Protein–Ligand Interactions: Induced Fit

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Secondary article

Article Contents

Protein–ligand interactions include two major components: protein folding and binding mechanisms; and hinge bending conformational transitions.

Introduction

Molecules function through their intermolecular associations. Hence, over the years, binding mechanisms have been the focus of intense research. The mechanisms of binding have been classified according to the extent and type of the motion that takes place.

Some intermolecular associations have been viewed as rigid, whereas others have been considered flexible. The latter have been thought to adapt their corresponding molecular structures to fit, and optimally bind, their incoming molecular partner.

Around the turn of the twentieth century, Emil Fischer proposed the 'lock and key' mechanism for protein binding. This mechanism can be best illustrated by the enzyme substrate-binding process. The enzyme active site was believed to be a rigid and sturdy lock, with an exact fit to only one substrate (key). The specificity of enzymatic catalysis was believed to be the outcome of matching the lock to the key. This simplistic process had been accepted as the universal mechanism for enzyme ligand/substrate binding for more than half a century, until challenged by an alternative mechanism of 'induced fit' of Koshland in 1958. According to the induced fit theory, proteins need not be rigid locks. They can accommodate the substrate by flexibly adapting their substrate-binding site. The rigid and flexible binding modes have subsequently been distinguished by comparing the structures of the free, unbound, protein molecule with the structure when complexed with its ligand. If the structures are similar, the binding mode has been classified as belonging to the rigid, 'lock and key' type mechanism; if the structural comparison illustrates a relatively large conformational change, the binding mode has been considered to belong to the 'induced fit' category. These views of molecular associations have since been widely accepted.

We have shown that the 'new view' of protein folding (reviewed in Dill and Chan, 1997) implies the presence of an ensemble of conformational isomers of a protein in equilibrium with one another around the bottom of the energy funnel (reviewed in Ma *et al*., 1999; Kumar *et al*., 1999). During the binding of a substrate, the conformational isomer whose binding pocket shape is most complementary to the substrate conformation is selected. The equilibrium then shifts towards the conformation of the bound protein. This view of protein binding is fundamental to both the 'lock and key' and the 'induced fit' mechanisms of protein–substrate binding. It combines the concepts of 'complementarity' and 'flexibility' inherent in the Fischer and Koshland theories. In the language of the 'new view' of protein folding, the energy landscape, which is depicted by the folding funnel of a rigid protein such as that which binds it via the 'lock and key' mechanism, is likely to have a smooth bottom with a deep minimum. On the other hand, the energy landscape of a flexible protein, binding through the so-called 'induced-fit' mechanism, may be depicted by a rugged funnel bottom, with multiple minima separated by low barriers. Such minima, with low barrier heights separating them, depict the range of conformational isomers.

Conformational flexibility and structural fluctuations play an important role in enzyme activity. A large variety of internal motions extending over different time scales and

with different amplitudes are involved in the catalytic cycle. The conformational changes upon substrate binding, and particularly the hinge-bending motions that occur in enzymes consisting of two domains, have a substantial effect on the catalytic activity of enzymes.

Below we describe binding mechanisms within the framework of the 'new view' of protein folding. We illustrate how this simple concept replaces long held notions of both 'induced-fit' and 'lock and key' type binding. **Figure 1** depicts the principle of funnels, their bottoms and their fusion in binding. We provide a range of examples substantiating our view. We relate structure, funnel and reactivity. We focus primarily on hinge-type motions, frequently observed in protein–ligand associations. Our examples progressively depict such hinge-bending motions between domains and between subunits (Kumar *et al*., 1999). An example of hinge-based domain motion is given in **Figure 2**, by superimposing 'closed' and 'open' conformations. **Figure 3** illustrates an example of subunit hinged-based motion, again through superimposition of two conformations: 'closed' and 'open'.

