Minireview

Conformational change in substrate binding, catalysis and product release: an open and shut case?

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Abstract The role of conformational change in substrate binding, catalysis and product release is reviewed for 11 enzymes, for which crystal structures are available for the apo, substrate- and product-bound states. The extent of global conformational changes is measured, and the movements of the functional regions involved in catalysis and ligand binding are compared to the rest of the structure. We find that most of these enzymes undergo relatively small amounts of conformational change and particularly small changes in catalytic residue geometry, usually less than 1 Å. In some enzymes there is significant movement of the binding residues, usually on surface loops.

Keywords: Conformational change; Catalysis; Structure; Enzyme

1. Introduction

The problem of fully describing, and explaining, the catalytic power of enzymes has been a key focus of modern biochemistry. Part of this power derives from the nature of enzyme ligand binding. Enzymes must allow substrates to bind and products to be released efficiently. The conformational changes undergone by the enzyme during substrate binding, conversion of substrate to product and product release, is the subject of this review.

Structural biology has allowed an understanding of enzyme mechanism at the atomic level. There are over 10 000 X-ray crystal and NMR enzyme structures available in the PDB [1]. Many enzymes have multiple structures solved, each bound to a different ligand. These ligands can represent substrates, products, analogs of substrates and products, allosteric and competitive inhibitors and pharmaceutical compounds. By examining the conformation of an enzyme in these different structures, we can understand the way in which conformational change is an integral part of the catalytic cycle.

The importance of conformational change in enzyme catalysis has long been appreciated. The classic example is the theory of induced fit [2], which proposes a general mechanism of substrate binding whereby an 'open' form of the enzyme binds the substrate, and in doing so closes around the substrate into a 'closed' form. Catalysis takes place in the closed form and the enzyme opens again to release the product. This basic cycle has been seen in many different enzymes including triosephosphate isomerase (TIM), which uses a small hinged loop to close the active site [3], and kinases, which use two large lobes moving towards each other when the substrate binds [4]. A database of known macromolecular motions (including non-enzymatic molecules) has been set up [5], demonstrating that conformational change is very common, and that many different types of motion are seen.

Previous reviews have classified the types of motion observed [6] and summarized the roles that these motions play in catalysis [7]. Enzyme conformational change is classified into the two types mentioned above: domain motion, where two rigid domains, joined by a flexible hinge, move relative to each other; and loop motion, where flexible surface loops move to different conformations. These changes are thought to fulfil a number of roles in catalysis: enhanced binding of substrate, correct orientation of catalytic groups, removal of water from the active site and trapping of intermediates. In addition to these roles, there are also theories which describe a direct coupling of conformational change to the catalytic mechanism. These theories link the energy involved in the making and breaking of non-covalent bonds in the enzyme structure, to the catalytic mechanism and the energy changes there.

In this review we aim to understand more about the nature of conformational change in the catalytic cycle, and specifically its role in substrate binding and product release. To do this we examine 11 different enzymes with solved crystal structures of the apo (ligand free) form, substrate (or substrate analog) bound form and product (or product analog) bound form. In some cases a number of intermediate structures are also known, allowing a very detailed structural insight into the mechanism. The structures are treated as snapshots along the reaction co-ordinate, and changes between the structures are analyzed. The residues and/or cofactors responsible for catalysis are also known for each of the examples presented here, and so it is possible to see how conformational change affects the catalytic machinery.

