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Photobleaching of the resonance Raman lines of cytochromes in living yeast cells

Konstantin A. Okotrub, Nikolay V. Surovtsev *

Institute of Automation and Electrometry, Russian Academy of Sciences, Novosibirsk 630090, Russia

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ABSTRACT

The photobleaching of the resonance cytochrome Raman lines in living *Saccharomyces cerevisiae* cells was studied. The photobleaching rate versus the irradiation power was described by square function plus a constant in contrast to the linear dependence of the photoinjury rate. This difference distinguishes the cytochrome photooxidation from other processes of the cell photodamage. The square dependence is associated with the reaction involving two photogenerated intermediates while the constant with the dark redox balance rates. This work demonstrates a potential of Raman spectroscopy to characterize the native cytochrome reaction rates and to study the cell photodamage precursors.

1. Introduction

Under intensive light irradiation of a living cell the photodamage effects can occur. Photodamage can result into the change of cell properties, metabolism, or even the cell death. In some cases the cell photodamage can be the goal of the cell illumination, e.g. in the case of the photodynamic therapy. In this case researchers are interested to enhance the photodamage effect by the addition of the photosensitizes [1,2]. On the other hand, the irradiation light is used in some experimental techniques of cell biology serving as probing or auxiliary irradiation (e.g. Raman spectroscopy or optical tweezers [3,4]). In this case the photodamage is an undesirable effect which put additional limitations on the irradiation power, the wavelength, and the exposure time. Independently whether the photodamage is desirable or undesirable effect the knowledge about microscopic photodamage mechanisms is of high importance. A lot of works are devoted to study the photodamage problem [3–10]. It is believed that in many cases the photodamaging factors are related to generation of the reactive oxygen species (ROS) [4,6,9]. Also the photodamage can occur via the direct light absorption by DNA and/or proteins [11,12].

Different techniques and methods are used to characterize the cell photodamage. The most commonly used approaches include the visual control of the cell state under illumination [3–8,13,14], photoluminescence methods [10,13–16], EPR techniques [9,17,18] and others. It was shown that for the low light intensity the photoinduced injury rate obeys the linear dependence on the irradiation power [4,6,8,13,16]. In this case the photoinduced injuries and the cell lifetime are defined by product of the irradiation power and the exposition time, or in other words by the exposition energy. The particular photodamage parameters depend on the irradiation wavelength [4,6,7]. The experimental techniques based on the visual characterization of cells or on integral characteristics do not provide the information about the microscopic mechanisms of the cell photodamage.

Raman spectroscopy has capability to provide information about the chemical content [19]. The potential of Raman spectroscopy for characterization the cell activity was demonstrated recently by series of studies [20–23]. Another outstanding application of the Raman spectroscopy for cells is the studies of the Raman spectra of cytochromes [24–33]. The enhanced Raman intensity of reduced (Fe^{2+}) state of *b* and *c* cytochromes under the green light excitation relates to the resonance scattering near the absorbance edge [34]. The Raman intensity of the oxidized cytochrome state (Fe^{3+}) is lower by few orders for the green light excitation. Cytochromes take part in the mitochondrion electron transport chain (ETC) as electron carriers. In living cells there is the stationary balance between the reduced and oxidized states of cytochromes. Change of the redox balance means abnormalities in the ETC work. Thus, the photodamage effect on ETC work can be studied by the resonance Raman scattering of cytochromes.

The present work is devoted to study the effect of photoinduced injuries in living yeast cells under the 532 nm irradiation by resonance Raman scattering technique. The photobleaching parameters of the cytochrome Raman spectra versus the laser intensity is studied. For the comparison the photoinduced injuries are characterized by the photoluminescence intensity. It will be shown that the laser power dependence of the photobleaching is different

* Corresponding author at: Pr. Ak. Koptyuga 1, 630090 Novosibirsk, Russia. Tel.: +7 383 3307978.
E-mail address: lab21@iae.nsk.su (N.V. Surovtsev).

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from the photoluminescence one. A simple model description of the photobleaching behavior is proposed.

2. Experimental

2.1. Sample preparation

Commercially available *Saccharomyces cerevisiae* instant yeast cells pellets (20 mg) were added to 5 ml of isotonic saline solution (0.9 wt.% NaCl). For each Raman experiment, the cell suspension was placed on fused silica slide, covered with a piece of mica (5–15 μm in thickness) and sealed with paraffin to prevent the sample desiccation.

2.2. Experimental setup

A home-made confocal Raman setup, based on a modified microscope (Orthoplan, Leitz) and a monochromator (SP2500i, Princeton Instruments) equipped with a CCD detector (Spec-10:256E/LN, Princeton Instruments) was used. The spectrometer wavelength calibration was done with a neon-discharge lamp. Raman scattering was excited by a solid-state laser (Millennia II, Spectra Physics) at 532.1 nm, the beam power was measured with a photodiode power meter (Lasercheck, Coherent). A 100 × air objective with NA = 0.75 and working distance 4.6 mm (PL FLUOTAR L, Leica Microsystems) was used to focus the laser beam to ~0.9 μm spot. For this spot the 1 mW laser irradiation corresponds to the intensity of about 0.13 MW/cm^2 for the average intensity and about 0.29 MW/cm^2 for the maximum.