Protein Folding and Binding Mechanisms

Proteins function through their intermolecular binding. Hence, we consider the implications of current understanding of protein folding for long-held notions on binding mechanisms. For this purpose, we focus on the consequences of the bottoms of the funnels. If the proteins are rigid, around the bottom there will be a single minimum or a few minima. Conversely, flexible molecules will display rugged funnel bottoms, with low barriers, corresponding to a range of conformations (Ma *et al*., 1999; Kumar *et al*., 1999).

The binding mechanism has often been assigned via a comparison of the structures crystallized both of the bound conformer and of the unbound, 'free' conformational isomer. If one observes a relatively large difference between the conformers, the mechanism has traditionally been assigned to the 'induced fit' category.

Conversely, if a small difference is observed, the mechanism has been assigned as belonging to the rigid 'lock and key' type binding. However, if we consider molecules as existing in ensembles of conformations, we can easily imagine that the isomer whose conformation is complementary to the ligand would bind to it. The conformers are in equilibrium in solution; hence, upon binding, and depletion of this conformation from the solution, there would be a shift in favour of that conformer. Consider the case of the crystal of the 'unbound' conformer: in practice, this isomer is actually also bound, except that in this case it is bound to its twin molecule. Thus, the conformer whose structure is determined in such a case is the one that is complementary to its twin in the crystal. On the other hand, the conformer that is crystallized in the so-called 'bound' (complexed) case is the one that is complementary to its respective ligand. Thus, the difference between the two conformations does not necessarily mean that in binding to the ligand the protein has undergone an 'induced fit' type of mechanism. Instead, it is the straightforward outcome of crystallizing different conformers. We stress, however, that here we discuss conformational changes observed in backbone movements. As discussed in a later section, on the local level, one may easily imagine that side-chain optimization between the interacting molecules will take place, favourably orienting the functional groups, to achieve an optimized stable association.

Thus, the presence of the ensemble of conformers around the bottom of the folding funnel explains binding mechanisms under the same, general principles. There is no

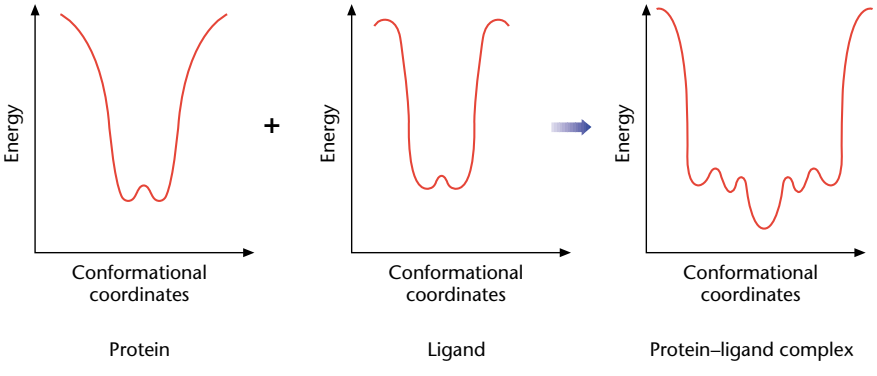

Figure 1 Schematic illustration of the landscapes of protein–ligand interactions. The energy landscape of the protein–ligand complex is a fusion between two individual funnel-like landscapes, corresponding to the protein and the ligand. In real protein–ligand associations, the energy landscapes for the protein, ligand and the protein–ligand complex will be much more rugged, depending upon the flexibility of the protein, the ligand and the protein–ligand complex.

