Biochemistry

 Volume 33, Number 22 June 7, 1994

Perspectives in Biochemistry

Structural Mechanisms for Domain Movements in Proteins†

Mark Gerstein,*,‡,§ Arthur M. Lesk,*,‖ and Cyrus Chothia*,‡,⊥

MRC Laboratory of Molecular Biology, Department of Haematology, Cambridge University, and Cambridge Center for Protein Engineering, Hills Road, Cambridge CB2 2QH, U.K.

Received February 15, 1994; Revised Manuscript Received April 1, 1994®

ABSTRACT: We survey all the known instances of domain movements in proteins for which there is crystallographic evidence for the movement. We explain these domain movements in terms of the repertoire of low-energy conformation changes that are known to occur in proteins. We first describe the basic elements of this repertoire, hinge and shear motions, and then show how the elements of the repertoire can be combined to produce domain movements. We emphasize that the elements used in particular proteins are determined mainly by the structure of the interfaces between the domains.

Nearly all large proteins are built from domains (Wodak & Janin, 1981), and large relative movements of domains provide spectacular examples of protein flexibility. Domain motions are important for a variety of protein functions, including catalysis, regulation of activity, transport of metabolites, formation of protein assemblies, and cellular locomotion. Domains often close around a binding site between them. Generally, the presence of bound substrates stabilizes a closed conformation, and their absence favors an open conformation. Consequently, domain motions illustrate induced fit in protein recognition (Koshland, 1958).

Most of our information on the mechanisms of domain movements has come from X-ray crystal structures of open and closed conformations of particular proteins. The results of early investigations were reviewed by Janin and Wodak (1983) and by Bennett and Huber (1984). Since then, a considerable amount of new information has become available, and we review here the portion of this information that concerns structural mechanisms of domain closure.

In catalysis, domain closure often excludes water from the active site and helps position catalytic groups around the substrate. It also traps substrates and prevents the escape of reaction intermediates (Anderson *et al.*, 1979; Knowles, 1991). Domain closure, therefore, must be fast, and the transition between open and closed forms cannot involve high-energy barriers. Protein interiors, however, have features that place strong constraints on their possible conformational changes: they are close-packed with main chains and side chains in preferred conformations and with buried polar groups hydrogen bonded. In the first part of this review, we discuss the repertoire of possible low-energy conformational changes that are available to proteins, *i.e.*, their intrinsic flexibility. In the second part we describe how this repertoire of low-energy conformational changes are used to produce domain movements in particular proteins.

THE INTRINSIC FLEXIBILITY OF PROTEINS

The intrinsic flexibility of proteins is taken here to mean the ability of different *segments* of the protein to move in relation to one another with only small expenditures of energy. Analysis of protein crystal structures has shown that this intrinsic flexibility can take two forms: hinge motions in strands, β-sheets, and α-helices that are not constrained by tertiary packing interactions and shear motions between close-packed segments of polypeptide (Figure 1; Table 1).

(A) Hinge Motions in Strands, Sheets, and Helices Not Constrained by Packing Interactions. (1) β-Strands. The most basic motion of a polypeptide chain is a few large changes in main-chain torsion angles in a localized region, *i.e.*, at a

† Supported by Damon Runyon–Walter Winchell Fellowship DRG-1272 (M.G.) and the Kay Kendall Foundation (A.M.L.).

‡ MRC Laboratory of Molecular Biology.

§ Present address: Beckman Center for Structural Biology, Department of Cell Biology, Stanford Medical School, Stanford, CA 94305.

‖ Department of Haematology, Cambridge University.

⊥ Cambridge Center for Protein Engineering.

® Abstract published in *Advance ACS Abstracts,* May 1, 1994.

0006-2960/94/0433-6739$04.50/0

Table 1: Comparison of Hinged vs Shear Mechanisms for Domain Closure

	shear mechanism	hinged mechanism
simple example	citrate synthase	lactoferrin
main-chain packing	constrained by close packing	free to kink
main-chain torsions	many small changes	a few large changes
motion overall	concatenation of small local motions	identical to twisting at hinge
motion at interface	parallel to plane of interface (shear)	perpendicular to plane of interface, exposing and burying surfaces
side-chain packing	same packing in both forms	new contacts created; packing at base of hinge crucial
side-chain torsions	mostly small changes	some large changes

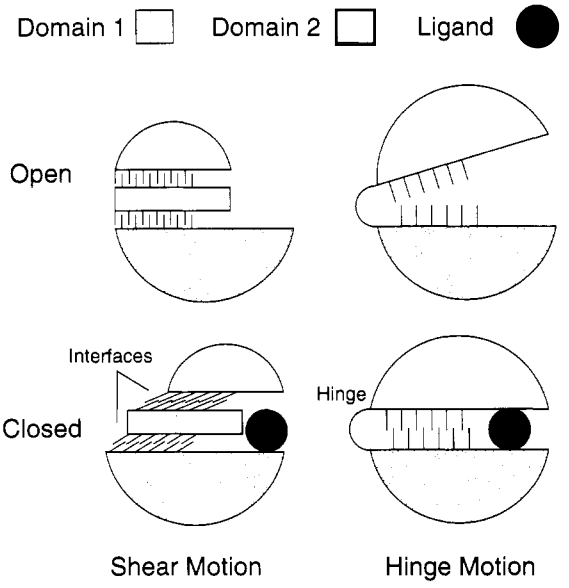

FIGURE 1: Hinged and shear mechanisms for domain closure. See Table 1 for a summary of the characteristics of both mechanisms.

hinge. The deformation of an extended strand is the simplest hinge motion because its only constraint is that the torsion angles of the strand remain in the allowed regions of the Ramachandran diagram. Consequently, its torsion angle changes can be very large and the resulting motion can rotate the polypeptide chain up to 60°. As shown in Figure 2A, in lactate dehydrogenase two adjacent torsion angle changes rotate a strand by ~35° in a direction not accessible by a single change (Gerstein & Chothia, 1991).

(2) β-Sheets. Two strands connected in a β-sheet can move together like the hinges on a door. However, the necessity that the strands remain hydrogen bonded together provides an additional structural constraint beyond the limitations of the Ramachandran diagram. As shown in Figure 2B, for the hinged sheet in lactoferrin this additional constraint means that in both strands the rotation axes of the principal torsion angle changes must be nearly parallel to each other and to the axis of the overall rotation of the sheet (Gerstein *et al.*, 1993b). Three large (>30°) torsion angle changes produce the bulk of the motion, rotating the sheet by 53°.