In Raman scattering experiment a single cell was exposed by a constant power of the laser beam, which simultaneously served as the excitation of Raman scattering. The sequence of Raman spectra (~40 scans) was measured with the integration time optimized to the particular photobleaching rate, the integration time being 1 s for ~20 mW of laser irradiation and 30 s for ~1 mW. Evolution of the Raman spectrum of the cell under irradiation was studied.

3. Results

Fig. 1 represents the Raman spectrum of the yeast cells and its evolution under the laser irradiation. In this spectrum the contributions of CH deformation (1440–1460 cm^{-1}), amide (1656, 1555 cm^{-1}), phenylalanine (1004 cm^{-1}) and nucleic acids (785 cm^{-1}) modes can be identified. The lines at 749, 1129, and 1585 cm^{-1} seen in Fig. 1 are known as the most intensive lines of the resonance Raman spectrum of *b* and *c* cytochromes [35,36]. The peak at 703 cm^{-1} in Fig. 1 is contributed by the mica slice covering the sample.

As it is seen from Fig. 1 the contribution of the cytochrome lines decreases with the laser exposition, while the intensities of the rest Raman lines do not change. This decrease is associated with the decrease of the reduced cytochrome concentration. We followed the intensity of the line at 749 cm^{-1} to estimate the amount of the reduced cytochrome in the yeast cell. This line corresponding to the heme perrole rings breathing mode [35] is intensive and placed apart the other cellular lines.

For the quantitative analysis the intensity of the line at 749 cm^{-1} was found taking into account the mica spectrum contribution and the background interpolated by a straight line. The relative contribution of cytochrome into the single cell Raman spectrum was characterized by the intensity ratio of the line at 749 cm^{-1}, I_{749}, and of the deformational CH mode (δCH), I_{CH}. This ratio can vary among the different yeast cells or even different cell parts, but it is independent of the absolute Raman intensity and, therefore, is convenient for the characterization of the single cell during the irradiation (for example, it eliminates the effect of change of the light absorption by the cell).

The Raman spectra of yeast cells are placed on some photoluminescence background. Typically, the photoluminescence background is low at the beginning of the irradiation. However, after some laser exposition the photoluminescence background starts to increase. This increase is caused by some fluorophores resulted from photoinduced processes in the cell. Prolonged laser exposure leads in the cell degradation, resulting in the formation of visual defects such as holes or black points. The spectra from the photoinduced defects are characterized by the high level of the photoluminescence background. It is natural to relate the fluorophore increase under irradiation with increase of the photodegradation events. Thus we propose to follow the cell photoderagation by the study of the temporal dependence of the photoluminescence intensity. The photoluminescence intensity was characterized by the integral signal in the range 550–634 nm. The temporal evolution of the photoluminescence intensity of a yeast cell under the laser irradiation is represented in Fig. 2a. It is seen that the photoluminescence intensity is characterized by an abrupt increase at τ_{PL} associated with the photodegradation. Since τ_{PL} characterizes the time needed to reach the critical photoinjury level, inverse τ_{PL} reflects the rate of the photodamage accumulation.

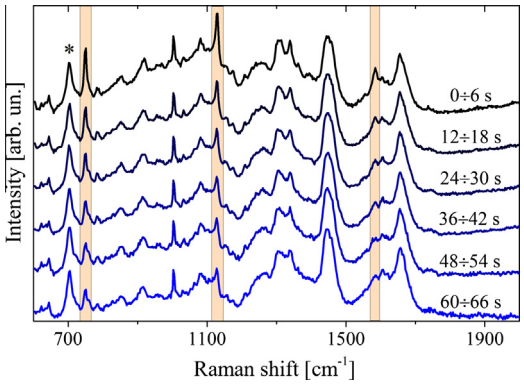

Fig. 1. Time-resolved Raman spectra of living yeast cells for 10 mW laser power. The spectra are vertically shifted for convenience. The shadow stripes mark the Raman peaks of cytochrome. The asterisk denotes the contribution from the mica slice.

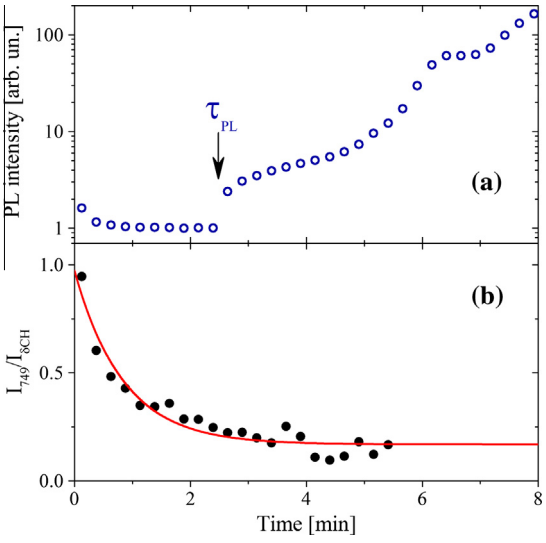

Fig. 2. Representative time dependence of the photoluminescence intensity (a) and the I_{749}/I_{CH} ratio (b) under 4 mW laser irradiation. The onset of the drastic photoluminescence increase is denoted by the arrow in (a). The exponential decay fit is shown by the solid line in (b).