased from Riedel-de Haën. Yeast extract, peptone from casein and ultrapure, granulated agar-agar were purchased from Merck (Darmstadt, Germany). Peptone, yeast extract and glucose were of 'microbiological' grade. The other chemicals were of GPR grade. For isotope ratio mass spectrometry high purity class gasses were used: He (5.0); N_2 (5.0); O_2 (4.6); (all purchased from Elme Messer Gaas, Lithuania) and CO_2 (purchased from AGA, Lithuania).

2.2. Cell preparation and modification with Ppy

The modification procedure has been described in detail previously [17]. In short, *Saccharomyces cerevisiae* yeast cells (Y00000; *BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) (Euroscarf, Germany) were cultivated aerobically overnight (for 20–24 h, 30 °C, YPD media: 1% yeast extract, 2 % peptone and 2% glucose), harvested then washed with neutral (pH 7.0) 0.1 M phosphate buffer saline (PBS). Cells were then incubated with shaking for 22–24 h in the same PBS buffer containing 0.2 M of glucose and differing concentrations of $K_4[Fe(CN)_6]$ (0.02–0.08 M) plus differing concentrations of pyrrole monomer (0.01–0.5 M). Harvested yeast cells and Ppy were washed from the reaction mixture and resultant bio-composites used in further experiments. Additionally in the isotope labelling mixtures both unlabelled pyrrole and pyrrole labelled with ^{15}N (400–4000 ‰) were used for polymerization. For pyrrole adhesion experiment cells were incubated as previously described, with the exception that the $K_4[Fe(CN)_6]$ was not included. We refer to 'unmodified' yeast cells to indicate cells, which were incubated in the same conditions except that the pyrrole monomer was absent.

2.3. Isotope ratio mass spectrometry

Detailed methods of the isotope ratio mass spectrometry have been described previously [18]. In brief, nitrogen isotope ratios in the bio-composites were measured using a Flash EA1112 elemental analyser (EA) coupled to an isotope ratio mass spectrometer (IRMS, Thermo delta plus advantage) *via* a ConFlo III interface (Thermo Electron GmbH, Germany). Yeast cells were freeze-dried, prior to being weighed into tin-based capsules and combusted in the elemental analyser in the presence of excess oxygen; helium (grade 5.0) was used as the carrier gas. The gases, produced by combustion, passed sequentially through an oxidation column comprising of CrO_3 granules at 1020 °C, a reduction column of copper wires at 650 °C, then a magnesium perchlorate water trap. The resultant N_2 and CO_2 were separated by the chromatography column in the EA and transferred to the ConFlo III interface. This interface allowed introduction of CO_2 and N_2 calibration gases from laboratory cylinders into the IRMS. The measured stable carbon and nitrogen isotope ratios are reported in the VPDB scale and in the air-N_2 scale respectively. Reference material caffeine IAEA 600, $\delta^{13}C = -27.771‰_{VPDB}$, $\delta^{15}N = 1\ ‰_{ai\ N2}$ [19] was used for the laboratory N_2 and CO_2 cylinder calibration.

2.4. Isotope mixing model

If the isotope ratios of the starting substrates are known, isotope ratio mass spectrometry allows determination of the amount of "substrate" material in the reaction product [20]. In order to determine polymer mass and biomass amounts, an isotope mixing model was applied. When isotopic composition of the initial substrates polypyrrole and yeast cells (respectively: δ_{PPy} and δ_Y) and isotopic composition of the composite material (δ_{sample}) are known, it can be expressed as:

$$\delta_{SAMPLE} = \left(\delta_{PPy}\right) * f_1 + \left(\delta_Y\right) * f_2 \quad (1)$$

and

$$f_1 + f_2 = 1 \quad (2)$$

where f_1 and f_2 are the fractions of the polypyrrole and Yeast cells in the sample, respectively. Combining Eqs. (1) and (2) gives:

$$f_1 = \left(\delta_{SAMPLE} - \delta_Y\right) / (\delta_{PPy} - \delta_Y) \quad (3)$$

$$f_2 = 1 - f_1 \quad (4)$$

The δ notation is used in isotope ratio mass spectrometry to describe the sample isotope ratio compared to an internationally accepted isotope standard and is expressed in parts per million. In the case of the nitrogen isotopes, it would be:

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000 \quad (5)$$

where R_{sample} is isotope ratio in the sample, and $R_{standard}$ is isotope ratio according to the international standard (defined by IAEA).