2. Review of enzyme structures

The enzymes and PDB files used in this review are summarized in Table 1. For each enzyme the structure of the apo form

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Table 1
Enzymes and PDB files used in the analysis

Enzyme	PDB files					Motion observed				Notes
	E	ES	ES*	EP	ESP	Loop	Domain	Sidechain	2° Structure	
Napthalene dioxygenase	1O7H	1O7N	–	1O7P	–	–	–	–	–	No significant motion observed [10]
Hal2p	1K9Z	1KA1	–	1KA0	–	–	–	–	–	No significant motion observed [11,12]
Aconitase	1AMJ	1C96	–	1C97	–	–	–	–	–	No significant motion observed [13,14]
Chalcone synthase	1BI5	1CML	–	1CGK	–	–	–	•	–	PHE215 sidechain rotates through a number of different conformations [15]
Peptide deformylase	1BS5	1LRU	–	1BS8	–	–	–	•	–	LEU91 rotates to occupy the space left by the leaving formate group [16,17]
P450cam	1PHC	1DZ8	–	1NOO	–	•	–	–	–	Mainchain carbonyl oxygen of ASP251 moves to accommodate a bound water molecule on substrate binding [18]
Protein farnesyltransferase	1FT1	1D8D	–	1KZP	1KZO	•	–	–	–	LYS164 moves ~1 Å on substrate binding, small motions in other loop residues [19]
Thymidylate synthase	3TMS	1BJG	2TSC	1TYS	–	•	–	–	–	C-terminal tail moves to cover active site [20–24]
Dethiobiotin synthase	1BYI	1DAH	–	1DAF	–	•	–	•	–	THR11 moves ~1 Å and rotates on substrate binding, also other loop movements [25,26]
Methylmalonyl-CoA mutase	2REQ	4REQ	–	6REQ	–	•	•	•	–	Large loop and domain motion on substrate binding, TYR89 rotates also [27,28]
Dihydrofolate reductase	5DFR	1RA2	–	1RX4	1RX6	•	•	•	•	MET20 loop adopts four conformations: unordered, closed, open and occluded. Sub-domain motion acts to open and close the active site [29]

E is the resting state of the enzyme with no ligands bound. 'ES' is the structure with substrate bound. 'ES*' is the structure with activated substrate bound. 'EP' is the structure with product bound. 'ESP' is the structure with product and fresh substrate bound.

(labelled E), substrate-bound form (labelled 'ES') and product-bound form (labelled 'EP') is known. In protein farnesyltransferase (FTase) and dihydrofolate reductase (DHFR) the apo form is not part of the usual catalytic cycle, instead the binding of fresh substrate causes the release of product from the previous cycle. In both cases, the structure with both substrate and product is known (labelled ESP). In thymidylate synthase (TS) the folate substrate binds in an unreactive conformation and then opens to form a reactive complex. This structure is labelled as ES*.

The observed conformational changes between states are categorized into four different types of motion:

1. *Loop motions*: Movements of small (2–10 residues) segments of structure.
2. *Domain motions*: Movements of protein domains, connected by a hinge region.
3. *Sidechain rotation*: Rotation of sidechains which alters the position of the functional atoms of the sidechain.
4. *Secondary structure change.*

In addition, only those motions that effect the conformation of the active site are reported. All of the enzymes show sidechain rotation and loop motions in some parts of the structure, but these are not considered significant if they are not part of the active site. It may be that these motions have other roles such as allostery or are simply background noise.

Significant movement of active site loop regions is seen, on substrate binding, in methylmalonyl-CoA mutase (MUT), DHFR, TS, dethiobiotin synthase (DTBS), FTase and cytochrome P450cam (P450cam). These movements are characterized by a general closing of the active site, with the surface loop regions moving in towards the rigid core of the protein, closing over the bound substrate.

MUT is unique in this set of enzyme, in the extent of the conformational change it undergoes, some residues move over 10 Å when the substrate binds. The conformational changes include loop movements, and domain movements, which form the substrate binding $(\alpha\beta)_8$ barrel and close the active site to exclude solvent.

DHFR also undergoes important conformational change. It uses the MET20 loop to control access to the NADPH binding pocket of the active site. As well as moving to block and cover the NADPH binding site, the MET20 loop undergoes changes in secondary structure, changing from disordered in the apo form, to β-sheet in the closed form, a 3_{10} helix in the occluded form and an ordered loop in the open form. There is also domain motion in DHFR, the two sub-domains move towards

each other by ~1 Å, closing the active site cleft around the bound substrates.