(3) α-Helices. Hinges in α-helices present a contrasting story. Because residues in helices are subject to more severe hydrogen-bonding and steric constraints than those in sheets, their torsion angles are restricted to a smaller region of the Ramachandran diagram. Thus, if residues are to remain in a helical conformation, the possible changes in their torsion angles are correspondingly smaller than those of residues in an extended conformation, and the deformation of helices must be spread over more residues than the deformation of sheets. Such spread-out helical deformations can produce bending motions: eight torsion angle changes between 9° and 15° in the C-terminus of a helix in a mutant lysozyme bend its end to produce a shift of 3.3 Å (Dixon *et al.*, 1992; Figure 2C). Similar deformations can also stretch a helix: six torsion angle changes over four residues at the N-terminus of a helix in lactate dehydrogenase tighten the helix up from an α to a 3_{10} conformation and stretch it by 3.3 Å (Gerstein & Chothia, 1991).

A different situation occurs in those helices that contain kinks, which often involve prolines. The disruption in the normal pattern of hydrogen bonding, and hence in the constraints on the helix, allows larger torsion angle changes. As shown in Figure 2D, such large torsion angle changes have been found in the proline-kinked helix in adenylate kinase.

The interconversion of helical and extended conformations is also possible and has been found in calmodulin (Ikura *et al.*, 1992; Meador *et al.*, 1992, 1993) and triglyceride lipase (Derewenda *et al.*, 1992). While such an interconversion may involve crossing energy barriers somewhat higher than those in the motions discussed above, it permits large torsion angle changes and large deformations. In calmodulin, torsion angle changes in five residues in the middle of a long helix split it into two smaller helices, separated by four residues of extended strand. These two small helices are inclined at an angle of ~100°.

(B) Limited Shear Motions of Close-Packed Segments of Polypeptide. The preceding discussion of hinges considered only the effects of structural constraints intrinsic to β-strands, β-sheets, and α-helices—*i.e.*, constraints arising from the requirements of secondary structure. The interactions that stem from tertiary structure provide even more severe structural constraints. Most of the atoms in a protein are partially buried and closely packed—in particular, most of the main chain is buried beneath layers of side chains. This close packing precludes large torsion angle changes and hence hinges. Indeed, a structural requirement for a residue to act as a hinge is that it have few tertiary structure packing constraints on its main chain.

As shown in Figure 3, we can divide movements of close-packed segments of polypeptide into those that are perpendicular to an interface and those that are parallel. Hinges from outside the region of the interface can produce a motion perpendicular to the plane of an interface (so the interface exists in one conformation but not in the other, as in the opening and closing of a book). As discussed below, this sort of motion can be driven by ligands stabilizing a closed conformation. Motions parallel to the plane of the interface are limited by the packing contacts involving the interdigitation of side chains. Large shifts of close-packed segments of polypeptide would require switching between different interdigitating configurations. Although such packing changes are seen at the subunit interfaces of allosteric proteins (Perutz, 1989), they have not been observed, so far, in domain closure. This is probably because such motions involve crossing high-energy barriers and would not occur with sufficient rapidity.

Small shear motions (Figure 3) that do not involve repacking of the interface are commonly involved in domain closure and have the following characteristics: (1) Interdigitating side chains accommodate shear motions, mostly, by small (<15°)

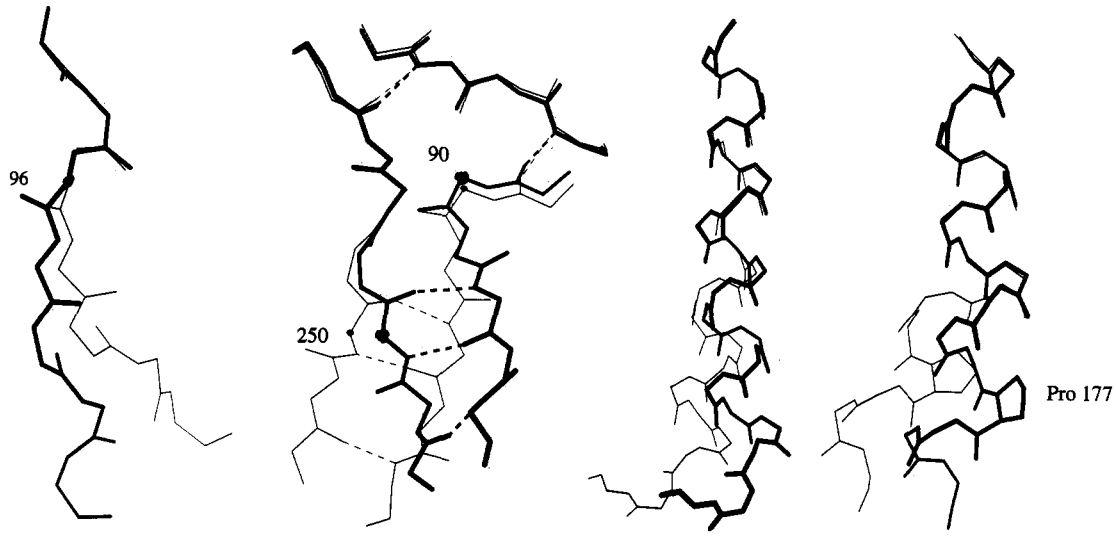

FIGURE 2: Hinge motions in strands, sheets, and helices. (A, far left) A hinge in lactate dehydrogenase is an example of a isolated hinge in a strand. Changes in two torsion angles ($\Delta\phi(96) = 36°$ and $\Delta\phi(97) = 40°$) are responsible for rotating the polypeptide chain ~35°. (B, middle left) The hinges in lactoferrin are an example of the coupling of two simple hinges together in a sheet. The hinges move through three large torsion angle changes, and the rotation axes for these torsion angle changes are inclined less than 20° with respect to the axis of the overall motion. (In the strand on the left $\Delta\psi(250) = -33°$ and $\Delta\phi(249) = 30°$; in the strand on the right $\Delta\psi(90) = 49°$.) Small conformational changes in adjacent residues help maintain the integrity of the β-sheet structure. As evident in Figure 6, the hinges have few main-chain packing constraints on them. (C, middle right) The interdomain helix in lysozyme is an example of a bending helix. It bends through the coordinated action of eight torsion angle changes between 9° and 15°, shifting the Cα atom at the C-terminal end of the helix by 3.3 Å. (D, far right) The helix linking the two domains in ADK is an example of a kinking helix. A torsion angle change in the residue three before Pro 177 ($\Delta\phi = -53°$) causes the helix to deform in a direction perpendicular to its original kink.

