STRUCTURAL CHEMISTRY

Annual Research Highlights

(1) Molecular Level Pursuit of a Spontaneous Cell Death Process by Time-resolved Raman Mapping

It is known that a particle called dancing body (DB) occasionally appears and moves vigorously in a vacuole of a budding yeast (*S. cerevisiae*) cell. We found that once a DB was formed, the vacuole was eventually lost and the cell died subsequently. This spontaneous death process of a budding yeast cell was studied at the molecular level by time-resolved Raman mapping experiments (Fig. 1). Between 0 min and 5h 50 min, the cell was in the normal state. The "Raman spectroscopic signature of life" at 1602 cm^{-1} showed high metabolic activity in mitochondria, and phosphlipids (1440 cm^{-1}) and proteins (1002 cm^{-1}) were localized in the outside of the vacuole. At 6 h, a dancing body suddenly appeared and, at the same time, the "Raman spectroscopic signature of life" became very weak, indicating the concomitant lowering of the metabolic activity. At 8 hr 41 min, the metabolic activity entirely stopped but the distribution of phospholipids and proteins still remained unchanged. At 9 hr and 31 min and later, the vacuole was lost and the molecular distribution became random, indicating that the cell was not living any more. In this way, the spontaneous cell death process following the DB formation was traced successfully by time-resolved Raman mapping. This result is of great interest regarding the definition of **cell death at the molecular level**.

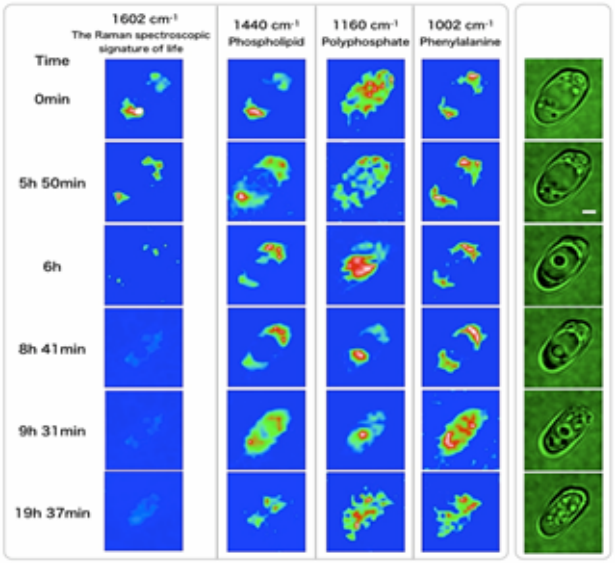

Fig. 1 Time-resolved microscopic images (right, green) and Raman mapping images of a budding yeast cell.

1.(1)-4) *J. Raman Spectrosc.,* **36,** 837-839 (2005).

(2) Ultrafast Association/Dissociation Dynamics of Magnesium Sulfate in Water

Our understanding of thermal reactions is much less advanced compared with photochemical reactions. For photochemical reactions, various ultrafast time-resolved spectroscopies are available and they provide detailed information on their reaction mechanism. This is not the case with thermal reactions, which can not be triggered by light. As an alternative to the time domain approach using time-resolved spectroscooies, we have been developing a frequency domain approach based on vibrational band shape analysis. We derived a formula from the Kubo-Anderson theory that describes the band shapes under the asymmetric limit of the two frequency exchange model. According to this formula, the vibrational band shape is a Lorentzian with a peak shift $\Delta\omega$ and a band width increase $\Delta\Gamma$, which are the functions of the exchange rates between the two frequencies. From the concentration dependence of the totally symmetric SO_4 stretch band of magnesium sulfate in water (Fig. 2), we were able to determine the **association/dissociation dynamics** as shown in Fig. 3.