TS uses the C-terminal tail, rather than a loop, to close the active site. The tail moves to cover the active site, serving to exclude the bulk solvent and trap intermediates. DTBS uses three small loops to bind the substrate and ATP, with motions of ~2 Å. FTase undergoes very small loop motions in the active site (~1 Å), these small motions are still important however, since one of these loops contains the catalytic lysine which is moved into the correct alignment with the substrate. P450cam undergoes a small rearrangement of the peptide mainchain to allow binding of a catalytic water molecule. This motion is classified as a loop motion here, though it is much smaller than the other motions.

There are significant rotations of functional sidechains in DTBS, MUT, DHFR, peptide deformylase (PDF) and chalcone synthase (CHS). In DTBS and DHFR, catalytic residues, a threonine and methionine, respectively, rotate so that their sidechain is correctly placed near the substrate. In CHS and PDF, hydrophobic residues, a phenylalanine and leucine, respectively, rotate so as to form close Van Der Waals contacts with the substrate and intermediates. In MUT, TYR89 rotates so as to knock the adenosyl cofactor off the cobalt atom, generating the adenosyl radical.

In general, the change seen between the substrate- and product-bound forms is small, and there are only obvious functional changes in P450cam, TS and PDF. In P450cam the bound water molecule accommodated in the substrate-bound form, is not present in the product-bound form and the peptide mainchain moves back into its previous conformation. In TS and PDF, there are suggestions that steric clashes between the product and the enzyme may encourage product release. In TS the extra methyl group of dTMP clashes with a bound water molecule and in PDF an active site leucine rotates such that the sidechain replaces the leaving formyl group.

For each enzyme, the catalytic and binding residues are known. The binding residues are defined as any residue with any atom within 4 Å of a bound substrate. The catalytic residues are extracted from the literature following the definition of catalytic used by Bartlett et al. [8]. A residue is defined as catalytic if any of the following are true:

1. Direct involvement in the catalytic mechanism, e.g., as a nucleophile.
2. Exerting an effect, that aids catalysis, on another residue or water molecule which is directly involved in the catalytic mechanism.
3. Stabilization of a proposed transition-state intermediate.
4. Exerting an effect on a substrate or cofactor which aids catalysis, e.g., by polarizing a bond which is to be broken.

The catalytic residues of four of the enzymes are shown in Fig. 1. In each case, the catalytic residues are extracted from the three structures and the C-α atoms are superposed using ProFit [9]. We see in Fig. 1A the multiple conformations of the catalytic phenylalanine sidechain in PDF. This is an example of sidechain rotation without loop motion. In Fig. 1B, we see a change in the peptide bond joining the catalytic aspartate and threonine residues. This moves the mainchain carbonyl oxygen ~2 Å and allows binding of an ordered water molecule. In Fig. 1C, we see an example of sidechain rotation and loop motion. The catalytic threonine of DTBS moves ~1 Å and rotates to correctly position the hydroxyl group. Finally, in Fig. 1D we see the active site of MUT, which undergoes the largest conformational change in this sample. There are large domain and loop motions as well as sidechain rotation of the catalytic tyrosine which acts to generate the adenosyl radical required to start the reaction.

