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Previews

Crystallography: Embracing Conformational Flexibility in Proteins

Alcohol cosolvents in conjunction with protein crosslinking can be used to stabilize functionally important structures in a signal transduction molecule.

It is estimated that about half the protein sequences in the Swiss Protein Database contain extended amino acid segments that are predicted to be flexibly disordered. These disordered regions can either be local or global and are typically inaccessible to protein crystallography. The trouble is that many of such regions are found to be critical for protein function (Wright and Dyson, 1999), as they become structured in particular environments or on interaction with ligands and other proteins. Using a wide range of solution conditions it has, nevertheless, been possible to crystallize a considerable number of such proteins by stabilizing intact polypeptides or selected fragments in well-defined conformations. The extent to which these conformations are representative of functionally important structures is frequently not clear given a single crystal. However, proteins can often be crystallized in different space groups, or can be cocrystallized with ligands or with other binding partners. Comparison between the different crystal structures typically provides compelling insights into protein function.

What if, however, the appropriate ligand or protein binding partner is not known or if the complex proves resistant to crystallization? The paper by Buhrman et al. in this issue of *Structure* (Buhrman et al., 2003) suggests a novel and general strategy that may allow a functional characterization of local disordered segments in crystalline proteins. As a test case, Mattos and coworkers examined the well-known conformational transition in the switch II region of the GTPase Ras. GTPases are understood to function as molecular switches in a variety of molecular signaling networks. Ras can alter between several conformational states depending on the nature of the nucleotide (GDP or GTP analogs) and on the regulatory or effector proteins that are bound. Specifically, the crystal structure of Ras-GDP is disordered in two regions, switch I (residues 32–38) and switch II (residues 59–72), while the GTP-bound form is ordered in switch I but retains multiple conformations in switch II. This latter region becomes ordered when Ras interacts with protein binding partners. With this crystallographic information in mind, Mattos and coworkers set out to explore the effects that alcohol cosolvents have on the conformation of the switch II region in Ras.

The strategy and findings reported in this issue of *Structure* can be summarized as follows: grown Ras crystals were transferred to the crystallization buffer containing 1% glutaraldehyde, which randomly crosslinked several lysine side chains within and between molecules in the crystal lattice. The crosslinked proteins, thus stabilized, could be transferred into alcohol cosolvents (50% v/v 2,2,2-trifluoroethanol, known as TFE, 60% v/v 1,6-hexanediol, or 50% isopropanol) with minimal damage to the crystal and little change to the overall protein structure. The switch II region, however, was found to be ordered in TFE and 1,6-hexanediol soaked crystals. Most significantly, the residues were structured into a conformation closely resembling that adopted by the switch II region when Ras interacts with RalGDS, PI_3 kinase, or RasGAP. The results show that the cosolvents helped to order an intrinsically disordered segment of the Ras protein into a biologically relevant structure. The following aspects of this report are remarkable.

Crystal formation appears to require protein conformations that are well defined and rigid. However, some crystallizations can incorporate proteins with extensive regions that are conformationally flexible (e.g., Xiao et al., 2002; Huntington et al., 2000). In the 166 residue fragment of the Ras-GDP protein, a total of 21 residues are disordered in the switch regions (Buhrman et al., 2003). Crystallization screens frequently contain alcohol cosolvents, typically isopropanol or 2-methyl-2,4-pentanediol (MPD) as protein stabilizers with concentrations up to 30% v/v (e.g., Jeruzalmi and Steitz, 1997). The effects of alcohols on protein structure and function have been extensively researched (Mattos and Ringe, 2001; Hirota et al., 1998; Buck, 1998; Anand et al., 2002), and it is clear that in certain cases nonnative and functionally irrelevant conformations of proteins are stabilized and crystallized (e.g., Xiao et al., 2002). Alcohol cosolvents, such as the popular TFE, disrupt hydrophobic interactions while promoting local hydrogen bonding in proteins. These effects may be modified by other solution components and by crystal packing, but for the majority of polypeptides, considerable cosolvent concentrations (e.g., $>$10% TFE v/v) are needed to cause observable structural changes in the context of a folded protein. Exceptions to this exist, such as the GTPase binding domain of WASP, which adopts a biologically relevant structure in the presence of low cosolvent ($<$5% TFE; Kim et al., 2000). Therefore, it will be interesting to further explore the use of TFE and other cosolvents, including the recently popular trimethylamine N-oxide (TMAO), in crystal screens at intermediate concentrations. It is known that high concentrations of alcohol cosolvent, such as 50% v/v TFE, disrupt the hydrophobic cores and thus the tertiary structure in most proteins (Buck, 1998), and are therefore unlikely to help protein crystallization.