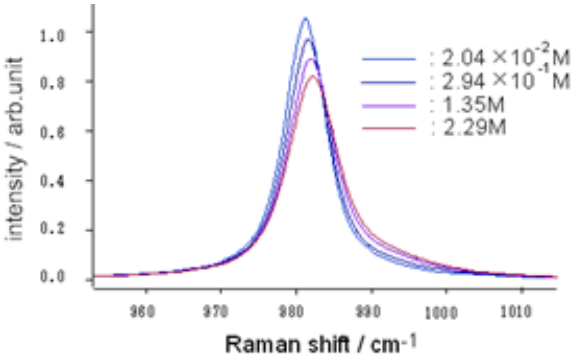

Fig. 2 Concentration dependence of the SO_4 symmetric stretch Raman band of magnesium sulfate in water.

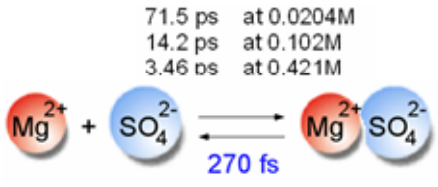

Fig. 3 Association/dissociation dynamics of magnesium sulfate in water.

1.(1)-6) *J. Chem. Phys.* **123,** 34508 (2005).

構 造 化 学 研 究 室

研究ハイライト

(1) 時間分解ラマンマッピングによる出芽酵母自然死過程の分子レベル追跡

出芽酵母(*Saccharomyces cerevisiae*)の液胞には、Dancing Body (DB)と呼ばれる顆粒が時折出現し、激しく動き回ることが知られている。我々は、DBが出現するとその後液胞が潰れ細胞内が無秩序になり、最終的に細胞死に至ることを見出した。この細胞死過程を、時間分解ラマンイメージングにより分子レベルで追跡した（図 1)。波数 1602 cm^{-1} の“生命のラマン分光指標”のイメージから、0 分から 5 時間 50 分の間では，ミトコンドリアが活発に代謝活動を行っており、リン脂質とタンパク質は液胞外に局在していることがわかる。6 時間後、DB が突如出現し、それと共にミトコンドリアの代謝活性が著しく低下する。8 時間 41 分後にはミトコンドリアの代謝活性が完全に消失し、分子レベルでは細胞が死んでいるとみなせるが、その他の物質分布は依然変化しない。9 時間 31 分以後には、物質分布が乱雑になり、細胞がもはや生きていないことが明らかである。このようにして、ラマン分光により DB の出現､それと同期したミトコンドリア代謝活性の消失、液胞の構造崩壊など一連の細胞自然死過程を時々刻々追跡することができた。この実験結果は、**分子レベルで細胞死**をどのように定義するかを考えるうえで大変興味深い。

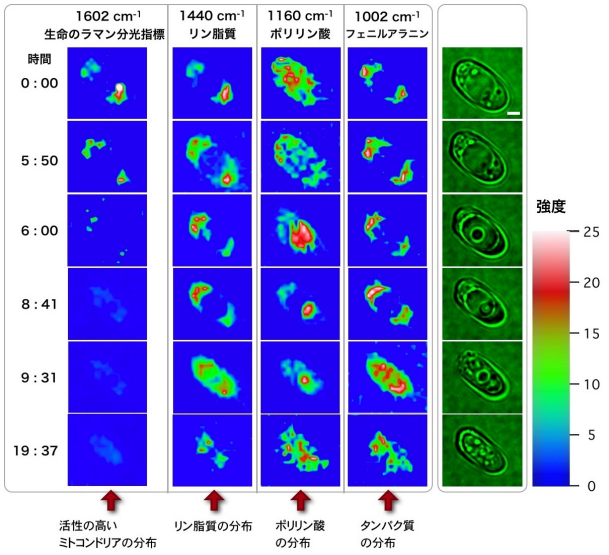

図1. 出芽酵母の時間分解顕微鏡像およびラマンマッピング像

1.(1)-4) *J. Raman Spectrosc.*, **36,** 837-839 (2005).