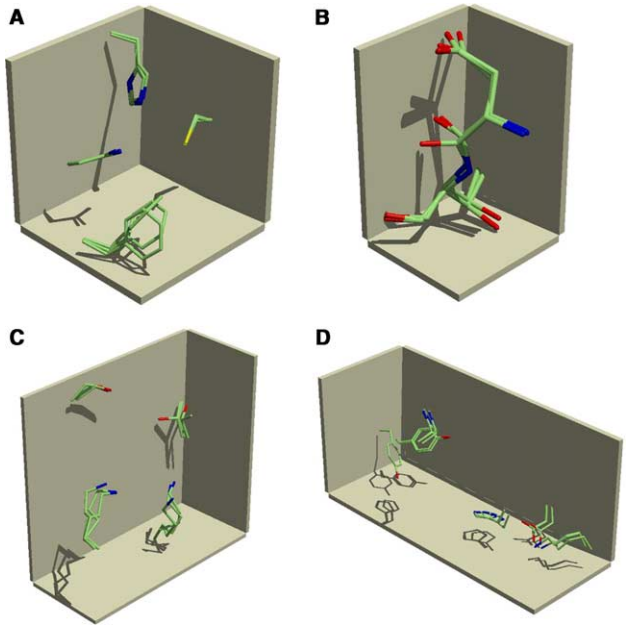

Fig. 1. Four examples of superpositions of catalytic residues from different stages of the reaction cycle. A: The catalytic residues of PDF, the multiple conformations of the phenylalanine can be seen at the bottom. B: P450cam, the central carbonyl oxygen can be seen in two different conformations which allows binding of an ordered water molecule in the substrate-bound form. C: DTBS, the threonine in the top right moves ~1 Å on substrate binding and rotates to correctly position the hydroxyl. D: MUT, all the residues move, but the tyrosine on the left hand side is the most important, rotating as well as moving to generate the adenosyl radical.

3. Analysis

To measure the extent of conformational change between two states of an enzyme, we use root mean square deviation (RMSD). RMSD is measured using a superposition of C-α atoms performed using the ProFit program [9]. RMSD provides a convenient, simple measure of the changes between two structures, however it is dependent on the number of atoms used to make the superposition and the physical size of the enzyme. Comparisons of RMSDs between enzymes have to be made with care therefore.

For each enzyme, a simple reaction cycle is constructed comprising the apo form, the substrate-bound form and the product-bound form. In FTase and DHFR, product release is only achieved by binding of fresh substrate and so the apo form is not part of the normal in vivo reaction cycle; in these cases the enzyme–substrate–product complex is used instead of the apo form.

In Fig. 2, RMSD is measured between each structure in the cycle and a triangle drawn such that each vertex represents a structure and the length of the edge connecting two vertices is proportional to the RMSD between those two structures. The triangles, which represent each enzyme, are listed in the same order as the enzymes in Table 1.

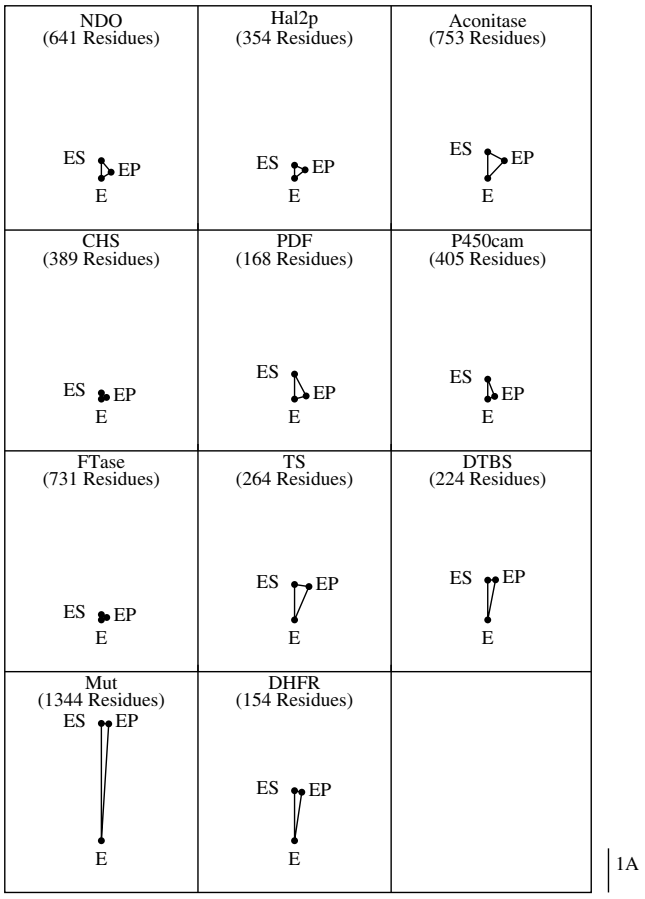

Fig. 2. Triangles representing the conformational change undergone by each enzyme. Each side of a triangle represents the RMSD between the structures represented by each vertex. RMSD is calculated by performing a superposition of C-α atoms. For DHFR and FTase, the structure with product and fresh substrate bound is used instead of the apo form, as this better represents the in vivo enzyme cycle. For TS the structure with activated substrate is used as the structure-bound form. A line representing a 1 Å RMSD is shown as scale.