Mattos and coworkers added alcohol cosolvents to pregrown crystals, and crosslinking of Ras was found to be absolutely essential. Crystals transferred to 50% v/v TFE without prior stabilization, for example, are severely damaged (C. Mattos, personal communication). While the extent of crosslinking and concentration of alcohol cosolvent that is necessary to observe the con-

formational change are the subject of detailed ongoing studies, the crosslinking and late addition cosolvent clearly limit perturbation to the protein structure and to the crystal. How limited are conformational changes in the crystalline environment? In general, only slight changes in protein conformation are allowed that are distant from crystal contacts and do not damage the crystal lattice. Nevertheless, a number of investigators were able to interpret small changes in crystal structures as a function of temperature and pressure in terms of protein function and folding (Urayama et al., 2002; Jacob et al., 1998). Additionally, significant progress has been made in the structural characterization of protein catalytic cycles that include limited conformational changes (Petsko and Ringe, 2000). The timescale and to some extent the nature of the conformational changes are, however, expected to be affected by the crystal contacts (Zhu et al., 1992). Because the majority of the protein is restrained in the native structure, the strategy presented by Buhrman et al. has the advantage that conformational effects of the cosolvent are likely to be limited to regions of the protein that remain flexible in the crystal, such as loops or residues in active site clefts.

A critical aspect of the study by Mattos and coworkers is the clear demonstration that the cosolvent-stabilized structure is functionally relevant. Significant effects of the cosolvent on the structure are confined to the switch II region. Furthermore, the structure that is stabilized is closely identical to that seen in Ras when bound to its regulatory or effector proteins. Only few reports exist where a relationship between cosolvent-stabilized structure and function has been demonstrated as clearly (Buck, 1998). This relationship is consistent with the general observation that alcohol-based cosolvents are not indiscriminate in their effect but that the structures that are stabilized reflect intrinsic conformational preferences of the polypeptide chain. It is likely that such alternative conformations of the protein have been preserved in evolution for the purpose of folding or function. An increased repertoire of solution conditions for crystallization trials and the ability to explore the flexibility of proteins in crystals by the use of alcohol cosolvents and crosslinking, as proposed here, will allow us to study different conformational states of protein segments. The automation that is possible will provide many high-resolution structures as part of this strategy. Homology modeling and other computational techniques will, in conjunction with additional experiments, suggest the functional importance of the conformational states thus sampled.

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Selected Reading

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Mycobacterial ABC Transport System: Structure of the Primary Phosphate Receptor

The crystal structure of the mycobacterial PstS-1, the primary phosphate receptor of the ABC transport system, reveals a number of features that account for the tight binding and high specificity along with the close similarity to the phosphate binding protein of *Escherichia coli.*

The capture of essential small molecules and their transport into microorganisms is a fertile area of research, particularly with novel information coming out on ABC transport systems, such as that for phosphate (Torriani, 1990). ABC-type transport systems are responsible for importing a variety of small molecules such as various essential amino acids, sugars, and anions.

The binding proteins that act as initial receptors for such systems in Gram-negative bacteria are well understood in terms of their ligand binding and structures (Quiocho, 1991). These proteins may be expressed in response to starvation for the particular essential molecules. In *Escherichia coli* and other Gram-negative bacteria, an N-terminal peptide directs the protein to the