(2) 水溶液中の硫酸マグネシウムの超高速会合/解離ダイナミクス

熱反応に対する我々の理解は、光化学反応に比べて進んでいない。光化学反応は、種々の超高速時間分解分光法によって調べることが可能で、その機構に関して詳細な情報を得ることができる。しかし、光による同期が不可能である熱反応には、この手法を適用することができない。時間分解分光を用いた時間領域のアプローチに代わるものとして、我々は振動バンド形の解析に基づく振動数領域のアプローチを追及してきた。Kubo-Andersonの理論を、2 振動数交換モデルの非対称極限に適用し、振動バンド形の表式を導出した。この式に従えば、バンド形は振動数シフトΔω とバンド幅の増加ΔΓで表されるローレンツ関数であり、これらの量は交換する 2 振動数間のダイナミクスと関係づけられる。この式に基づいて硫酸マグネシウム水溶液のSO_4全対称伸縮ラマンバンドの濃度依存性（図2）を解析することにより、**超高速会合/解離ダイナミクス**に関する定量的情報を得ることができた（図3）。

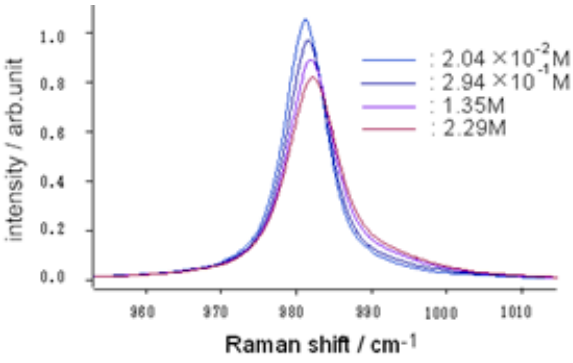

図2. 硫酸マグネシウム水溶液の.SO_4全対称伸縮振動ラマンバンドの濃度依存性

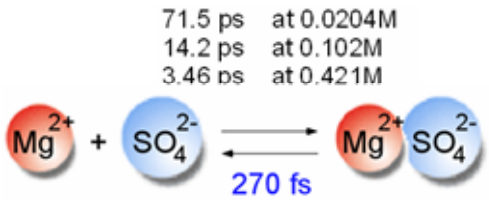

図3. 水溶液中の硫酸マグネシウムの超高速会合/解離ダイナミクス

1.(1)-6) *J. Chem. Phys.* **123,** 34508 (2005).

1. 原著論文

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3) Kensuke Tono, Hiroshi Kondoh, Yasuhiro Hamada, Takahiro Suzuki, Kotatsu Bito, Toshiaki Ohta, Shin Sato, Hiro-o Hamaguchi, Akira Iwata and Haruo Kuroda, "Photoinduced Processes of Solid Aromatic Compounds by Mid-IR Free Electron Laser", *Jpn. J. Appl.Phys.* **44,** 10 7561-7567 (2005).
4) Yasuaki Naito, Akio Toh-e and Hiro-o Hamaguchi, "In vivo time-resolved Raman imaging of a spontaneous death process of a single budding yeast cell", *J. Raman Spectrosc.,* **36,** 837-839 (2005).
5) Motohiro Banno, Shin Sato, Koichi Iwata and Hiro-o Hamaguchi, "Solvent-dependent intra- and intermolecular vibrational energy transfer of $W(CO)_6$ probed with sub-picosecond time-resolved infrared spectroscopy", *Chem. Phys. Lett.,* **412,** 464-469 (2005).
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7) Hideaki Kano and Hiro-o Hamaguchi, "Ultrabroadband (>2500 cm-1) multiplex coherent anti-Stokes Raman scattering microspectroscopy using a supercontinuum generated from a photonic crystal fiber", *Appl. Phys .Lett.* **86,** 121113 (2005).
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10) Shinsuke Shigeto, Hideaki Kano, and Hiro-o Hamaguchi, "Cascading third-order Raman process and local structure formation in binary liquid mixtures of benzene and n-hexane", *J. Chem. Phys.,* **122,** 064504 (2005).
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13) Hideaki Kano and Hiro-o Hamaguchi, "Near-infrared coherent anti-Stokes Raman scattering microscopy using supercontinuum generated from a photonic ctystal fiber", *Appl. Phys. B,* **80,** 243-246 (2005).

2. 総説・解説

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