Folding and binding

Editorial overview

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Folding and binding are two of the most fundamental aspects of protein behaviour. Biological function is generally possible only when a protein is folded into a specific three-dimensional conformation; unfolded proteins are impotent. Biological function also involves the interaction with other molecules: structural proteins assemble into rigid complexes; enzymes bind their substrates and products; transport proteins bind their ligands; redox proteins bind electron donors and acceptors; and receptors bind hormones and other effectors.

Protein folding and binding are two manifestations of the same phenomenon: the recognition by several protein atoms of another group of atoms that is complementary in shape and physical properties. In the case of protein folding, the two groups of atoms are part of the same polypeptide chain, whereas in binding, the ligand is the second group. Nevertheless, the nature of the physical interactions seems to be basically the same in both cases. The greatest difference between folding and binding is that, in the case of folding, the protein atoms do not start out with a fixed conformation. Folding involves the interaction of two initially flexible surfaces, whereas in binding, generally only the ligand is substantially flexible.

A proper foundation for understanding protein-folding and protein-binding reactions requires an understanding of the nature of the physical interactions between atoms, and how these interactions are used to stabilize folded conformations and protein–ligand complexes. A few years ago, most workers in the field would have said that the major contribution to protein stability resides in the hydrophobic effect, and that polar interactions, including hydrogen bonding, do not contribute greatly to protein stability, because of the competing effects of water. At present, however, the subject of protein stability is in an apparent state of disarray, mostly as a result of new data and a more detailed analysis of the hydrophobic effect. The most recent line of reasoning, reviewed by Creighton (pp 5–16), concludes that the hydrophobic interaction is important but that the predominant factor that stabilizes folded-protein conformations is intramolecular hydrogen bonding. A paradigm shift has not yet occurred, however, as the new view has not been universally accepted, and a vigorous, constructive debate would be welcome.

Fortunately, the influx of confusion has been accompanied by many informative experimental results that illustrate clearly the power of mutational analysis of protein stability (Matthews, pp 17–21), especially when combined with detailed structural and thermodynamic analyses. The energetic contributions of hydrophobic, electrostatic, and other interactions to protein stability are beginning to be evaluated in this manner, as is the importance of packing of atoms within the protein interior. Indeed, it seems reasonable to expect that several of the controversies surrounding protein stability will soon be resolved.

In order to understand the process of folding, it is clear that structural information about intermediate stages in the process is required. The importance of NMR for this purpose is unmistakable, as summarized by Dobson (pp 22–27). Recent advances in NMR spectroscopy, especially the combination of isotope labeling with heteronuclear methodologies, are a most welcome addition to the techniques that are useful in studying protein folding. The available data on folding intermediates point towards the importance of native-like structures, even at early stages of folding, which would support models of folding based upon hierarchies of protein structure. Whether isolated secondary structures are sufficient for cooperative folding, or entire subdomains with both secondary and tertiary structure elements are required, remains an open question. In addition, more data and new approaches are needed to address the importance of non-native intermediates in protein folding. Bearing in mind the current activity in studying stable partially folded conformational states, trapped kinetic intermediates, protein fragments and subdomains, answers should soon be available.

Much attention has focused recently on a third conformational state of proteins, commonly referred to as the 'molten globule'. As reviewed by Dobson (pp 22–27) and Matthews (pp 28–35), this collapsed conformation can be populated in equilibrium conditions (such as at extremes of pH and/or ionic strength or at intermediate denaturant concentrations) with certain proteins. It may also correspond to an early kinetic intermediate in at least some protein-folding reactions. The molten-globule forms of proteins seem to be compact and contain a high degree of secondary structure, generally similar in content to the native conformation, but with much more flexibility. A major question is how specific are the interactions within the interior of the molten globule, and

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detailed structural studies that aim to answer this question are in progress. The molten-globule states exhibit a substantial tendency to aggregate, however, so care will need to be taken in order to avoid producing artifacts of this type when studying them.

Molecular chaperones, proteins that have been implicated in the folding, assembly and maturation of proteins *in vivo* have been the subjects of recent intense investigation. Some cell biologists have been so bold as to suggest that the physical chemists have completely 'missed the boat' and that the key to protein folding lies in understanding how these chaperones work. So far, however, the available data indicate that the primary roles of chaperones are to prevent folding and aggregation, as summarized by Schmid (pp 36–41). As noted above, unfolded proteins and folding intermediates such as molten globules have a strong tendency to aggregate. Therefore, binding to a chaperone could serve a 'folding helper' function, not by actively directing folding but rather by preventing an off-pathway aggregation reaction. Other chaperones seem to prevent folding of certain proteins so that they may be translocated across a membrane before folding. It is important to note that, as yet, there is no example of a protein that folds to a non-native but specific conformation (i.e. excluding aggregation phenomena) in the absence of a chaperone.

X-ray crystallography is an extremely powerful method for studying protein-binding reactions in structural terms, so our understanding of binding is much more advanced than that of protein folding. The overall goal is to understand the specificities of binding reactions; one needs, therefore, to understand differential affinities. Recently several new crystal structures of protein complexes have been determined, as summarized by Janin (pp 42–44), and the exciting prospect of learning how binding of a ligand induces an allosteric change in an enzyme is becoming a reality. For both proteinase–protein inhibitor interactions (Bode and Huber, pp 45–52) and antibody recognition (Chothia, pp 53–59), the crystallographic data emphasize the importance of complementary surfaces in protein recognition processes. Indeed, packing interactions at the interface seem to play a crucial role, and there seems to be little rearrangement of protein surfaces upon the binding of ligands or other proteins, at least for these two classes of protein-binding reactions.

The recent data on antigen–antibody complexes should serve to dispel the very many suggestions that have been made that the immunoglobulin binding reaction is fundamentally different from other protein–ligand and protein–protein interactions; clearly, it is not. The numerous proteinase–protein inhibitor structures that have been determined recently illustrate vividly the diversity of ways in which proteinases may be inhibited by other proteins. The only common feature is that the inhibitors make the proteinase active site inaccessible to substrates. These systems are ideally suited for dissecting the energetics of protein–protein interactions, so we expect to hear much more about them in the future.

The questions considered here have been recognized for a long time as being fundamental. The reviews demonstrate that techniques are now available to provide us with definitive answers. Indeed, protein folding and binding have quickly become, and are likely to remain, some of the most active research areas in molecular biology.

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