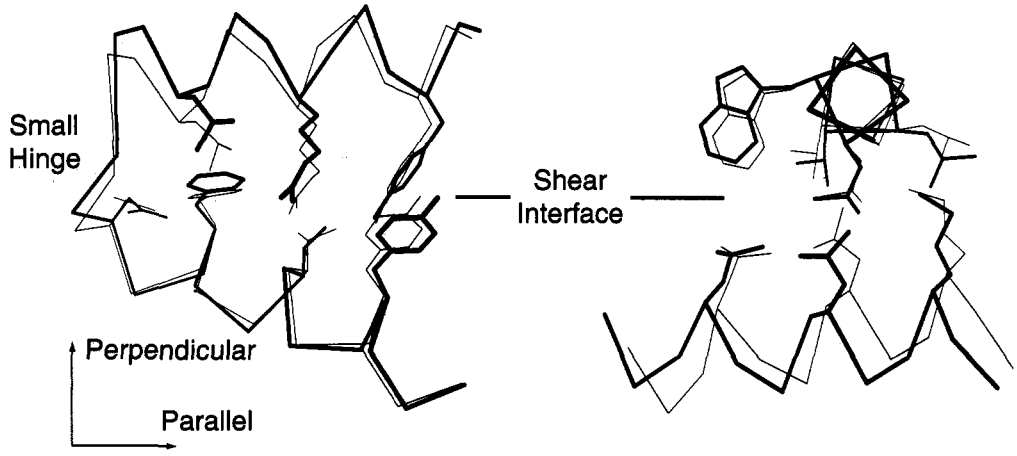

FIGURE 3: Shear motions involve interfaces. Two examples taken from citrate synthase show helix–helix interfaces undergoing a shear motion. The two labeled axes show the direction of parallel and perpendicular motion at an interface. (Left) The QP helix–helix interface illustrates how small hinges in linking peptides function in shear motions. Helix Q shifts 1.4 Å and rotates 13° relative to helix P. (Right) The NQ helix–helix interface shows a crossed-helix packing and a slightly larger motion than at the QP interface. Helix N shifts 1.8 Å and rotates 11° relative to helix Q. There are many close-packed side chains forming the N–Q interface, which just rock slightly in the shear motion.

changes in side-chain torsion angles. They keep the same overall rotamer configuration and move among conformational states of nearly the same energy without crossing large energy barriers. Occasionally, they may change to a different rotamer conformation (*i.e.*, to a different local minimum) with large rotations (>100°). (2)The main chain of each segment in a shear motion does not deform appreciably. In the case of helices, the root mean square difference in the positions of their main-chain atoms in the open and closed forms is typically 0.15–0.25 Å; for loops the difference is slightly larger. This rigidity, combined with "rocking" movements of side chains, implies that the interface itself shears. (3) The segments shift and rotate relative to each other by no more than 2 Å and 15°, amounts likely to be the limits of low-energy conformational adjustments. Except at very small interfaces, larger movements than these require the combination of several shear motions.

These characteristics were initially deduced from the analysis of protein crystal structures (Chothia *et al.*, 1983; Lesk & Chothia, 1984). A similar, and in some ways more detailed, picture of shear motions has recently emerged through physical studies and computational simulations (Elber & Karplus, 1987; Rojewska & Elber, 1990; Frauenfelder *et al.*, 1991).

SHEAR AND HINGE MOTIONS UNDERLIE DOMAIN-MOTION MECHANISMS

The characteristics of the two basic mechanisms of protein flexibility, hinge and shear motions, are summarized in Figure 1 and Table 1. These two mechanisms constitute a repertoire of conformational changes that can be used in a great variety of protein motions. Here we describe their use in the motions of whole protein domains, *i.e.*, in the relative motion of discrete linked units that consist, in most cases, of at least 100 residues.

Hinge and shear mechanisms are also involved in the motion of small protein fragments, for example, when individual loops or helices move relative to each other. In Table 2 we summarize the current crystallographic evidence for hinge and shear mechanisms in both domain motions and smaller motions. It is important to realize that hinge and shear motions are ideal paradigms for describing large domain motions. A real domain motion will often have a combination of both motions, *i.e.*, hinges in one part of the protein and shearing interfaces elsewhere. Nevertheless, many domain motions can be described as occurring predominantly by a hinge or a shear mechanism.

As shown in Figure 1, proteins that have a predominantly hinged domain motion usually have two domains connected by linking hinge regions that are relatively unconstrained by packing. A few large torsion angle changes are sufficient to produce almost the whole domain motion. The rest of the protein rotates essentially as a rigid body, with the axis of the overall rotation passing through the linking hinge regions.

Since an individual shear motion is small, a single one is usually not sufficient to produce a large domain motion. Usually, a number of shear motions combine to give a large effect—in a similar fashion to each block in a stack sliding slightly to make the whole stack lean considerably. (The peptides that link the shearing segments have small main-chain torsion angle changes to accommodate the relative movements.)

Proteins with shear motions tend to have certain architectural features. First, they often have layered architectures with one layer sliding over another. Second, though shear motions have been found at many different interfaces (*i.e.*, helix–helix, sheet–helix, loop–sheet, and loop–helix), helix–helix interfaces are most commonly used. The helices involved in shear motions are usually crossed. That is, they are usually oriented in a more perpendicular than parallel fashion (interhelical angle 60°–90°). Such crossed geometries are unusual in that helix–helix packings tend to be more parallel. Crossed helices will obviously have a smaller and more accommodating interface than parallel helices, and this is perhaps the reason for their preferential involvement in shear motions.

Table 2A lists all instances of crystallographically resolved domain motion, *i.e.*, proteins that have been solved in two or more conformations. With the notable exception of the immunoglobulins, almost all large domain motions can be understood in terms of hinge and shear motions. Table 2B lists proteins for which a domain closure mechanism can be inferred. The structures of these proteins have been determined in only one conformation. However, each has a structure similar to that of a protein with a well-characterized domain motion, *i.e.*, one listed in Table 2A, and is expected to move using the same mechanisms.

