Elucidation of Dynamical Structures of Biomolecules toward Understanding the Mechanisms Underlying Their Functions

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Our biomolecular studies are based on detailed analyses of structures and dynamics of various biological macromolecules and their complexes at atomic level, primarily using nuclear magnetic resonance (NMR) spectroscopy. In particular, we conducted studies aimed at elucidating dynamic structures of glycoconjugates and proteins for integrative understanding of the mechanisms underlying their biological functions. For this purpose, we use multidisciplinary approaches integrating the methodologies of molecular and cellular biology and nano-science along with molecular spectroscopy.

1. NMR Characterization of the Interaction between Amyloid β-Peptide and Ganglioside Clusters[1,2)]

Gangliosides are targets for a variety of pathologically relevant proteins, including amyloid β (Aβ), an important component implicated in Alzheimer's disease (AD). To provide a structural basis for this pathogenic interaction associated with AD, we conducted NMR analyses of the interactions of Aβ with ganglisoides using lyso-GM1 micelles as a model system. Our NMR data revealed that the sugar-lipid interface is primarily perturbed upon binding of Aβ to the micelles, underscoring the importance of the inner part of the ganglio-side cluster for accommodating Aβ in comparison with the outer carbohydrate branches that provide microbial toxin- and virus-binding sites. To provide a more detailed understanding of the interaction mode of Aβ and gangliosides, we observed NMR peaks originating from isotopically labeled Aβ bound to the micelles. The secondary chemical shifts of the polypeptide backbone indicated that Aβ forms discontinuous α-helices upon binding to the gangliosidic micelles, leaving the remaining regions disordered. TROSY-based saturation transfer analyses revealed that Aβ lies on hydrophobic/hydrophilic interface of the ganglioside cluster exhibiting an up-and-down topological mode in which the two α-helices and the C-terminal dipeptide segment are in contact with the hydrophobic interior, whereas the remaining regions are exposed to the aqueous environment (Figure 1). These findings suggest that the ganglioside clusters provide a unique platform at their hydrophobic/hydrophilic interface for binding coupled with conformational transition of Aβ molecules, rendering their spatial rearrangements restricted to promote specific inter-molecular interactions.

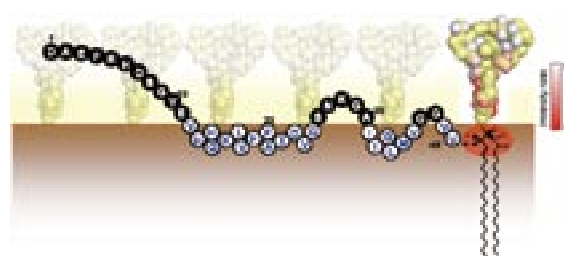

Figure 1. Schematic drawing of Aβ(1–40) lying on the hydrophobic/hydrophilic interface of ganglioside clusters. The amino acid residues exposed to the hydrophilic and hydrophobic milieus are represented by closed and open circles with single-letter codes. The sugar hydrogen atoms coloured in red and the ceramide hydrogen atoms located within the red ellipse area exhibit lower peak intensity ratios, indicating that they are in close spatial proximity of the C-terminus of Aβ(1–40).

2. Structural Basis of the Protein-Fate Determination in Cells[3–6)]

In eukaryotc cells, folding of nascent proteins is assisted by complexes of molecular chaperones in the endoplasmic

reticulum (ER). Correctly folded proteins are transferred from the ER to the Golgi complex on the secretion pathway, while terminally malfolded proteins are transferred from the ER to the cytosol and subsequently degraded in the ubiquitin (Ub)- and proteasome-mediated proteolytic pathway. We conducted structural biology studies of the ER chaperones, the cargo receptors, and the ubiquitinating enzymes to elucidate the mechanisms underlying the protein-fate determination in cells.

Protein disulfide isomerase (PDI) is a major protein in the ER, operating as an essential folding catalyst and molecular chaperone for disulfide-containing proteins by catalyzing the formation, rearrangement, and breakage of their disulfide bridges. By combined use of NMR and small-angle X-ray scattering methods, we reveal the redox-dependent domain rearrangement of PDI coupled with exposure of its substrate-binding hydrophobic surface spanning the *b*' and *a*' domains (Figure 2A). On the basis of these data, we propose a mechanistic model of PDI action; the *a*' domain transfers its own disulfide bond into the unfolded protein accommodated on the hydrophobic surface, which consequently changes into a *closed* form releasing the oxidized substrate.

The intracellular lectin ERGIC-53 and the EF-hand Ca^{2+}-binding protein MCFD2, which have been identified as products of the causative genes of combined deficiency of coagulation factors V and VIII (F5F8D), form a complex operating as cargo receptor of these coagulation factors. We determined the 3D structure of ERGIC-53 complexed with MCFD2 by X-ray crystallographic analysis in conjunction with NMR and ultracentrifugation analyses (Figure 2B). The interaction is independent of sugarbinding of ERGIC-53 and involves most of the missense mutation sites of MCFD2 so far reported in F5F8D. Comparison with the previously reported uncomplexed structure of each protein indicates that MCFD2 but not ERGIC-53-CRD undergoes significant conformational alterations upon complex formation. These findings provide a structural basis for the cooperative interplay between ERGIC-53 and MCFD2 in capturing FV and FVIII.

A key question in ubiquitination is how E2-E3 complexes can deal with the various acceptor sites distributed on the substrate surface to perform multiple ubiquitination and/or poly-Ub elongation. We solved the crystal structure of an intermediate of the UbcH5b E2 enzyme conjugated with Ub which is assembled into an infinite spiral through the backside interaction (Figure 2C). This active complex may provide multiple E2 active sites, enabling efficient ubiquitination of substrates. Indeed, biochemical assays support a model in which the self-assembled UbcH5b~Ub can serve as a bridge for the gap between the lysine residue of the substrate and the catalytic cysteine of E2.

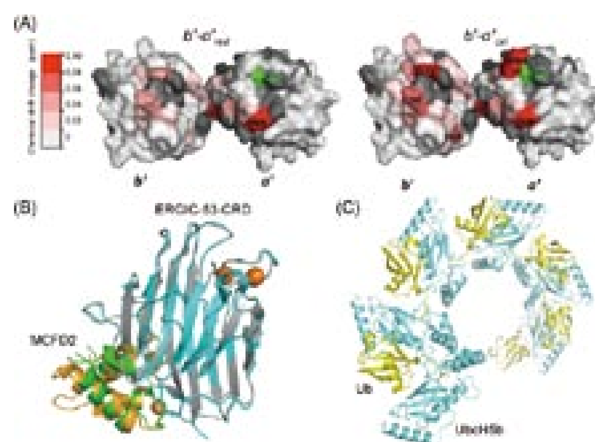

Figure 2. (A) Mapping on the 3D model of *b*'-*a*' (reduced form) of the residues whose chemical shift were perturbed upon binding with mastoparan (as a model ligand) in the reduced (left) and oxidized (right) forms of *b*'-*a*'. Red gradient indicates the strength of the perturbation. The proline residues and the residues whose ^{1}H–^{15}N HSQC peak could not be used as a probe due to broadening and/or overlapping are shown in gray. The catalytic cystein residues appear in green. (B) 3D structures of MCFD2 and ERGIC-53-CRD. Uncomplexed structures of rat ERGIC-53 (cyan) and human MCFD2 (orange) are superimposed on our crystal structure of the complex between human ERGIC-53 (gray) and MCFD2 (green). The bound calcium ions are shown as spheres. (C) Formation of the self-assembly of the UbcH5b~Ub conjugate through the backside interaction.

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Award

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