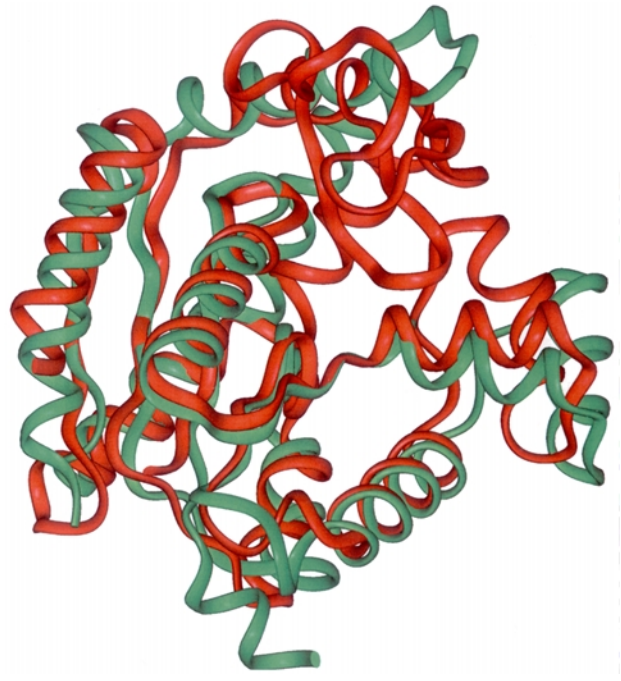

Figure 2 Ribbon diagram showing domain motion in adenylate kinase. Unbound adenylate kinase is shown in green. Adenylate kinase bound with the inhibitor Ap_5A is shown in red.

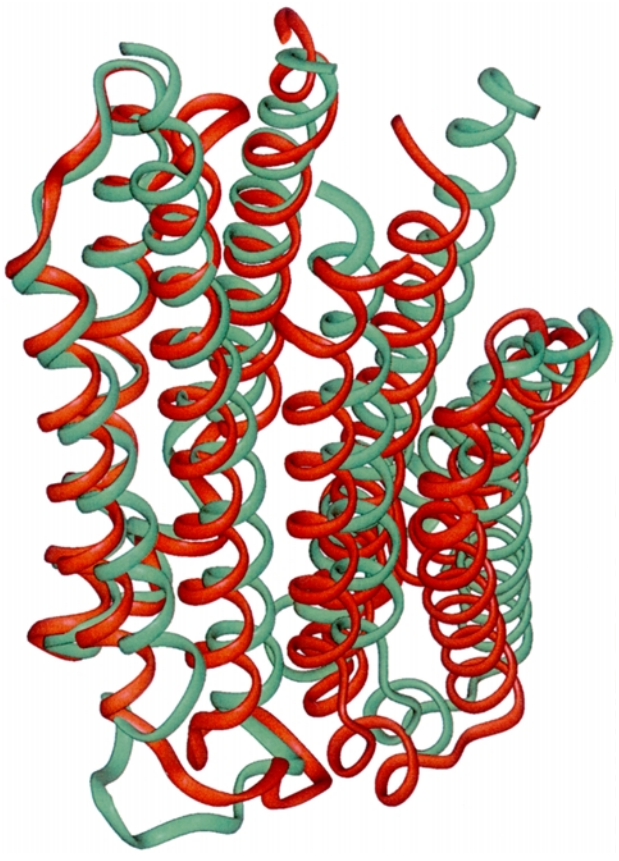

Figure 3 Ribbon diagram showing subunit motion in aspartate receptor. Only the ligand (aspartate) binding domains are shown. Unbound aspartate receptor is shown in green. Aspartate-bound aspartate receptor is shown in red. The orientation of the subunits in aspartate receptor changes upon aspartate binding.

need to invoke the 'lock-and-key' or the 'induced fit' for intermolecular associations. We should simply consider the variability of the conformers. In all cases, the conformer that binds is the one most favourable and complementary, with the equilibrium shifting in its favour. Different conformers may well bind different ligands. Thus, the larger the flexibility, the wider the scope of binding specificity. Nevertheless, since in solution side-chains, and to a lesser extent backbone, move, some induced fit is not precluded, optimizing the receptor–ligand interactions. This would optimize the packing at the intermolecular interface.

Similarly, around the bottom of the binding funnel there may be an ensemble of conformations of the bound, multimolecular assemblies. These will further bind the next approaching ligand. Here, the already bound, multimolecular conformer, whose structure is most favourable for further binding, is the one that will associate. Hence, the principles are universal.

