Structural Analyses of Biological Macromolecules by Ultra-High Field NMR Spectroscopy

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Our research seeks the underlying molecular basis for the function of biological macromolecules. In particular, we are interested in the function of molecular machines that work in the cellular processes involving protein folding, transport and degradation, and of glycoproteins playing important roles in the humoral and cellular immune systems. By use of ultra-high field NMR spectroscopy, we aim to elucidate the three-dimensional structure, dynamics, and interactions of proteins and glycoconjugates at the atomic level. Here we report stable-isotope-assisted NMR studies of IgG-Fc glycoprotein, NEDD8 and protein disulfide isomerase.

1. Structural Comparison of Fucosylated and Nonfucosylated Fc Fragments of Human Immunoglobulin G1[1)]

Removal of the fucose residue from the oligosaccharides attached to Asn297 of human immunoglobulin G1 (IgG1) results in a significant enhancement of antibody-dependent cellular cytotoxicity (ADCC) via improved IgG1-binding to Fcγ receptor IIIa (FcγRIIIa). To provide a structural insight into the mechanisms of affinity enhancement, we determined the crystal structure of non-fucosylated Fc fragment and compared it with that of fucosylated Fc. The overall conformations of the fucosylated and non-fucosylated Fc fragments were similar except for hydration mode around Tyr296. Stable-isotope-assisted NMR analyses confirmed the similarity of the overall structures between fucosylated and non-fucosylated Fc fragments in solution. These data suggest that the glycoform-dependent ADCC enhancement is attributed to a subtle conformational alteration in a limited region of IgG1-Fc. Furthermore, the electron density maps revealed that the traces between Asp280 and Asn297 of our fucosylated and non-fucosylated Fc crystals were both different from those in previously reported isomorphous Fc crystals.

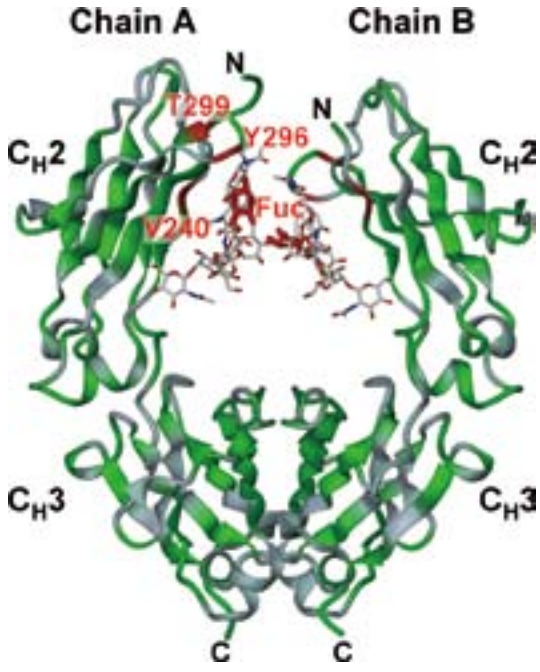

Figure 1. Mapping on the crystal structure of Fuc (+) of the amino acid residues showing the chemical shift difference between Fuc (+) and Fuc (–). The chemical shift differences are quantified for each residue according to the equation $(0.2\delta N^2 + \delta H^2)^{1/2}$, where δN and δH represent the differences in nitrogen and proton chemical shifts between Fuc (+) and Fuc (–). The amino acid residues showing and not showing observable chemical shift differences $[(0.2\delta N^2 + \delta H^2)^{1/2} > 0.1$ ppm] are colored red and green, respectively. The Fuc residues are colored magenta.

2. Direct Interactions between NEDD8 and Ubiquitin E2 Conjugating Enzymes Upregulate Cullin-Based E3 Ligase Activity[2)]

Although cullin-1 neddylation is crucial for the activation of SCF ubiquitin E3 ligases, the underlying mechanisms for NEDD8-mediated activation of SCF remain unclear. We demonstrated by NMR and mutational studies that NEDD8 binds the ubiquitin E2 (UBC4), but not NEDD8 E2 (UBC12). Our data imply that NEDD8 forms an active platform on the SCF complex for selective recruitment of ubiquitin-charged E2s in collaboration with RBX1, and thereby upregulates the E3 activity.

