LETTER

The “Raman spectroscopic signature of life” is closely related to haem function in budding yeasts

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1. Introduction

Recent progress in lasers, optical multichannel detectors and other optoelectronic devices has facilitated the extensive use of Raman spectroscopy in studying biological systems [1]. In our previous work using living yeast cells as model organism, we have detected a still unassigned unique Raman signal at 1602 cm⁻¹ that has been shown to reflect cell metabolic activity. This result suggests that the molecule giving rise to the “Raman spectroscopic signature of life” is closely related to haem functions in the cell. Besides yeasts, the signature is also observed in rat liver mitochondria [8] and HELA cells [9] as well, suggesting it to be a Raman signal that universally exists in most kinds of cells.

The fact that the “Raman spectroscopic signature of life” diminishes after adding KCN or NaN₃ suggests that it originates from a molecular species involved in a metabolic pathway that requires the utilization of molecular oxygen through haem proteins. To further discuss the origin of this signature, we expect that yeast mutants deficient of haem synthesis such as HEM1 disrupted yeasts (hem1Δ strain) would be helpful. The HEM1 gene encodes δ-aminolevulinate (ALA) synthase, the enzyme which catalyzes the first step of the haem synthetic pathway [10]. The disruption of this gene would lead to haem deficient and unviable cells unless the cells are pro-

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vided with δ-aminolevulinate (ALA), the product of ALA synthase, or both ergosterol and unsaturated fatty acids [11]. Therefore, it is clear that hem1Δ yeasts are truly deficient in haem biosynthesis and no other metabolic pathways could compensate for it. In this study, we report the Raman spectroscopic study of the wild type and the hem1Δ yeasts to clarify how haem function affects the “Raman spectroscopic signature of life” in yeasts.

2. Experimental

2.1 Yeast strains and culture conditions

The yeast strains used in this study are listed in Table 1. The wild type strains are cultured aerobically at 30 °C in 2% peptone, 2% D-glucose and 1% yeast extract (YPD medium). All hem1Δ yeasts are cultured in the same condition except that 80 μg/ml ALA is supplied in the YPD medium. The hem1Δ yeasts not supplied with ALA are obtained by culturing hem1Δ yeasts in YPD medium without ALA for three days before Raman spectroscopy experiments.

2.2 Raman micro-spectroscopy experiment

The Raman micro-spectroscopy setup used in this study is the same as the one reported previously [2]. In brief, the pump wavelength is 785 nm and the laser power at the sample point is 18 mW. The exposure time is 100 seconds. Quartz coverslips and slides are used in order to avoid fluorescence from the glass. The Raman spectra of S. cerevisiae do not have strong spatial dependence within the cell, most probably because of the laser trapping effect. Therefore, we always measure the cells at the place where the laser trapping of the whole cell occurs. It is also the place where we can obtain the highest signal-to-noise Raman spectrum of the cell.

3. Results and discussion

In most of our previous studies, we used the industrial W4 tetraploid yeast strain as our model system. However, tetraploid strains are not suitable for gene knockout experiments. Here we introduce haploid S. cerevisiae for our study. Figure 1 compares the optical images and the Raman spectra of wild type tetraploid and haploid yeast cells. Both the size of the cell and the intensity of the “Raman spectroscopic signature of life” show clear dependence on the cell ploidy. Tetraploid cells are generally larger in size and have a stronger 1602 cm⁻¹ band than haploid cells. The reason for this variance in the 1602 cm⁻¹ signal could be the difference in the metabolic state of the cells. Most of the industrial yeast strains are tetraploid because they grow and ferment more actively than other yeast cells. This serves as another piece of evidence that the intensity of the “Raman spectroscopic signature of life” is dependent on the metabolic state of the cells.

Since the spectra of a and α wild type haploid cells are almost identical, we have chosen only α type haploid cells for further experiments. Figure 2a–d shows the optical image and Raman spectra of wild type and hem1Δ yeasts cultured in YPD medium without any supplement. We have measured 30 different cells from 6 independent batches of culture and none of the hem1Δ yeasts showed the “Raman spectroscopic signature of life” at 1602 cm⁻¹. The strain also displays growth arrest at early stage so that its OD₆₀₀ never reaches the same level as wild type strains or hem1Δ strain supplied with ALA (data not shown, refer to [11]). This result is consistent with our expectation that the 1602 cm⁻¹ signal is closely related to haem function.
Besides the 1602 cm\(^{-1}\) signal, the 1656 cm\(^{-1}\) Raman band in hem1\(\Delta\) strain is no more visible and a new band appears at 1668 cm\(^{-1}\) as shown in Figure 2d. It is worth noting that besides the Raman band mentioned above, two new peaks appeared at 1380 cm\(^{-1}\) and 1330 cm\(^{-1}\). The 1668 cm\(^{-1}\), 1380 cm\(^{-1}\) and 1330 cm\(^{-1}\) Raman bands correspond well with the Raman spectrum of squalene (Figure 2e, [12]). Therefore, it is clear that the 1656 cm\(^{-1}\) Raman band from C=C double bond stretch of unsaturated fatty acids [2] no more exists in hem1\(\Delta\) cells, as haem deficient yeast could not synthesize unsaturated fatty acids [11], and the 1668 cm\(^{-1}\) signal comes from the C=C double bond stretch of squalene. Squalene is the precursor of many lipid structures in yeasts that requires haem protein in their synthetic process [13]. Since hem1\(\Delta\) cells could not synthesize haem groups properly, it is reasonable that squalene is accumulated in the hem1\(\Delta\) yeast cell. We believe this is the first in vivo observation of the accumulation of squalene in hem1\(\Delta\) yeast cells. Similar results have been reported by Spanova et al. using the lipid extraction technique [14].

It is reported that the haem synthesis pathway of hem1\(\Delta\) strains could be recovered by supplying ALA to the yeasts [11]. However, it has been difficult to determine whether hem1\(\Delta\) strains supplied with ALA has indeed the same metabolic state as wild type cells. Here we propose Raman spectroscopy as a useful method to analyze the general metabolic status of the two strains. As shown in Figure 3, the Raman spectra of wild type and ALA supplied hem1\(\Delta\) cells are basically identical, suggesting that the haem synthesis pathway has been fully recovered in the mutant strain.

4. Conclusion

In this paper we have shown the dependence of the “Raman spectroscopic signature of life” on the metabolic activity and the haem synthesis of the cell. It is the first gene knockout experiment for the elucidation of the 1602 cm\(^{-1}\) Raman band and has successfully confined the candidate molecular species that gives rise to the signal downstream of the haem synthetic pathway. Together with our previous results, in which the signal was inhibited by KCN [2] and NaN\(_3\) [7], it is clear that the haem-oxygen reaction is necessary for the 1602 cm\(^{-1}\) band to exist. Isotope substitution experiments also showed that the signal originates from a C=C double bond structure [15]. The information above has helped us to finally propose sev-
eral specific candidates of the “Raman spectroscopic signature of life” and we hope that the true origin of the signal will be elucidated soon.

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