Below, we illustrate the applicability of this principle to conformational transition through hinge-bending, a common transition observed in protein–ligand interactions and in enzyme catalysis. We illustrate this approach both for allosteric transitions and for motions that do not involve allosteric transition (Kumar *et al.*, 1999).

Hinge-bending Conformational Transitions

Protein motions have been very conveniently systematized on the basis of packing (Gerstein and Krebs, 1998), since atoms are very tightly packed in the interior of protein molecules. Groups of atoms can move with respect to each other only if there is a packing defect, or a cavity, that allows them to do so. Interfaces between groups of atoms, or between structural parts, are not smooth. Tight packing, mostly of side-chains, restricts the movements of the structural units if their internal packing is to be preserved. In hinge-bending motions, structural units move with respect to each other; however, while the packed arrangement inside the unit is preserved, the interunit packing at their interface is disrupted. It is important to note that the parts move as relatively rigid bodies with respect to each

other, rotating on their common 'hinge'. The motion that is observed is roughly perpendicular to the interface.

Hinge-bending motions differ from those classified as 'shear'. In shear movements the packing at the interunit, or intermolecular interface is maintained. Here, the structural units slide with respect to each other. Furthermore, while in hinge-bending movements a few large (twisting) changes may be observed, in shear movements many small changes, parallel to the plane of the interface, may be seen. In this article, we restrict ourselves largely to hinge-bending motions, which are the major ones observed in intermolecular associations. Next, we give some examples, related to protein–ligand intermolecular binding.

Some Specific Examples: Motions of Fragments and Domains

Here we give examples for motions of fragments, domains and subunits. We describe the hinge bending that is involved, between the two conformers – 'open' and 'closed'. We further provide examples of nonallosteric enzymes and allosterically regulated molecules.

Triose phosphate isomerase: motion of a fragment

Triose phosphate isomerase (TIM) is a glycolytic enzyme. It catalyses the interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The open and closed forms of the TIM loop have been analysed in detail. The loop has two hinges, and moves by about 7 Å. TIM is a β-barrel enzyme, with eight helices arranged around a barrel of eight strands. An 11-residue loop connects an inner strand with an outer helix. This flexible loop is closed when the substrate is bound at the active site of the enzyme. The sequences at the hinges in mutants revealed that the solutions to the hinge flexibility problem vary, with the preferences being sequence-dependent. In particular, Gly, the smallest residue, lacking a side-chain, is avoided, suggesting that unrestricted hinging is preferentially avoided if the enzyme is to be biologically functional (Sun and Sampson, 1998). TIM illustrates an example of a protein having a relatively smooth energy landscape with a few minima around the bottom. The structures of the enzyme when complexed with its substrate as compared to its unbound form are similar, with the exception of small loop movements.

TIM functions as a dimer. The monomeric form is inactive and thermodynamically unstable. The unfolded TIM monomers are susceptible to proteolytic digestion and thiol oxidation, while native TIM is resistant to both. The dimeric form of TIM reduces the frequency of subunit unfolding by several orders of magnitude, thereby enhancing the chemical stability of the protein. Stability appears to be the main reason for the dimerization. However, a stable monomeric variant of trypanosomal TIM (monoTIM) has also been detected. MonoTIM has lower, though still significant, catalytic activity. In the dimer, the two subunits function independently. The conformational change is largely intrasubunit. Hence, the funnel of TIM is the result of the coupling of the funnels of its two subunits. The coupling, though weak, is crucial for the stability of the dimer.

To explore the origin of the large-scale motions of the flexible loop in triose phosphate isomerase (residues 166 to 176) at the active site, several simulation procedures have been employed, both for the free enzyme *in vacuo* and for the free enzyme with some solvent modelling. These procedures include high-temperature Langevin dynamic simulations, sampling by a 'dynamics driver' approach, and potential-energy surface calculations. The simulations and analyses indicate that in the context of a spontaneous opening of the free enzyme, the motion is a rigid-body type. The interaction between residues Ala176 and Tyr208 was observed to be crucial in the loop opening/closing mechanism.