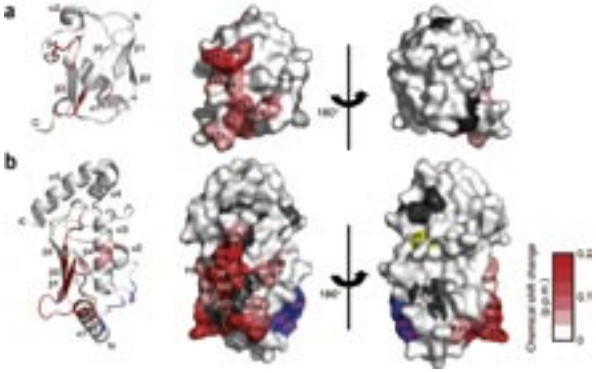

Figure 2. Identification of the binding sites on NEDD8 and UBC4. (**a**,**b**) Mapping of the perturbed residues of NEDD8 (**a**) and UBC4 (**b**) upon binding to each other. Residues are highlighted in red on the crystal structures of NEDD8 and UBC4. Red gradient indicates the strength of the perturbation. Blue, residues involved in the interaction with the RING-finger domain in the crystal structure of c-Cbl (PDB 1FBV); gray, prolines; yellow, catalytic cysteine (C85).

3. NMR Assignments of the *b*' and *a*' Domains of Thermophilic Fungal Protein Disulfide Isomerase[3)]

Protein disulfide isomerase (PDI) is a folding assistant in the endoplasmic reticulum that catalyzes the formation, breakage and rearrangement of disulfide bonds of its substrate proteins. PDI comprises four structural domains, *a*, *b*, *b*', *a*' plus C-terminal extension. To gain insight into the functions of PDI, we initiated NMR structure determinations of the *b*' and *a*' domains of thermophilic fungal PDI expressed in *E. coli*. Backbone NH signals of these domains were completely assigned except for His367 in the *a*' domain. In total, 87% (*b*' domain) and 86% (*a*' domain) of the observable proton signals were assigned. The secondary chemical shifts indicate that both domains assume thioredoxin folds.

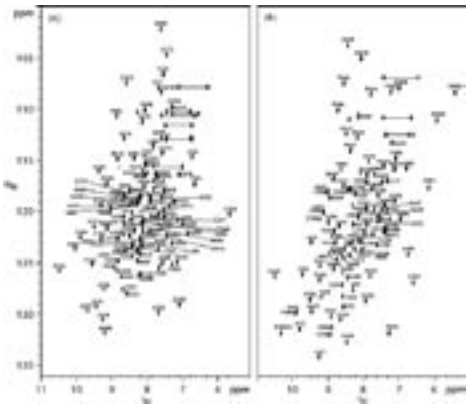

Figure 3. ^{1}H-^{15}N HSQC spectra of 1 mM uniformly $^{13}C/^{15}N$-labeled *b*' domain (a) and *a*' domain (b) of thermophilic fungal PDI in the presence of 10 mM [$^{2}H_{10}$]dithiothreitol. Backbone amide cross peaks are indicated with assignments. The residue numbering of intact PDI was applied for each domain.

References

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2) E. Sakata, Y. Yamaguchi, Y. Miyauchi, K. Iwai, T. Chiba, Y. Saeki, N. Matsuda, K. Tanaka and K. Kato, *Nat. Struct. Mol. Biol*. **14**, 167–168 (2007).
3) M. Nakano, C. Murakami, Y. Yamaguchi, H. Sasakawa, T. Harada, E. Kurimoto, O. Asami, T. Kajino and K. Kato, *J. Biomol. NMR* **36**, 44 (2006).

Awards

UTSUMI, Maho; Physical Pharma Forum 2007 (The Pharmaceutical Society of Japan, Division of Physical Sciences) Award of Superior Excellence.
UTSUMI, Maho; 71st The Japanese Biochemical Society, Chubu Branch, Award for Young Scientists.

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