EXAMPLES OF SHEAR DOMAIN MOVEMENTS

(A) Citrate Synthase. Citrate synthase is one of the clearest examples of a domain closure occurring through shear motions. The molecule is a dimer, and each monomer comprises a large domain, containing 15 helices, and a small domain, containing five helices, with the active site cleft between them (Figure 4). The domain closure involves the small domain closing over the large one, burying the substrates in the active site (Remington *et al.*, 1982). An extensive interface between the large and small domains prevents closure taking place through a hinge mechanism. As shown in Figure 4, closure is produced by the summation of many small shear motions

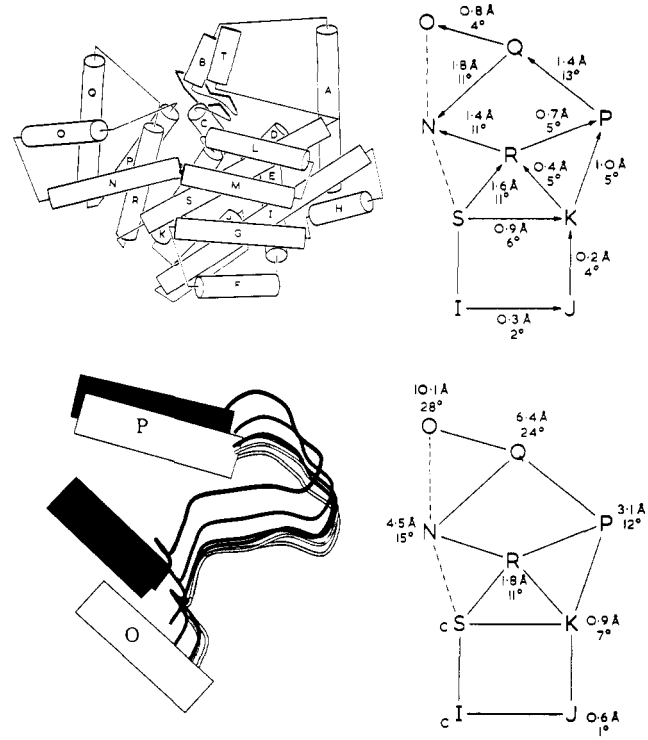

FIGURE 4: Shear motions in citrate synthase. (Top left) Cartoon of one subunit of citrate synthase. α-Helices are represented by cylinders. The small domain contains helices N, O, P, Q, and R. (top right) Schematic showing the relative movements of the principal helices in citrate synthase. [This figure is adapted in part from Lesk and Chothia (1984).] Each helix is represented by its letter, and the lines indicate the existence of helix–helix packings in both the open and closed forms. The shifts and rotations show local changes in the positions of pairs of packed helices (i.e., the movement in one helix in a pair relative to the other). (Bottom right) The overall effect of the helix movements. The same conventions as in the top right schematic apply, but the shifts and rotations shown now are those required to superimpose equivalent pairs of helices after the open and closed forms have been superimposed on the core of the large domain. Many small motions add up to shift helix O by 10.1 Å and rotate it by 28°. (Bottom left) Incremental motion in shear domain closure is shown by Cα traces of the OP loop: black is the apo form; white, the holo form; gray, the cumulative effect of motion over the K, P, and then Q helix–helix interfaces. (The apo form was fit to the holo form, first on the core and then on the K, P, and Q helices.)

between pairs of packed helices (Lesk & Chothia, 1984). The overall motion results in a helix on the far side of the small domain shifting by 10 Å and rotating by 28°, thereby moving an adjacent loop over the active site. Each local shear motion involves one helix moving relative to a neighboring helix by main-chain rotations and shifts of up to 13° and 1.8 Å. To a good approximation, the main chain of each helix moves without deformation as a rigid body. The shear motions are facilitated by small deformations in the loops linking the helices.

There are over 50 distinct helix–helix interfaces in the citrate synthase dimer. Depending on the angle between neighboring helices, these interfaces can be categorized as having roughly parallel helices, roughly perpendicular ones, or orientations in between. The interfaces between many of the moving helices tend to be roughly perpendicular, or "crossed", while the helices that are relatively motionless tend to have a more parallel packing.

(B) Aspartate Amino Transferase. In citrate synthase the domain closure is the cumulative result of many shear motions. In aspartate amino transferase (AAT) the domain motion is mainly the result of just two shear motions, which occur in

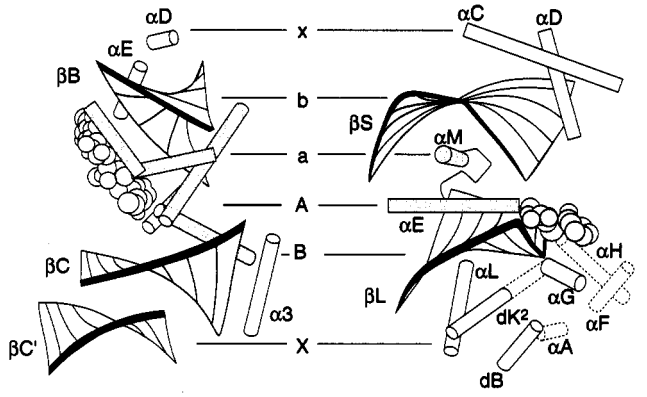

FIGURE 5: XBAabx layering in hexokinase and other proteins. XBAabx layering [see Examples of Shear Domain Movements (C)] is shown graphically by schematics of GAPDH (left) and hexokinase (right). Helices are drawn as narrow cylinders (radius 1.0 Å); sheets are represented as sheets as opposed to collections of strands; and substrates are drawn in CPK representation. (GAPDH is shown in its closed form with its actual ligand. Hexokinase is shown with the inhibitor *o*-toluoylglucosamine.)

perpendicular directions (McPhalen *et al.*, 1992). AAT has an active site situated between a large and a small domain, and on substrate binding the small domain closes over the active site. The major shear motion involves a 13° rotation of the core of the small domain relative to the large one. A secondary shear motion moves a helix on one side of the small domain in a direction perpendicular to both the interdomain interface and the direction of the other shear motion. With a 1.2-Å shift and a 10° rotation, it "drops down" to cover the active site.

The shear motions in AAT are facilitated by a hinge motion in a long interdomain helix. This helix is kinked by 17° in the open form and changes its kink angle by 12° on closure.

(C) Glyceraldehyde-3-phosphate Dehydrogenase, Alcohol Dehydrogenase, and Hexokinase. In the previous two examples, domain closure involved motions spread throughout a domain. Here we describe three examples where the major shear motion occurs at the interface between the domains with subsidiary motions on one or both sides of this region.

Because the enzymes hexokinase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and alcohol dehydrogenase (ADH) share many common architectural features, their domain movements proceed through similar mechanisms (see Table 2 for detailed references). These enzymes have three moving layers in one domain that shift relative to three rigid layers in the other domain. This distinctive layering pattern is of the form XBAabx, where a, b, and x are the three moving layers and X, B, and A are the rigid layers (Figure 5). The interface between the two middle layers is where the major shear motion occurs. One layer of helices from the mobile domain (a) slides over a layer of helices from the motionless domain (A). Helices in these two layers, which in a sense form gears upon which the domains slide, are often crossed, as is dramatically illustrated for the case of hexokinase in Figure 5. Near the a and A layer helices, the ligand binds in the interdomain cleft. Packed onto either side of the central layers of helices (a and A) are sheets (b and B) from the mobile and motionless domains, respectively. The mobile sheet (b) forms a second moving layer, which slides over the helices, and packed onto the other face of this sheet is a third layer (x) which moves with the sheet. Symmetrical to this third moving layer (x), a third motionless layer (X) is packed onto one side of the static sheet (B). (Layer x is made up of helices in hexokinase and GAPDH and of helices and a sheet in ADH, and layer X is made up of helices in hexokinase and ADH and of a sheet from another subunit in GAPDH.)