Phosphoglycerate kinase: motion of domains

Phosphoglycerate kinase (PGK) is critically important for most living cells. It is needed both for ATP generation in the glycolytic pathway of aerobes and for fermentation in anaerobes. In many plants the enzyme is involved in carbon fixation. Like other kinases, PGK folds into two distinct domains. These undergo a large hinge-bending motion upon catalysis. The active site is located in the interdomain cleft. The monomeric enzyme catalyses the transfer of the C1-phosphoryl group from 1,3-bisphosphoglycerate to ADP to form 3-phosphoglycerate (3-PG) and ATP. For many years the conformation of the enzyme during catalysis has been unclear. The large distance between the binding sites for 3-PG and ATP, deduced from the crystallographic structures of the binary complexes, has suggested that this enzyme undergoes a hinge-bending domain motion from an open to a closed conformation during its catalytic activity.

Direct experimental evidence for the 'closed' conformation in the presence of both substrates has been obtained. The crystal structure of PGK from the hyperthermophilic *Thermotoga maritima* (TmPGK) represents the first structure of an extremely thermostable PGK. The crystal structure of TmPGK was determined to 2.0 Å resolution. It is a ternary complex with two products, 3-PG and the AMP-PNP (adenylylimidodiphosphate) analogue. The complexed structure has a closed conformation with a substantially smaller interdomain angle. The distance between the two bound ligands is 4.4 Å. The closed conformation constitutes the active conformation of the

enzyme. The structure illustrates that there is an interdomain salt bridge between residues Arg62 and Asp200. This salt bridge contributes to holding the two domains in the closed state. Lys197 contributes towards the stabilization of the transition state phosphoryl group. Comparison of TmPGK with its mesophilic homologues reveals that the higher rigidity of TmPGK is achieved via the larger number of intramolecular interactions, such as a larger number of salt bridges, and further stabilization of the helix–loop regions. An additional ternary complex of PGK has been solved. This PGK ternary complex exhibits a dramatic closing of the large cleft between the two domains. This closure brings the two ligands, 3-PG and ADP into close proximity. This structure has confirmed that PGK is a hinge-bending enzyme.

Phosphoglycerate kinase is an example of a protein with strong domain–domain interaction. Several PGK mutants have been studied with single tryptophans at various locations. These intrinsic fluorescent probes were used to investigate the extent and delocalization of conformational changes taking place upon the binding of 3-PG, 1,3-diphosphoglycerate, ADP, ATP and PNP-AMP (nonhydrolysable analogue of ATP), and upon the concomitant binding of 3-PG and PNP-AMP. Only those probes that are situated at the hinge, and in the parts of each domain that are close to the hinge, manifest substrate-induced conformational changes. The binding of substrates to one domain was observed to induce spectral perturbation of the probes in the second domain. This indicates transmission of conformational changes between the domains.

The strong interactions between the domains is also observed in the activity of PGK. To determine the role of the C-terminal helix in the folding and stability of yeast phosphoglycerate kinase, a mutant lacking the 12 C-terminal residues (PGK Δ404–415) has been constructed. The conformation of the mutant is very similar to that of the wild-type protein. However, it has a very low activity. The deletion of the C-terminal helix results in an increase in the flexibility of the whole protein, and hence a decrease in stability. Qualitatively, the structural properties of the shortened protein are very similar to those of the isolated domains. The C-terminal part of the yeast enzyme is apparently not necessary for most of the initial folding steps. However, it locks the C-domain onto the N-domain, ensuring full enzyme activity. Nevertheless, the interdomain interactions are best described as the sensitivity of the PGK catalytic activity to conformational changes, though not in terms of interdomain interaction energy. The actual contacts between the domains are relatively weak. Contacts formed either between domains or with the interdomain helix are made only in the folded ground state, and do not constitute a separate step in the folding mechanism. Numerous studies illustrate that the folding–unfolding of PGK is a sequential, multistep process. It is interesting that the hinge motion exists in the folding intermediate. Thus, the funnel of PGK illustrates a simple merging of the two corresponding subfunnels of its domains (Kumar *et al*., 1999).

