The cellular health condition severely affects protein folding state in mammalian cells

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In-cell NMR spectroscopy is a promising non-invasive technique to investigate the structure and dynamics of biomolecules at the atomic level in living cells. In this study, we investigated the correlation between the cellular health condition and the protein folding state in living mammalian cells by using in-cell NMR spectroscopy with the bioreactor system[1].

In a typical in-cell NMR experiment, cells exist in an anaerobic high-density suspension (approximately 1 × 10^8 cells/mL) within the sample tube, in which nutrients can be rapidly depleted to induce the cell death within a few hours. In order to overcome this issue, bioreactor systems have recently been developed to maintain appropriate cell culture conditions during the experiments by continuously supplying fresh medium[2,3]. As it was difficult for us to reproduce the method as reported, especially with respect to the cell immobilization by gel encapsulation, we employed the centrifuge-based alginate gel encapsulation method[4] for cell immobilization. This method allowed us to easily obtain cell beads with highly reproducible sizes and distributions. Figure 1 illustrates the schematic overview of our bioreactor system for in-cell NMR experiments. Gel encapsulated HeLa cell beads were packed into the custom-built NMR tube, and culture medium was supplied to the HeLa cell beads from the bottom, through the tip of the custom-built inner tube made from a Pasteur pipette.

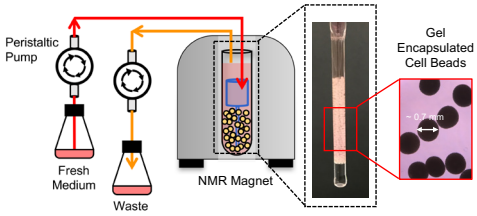

Figure 1. Schematic overview of our bioreactor system for in-cell NMR experiments.

We selected human Adenylate Kinase 1 (hAK1) as a target, which is a cytosolic enzyme catalyzing the reversible phosphoryl transfer reaction of adenine nucleotides in cells and is well known to undergo the large conformational change by the opening and closing of domains depending on the bound nucleotide during the enzymatic cycle. We first performed a series of in-cell NMR experiments of hAK1 in a high-density HeLa cell suspension without a fresh medium supply. The time-dependent change of the 2D ^{1}H-^{15}N HSQC spectra suggest that the deterioration in the cellular health conditions induces the protein unfolding during the experiments (Fig. 2A-D). We then performed in-cell NMR experiments with a fresh medium supply. At least for 12 hours, the hAK1 exhibited a relatively well-resolved NMR spectrum (Fig. 2E), whose cross-peak pattern is basically the same as that in the ATP saturated state *in vitro* (Fig. 2F), demonstrating that hAK1 adopts an ATP-bound folded conformation in HeLa cells. We inferred that a stress induced denaturation of hAK1 in HeLa cells occurs due to a simple acid denaturation by being translocated into lysosomes possibly through Chaperon-Mediated Autophagy mechanism or a combination of various physicochemical properties under the stressful cellular environment, for example, cytosolic acidification, quinary interactions of bio-macromolecules, and so on.

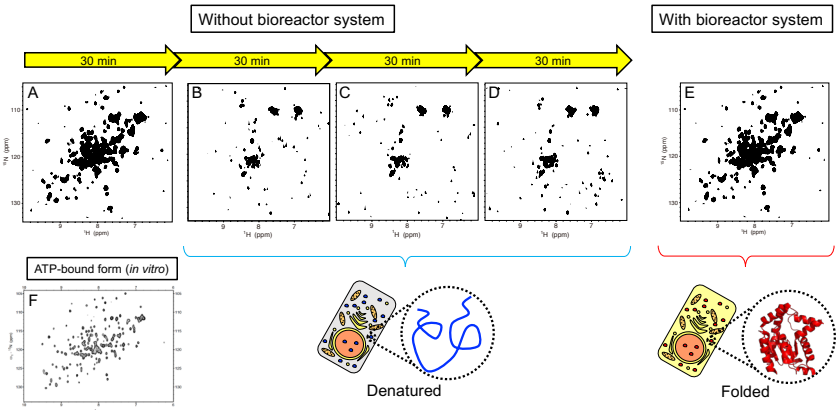

Figure 2. In-cell NMR experiments of hAK1. Time course of the 2D ^{1}H–^{15}N SOFAST–HMQC spectra without a fresh medium supply (A-D) from the start of the experiments (30 min interval). (E) In-cell NMR spectrum with a fresh medium supply. (F) *in vitro* reference spectrum in the presence of ATP in excess.

These results indicate that the proper regulation of the physiological condition of the cells is crucial when investigating protein behaviors in cells by in-cell NMR. Our work provides a critical guide to in-cell NMR analyses, not only for protein structural analyses at an atomic level in mammalian cells but also for their potential applications in the future, such as drug candidate screening and molecular diagnoses of biopsy samples.

References

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