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Substrate-Induced Unfolding of Protein Disulfide Isomerase Displaces the Cholera Toxin A1 Subunit from its Holotoxin
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To generate a cytopathic effect, the catalytic A1 subunit of cholera toxin (CT) must be separated from the rest of the toxin. Protein disulfide isomerase (PDI) is thought to mediate CT disassembly by acting as a redox-driven chaperone that actively unfolds the CTA1 subunit. Here, we show by isotope-edited Fourier transform infrared spectroscopy and circular dichroism that PDI itself unfolds upon contact with CTA1. The substrate-induced unfolding of PDI provides a novel molecular mechanism for holotoxin disassembly: we postulate the expanded hydrodynamic radius of unfolded PDI acts as a wedge to dislodge reduced CTA1 from its holotoxin. The oxidoreductase activity of PDI was not required for CT disassembly, but CTA1 displacement did not occur when PDI was locked in a folded conformation or when its substrate-induced unfolding was blocked due to the loss of chaperone function. Our data establish a new property of PDI that is required for cholera intoxication and may be linked to its function as a chaperone that prevents protein aggregation.

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Positively Charged Redox Agents Accelerate Disulfide Coupled Protein Folding
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Multi disulfide-containing-proteins are folded into the native conformations by the assistance of redox molecules, such as glutathione and cysteine *in vivo*. Because of this, the glutathione redox system is also generally used in *in vitro* folding. However, *in vitro* disulfide-coupled protein folding is often problematic since the folding intermediates are sometimes prone to aggregation. Particularly, mis-bridged disulfide isomers that possess relatively long half-lives expose their hydrophobic surfaces to solvents, resulting in non-specific aggregation during folding. In this study, to overcome this issue and investigate the mechanism of disulfide-coupled protein folding, a series of thiol reagents was prepared and their ability to regulate the folding of disulfide-containing proteins was examined.We recently reported that Arg-Cys-Gly (RCG) accelerated the folding of disulfide-containing-proteins and proposed that positively-charged redox reagents are effective in promoting disulfide-coupled protein folding. However, the mechanism of folding reaction involved in the formation of the cross disulfide species during folding remains to be studied in detail. Therefore, to clarify the disulfide shuffling mechanism of the cross disulfide species, we focused on the positive charge and steric hindrance of the thiol reagents. Lysozyme (4 disulfides) and prourogunaylin (3 disulfides) were used as model proteins to investigate the folding ability of the series of thiol reagents. The reduced/denatured proteins were folded in the presence of 2-aminoethanethiol (charge: +1), 2-mercaptoethanol (0), or 3-mercaptopropionic acid (−1). Among the reagents tested, 2-aminoethanethiol was superior for enhancing the folding reaction, indicating that the positive charge is effective for disulfide-coupled protein folding. The hydrophobicity and steric hindrance of the thiol reagents were also discussed for the folding reaction of lysozyme and purouroguanylin. The results will be discussed in this paper.

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Regulation of Disulfide Coupled Folding of De Novo Designed Precursor Protein
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Uroguanylin, a peptide consisting of 16 amino acid residues and 2 disulfide bonds, participates in salt and water homeostasis in mammals and the correct disulfide pairings are absolutely required for its biological activity. However, uroguanylin is not able to fold into its native conformation by itself, and its disulfide-coupled folding is mediated by the pro-peptide region in the precursor protein. Thus, the pro-peptide region of uroguanylin serves as an intramolecular chaperone.
We previously proposed that a relationship existed between the partial folding of the bioactive conformation of the mature peptide hormone and the disulfide-coupled folding of the precursor protein. X-ray crystallography data for prouroguanylin revealed that the Asp72 interacts with the receptor-binding site (Asn79-Val80-Ala81). This implies that the Asp72 residue plays an important role in the folding of prouroguanylin by stabilizing the bioactive site. To address this issue, we studied the roles of the Asp72 residue in precursor proteins, prouroguanylin and a de novo designed protein (pro-NDD-hybrid protein).
To investigate the role of hydrogen bonding between the Asp72 and Asn79 residue for the precursor folding, a series of mutant proteins was prepared using an E. coli expression system and the folding of these molecules was examined using the typical glutathione redox system.
The replacement of the Asp72 residue with Gly had no effect on the folding of prouroguanylin but dramatically influenced the folding of the pro-NDD-hybrid protein. The results revealed that the Asp72 residue is important in stabilizing the native conformation. Thus the Asp72 residue plays an important role in the intra-molecular chaperone function of the pro-peptide region. Furthermore, to evaluate the role of the Asp72 residue in in vivo folding, mutant proteins were also expressed in human 293T cells. The results will be discussed in this paper.

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Revisiting Structural Hieracrchy: A Fluorescence Investigation of Unfolding of an Oligomeric Protein
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α-crystallin is a multimeric lens protein with chaperone-like function and is responsible for maintaining lens transparency. The structural stability and unfolding-refolding properties of this protein are believed to be important for its function. We undertook a multi-probe based fluorescence approach to explore the changes in the various levels of organization of α-crystallin at different urea concentration region. Steady-state fluorescence emission and quenching studies using extrinsic and intrinsic fluorescence probes reveal that at 0.6 M urea a compact structural intermediate is formed which has a native-like secondary structure with minimal yet detectable tertiary structure perturbation associated with enhanced surface exposure of hydrophobic groups, possibly arising from interfacial structure meltdown. At 2.8 M urea the tertiary interactions undergo almost complete collapse with partial disintegration of secondary and quaternary structure. Investigation of surface solvation with picosecond resolved fluorescence transients of acrylodan covalently tagged to α-crystallin reveals a dry native-like core of α-crystallin at 0.6 M urea compared to enhanced water penetration at 2.8 M urea and extensive solvation at 6 M urea. Temperature dependent subunit exchange kinetics reveal decrease of activation energy for the subunit exchange process by 22 kJ mol-1 on changing urea concentration from 0 to 0.6 M compared to over 75 kJ mol-1 on changing urea concentration from 0 to 2.8 M. Dynamic light scattering study indicates swelling at 0.6 M urea, however oligomerization is retained as observed from sedimentation equilibrium experiment. At 2.8 M urea the oligomeric size is reduced and a monomer is produced at 6 M urea. These data clearly demonstrates that tertiary structure dissolution precedes oligomeric degradation. Such non-hierarchical structure dissolution indicates the possibility of tertiary contact formation to be a rather later folding event in case of large oligomeric proteins likes α-Crystallin.

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Knotting a Protein in Explicit Solvent
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Today it is well accepted that knotted proteins exist, however it is not known why such structures exist and what is function of knots. Knots in proteins behave in different why than commonly known knots in polymers and constantly surprise experimentalists and theoreticians. So far it has been shown experimentally only for two proteins that they can self tie, however folding mechanism is not understood. Moreover currently we do not even know how to untie proteins. Some light can be shed on these puzzles by structure based model (SBM), which were very successful in the case of "ordinary" proteins. In our previous works we shown that for some proteins folding into knots involves threading the protein terminal across a twisted loop via a slipknot configuration (as in shoelaces).
Here we report for the first time unbiased explicit-solvent simulations of the knotting dynamics of a protein. In simulations totalling 40 μs with the Amber99sb forcefield, we have found that five out of fifteen simulations reach the knotted native state when started from unknotted, slipknotted intermediates. Comparison of explicit-solvent to structure-based simulations shows that similar native contacts are responsible for threading the slipknot through the loop, however competition between native and non-native salt bridges during threading results in increased energetic roughness.
These results, firstly, support the existence of an underlying free energy bias able to overcome the entropically-unfavorable constraints of the C-terminus