Nevertheless, despite the overwhelming evidence regarding the hinge-bending in PGK, a different case has also been found. The ternary complex of the R65Q mutant of yeast 3-phosphoglycerate kinase (PGK) with magnesium 5′-adenylylimidodiphosphate (Mg AMP-PNP) and 3-phospho-D-glycerate (3-PG) has been solved by X-ray crystallography to 2.4 Å resolution. The yeast PGK consists of two domains. The 3-PG binds a patch of basic residues from the N-terminal domain, and the Mg AMP-PNP interacts with residues from the C-terminal domain. The two ligands are separated by about 11 Å across the interdomain channel. The R65Q mutant of yeast PGK is very similar in structure to the PGK from horse, pig and *Bacillus stearothermophilus*. In particular, the most significant tertiary structural differences among the yeast R65Q, equine, porcine and *B. stearothermophilus* PGK structures occur in the orientations of the two domains with respect to each other. Nevertheless, the relationships between the observed conformations of the yeast PGK are inconsistent with a 'hinge-bending' behaviour that would close the interdomain cleft. Hence, it has been proposed that the available structural and biochemical data on yeast PGK may indicate that the stretch of basic residues represents the site of anion activation and not the catalytically active binding site for 3-PG.

Adenylate kinase

It is critically important for kinases that they shield their catalytic centres from water, to avoid becoming ATPases. Hence, it is not surprising that large differences are observed between the bound and the unbound structures (Schulz *et al*., 1990). There are two interdomain linkages and four hinges. The first pair of hinges shows a 60° rotation, and the second pair a rotation of 30°. Thus, the total rotation of the domain is around 90°. This mobile domain is connected to the molecule through two helices, which are oriented in an antiparallel fashion and are packed together. In the closed form, deformations are observed near the hinges, at both termini of the helices. The deformations are the outcome of rotations of three torsion angles at each joint. The regions between the hinge-joints, the two helices and the remainder of the mobile domain move as relatively rigid bodies. Energy landscapes and funnels for proteins exhibiting domain motion can, in general, be expected to have a higher degree of complexity. The funnel of adenylate kinase can be described as a fusion of two energy funnels. One of these funnels is smooth, with only relatively few minima around the bottom. This funnel would correspond to the rigid domain of adenylate kinase. The mobile domain has a complex energy funnel with several isoenergetic minima around the bottom. In this sense, funnels for adenylate kinase can be schematically

represented by **Figure 1**. Fusion of these two funnels yields a semiflexible adenylate kinase funnel, which in turn is capable of producing motion in the mobile domain, independently of the rigid domain. The conformational change that is observed can then be rationalized on the basis of such a funnel.

Adenylate kinases undergo large conformational changes during their catalytic cycles. Domain motions occur largely independently from each other. The structure of the unligated adenylate kinase has been solved and compared with that of the same enzyme when ligated to an inhibitor mimicking both substrates, ATP and AMP. This comparison has illustrated that upon substrate binding, the enzyme increases its chain mobility in a region that is remote from the active site. The change of the enzyme activity was also observed to coincide with that of the rate of ANS binding during denaturation by low concentration of the denaturants. This suggests that the activation of adenylate kinase by denaturants may be due to an increase in conformational flexibility at its active site.