In addition to its shear motion, ADH also has two well-defined hinge points (Eklund *et al.*, 1981; Colonna-Cesari *et al.*, 1986).

As discussed in Table 2B, a number of other proteins have XBAabx architectures similar to those of hexokinase, GAPDH, and ADH but have not yet been solved in multiple conformations. These proteins include phosphoglycerate kinase (PGK), actin, and heat-shock protein. There is experimental evidence that these proteins may undergo domain movements [*e.g.*, for PGK, see Mas *et al.* (1987, 1988)], and they would be expected to use mechanisms similar to those of hexokinase, GAPDH, and ADH. Moreover, a model-building study done on PGK (Blake *et al.*, 1986) predicts that the domain movement will involve the shearing of the two central helices, a conclusion similar to that implied by our comparisons.

(D) trp Repressor. In the previous sections we described examples of domains closing around substrates. In the *trp* repressor, the binding of a ligand stabilizes a more open conformation. The *trp* repressor is a small protein that regulates three operons involved in the synthesis of tryptophan. It is a dimer, and each subunit contains six helices, divided between two domains. The central core of the molecule is formed from four helices from each subunit. On either side of this core, helix–turn–helix motifs form two symmetrically arranged DNA "reading head" domains. Between the central core and the reading-head domains, there are two binding sites for L-tryptophan, which need to be filled for *trp* repressor to recognize DNA (Zhang *et al.*, 1987). Comparison of the holo and apo forms of the repressor (Lawson *et al.*, 1988) shows that the binding of L-tryptophan shifts Cα atoms in the reading head domain by up to 4 Å. These shifts are produced by separate shear motions of the two helices in the reading-head domain (0.75–1.5 Å, 5–20°). These helix motions move the reading-head domains further apart than they are in the apo form so they are correctly separated to bind DNA.

EXAMPLES OF HINGED DOMAIN MOVEMENTS

(A) Tomato Bushy Stunt Virus. An example of a very simple hinge motion is found in the coat protein of tomato bushy stunt virus (Olsen *et al.*, 1983). This spherical virus contains 180 subunits arranged with icosahedral symmetry on a $T = 3$ lattice. Each subunit, in turn, contains two major domains, the shell (S) and projection (P) domains, that are linked by a peptide in an extended conformation. The symmetry of the virus requires each subunit to fit into one of three different packing environments. One of the principal mechanisms for accommodating the different environments is a relative movement of the two domains by ~22°. This movement involves a simple hinge in the peptide connecting the S and P domains (Olsen *et al.*, 1983).

(B) Calmodulin. Like the TBSV coat protein, the motion in calmodulin involves a single deformation. The unligated form of calmodulin contains two globular domains, connected by a long helix (Babu *et al.*, 1985). NMR and X-ray structures of ligated calmodulin show the molecule binding to peptide helices with different sequences and the two domains closing around the peptide far enough to make contact with each other (Ikura *et al.*, 1992; Meador *et al.*, 1992, 1993). As discussed above [The Intrinsic Flexibility of Proteins (A) (3)], in this motion, the long interdomain helix, which is known to have only marginal stability in solution (Ikura *et al.*, 1992),

Table 2: Proteins That Undergo Domain Movements

(A) Proteins for which open and closed conformations are known [a] [b]

(i) Domain motion is predominantly shear

Citrate Synthase [c]	1CTS 3CTS	Remington *et al.*, 1982; Lesk & Chothia, 1984	Shear motions at many helix-helix interfaces shift mainchain atoms up to 10 Å
Aspartate Amino Transferase (AAT) [c]	9AAT 1AMA	McPhalen *et al.*, 1992	Shear motion at 2 interfaces combined with hinge in a kinked helix.
Trp Repressor [c]	1WRP 2WRP 3WRP	Lawson *et al.*,1988	Shear motion between 2 helices adjusts position of helix-turn-helix reading head domain to enable it to bind DNA
Hexokinase [c]	2YHX 1HKG	Bennett & Steitz, 1978, 1980; Lesk & Chothia, 1984	Shear motion with XBAaba layering. Prominent crossed helices at interdomain interface.
Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) [c]	1GD1 2GD1	Skarzynski & Wonacott, 1988	Shear motion with XBAaba layering.
Alcohol Dehydrogenase (ADH) [c]	8ADH 6ADH	Eklund *et al.*,1981; Colonna-Cesari *et al.*, 1986	Shear motion with XBAaba layering and 2 hinges.
Endothiapepsin	4APE 5ER2	Sali *et al.*, 1989; 1992	Small shearing motion at 1 interface between domains (17° rotation and 1 Å displacement)

(ii) Domain motion is predominantly hinge

Tomato Bushy Stunt Virus (TBSV) Coat Protein [c] [e]	2TBV	Olson *et al.*, 1983	1 interdomain linkage,1 hinge, ~22° rotation.
Lactoferrin [c]	1LFH 1LFG	Anderson *et al.*, 1990; Gerstein *et al.*, 1993b	2 interdomain linkages, 2 hinges (in a β-sheet), 53° rotation. See-saw between two interfaces.
Maltodextrin Binding Protein (MBP) [c]	1OMF 2MBP	Sharff *et al.*, 1992;	3 interdomain linkages, 3 hinges, 35° rotation.
Lysine/Arginine/Ornithine (LAO) binding protein [c]	1LST	Oh *et al.*, 1993	2 interdomain linkages, 2 hinges, 52° rotation.
T4 lysozyme mutants: Ile3→Pro & Met6→Ile [c]	1L96 1L97	Dixon *et al.*, 1992; Faber & Matthews, 1991	2 hinges, at either end of interdomain helix, produce rotations up to 32°.
Adenylate Kinase (ADK) [c] [g]	1AK3 1AKE	Schulz *et al.*, 1990; Gerstein *et al.*, 1993a	2 interdomain linkages and 4 hinges (one involves kinking helix). 60° rotation from 1st pair of hinges, 30° from 2nd pair, 90° total.
Catabolite Gene Activator Protein (CAP) [e]	3GAP	Weber & Steitz, 1987	1 interdomain linkage and 1 hinge. Comparison of subunits in the dimer reveals that the small domain has rotated ~30° closer to the large domain in one subunit.
cAMP-dependent Protein Kinase (catalytic domain) [c] [d]	1ATP 1APM	Karlsson *et al.*, 1993	1st set of hinges, involving 3 interdomain linkages, produces 12° rotation of domain cores (with ~3 Å shift). 2nd set of hinges produces further 6° rotation of a loop. 1 shearing interface between domains.
Calmodulin [c]	1CLL 4CLL 2BBM	Ikura *et al.*, 1992; Meador *et al.*, 1992, 1993	1 interdomain linkage, 1 hinge, ~150° rotation. Hinge involves long helix splitting into 2 helices (inclined at ~100°) with strand in between.
Glutamate Dehydrogenase		Stillman *et al.*, 1993	13° rotation of 1 domain relative to other