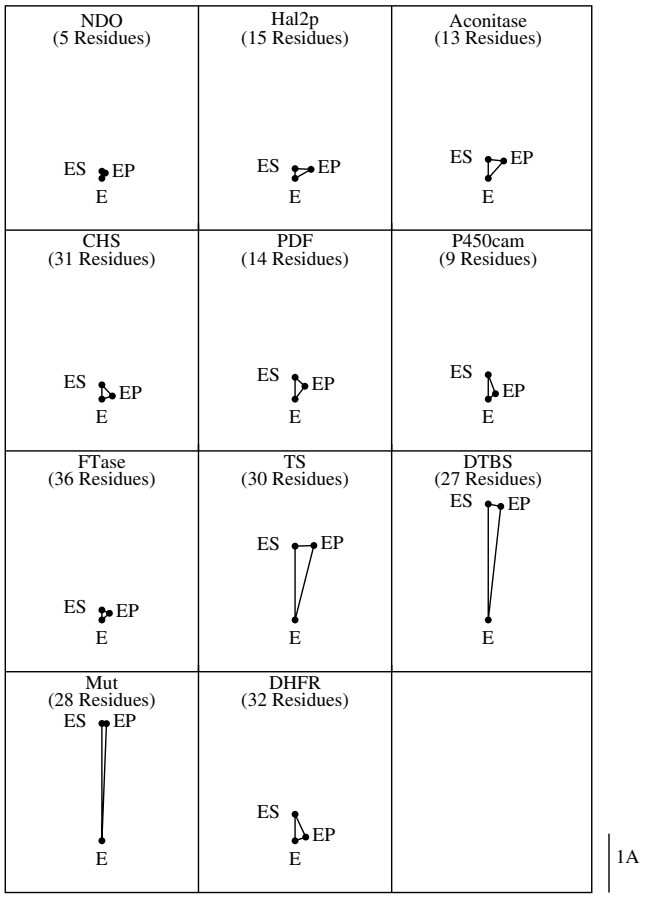

Fig. 3. Triangles representing the conformational change undergone by the binding residues of each enzyme. Each side of a triangle represents the RMSD between the position of the binding residues in the structures represented by each vertex. RMSD is calculated by performing a superposition of C-α atoms. For FTase and DHFR, the structure with product and fresh substrate bound is used instead of the E form, as this better represents the in vivo enzyme cycle. For TS the structure with activated substrate is used as the structure-bound form. A line representing a 1 Å RMSD is shown as scale.

In most cases, the RMSD is small (less than 1 Å), reflecting the localized nature of the motions observed. Usually conformational change is restricted to small loop regions, only a few residues in length, moving to close around a rigid core. Only MUT, with RMSD ~2.5 Å, and to a lesser extent DHFR, TS and DTBS, with RMSD ~1 Å undergo large-scale motions. In these enzymes the motion between the apo- and substrate-bound forms is much greater than the motion between the substrate- and product-bound forms, in contrast to those enzymes undergoing smaller motions, where the three sides of the triangle are usually similar in length.

In Fig. 3 the RMSD is calculated using a superposition of the C-α atoms of the binding residues only, and in Fig. 4 the RMSD is calculated using only the C-α atoms of the catalytic residues.

