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LETTER

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

Liang-da Chiu[1] *and Hiro-o Hamaguchi*[*, 1, 2]

[1] Department of Chemistry, the University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033 Japan
[2] The Department of Applied Chemistry, National Chiao Tung University, 1001 University Road, Hsinchu, Taiwan 300 R.O.C.

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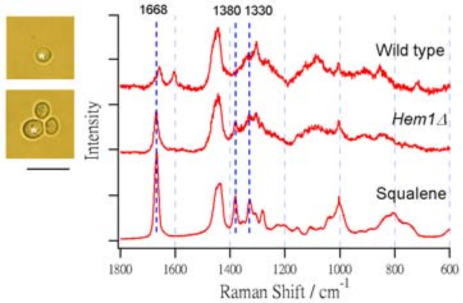
HEM1 gene encodes δ-aminolevulinate synthase that is required for haem synthesis. It is an essential gene for yeast survival. The Raman spectra of *HEM1* knockout (*hem1Δ*) yeast cells lacks a Raman band at 1602 cm^{-1} that has been shown to reflect cell metabolic activity. This result suggests that the molecule giving rise to the"Raman spectroscopic signature of life" is closely related to haem functions in the cell. High amount of squalene is also observed in the *hem1Δ* strain, which is another new discovery of this study.



The image and Raman spectra of wild type and *hem1Δ* yeasts compared with squalene

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}. We called it the "Raman spectroscopic signature of life" because it was proven to be an indicator of the cell metabolic activity [2–6]. It is established by now that the intensity of this band decreases after the cells are treated with potassium cyanide (KCN) [2] or sodium azide (NaN_3) [7]. H_2O_2 treatment also causes the signal to vanish in several minutes [4]. Besides yeasts, the signature is found 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 NaN_3 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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[*] Corresponding author: e-mail: hhama@chem.s.u-tokyo.ac.jp, Phone: +81 3 5841 4327, Fax: +81 3 3818 4327

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.

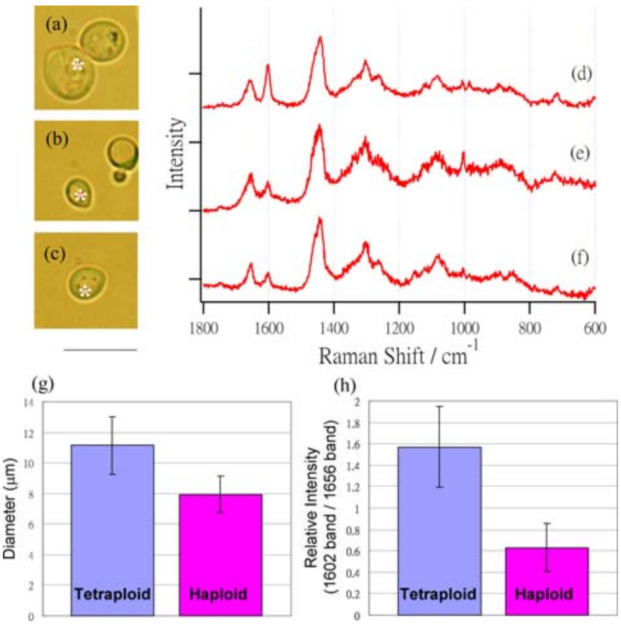

Figure 1 (online color at: www.biophotonics-journal.org) The optical images and Raman spectra of (**a**, **d**) tetraploid cells, (**b**, **e**) a type haploid cells and (**c**, **f**) α type haploid cells. The asterix mark represents the laser focus and the bar below (**c**) is the 20 µm scale bar for the three optical images. The average size (**g**) and the average intensity of the 1602 cm^{-1} band (**h**) of 15 tetraploid and 13 haploid cells are also shown.

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 OD$_{600}$ 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.

Table 1 The yeast strains used in this study.

Strain Name	Species Name	Ploidity	Source
W4*, **	*Saccharomyces cerevisiae* × *Saccharomyces bayanus*	Tetraploid	Suntory Ltd.
FY1679 a*	*Saccharomyces cerevisiae*	Haploid	Prof. Ferreira
FY1679 α*	*Saccharomyces cerevisiae*	Haploid	Prof. Ferreira
Hem1Δ α	*Saccharomyces cerevisiae*	Haploid	Prof. Ferreira [11]

* Wild type strains. ** Industrial strain.

Besides the 1602 cm^{-1} signal, the 1656 cm^{-1} Raman band in *hem1Δ* 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 *hem1Δ* 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 *hem1Δ* cells could not synthesize haem groups properly, it is reasonable that squalene is accumulated in the *hem1Δ* yeast cell. We believe this is the first *in vivo* observation of the accumulation of squalene in *hem1Δ* 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 *hem1Δ* strains could be recovered by supplying ALA to the yeasts [11]. However, it has been difficult to determine whether *hem1Δ* 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 *hem1Δ* cells are basically identical, suggesting that the haem synthesis pathway has been fully recovered in the mutant strain.

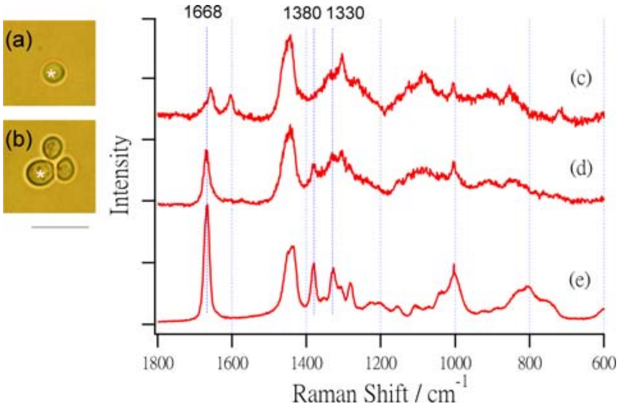

Figure 2 (online color at: www.biophotonics-journal.org) The optical images and Raman spectra of (**a**, **c**) wild type α strain and (**b**, **d**) *hem1Δ α* strain without any supplement. The asterix mark represents the laser focus and the bar below (**b**) is the 20 μm scale bar for the three optical images. Spectra (**e**) is the spectrum of squalene.

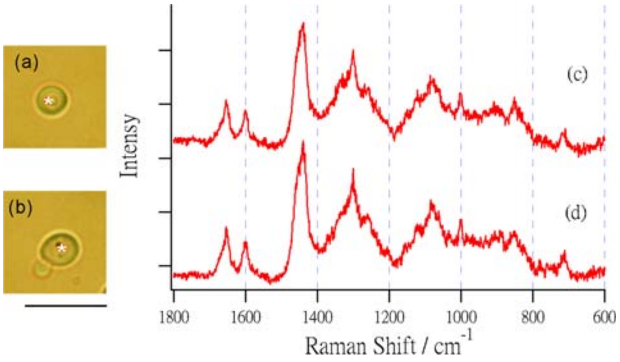

Figure 3 (online color at: www.biophotonics-journal.org) The optical image and Raman spectra of (**a**, **c**) wild type α strain and (**b**, **d**) *hem1Δ α* strain supplied with ALA. The asterix mark represents the laser focus and the bar below (**b**) is the 20 μm scale bar for the three optical images.

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 several 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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