(iii) Domain motion is not predominantly a hinge or shear mechanism

Immunoglobulins [c] [h]	2FB4 1MCP	Bennett & Huber, 1984; Lesk & Chothia, 1988;	Hinge motion in linking peptides. Ball & socket joint forms interface between domains. Range of rotations up to 50° allowed.
Serpins	5API 1OVA	Loebermann *et al.*, 1984 Engh *et al.*, 1990 Stein & Chothia, 1991 Mottonen *et al.*, 1992	Translation at a helix-sheet interface results in the transformation of the tertiary structure by insertion of strand into sheet.

(iv) Domain motion can not be fully classified at present [f]

HI V-1 Reverse Transcriptase	1HMI 1HVT	Kohlstaedt *et al.*, 1992; Jacobo-Molina *et al.*, 1993	Comparison of subunits shows very large rearrangement of 2 of the 4 domains which is accomodated by changes in loops and by unfolding of small 3 stranded β-sheet.
TATA-box Binding Protein (TBP) [e]	1TBP	Kim *et al.*, 1993a, 1993b; Chasman *et al.*, 1993	Twisting of a central sheet moves 2 domains ~10°.
Themolysin, Elastase, neutral proteases	1EZM 4TMN	Holland *et al.*, 1992; Thayer *et al.*, 1991	Bending interdomain helix
Elongation Factor Tu (EF-Tu) [d]	1ETU	Berchtold *et al.*, 1993; Kjeldgaard *et al.*, 1993	Internal loop movements similar to those in *ras* protein (below) lead to large domain rearragements (90° rotation, 40Å shifts)

Table 2: (Continued)

(B) Proteins for which only one conformation is known

(i) Domain motion is predominantly shear

Phosphoglycerate Kinase (PGK) [c]	3PGK	Harlos *et al.*, 1992	Similar to hexokinase (XBAabx layering)
Heat Shock Protein	1HSC	Flaherty *et al.*,1990	Similar to hexokinase (XBAabx layering)
Actin	1ATN	Kabsch *et al.*,1990; Flaherty *et al.*, 1991 [i]	Similar to hexokinase (XBAabx layering)
Aspartic Proteases, besides endothiapepsin: Penicllopepsin, Rhizopuspepsin, Chymosin, Porcine Pepsin	2APP 2APR 2PEP 3CMS 1PSG	Sali *et al.*, 1992	Similar to endothiapepsin

(ii) Domain motion is predominantly hinge

Sulfate & Phosphate Binding Proteins	1SBP 1ABH	Luecke & Quiocho, 1990; Pflugrath & Quiocho, 1988	Similar to MBP & lactoferrin. These are group-II periplasmic binding proteins.
Arabinose, Leucine, & Galactose Binding Proteins	2LBP 2GBP 1ABP	Gilliland & Quiocho, 1981; Vyas *et al.*, 1988, 1991; Sack *et al.*, 1989a,b	Similar to MBP & lactoferrin. However, these are group-I periplasmic binding proteins and are not as similar as group-II ones (above) are.
Transferrins (N-terminal lobe)	1TFD	Sarra *et al.*, 1990	Similar to lactoferrin
Guanylate Kinase (GDK)	1GKY	Stehle & Schulz, 1990	Similar to ADK
Porphobilinogen Deaminase	1PDA	Louie *et al.*, 1992	Domains 1 and 2 similar to lactoferrin

(iii) Domain motion can not be classified at present [f]

Myosin		Rayment *et al.*, 1993	Closure of a nucleotide-binding cleft, with similarities to that of ADK, hypothesized to produce movements > 50 Å
Transducin-α		Noel *et al.*, 1993	Similar movements to EF-Tu and ras expected

(C) Proteins known in two conformations which involve movements of fragments smaller than domains [a]

(i) Motion is predominantly shear

Insulin [d]	4INS	Chothia *et al.*, 1983	Helices shear by ~1.5 Å.
Thymidylate Synthase	3TMS 2TSC	Perry *et al.*, 1990; Montfort *et al.*, 1990	Small shear motion of helices packed onto central sheet.
Dihydrofolate Reductase (DHFR)	4DFR 5DFR	Bystroff *et al.*, 1991	Small (~3 Å) movement, shearing interface with hinges.

(ii) Motion is predominantly hinge

Annexin V	1AVR 1RAN	Sopkova *et al.*, 1993; Concha, *et al.*, 1993	Large movements of 2 loops and end of a helix moves a buried trp residue 18Å to surface.
Lactate Dehydrogenase (LDH)	6LDH 1LDM	White *et al.*, 1976; Gerstein & Chothia, 1991	Loop closure with 2 hinges, one in helix, moves Cα atoms ~11 Å
Triose Phosphate Isomerase (TIM)	2YPI 3TIM 6TIM	Lolis & Petsko, 1990; Joseph *et al.*, 1990; Wirenga *et al.*, 1991	Loop closure with 2 hinges moves Cα atoms ~ 7Å
Enolase	3ENL 7ENL	Lebioda & Stec, 1991	Loop movements of ~7 Å
HIV-1 protease	4HVP 3HVP 5HVP	Miller *et al.*, 1989; Fitzgerald *et al.*, 1990	Two large loop regions, that together comprise one quarter of the structure, move Cα atoms ~ 7Å
Foot and mouth disease virus [d]	1BBT	Parry *et al.*, 1990	Comparing variants of virus shows movement of a surface loop
Triglyceride Lipase	1TGL 4TGL	Derewenda *et al.*, 1992;	2 hinges on either side of a helix move Cα atoms up to12 Å. In one hinge a residue changes from an extended to a helical conformation.
Isocitrate Dehydrogenase [d]	3ICD	Stoddard & Koshland, 1993	Loop movements of ~2 Å
Malate Dehydrogenase (MDH) [e]	4MDH	Birktoft *et al.*, 1989	Comparison of subunits shows a loop closure similar to LDH, moving atom Cα atoms up to 8 Å.
ras Protein	4Q21 6Q21	Milburn *et al.*, 1990; Sclichting *et al.*, 1990	2 loop movements move Ca atoms up to 10 Å (one movement includes helix attached to loop).