Allosteric and Nonallosteric Enzymes

Aspartate carbamoyltransferase: motion of subunits in allosteric enzyme

Aspartate carbamoyltransferase is an allosteric enzyme, and its motion is an example of allosteric transition between R (relaxed) and T (taut) states. The N-terminal region of the regulatory subunit is important for controlling nucleotide binding, creating the high-affinity and low-affinity effector binding sites, and coupling the binding sites within the regulatory dimer. The enzyme catalyses the reaction between carbamoyl phosphate and L-aspartate to yield phosphate and *N*-carbamoylaspartate. The latter is a precursor in the synthesis of pyrimidines. Aspartate carbamoyltransferase consists of six regulatory subunits and of six catalytic subunits. The catalytic subunits, are arranged as a dimer of two trimers. On the other hand, the six regulatory subunits are arranged as three dimers, with each connecting the two sides of the catalytic trimer. Movement from a T to an R state involves rotation of about 15° of the regulatory dimer subunits, around their dimer axis. This movement is accompanied by a 12 Å separation of the catalytic trimer, and a 10° rotation about the threefold axis. Each catalytic subunit is composed of two domains, whose movement is coupled to the overall subunit motion. Hence, overall, this is an example of concerted subunit–domain motions. The energy landscapes in both the folding and binding funnels that correspond to proteins showing subunit motions have a much higher degree of complexity than those of proteins showing fragment and domain motions. Yet, owing to the analogy between the processes of folding and binding, these funnels can be schematically illustrated in a simple form. Each subunit is rigid and has a simple energy landscape. However, the complex is semiflexible, which allows subunit motion between the T and R states of the enzyme.

Aspartate receptor: motion of subunits in nonallosteric transition

While the subunit hinge-based motions described above are all related to allosteric transitions, there are other, similar hinge-bending movements between subunits that are not linked to allostery. The aspartate receptor of chemotaxis belongs to a large class of transmembrane proteins. These proteins contain an extracellular, ligand-binding domain, a cytoplasmic signalling domain, and a transmembrane domain. The ligand-binding domain has been crystallized in the presence and in the absence of aspartate. There are two subunits. Each subunit is a four α-helix bundle, with two long NH_2- and COOH-terminal helices, and two shorter helices. These form a cylinder 20 Å in diameter and 70 Å long. The substrate-binding site is at the interface between the two subunits. Comparison between the free and the bound structures has indicated that while the structures of the subunits are largely unchanged (except for some change in the conformation of one loop), the subunits change their orientation with respect to each other. The conformational change is apparently propagated through the membrane, and is involved in signal transduction.

Functional Groups and Binding Epitopes

The definitions of binding epitope vary. However, in general, the term refers to a recurring pattern of molecular surface, in a family of proteins, where in at least one family member the site is known to be a binding site. A similar binding epitope may also be found across family boundaries.

Considerable work has been carried out on the interactions and energetics of protein–protein binding, whether of the entire interface or focusing on the contributions of single residues. Single-residue contributions are particularly important for the comprehension of drug-resistant mutations. The principles that govern the interactions of protein–protein interfaces are still not fully understood. Experimental point mutations, alanine scanning mutagenesis, double mutation cycles and, on the computational side, the derivation and analysis of a dataset of protein–protein interfaces may well provide measures of the driving forces stabilizing protein–protein

interactions. Additionally, it may supply critical information necessary for ligand (inhibitor, drug) design.

It has been shown that the free energy of binding is not distributed evenly across interfaces. Experiments have shown that there are hot spots of binding energy consisting of a small subset of residues in the dimer interface. These hot spots are enriched in tryptophan, tyrosine and arginine, and are surrounded by a shell of energetically apparently less critical residues (Clackson and Wells, 1995; Bogan and Thorn, 1998). These have been suggested to be important by occluding bulk solvent from the hot spot. These conclusions have recently been reinforced by an analysis of families of related interfaces. Utilizing a sequence-order-independent structural comparison method, a comprehensive and systematic structural analysis of a dataset of families of protein–protein interfaces has been carried out. Since the structures of the families of these interfaces are geometrically similar, the dataset can be used to analyse the structural characteristics of protein–protein interfaces. In particular, it enables an examination of the similarity of the binding surfaces of the families, surface residue conservation, the major determinants of binding (hydrophobicity, electrostatic, etc.), and preferred, avoided, or neutral residues at each interface. These have important implications for both protein–protein binding and protein ligand design. An insight into these basic questions, along with the derived binding epitopes, enables searches for homologous potential sites in other structures, where the existence and/or location of these sites are unknown.

