NMR を用いた細胞構造生物学

Cellular structural biology by NMR

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Introduction

In vivo observations of 3D structures, structural changes, dynamics or interactions of proteins are essential for the explicit understanding of the structural basis of their functions inside cells. Recently we reported the first 3D protein structure calculated exclusively on the basis of information obtained in living *E.coli* cells [1,2]. We are currently addressing the structure determination of *Streptococcus* protein G B1 domain (GB1) overexpressed in *E. coli* cells, as an another demonstration of our approach.

Extending in-cell NMR to study proteins inside higher eukaryotic cells, and thus making this method more useful in medical and pharmaceutical researches, was another issue to be investigated. However, the low intracellular concentration of target proteins makes it difficult to perform detailed NMR analyses, such as backbone/side-chain resonance assignments, collection of various structural informations etc. By using two different eukaryotic systems, cultured human HeLa cells and Sf9 cultured insect cells, we have initiated methodological developments/optimisations in stable isotope labelling of target proteins, rapid measurement of multidimensional NMR spectra, improved data processing algorithms, etc.

Results and Discussion

The GB1 *E. coli* in-cell NMR samples suffer from much lower intracellular concentration of target proteins (~0.2-0.5 mM) compared to the previous *Thermus thermophilus* HB8 TTHA1718 case, thus further methodological optimisation was required for sample preparation, data processing, resonance assignments, NOE analysis and structure calculation. Particularly the newly introduced labelling procedure for side-chain methyl selective protonation, in which the ^{1}H-^{1}H dipolar relaxation between neighbouring methyl groups are minimised, provided much improved 3D ^{13}C/^{13}C-separated NOESY spectra for collecting long-range methyl-methyl distance restraints. In addition we found that significantly improved 3D NMR spectra can be reconstracted by employing a new maximum entropy procesure, Quantitative Maximum Entropy (QME) (D. O'Donovan, W. Boucher and E. D. Laue, unpublished results). Owing to these

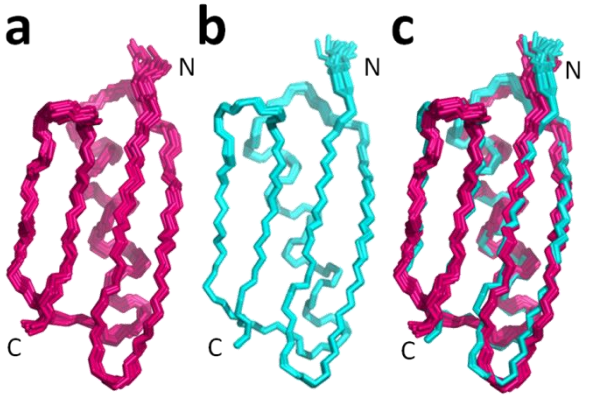

Fig. 1. NMR solution structure of protein G B1 domain in living *E. coli* cells. a, A superposition of the 20 final structures of GB1 in living *E. coli* cells, showing the backbone (N, C^{α}, C') atoms. The resulting structure is well-converged with a backbone RMSD of 0.51 Å to the mean coordinates. **b**, A superposition of the 20 final structures of purified GB1 *in vitro*. **c**, A comparison of GB1 structures in living *E. coli* cells and *in vitro*. The best fit superposition of backbone (N, C^{α}, C') atoms of the two conformational ensembles are shown with the same colour code in **a** and **b**.

methodological improvements, the resulting structure (the world's 2nd protein structure in living cells) is well-converged, and is similar to the structure that was determined independently *in vitro* from a purified sample (Fig. 1). Further refinement of the in-cell structure is in progress by introducing automated side-chain assignment procedure [3] into the structure calculations.