[a] When both open and closed forms are known, we refer to the papers that describe the structure comparisons. Further references to the individual open and closed structures can be found in these papers. [b] Allosteric proteins are not included because these proteins have motions that involve extensive repacking of interfaces (see Perutz, 1989 for a review). Such repacking involves high-energy conformational transitions distinctly different from the hinge and shear mechanisms. [c] Indicates proteins discussed in detail in the text. [d] Structures of 2 conformations have been solved but only 1 has been deposited in the Protein Data Bank. [e] Motion is evident in comparing different subunits in the asymmetric unit. Single data bank identifier applies for both forms. [f] It is not possible to classify some domain motions at present because full sets of coordinates or detailed analyses are not yet available. [g] ADK also has a shear motion when the first substrate, AMP, binds: i.e. in moving from the conformation of 3ADK to 1AK3, 3 helices with a crossed geometry shift 1-2 Å to rearrange the geometry of the nucleotide binding site slightly (Diederichs & Schulz, 1991). [h] Data bank indentifiers for only two of the many representative immunoglobulin stuctures are indicated. [i] This paper describes the structural similarity of actin and the heat-shock protein.

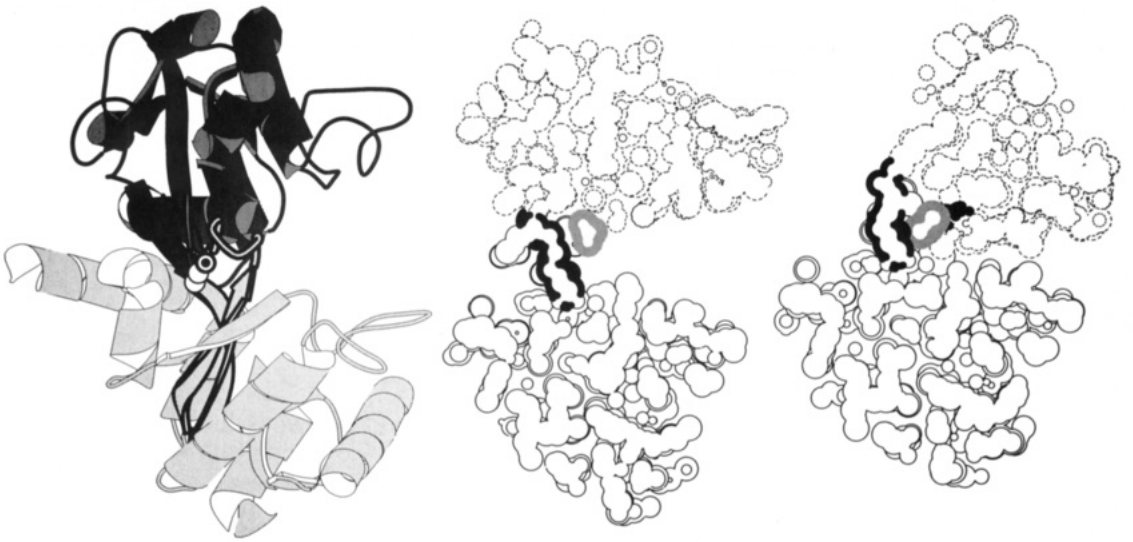

FIGURE 6: Hinge motion in lactoferrin. (Left) Cartoon of the two domains of lactoferrin (N1 and N2) in the open form (drawn with MOLSCRIPT; Kaulis, 1991). The origin of the rotation axis for the domain movements [see Examples of Hinged Domain Movements (A)] lies at the center of the figure. The view is down the rotation axis, which is indicated by a circle with a dot in it. N2 is shown in darker shading than N1, and the two antiparallel β-strands with the hinges are highlighted with bold lines. The Cα atoms of the residues with the largest movements (90 and 251) are indicated by empty circles. They lie in the middle of these strands and are very near the rotation axis in the open form. (Middle and right) Slices through the van der Waals envelope of the open and closed forms, respectively. N1 is shown by thin black lines; N2, by dashed black lines; the main-chain atoms of the hinge (89–92 and 249–252), by black shading; the side chain of Tyr 92, by a stippled gray line. Note the absence of packing constraints on the main-chain atoms of the hinge in the open form and the tight packing at the base of the hinge in the closed form.

partly unfolds to break into two helical segments connected by a hinge region in an extended conformation. The angle between the axes of the two helical segments is ~100°. As there is an additional twist around the helix axes, the total rotation of one domain relative to the other is more than 150°. Calmodulin can bind peptides with different sequences because of flexibility in the side chains that make contact with the peptide and by slightly shifting the relative placement of the domains through changes in the extent of the hinge region, which has consequently been dubbed "a variable expansion joint" (Meador *et al.*, 1993).

(C) T4 Lysozyme Mutants. Like calmodulin, two mutants of T4 lysozyme (Ile 3 → Pro and Met 6 → Ile) have a hinge motion involving a long interdomain helix. Crystals of these mutants grow in a number of different forms. Depending on the crystal form, their structures either are very similar to that of the wild type or differ from it by a *range* of rigid-body domain rotations up to 32° (Dixon *et al.*, 1992; Faber & Matthews, 1990). There are two main hinge points for the domain motion. They occur at the ends of the long helix that spans the domains. As discussed above, the second hinge involves small torsion angle changes spread throughout the C-terminal part of the helix (Figure 2C). As the location of the mutation is next to the hinge, the domain motion appears to be a consequence of the loss of close packing created by the mutation and is an example of hinged motion created by reducing the number of steric constraints.

(D) Lactoferrin and the Periplasmic Binding Proteins. Unlike the TBSV coat protein, lysozyme, and calmodulin, lactoferrin and the periplasmic binding proteins have two or three interdomain linkages, containing hinges. These proteins are examples of transport proteins that use domain closure to recognize and sequester small molecules.

Lactoferrin has two similar lobes, and each lobe, in turn, has two domains with an iron-binding site between them. Analyses of the open and closed forms of one of lobes give a detailed picture of the domain movements (Anderson *et al.*, 1990; Gerstein *et al.*, 1993b). Upon binding iron, the two domains move together, rotating 53° essentially as rigid bodies. The axis of rotation passes through the two β-strands linking the domains (Figure 6). As discussed above (Figure 2b), these strands contain distinct hinges, and as the rotation axes of the principal torsion angle changes are nearly parallel to the axis of the overall 53° rotation, the local motion in the hinges can be directly related to the overall domain closure.

