Inorganic phosphate dynamics and starvation induced necrosis in yeast cells by Raman Microspectroscopy

Yasuaki Naito[1], Venkatesh Kaliaperumal[1], and Hiro-o Hamaguchi[1, 2*]

[1]Department of Chemistry, School of Science, The University of Tokyo, Hongo 7-3-1 Bunkyo-ku Tokyo,113-0033, Japan and [2]Institute of Molecular Science and Department of Applied Chemistry, National Chiao Tung University, 1001 Ta Hsueh Road, Hsinchu 300, Taiwan

Inorganic phosphates (Pi) are indispensible for life as many biological processes involve formation or breaking down of phosphate ester bonds. Pi exist in different forms inside cells depending on the pH of the cellular compartment, transport proteins and enzymatic activities. For instance, the vacuolar compartment usually has an acidic pH and harbours dihydrogen phosphate ($H_2PO_4^-$), whereas the mitochondria have an alkaline pH and possess monohydrogen phosphate (HPO_4^{2-}). Raman microspectroscopy is able to detect these two forms of phosphates in living yeast cell vacuole and mitochondria respectively. In addition, the vacuole is also found to contain polyphosphates (polyP) which has its characteristic Raman bands[1]. In this study we report dynamical changes in the intracellular Pi concentration during starvation induced stress and cell death in a single yeast cell. To our knowledge, we are the first to report the feasibility of detecting subcellular Pi changes in single cells.

Based on Raman microspectroscopic images we have divided the yeast starvation response into three phases (Fig.1). In the early phase of starvation there occurs a decrease in the vacuolar polyP concentration (b-1 to b-2). One of the functions of polyP is to provide energy in the form of ATP during nutrient deprived conditions. The hydrolysis of polyP to form ATP leads to a decrease in the aqueous polyP Raman band at 1150 cm^{-1} during the initial phase of nutrient starvation.

In the intermediate phase of starvation there is a complete decline in the mitochondrial activity inferred from the Raman band at 1602 cm^{-1}(a-3). This is associated with the appearance of a crystalline polyP granule inside the vacuole with its characteristic peak at 1160 cm^{-1}(b-3). Divalent cations like calcium and magnesium lead to formation of insoluble polyPs. Under normal growth conditions

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long chain aqueous PolyPs are known to sequester divalent cations in soluble form. We presume that the hydrolysis of polyP during the early phase of starvation leads to an increase in the free divalent cation and phosphate concentrations in the cytoplasm which affects the mitochondria. Complete loss of mitochondrial integrity is likely to further increase the cytoplasmic cation and phosphate concentrations. Excess of divalent cations and low levels of polyP tend to produce the insoluble form of polyP inside the vacuole during this phase.

In the late phase response to starvation there is loss of cellular structure leading to cell death (b-4 to b-6, c-4 to c-6). Excess of divalent cations are likely to damage the membranes causing this effect. Thus, by Raman microspectroscopic mapping we could observe dynamical changes in the Pi concentrations at subcellular level and explain the sequence of molecular events occurring during necrotic cell death induced by starvation.

1. Naito, Y., Toh-e, A. & Hamaguchi, H. In vivo time-resolved Raman imaging of a spontaneous death process of a single budding yeast cell. Journal of Raman Spectroscopy 36, 837-839 (2005).

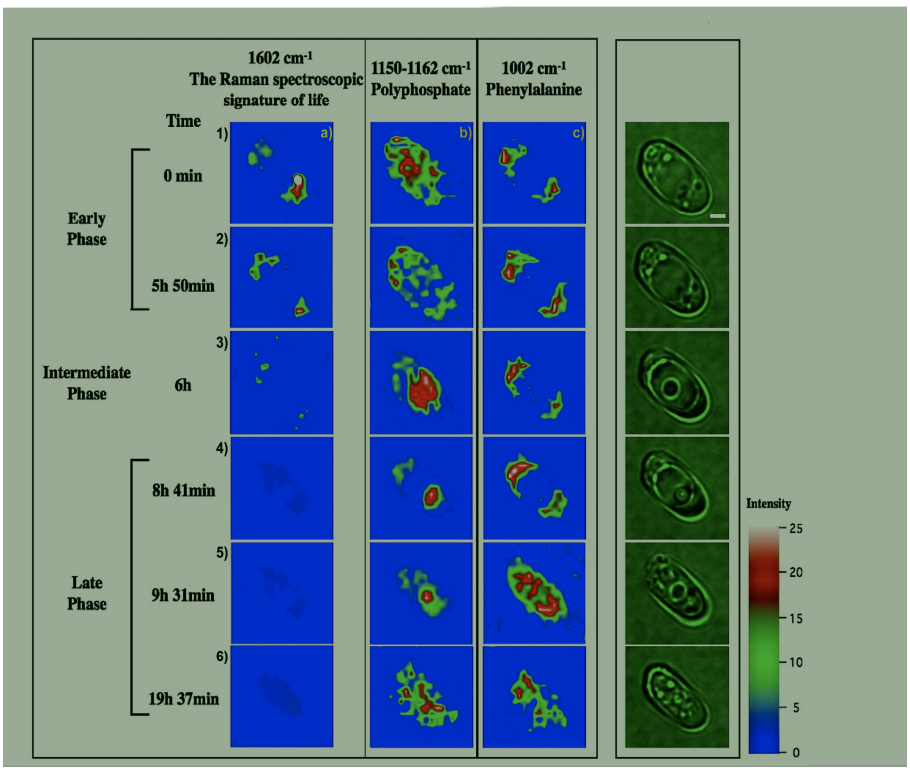

Fig.1
Raman mapping of characteristic Raman peaks during starvation and corresponding optical images

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