The pattern seen in Figs. 3 and 4 is similar to that seen in Fig. 2. A few enzymes (MUT, DTBS and TS) undergo relatively large motions of the binding and catalytic residues, whilst the remainders are relatively static. As with the whole protein RMSDs, the difference between substrate- and product-bound forms is small in most cases, though in both P450cam and DHFR the product-bound state has moved closer to the apo form.

To compare the flexibility of the catalytic and binding regions to the rest of the protein, we randomly select groups of residues from the structure and calculate the RMSD for those groups. Each group is chosen so that it has the same number of residues as the functional region and approximately the same size. One thousand different randomly selected groups are chosen from each structure and the RMSD is calculated for each. The percentile-rank (P) of the RMSD of the functional residues within the 1000 random samples is then calculated.

The distribution of absolute RMSD and the P value for the catalytic residues are shown in Fig. 5 and for the binding residues in Fig. 6. In each case the distribution of RMSDs for the E–ES and EP–E pairs peak at low RMSD, with an extended tail representing the examples of large conformational change. The ES–EP pair does not have a tail and tends to small values only, reflecting the consistently small changes we see between the substrate- and product-bound forms. The P values show that both the catalytic and binding residues are often

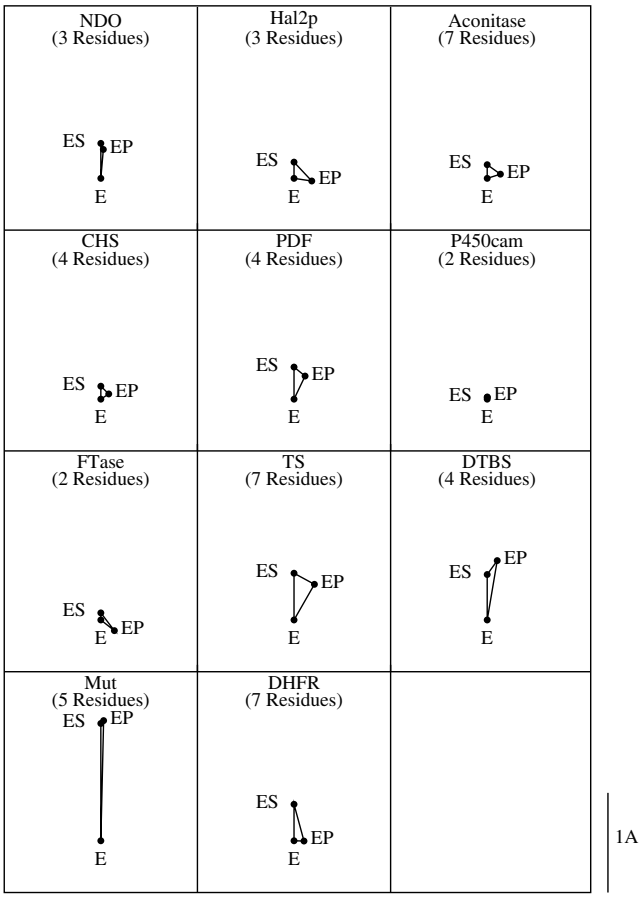

Fig. 4. Triangles representing the conformational change undergone by the catalytic residues of each enzyme. Each side of a triangle represents the RMSD between the position of the catalytic residues in the structures represented by each vertex. RMSD is calculated by performing a superposition of C-α atoms. For FTase and DHFR, the structure with product and fresh substrate bound is used instead of the E form, as this better represents the in vivo enzyme cycle. For TS the structure with activated substrate is used as the structure-bound form. A line representing a 1 Å RMSD is shown as scale.

amongst the most flexible parts of the structure, despite the small values of the RMSDs.

4. Discussion

One of the surprising results of this survey is the small size of the motions undergone by most of the enzymes in this sample. Only DHFR and MUT undergo large conformational change. DTBS and TS have a few loop regions which undergo localized changes, but the remaining seven enzymes undergo only very subtle conformational changes. However, even the small motions we see here could still be important for catalysis. It has been shown that movements of residues of less than 1 Å can alter the rate of catalysis by several orders of magnitude [30]. The precise relative positioning of residues in the Ser–His–Asp catalytic triad is an example of a system where movements of less than 1 Å could easily destroy catalytic power. The small movements observed for catalytic residues are encouraging for template based methods of structure annotation [31], which might use catalytic residues as the basis for templates and rely on them maintaining a fixed geometry.