There are a number of ways to locate an unknown active site on a known protein structure. Ringe (1995) surveyed approaches to the definition of 'what makes a binding site a binding site'. She presented some guidelines, and proposed that binding sites are generally depressions in the protein surface 'in which there is greater than average degree of exposure of hydrophobic groups'. These generally contain disordered, easily displaced water molecules. She proposed that conformationally flexible residues at the binding site may be expected to be particularly functional in replacing the disordered water by the competing ligand. In depth analysis indicates that active sites of enzymes are characterized primarily by large clefts. In most single-chain enzymes the ligand binds in the largest cleft. Thus, the active sites of enzymes can frequently be identified using geometrical criteria alone. Peters *et al*. (1996) have utilized 'alpha-shapes', an attractive computational geometry tool, in an automated search for ligand-binding sites on protein surfaces. This geometry-based approach also found a correlation between deep clefts and enzyme active sites. A correlation between patches of hydrophobic surfaces and binding sites has been noted (e.g., Clackson and Wells, 1995). On the other hand, protein–protein binding sites are characterized by more shallow, flat surfaces.

In general, molecules with similar surface motifs frequently have similar biological features. Local surface similarity in different proteins can lead to conclusions concerning their (similar) biological functions. Homology of a potential binding site to a known one facilitates its engineered change to further optimize it and to rationally design inhibitors or drugs.

Conclusions

Conformational transitions observed in the comparisons of 'open' and 'closed' structures are not the result of an induced fit binding mechanism. Rather, around the bottom of the folding funnels, flexible molecules exist in a range of conformational isomers. The conformation that is observed in the 'unbound' molecule is the one most favourable for binding in the crystal under these conditions. Upon its binding to the growing crystal, the equilibrium shifts toward this conformer. The conformation observed in the 'bound', complexed, state is the one most favourable for binding the ligand. Hence, upon binding the ligand, the equilibrium shifts in the direction of the conformer that is most complementary to the ligand. Furthermore, the two (or more) conformers that have been observed in the crystals are merely a sample of the isomers that populate the bottom of the funnel. The examples we have described illustrate conformers with a hinge-bending type of motion, in which the molecular parts move with respect to each other as relatively rigid bodies. Such structural motions represent low-energy transitions (as low as several per cm), and hence may take place in solution in the absence of the ligand.

The majority of the conformational changes in allosteric regulation involve subunit motions. Binding of the inhibitor favours the T (taut) state. Binding of the substrate or the activator favours the R (relaxed) state. Allostery is related to the concept of the funnels. However, as the system is larger, and more conformations are potentially possible, the funnels are more complex. Furthermore, in allostery, we should consider binding funnels (Kumar *et al*., 1999). In general, while the bottoms of the folding funnels are populated by ensembles of conformations of single protein molecules, the bottoms of the binding funnels are populated by an ensemble of bound conformations. For allosterically regulated proteins, there are additional considerations. First, allostery is often observed in a quaternary molecular assembly, involving several subunits. Second, the binding of the inducer needs to be considered, in addition to the substrate. Hence, allostery involves large multimolecular complexes, with higher-dimensional, complex funnels.

In summary, here we have shown that the 'new view' of protein folding can be extended to explain the fundamental concepts behind theories of induced fit and lock and key binding. Exploiting the similar nature of protein folding and binding processes, we show that the concept of the

energy funnel is equally useful in understanding protein function.

Throughout this chapter we have paid particular attention to the role played by conformational flexibility and structural fluctuations in enzyme activity. A large variety of internal motions, extending over different time scales and with different amplitudes, are involved in the catalytic cycle. Here we have focused particularly on conformational changes upon substrate binding, taking place in enzymes consisting of two domains. These have a considerable effect on the catalytic activity of enzymes. Such conformational transitions involving hinge-bending motions are very frequent, and consequently functionally important.

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