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Abstract of Thesis

Name (Nat Sakol)	
Title	NMR study of biomolecular localization in cells by the paramagnetic relaxation (常磁性緩和を用いたNMRによる生体分子の細胞内局在の研究)

Introduction:

The information of the locations of macromolecules inside the cell is crucial for understanding the function of macromolecules and biological processes inside the cell. This research introduces a new developed in-cell solid-state NMR technique for investigating this important issue in vivo environment of *Escherichia coli* cells by employing paramagnetism of gadolinium complexes to enhance magnetic relaxation rate of cellular proton. The spin diffusion of protons spreads out the effect of gadolinium ion and generates the locational dependence of relaxation rate. The appropriate gadolinium agent and concentration were studied. The locations of macromolecules were interpreted from the relaxation rates of macromolecules.

Materials and methods:

The distributions and toxicities of aqua gadolinium and Gd-DOTA complexes were studied by monitoring the water ^{1}H relaxation rate in non-labelled *E. coli* cell solution containing these paramagnetic agents.

To study the location dependence of relaxation rate by solid-state NMR, ^{13}C labeled cell sample was treated with the appropriate gadolinium agent and frozen. The relaxation ordered spectroscopy (ROSY) is the method for resolving the solid-state ^{13}C-NMR spectrum based on the ^{1}H relaxation rate. The relaxation rate of frozen cells was detected by ROSY pulse. The proton magnetization is saturated first and relaxes to the thermal equilibrium polarization along B_0 during recovery time τ_{rec}. Cross polarization pulse then transfers the ^{1}H magnetization to the ^{13}C magnetization. Therefore, the ^{1}H relaxation process is monitored as a function of τ_{rec} via high-resolution ^{13}C NMR spectra of biomolecules under magic-angle spinning.

The signals were assigned by comparing the experimental one-dimensional ^{13}C NMR spectrum with the simulated cellular NMR spectrum and cross peaks of two-dimensional ^{13}C NMR spectrum. The locational dependence was analyzed by spin diffusion model.

Results and discussion:

The proton buildup curves of *E. coli* solutions at 0 °C could be analyzed with a double exponential relaxation equation. This shows that most of gadolinium complexes were distributed in extracellular part of the sample. The gadolinium solution at 150 mM was high enough to distinguish between the intracellular and extracellular relaxation rate and provided good reproducible experimental results. Aqua gadolinium complex was shown to have weak permeability to cell membrane and binding to cellular components with a dissociation constant K_d of 1.0 mM, leading to cytotoxicity. Despite of the similar cell permeability of Gd-DOTA complexes, this agent exhibited much weaker binding to cell components leading to viability of the cells over a long experimental time of days.

The buildup curve for overall proton signal of the cell sample treated with 150 mM Gd-DOTA solution showed two relaxation times of 108 ms and 393 ms for extracellular and intracellular proton, respectively, at -60°C. The contributions of proton magnetization diffusing from intracellular and extracellular parts of the cell causes the locational dependence of proton relaxation rates of phospholipid, sugar and nucleotide molecules. The proton magnetization of lipids which compose the cell membranes relaxed quickly because of strong paramagnetic effect from the extracellular part, while that of nucleotide which are located deeply inside

the cell relaxed slowly due to small contribution of fast-relaxing magnetization diffusing from the extracellular part. The spin diffusion model computed using spin diffusivity of 0.8 nm^2/ms revealed that the Gd-DOTA cannot pass though the inner cell membrane. Therefore, this model could be used to analyze the relaxation rate of macromolecules locating within a 20 nm of the centre of inner cell membrane.

Conclusion:

This thesis showed high-resolution solid-state NMR provided the *E. coli* cellular site information semiquantitatively for biological macromolecules by using paramagnetism of Gd-DOTA as a relaxation contrast agent. Thus this methodology enables solid-state NMR spectroscopy to study cellular structure as well as biomolecular structure.

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論文審査の結果の要旨及び担当者

氏　名　（ Nat Sakol ）			
論文審査担当者		（職）	氏　名
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論文審査の結果の要旨

常磁性金属錯体による磁気緩和効果の増大を利用して，核磁気分光法によって生体分子の細胞内での位置に関する情報を得る方法を開発してその有効性を検証した。

論文前半では，細胞内外の常磁性金属錯体濃度を，溶液 NMR で計測することが示された。これには，水のプロトン磁気緩和速度が常磁性錯体濃度に比例することを利用した。細胞内生体分子の固体核磁気共鳴解析も併用して解析した結果，Gd^{3+}はリン脂質などと結合して細胞内では遊離状態では存在せず磁気緩和増大効果が弱く，さらに細胞毒性が強いこと，Gd-DOTA は生体分子との相互作用が弱く毒性も低いことから細胞解析に適していることがわかった。また，細胞内外で約 10 倍の常磁性錯体濃度差を作れることを明らかにした。

論文後半では，その細胞内外での常磁性錯体濃度差を利用して，固体核磁気共鳴法によってプロトンの磁気緩和速度を高分解能に生体分子ごとに計測することで，細胞内での位置情報を得られることが示された。^{13}C 核磁気共鳴の感度を高めるため，細胞は安定同位体で一様に標識したものを用いた。生体分子の運動性による磁気緩和を抑制して，細胞状態の変化を止めて長時間の計測を行うために実験は，－60 度以下の低温で行われた。この状態においては，プロトンのスピン拡散により，磁気緩和速度は主に常磁性錯体濃度のみで決まること，常磁性緩和効果は数十ナノメーター伝達すること，生体分子種を区別して位置情報を得られることが，実験とその磁気緩和シミュレーション解析によって示された。

この実験と解析は，スピン拡散効果が相対的に強くなる極低温の条件で細胞膜表面から 100 ナノメーター程度までの距離を計測できること，核磁気共鳴により生体分子構造と細胞構造を同時に解析できることを示すものである。特に本方法は，核スピン分極を 1000 倍以上増大させる高磁場動的核分極法や分解能を向上させる多次元固体 NMR 法と併用することで，解析能力がさらに向上することが期待できる。よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。