4B09

Behaviour of the “Raman Spectroscopic Signature of Life” in Isolated Mitochondria

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【序】

The “Raman spectroscopic signature of life” is a Raman band at 1602 cm^{-1} that sharply reflects the metabolic activity of cell mitochondria[1,2,3]. It is well established with time- and space-resolved Raman spectroscopy that the intensity of this signature decreases when haem protein inhibitors are added to living yeast cells[1]. Haem protein inhibitors such as KCN or sodium azide could bind to the iron centre of the haem group and block all the reactions that are related to haem groups. One famous example is in the process of respiration, oxygen reacts with the haem group of the cytochrome c oxidase as in the chemical reaction formula:

$$4Fe^{2+}\text{-cytochrome c} + 8H^{+}_{in} + O_2 \rightarrow 4Fe^{3+}\text{-cytochrome c} + 2H_2O + 4H^{+}_{out}$$

By adding the inhibitors, this reaction is blocked and many reactions such as the respiratory electron transport chain as well as the TCA cycle are also hampered. It is clearly proved that by our previous work that the intensity changes of the “Raman spectroscopic signature of life” could reflect physiological changes of these metabolic processes *in vivo*. Here we report the similar behaviour of the “Raman spectroscopic signature of life” in isolated mitochondria as in living yeast cells.

【実験】

A confocal Raman microspectrometer with a tunable Ti:Sapphire laser set at 785 nm excitation wavelength was used for the experiment. The slide glasses and coverslips used in this work were all made by quartz in order to avoid the strong fluorescence of glass. The isolation of mitochondria was performed according to a combination of two previously established protocols[4,5]. The final concentration of sodium azide for the respiration inhibition experiment was 50 μM in the mitochondria suspension.

【結果と考察】

The Raman spectrum of isolated mitochondria and the space-resolved Raman spectrum of mitochondria in a living yeast cell are compared in Fig. 1. The overall feature seems to be the same in the two spectra, indicating that the spectra obtained from living yeasts comes predominantly from mitochondria. Continuous Raman spectroscopic observation shows that the intensity of the 1602 cm^{-1} band decreases when sodium azide is added to

(b)

isolated mitochondria (Fig. 2). This confirms the existence of the "Raman spectroscopic signature of life" in isolated mitochondria and that stepwise isolation could serve as a feasible approach to elucidate its origin.

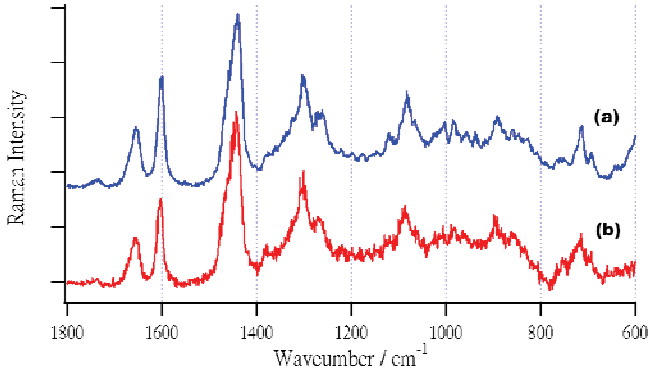

Figure 1. The Raman spectra of (a) yeast and (b) isolated mitochondria.

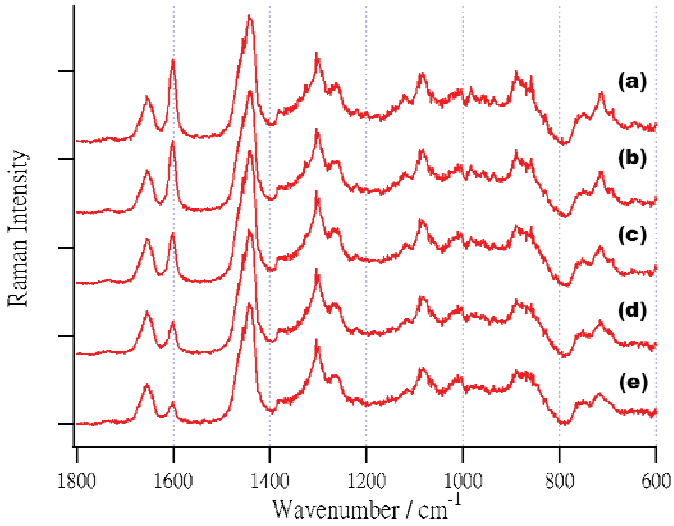

Figure 2. Time resolved Raman spectra of isolated mitochondria after adding sodium azide. The Raman spectrum obtained (a) immediately, (b) 3, (c) 11, (d) 19, and (e) 30 minutes after the addition of sodium azide.

1. Huang YS, Karashima T, Yamamoto M, Hamaguchi H *Biochemistry* **2005**, *44*, 10009-10019.
2. Naito Y, Toh-e A, Hamaguchi H *J. Raman Spectrosc.* **2005**, *36*, 837-839.
3. Huang YS, Nakatsuka T, Hamaguchi H *Appl. Spectrosc.* **2007**, *61*, 1290-1294.
4. Yamamoto M, Ohya Y *Yeast Lab Manual*; Springer Japan: Tokyo, 1998.
5. Yaffe MP *Methods. Enzymol.* **1991**, *194*, 627-643.