One possible bias in this dataset is that the structures we have chosen are self-selected to be those which undergo minor conformational changes. There are two reasons for this: first, rigid enzymes are likely to be easier to crystallize in multiple states, and second, in enzymes which do undergo large change, it may be that soaking the ligand into the apo form destroys the crystal, and so crystal structures cannot be obtained in this way. In this set, six of the eleven enzymes had structures generated by soaking the ligand into crystals of the apo form, and five by crystallization of the ligand-bound form.

It is possible that the available structures are only revealing part of the story. Certainly in two cases: P450cam and aconitase, the crystal structures cannot be giving the full picture, as the active site is entirely closed even in the apo form. In these enzymes, there must be hidden flexibility in the structure that the static snapshots given by X-ray crystallography do not detect. However, we believe that in the majority of cases the crystal structure does represent a true picture of the enzyme at that point in the reaction cycle.

It might be expected that there would be a correlation between the size of conformational change and the size of the substrate, with large substrates requiring large motions of the enzyme. We do not see such a correlation here. FTase, for instance, binds two large substrates: farnesyl diphosphate (FPP) and a peptide; but has some of the smallest RMSDs in the sample. Similarly, CHS and PDF bind large substrates with relatively little conformational change. However, we do note that all the enzymes binding small substrates, such as P450cam, NDO and Aconitase, undergo small changes in enzyme conformation.

Do the triangle diagrams of the catalytic cycle tell us anything about the Gibbs free energy profiles of these enzymes? The diagrams only deal with the conformational change of the

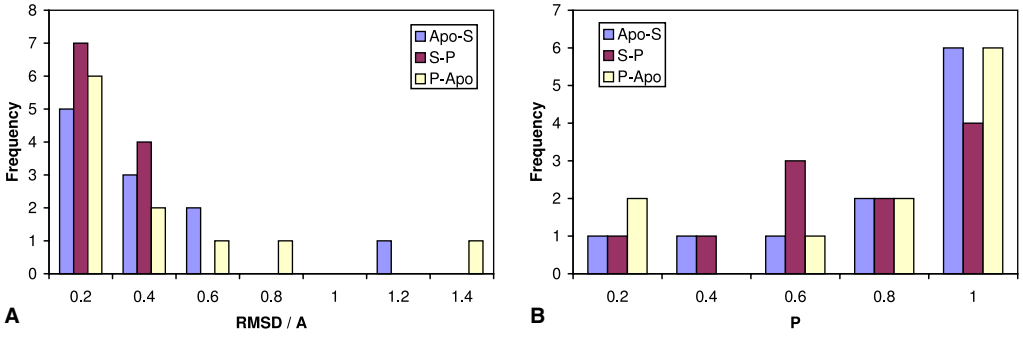

Fig. 5. Graphs of RMSD and *P* for the catalytic residues. (A) RMSDs for catalytic residues; (B) *P* values for catalytic residues.

