Structure
Previews

Proteins Do Not Have Strong Spines After All

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In this issue of *Structure*, Berkholz et al. show that the detailed backbone geometry of proteins depends on the local conformation and suggest how this information can be practically used in modeling and refining protein structures.

From the time when Linus Pauling, confined to bed by a flu, constructed paper models of the α helix (Pauling et al., 1951), it has been assumed that proteins are built from rigid peptide units and that the differences in their backbone conformations result only from different torsion angles, Φ and Ψ, around the N-C_α and C_α-C bonds, respectively. This assumption is routinely used in protein crystallography and other methods of protein-model building, and is the basis for setting up the standard sets of values for various types of protein bond lengths and bond angles, of which the most popular is the E&H library (Engh and Huber, 2001). The library values of these geometrical parameters are used for construction of the initial (or theoretical) models of proteins and as a priori knowledge in the restrained refinement of atomic coordinates against X-ray (and/or neutron) diffraction data. The bond lengths and angles and some other parameters (planarity, chiral volumes, etc.) within the protein main and side chains are then restrained, with appropriate weights, to be close to the library targets, but in general the torsion angles (except ω-angles around the C-N bonds of peptides) are not restrained. Not all combinations of the backbone conformational angles Φ/Ψ are possible, at least for nonglycine amino acids. Ramachandran was the first one to realize that the presence of the C_β atom limited the allowed, clash-less conformation of each dipeptide within the protein chain to certain regions of the plot that now bears his name (Ramachandran et al., 1963). Each point on the Ramachandran plot corresponds to the combination of a pair of the Φ/Ψ angles, and only some parts of the plot are populated for non-glycine residues.

The early X-ray structures of proteins were analyzed at rather modest resolution, with the first 1.0 Å structure deposited in the PDB only in 1984 (Wlodawer et al., 1984), and the second one almost a decade later (Dauter et al., 1992). However, the number of atomic resolution structures available in the PDB now exceeds 350, almost all of them deposited in the last 10 years. The number of observed reflection intensities at the resolution of 1 Å or better is significantly higher than the number of refined atomic parameters, and, as a consequence, the geometry of the refined model (at least in the well-behaving parts of the structure) does not reflect the restraint targets, but tends to represent the unbiased experimental values. Based on the analyses of such structures, several investigators observed that the unbiased values of geometrical parameters may considerably differ from their target values (Jaskolski et al., 2007). One of the current widely accepted features is the nonplanarity of the peptide planes, with some ω-angles differing by more than 20° from the ideal *trans*- or *cis*-conformations (Wilson et al., 1998).

Previously, Karplus (1996) suggested that some aspects of the geometry of protein backbone depend on the conformational context, resulting in the correlation of the departure from the library values and the place that a peptide occupies on the Ramachandran plot. In this issue of *Structure*, Berkholz et al. (2009) present conclusions of a detailed statistical analysis of the backbone geometry in a large number of atomic resolution protein crystal structures. Even at an ultra-high resolution, the accuracy of atomic coordinates does not allow to convincingly extract dependencies between secondary conformation and backbone bond lengths. However, the main-chain bond angles display very strong correlation with the backbone torsion angles, elegantly presented by Berkholz et al. (2009) in the form of colored Ramachandran plots. For example, the preferred values of the N-C_α-C angle for α helices and β sheets differ by about 7°. Forcing this angle to be wrong by 7° involves a shift of atomic position by about 0.2 Å ($\sin 7° \times 1.5 = 0.18$), which may lead to significant distortion of the protein chain. As pointed out by Holmes and Tsai (2004), replacement of experimental bond angles with the ideal ones while holding the Ramachandran angles fixed leads to models departing from their targets by as much as 6 Å!

However, it is one thing to notice and report a phenomenon, but it is quite another to do something to counteract it. Berkholz et al. (2009) not only point out that the geometry targets should not have fixed, constant values, and should depend on the stereo-chemical context of a particular peptide, but also suggests a way to apply this knowledge in practice. Based on their analysis, the authors constructed the conformation-dependent library of geometrical targets, which can be used as restraints for the refinement of protein models against X-ray or NMR data, for structure prediction, and for constructing theoretical models of proteins. The use of such a library does not involve any significant complication in practice in terms of programming or computing time and does not decrease the ratio of observations to parameters. Hopefully, the structural biology community will soon adopt the ideas presented by Berkholz et al. (2009). It seems that proteins, like humans, do not always have strong spines.

REFERENCES

Berkholz, D.S., Shapovalov, M.V., Dunbrack, R.L., and Karplus, P.A. (2009). Structure *17*, this issue, 1316–1325.

Dauter, Z., Sieker, L.C., and Wilson, K.S. (1992). Acta Crystallogr. B *48*, 42–59.

