Site-Specific Conformational Probes of Misfolded Proteins

by

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ABSTRACT

The proper folding of proteins into complex 3D folded structures is essential for a myriad of cellular functions, while defects in this process are associated with several devastating neurodegenerative disorders (e.g., Alzheimer's and prion diseases). The seminal event in these diseases is the misfolding and aggregation of proteins into either β-sheet rich amyloid fibrils or related oligomeric intermediates (or both). Subtle differences in the structure of aggregated isoforms lead to dramatic differences in their biological activity, yet elucidating such structural differences at the level of individual residues is extremely difficult.

Towards understanding the molecular basis of aggregation, we developed and employed novel site-specific approaches to investigate structure of misfolded proteins. We first elucidated how differences in structure of infectious prion conformers govern their ability to establish and overcome species barriers. Fungal prions from *Saccharomyces cerevisiae* and *Candida albicans*, like their mammalian counterparts, form different prion strain (amyloid) conformations with unique capacities to overcome species barriers. Therefore, we investigated the relationship between the structure of a promiscuous yeast prion strain conformation and its ability to selectively infect the two yeast species. Using fluorescent labeling techniques and proline scanning mutagenesis, we found that the specific infectivity of this prion strain conformation is due to selective folding of the *C. albicans* cognate prion recognition sequence within the amyloid core, while the corresponding recognition sequence for *S. cerevisiae* is excluded from the amyloid core.

We also developed a novel motif-grafting strategy for generating conformation- and sequence-specific antibodies for evaluating structural features of misfolded proteins. We grafted peptide segments known or predicted to regulate the assembly of several amyloidogenic proteins into the antigen binding loops of antibodies and evaluated antibody binding to aggregated isoforms. We showed that the same self-complementary interactions that mediate amyloid assembly can be harnessed by our motif-grafting strategy and enable antibodies to immunoreact in amyloidogenic proteins. We found that Grafted AMyloid-Motif antiBODIES (gammabodies) are powerful structural probes for illuminating conformational differences.

Expanding on our design of novel antibodies, we established systematic approaches to optimize solubility and binding properties of antibodies. We first investigated the impact of charged mutations at the edges of binding loops and found that they endow antibodies with increased solubility and aggregation-resistance under denaturing conditions. We used these principles to also create multidomain antibodies that have high binding affinity. We expect that our discovery will guide the design and selection of antibodies that not only possess high affinity and conformational stability, but also extreme resistance to aggregation.

Our methods used to investigate amyloids are adaptable and general to investigating protein structure. Our approach serves to complement current technologies employed to investigate the structure-function relationship of both misfolded and natively folded proteins. We contributed several simple key principles in protein design which can be used to elucidate the molecular basis of protein assembly.