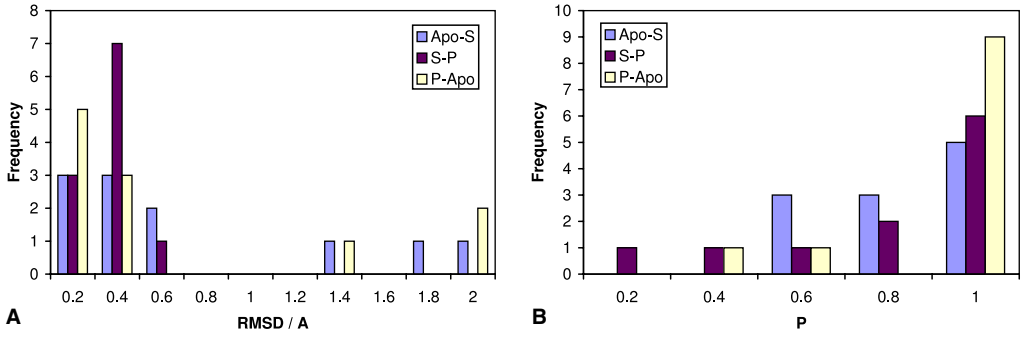

Fig. 6. Graphs of RMSD and *P* for the binding residues. (A) RMSDs for binding residues; (B) *P* values for binding residues.

enzyme, and so can only describe the reaction from the proteins point of view, which forms just one component of the total free energy. Also the RMSD of a conformational change is not proportional to the energy involved in making that change. Unravelling an alpha helix, for example, may take more energy than moving a surface loop, though the RMSD may well be greater for the loop motion.

Given these caveats, what would we expect to see in terms of conformational change from an energy perspective? The free energy profile found by Albery and Knowles [32] for TIM shows that the apo form has the lowest free energy, followed by the substrate-bound species, followed by the product-bound species. Given this pattern, and assuming that conformational change is related to free energy change, we would expect to see a triangle with EP–E as the longest side, representing the large energy change between the product bound and resting states. Looking at Fig. 2 we do not see any examples of triangles with this shape. In fact, what we see is that in those enzymes which do undergo significant induced fit (TS, DTBS, MUT and DHFR), the ES–EP side is short, leading to E–ES and EP–E sides of similar length. For enzymes undergoing smaller conformational change, some have a similar length for all three sides, whilst others have short EP–E sides. The RMSDs of the binding residues shown in Fig. 3 show a similar pattern.

These observations suggest that the increase in free energy from the ES to the EP form is not spent in further conformational change of the enzyme, but in some form of strain on the substrate. In this study, we have only analyzed the motion of the enzyme, not the changes undergone by the substrates, so we do not observe this aspect of the reaction. It is clear that in some cases, the conformational change of the substrate is extremely important. In FTase for example, the two substrates are bound with the acceptor carbon of the farnesyl and the attacking cysteine sulfur of the peptide 7 Å apart. The reaction must therefore involve substantial motion of the substrates during catalysis. The enzyme itself appears not to have moved between the substrate- and product-bound states, but we cannot rule out the possibility that there is motion between the static snapshots given by the crystal structures.

The final question we consider is whether there is a conflict between the requirement for enzymes to precisely position their functional groups and the conformational change required by theories of induced fit. Intuitively, it would seem harder to precisely position residues that are in a flexible part of the enzyme than residues in the rigid core. An obvious solution to this apparent contradiction is to restrict induced fit motions to surface loops which can close over the catalytic machinery located at the base of the active site. There is a suggestion of this in the results obtained by Bartlett et al. [8] which showed that catalytic residues generally have small solvent exposure and so do not lie on surface exposed loops.

Examining the 11 examples here, we do see that most of the conformational change occurs in flexible loop regions, and that most of the catalytic residues do not lie on these loops. There are four notable exceptions though: FTase, DTBS, DHFR and MUT, all have some rigid catalytic residues, but also rely on one catalytic residue lying on a mobile loop. Looking at the *P* values for the catalytic and binding residues, which measure the conformational change seen in these residues compared to the rest of the protein, we find that the average *P* value for the catalytic residues is 0.67 compared to 0.77 for the binding residues. This suggests that the binding residues are more flexible than the catalytic residues, however, this difference is not significant for a data set of this size. A larger data set would be required to confirm this difference.

In summary, we find that most enzymes undergo small motions upon substrate binding. In those cases where there is a large motion, there is relatively little change between the substrate- and product-bound forms. We hypothesize that the free energy change between the substrate- and product-bound states is accounted for by changes in the substrates, not motions in the enzyme. We also find, for this small sample, that the catalytic machinery tends to be preformed, with only a single flexible residue in some cases.

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