Engh, R.A., and Huber, R. (2001). M.G. Rossman and E. Arnold, eds. (Dordrecht: Kluwer Academic Publishers), pp. 382–392.

Holmes, J.B., and Tsai, J. (2004). Protein Sci. *13*, 1636–1650.

Jaskolski, M., Gilski, M., Dauter, Z., and Wlodawer, A. (2007). Acta Crystallogr. D Biol. Crystallogr. *63*, 1282–1283.

Karplus, P.A. (1996). Protein Sci. *5*, 1406–1420.

Pauling, L., Corey, R.B., and Branson, H.R. (1951). Proc. Natl. Acad. Sci. USA *37*, 205–211.

Ramachandran, G.N., Ramakrishnan, C., and Sasisekharan, V. (1963). J. Mol. Biol. *7*, 95–99.

Wilson, K.S., Butterworth, S., Dauter, Z., Lamzin, V.S., Walsh, M., Wodak, S., Pontius, J., Richelle, J., Vaguine, A., Sander, C., et al. (1998). J. Mol. Biol. *276*, 417–436.

Wlodawer, A., Walter, J., Huber, R., and Sjolin, L. (1984). J. Mol. Biol. *180*, 301–329.

How ATPases Unravel a Mystery

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Principles of intracellular protein degradation remain among the most challenging questions in cell biology. Here, we discuss Wang and colleagues' crystal structure elucidation of the intermediate domain of Mpa, a regulatory particle of Mtb proteasome, the core proteolytic machinery of *Mycobacterium tuberculosis*.

The strictly regulated degradation of proteins in eukaryotes is performed by the ubiquitin-proteasome pathway, which plays an intrinsic role in many intracellular functions. To maintain this uncompromising regulation of eukaryotic protein degradation, the substrate proteins of interest are tagged by means of a series of ligases, with a 76-residue protein named ubiquitin. To mark the protein of interest for degradation, ubiquitin molecules have to be sequentially bound to form a polyubiquitin chain (Hershko and Ciechanover, 1986). The heart of this nonlysosomal protein degradation pathway is a highly complex hydrolyzing machinery, known as the 26S proteasome. This multifunctional enzymatic complex is composed of a 20S proteasome core particle (CP), with a molecular mass of approximately 700,000 Da, and two regulatory particles (RP), the 19S caps. The CP imbeds its hydrolytic sites in a refined cylindrical structure composed of different α and β subunits arranged in an $\alpha_{1\text{-}7}\beta_{1\text{-}7}\beta_{1\text{-}7}\alpha_{1\text{-}7}$-stoichiometry, whereas the 19S cap is composed of a base and a lid subcomplex. The base is mainly composed of six distinct AAA^+-ATPase subunits, among others, involved in the unfolding and translocation of protein substrates, while the lid, a complex of at least eight non-ATPase subunits, is implicated in the recognition and ubiquitin tag removal (Voges et al., 1999). It is not surprising therefore that both CP and RP were compelled to evolve synergistically, as the CP itself is a very unspecific protease that needs a strict regulation.

Proteasome-mediated protein degradation was initially considered to be a eukaryote-exclusive process as prokaryotes do not express ubiquitin. However, an interesting link between prokaryotic and eukaryotic protein degradation pathways arose with the identification of HslVU, an operon in *Escherichia coli*. HslVU is composed of: (1) HslV, a proteolytic homo-oligomeric ring system, in which the subunit shares 20% sequence similarity as well as a conserved topology with proteasomal active β-type subunits; and (2) HslU acting as the ATP-dependent regulatory particle. Notably, ATP-dependent proteases from prokaryotes and eukaryotes have hexameric ring structures and seem to operate along similar principles, despite the lack of ubiquitin in the prokaryotic system. It was then shown that prokaryotes replace ubiquitin with a defined linker peptide as their specific labeling tag for selective protein degradation (Keiler et al., 1996). Precise ATP-dependent protein destruction therefore appears to be a common principle among all three kingdoms of life, with prokaryotes holding a much simpler architecture in both CP and RP.

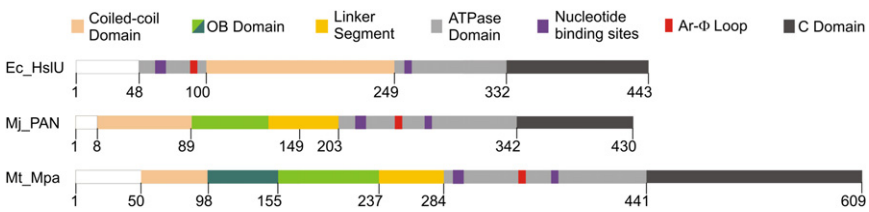

Figure 1. Location of the Different Domains in the Protein Sequence of HslU (*E. coli*), PAN (*M. jannaschii*), and Mpa (*M. tuberculosis*)