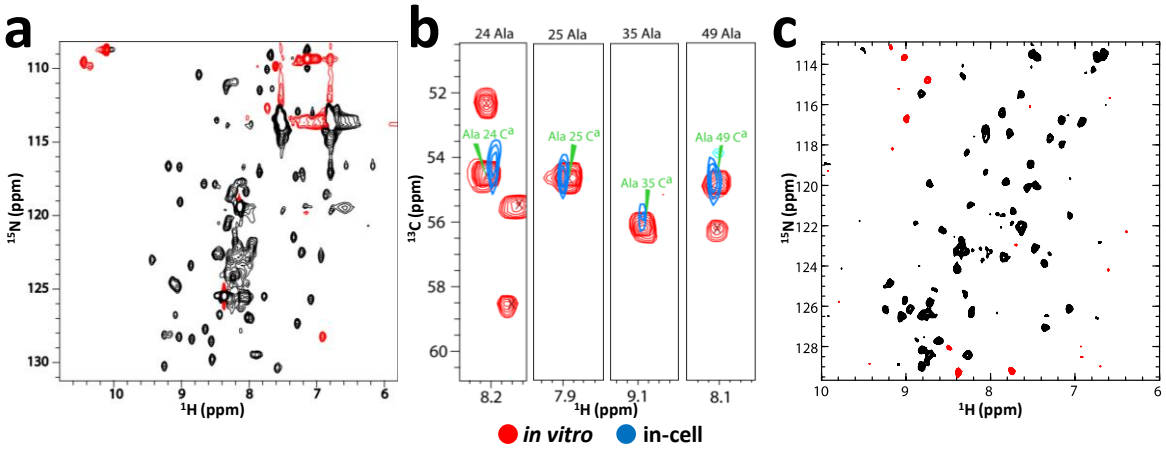

Fig. 2. In-cell NMR spectra of proteins inside living eukaryotic cells. a. The 2D ^{1}H-^{15}N HSQC spectrum of GB1 in Sf9 cells. **b**. 2D ^{13}C-^{1}H^{N} slices from the 3D HNCA spectrum of alanine-selectively ^{13}C-labelled and uniformly ^{15}N-labelled GB1 in SF9 cells (blue crosspeaks) overlaid with corresponding slices from the 3D HNCA spectrum of uniformly ^{13}C/^{15}N-labelled GB1 *in vitro* (red crosspeaks). In 3D HNCA intraresidue and interesidue ^{13}C^{a}-^{15}N-^{1}H^{N} correlations are observed, thus commonly used for backbone resonance assignment. **c**. The 2D ^{1}H-^{15}N SOFAST-HMQC spectrum of TTHA1718 in living HeLa cells. For all 2D/3D NMR experiments the indirectly acquired dimensions were measured with a nonlinear sampling scheme and processed with maximum entropy using Azara v2.8 software (W. Boucher, www.bio.cam.ac.uk/azara).

Baculovirus systems in cultured insect cells (e.g. Sf9 cells) are very widely used for expressing recombinant proteins with eukaryotic protein production machinery. By employing this system we initiated in-cell NMR studies of some of our model proteins expressed in cultured Sf9 cells. After optimising labelling procedure, we could measure high resolution 2D ^{1}H-^{15}N correlation spectra of target proteins (Fig. 2a). In all eukaryotic in-cell NMR studies published so far, target proteins have been incorporated from outside of cells. Our results are, therefore, the world's 1st examples of in-cell NMR spectra of proteins expressed in eukaryotic cells. Further we succeeded in measuring 3D triple resonance NMR spectra of GB1 in Sf9 cells (Fig.2b). Our results suggest the possibility that the approach we used for the structure determination of proteins inside *E. coli* cells can also be applied to this Sf9 system.

Inomata et al. introduced an innovative method that enables in-cell NMR in human cells by which the target protein is delivered into the cells by tagging it with a cell-penetrating peptide [4]. We have initiated modification and optimisation of the existing protocol aimed at measuring NMR spectra for resonance assignments and collecting various structural informations. Fig. 2c shows an example of in HeLa cell 2D SOFAST-HMQC spectra. In this presentation we report our recent results on two model proteins, TTHA1718 and GB1, and discuss the vision for the structural analysis of proteins inside human cultured cells.

References:

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