The two domains make different packing contacts in the open and closed forms. In the open form the contacts are on one side of the hinges, and in the closed form they are on the other side. Pivoting about the hinges produces a seesaw motion between the two interfaces: when the domains close, residues in the interface on one side of the hinges become buried and close-packed, and residues on the other side become exposed. The situation is reversed on opening.

Lactoferrin shares a similar structure, topology, and binding site construction with the group II periplasmic binding proteins (Baker *et al.*, 1987). For two of these binding proteins, maltodextrin-binding protein and LAO-binding protein (Sharff *et al.*, 1992; Spurlino *et al.*, 1991; Oh *et al.*, 1993), structures have been determined for both the open and closed forms, and the mechanism of domain movement appears to be similar to that in lactoferrin. The domain motion in the maltodextrin-binding protein is a 35° rotation about an axis through the hinge region, and there are large, localized torsion angle changes in the three peptides linking the domains. The positions of two of the hinges are structurally equivalent to those of the lactoferrin hinges. In the LAO-binding protein there is a 52° rotation of the two domains, which involves only a few large torsion angle changes in a region structurally equivalent to the lactoferrin hinge.

(E) Adenylate Kinase. A more complex and extensive hinge motion is seen in the large variants of adenylate kinase. This enzyme has two nucleotide binding sites, and crystal structures have been solved with both sites, a single site, and no sites filled (Schultz *et al.*, 1974, 1990; Diederichs & Schulz, 1991; Müller & Schulz, 1992). The major conformational change,

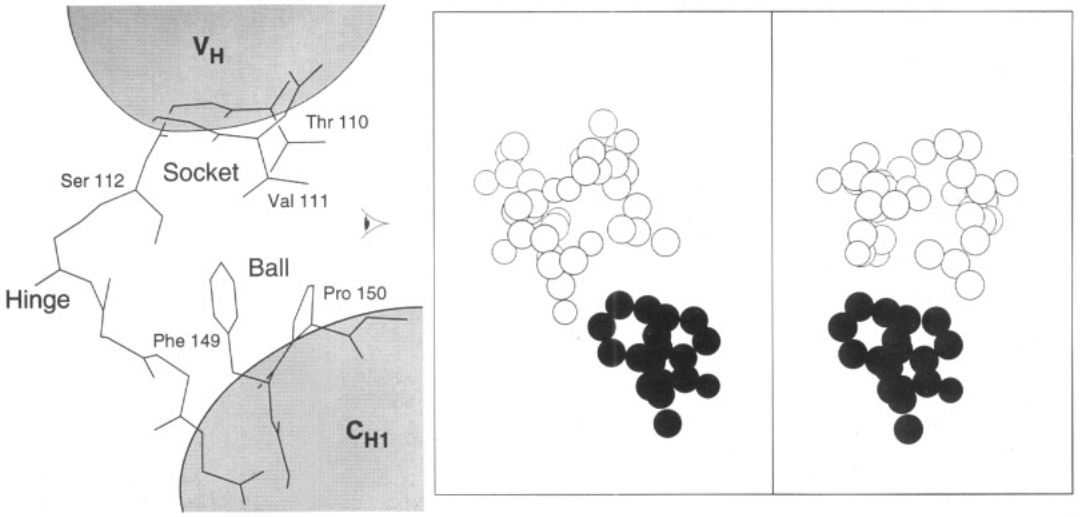

FIGURE 7: Ball-and-socket motion in the immunoglobulins. (Left) The conserved V_H–C_{H1} contacts and the switch (hinge) peptides. Three V_H residues (11, 110, and 112) form a "socket", and two C_{H1} residues, 149 and 150, form a "ball". The view is such that the motion of the V dimer relative to the C dimer is perpendicular to the page. (Middle and Right) The movement of the ball-and-socket joint. The five side chains in the joint are represented by spheres drawn at one-half van der Waals radius. White spheres indicate the socket, and black ones the ball. The orientation is roughly perpendicular to that in the left figure (see the eye symbol there). The middle figure shows the packing that occurs when the domains are fully extended (*i.e.*, 180° elbow angle), and the right figure shows the packing that occurs when the domains are close enough to be in contact (i.e., 135° elbow angle).

which occurs when the second substrate binds, rotates the smaller of the two domains ~90° relative to the larger one and shifts main-chain atoms up to 32 Å. The small and large domains are linked by two helices, and on closure, conformational changes take place in four hinges at the N and C termini of these linking helices (Gerstein *et al.*, 1993b). Two of these hinges have simple motions; a third hinge requires motion throughout an extended loop; and a fourth hinge (Figure 2D) occurs in the middle of a proline-kinked helix. The four hinges have few packing constraints on their main chain. One pair of hinges is responsible for one-third of the total rotation, and the other pair, for two-thirds.

(F) cAMP-Dependent Protein Kinase. Like ADK, the catalytic subunit of cAMP-dependent protein kinase has an elaborate multipart hinged motion, which involves at least five distinct hinges, split into the two sets. Containing two domains, one large and one small, the structure of the catalytic subunit has been solved in binary and ternary complexes with an inhibitory peptide and in an apo form (Knighton *et al.*, 1991; Karlsson *et al.*, 1993). In a comparison of the apo form with either complex form, the core of the small domain rotates ~12° relative to that of the large one. The small domain is principally connected to the large domain through three roughly parallel peptide linkages, which deform as hinges upon closure. In addition, through the deformation of two more hinges a loop in the small domain near the binding pocket rotates a further 6° down into the interdomain cleft. Partly because of the size of the interdomain cleft, which has to accommodate a 15-residue peptide, the protein kinase motion does not involve an extensive interdomain interface. There is, however, one helix in the small domain which moves in a shear fashion to maintain its contacts with the large domain throughout the motion.

THE BALL-AND-SOCKET MOTION IN THE IMMUNOGLOBULINS

The domain motion observed in the immunoglobulins involves, so far as is known at present, a unique combination of hinge and shear motions. In the immunoglobulins the V_L domain is linked by an extended peptide to the C_L domain, and V_H is similarly linked to C_{H1}. V_L and V_H pack together, as do C_L and C_{H1}. The V_L–V_H dimer can freely rotate, relative to the C_L–C_{H1} dimer, over a range of ~50° in a manner described as "elbow motion".

Elbow motion involves localized deformations in the two peptides that link the V and C dimers (Bennett & Huber, 1984). These deformations are similar to those in the hinged domain closures described in the previous sections. However, the elbow motion also involves an unusual type of shear motion: two large residues in C_{H1}, a Pro and a Phe, pack closely together, forming a "ball", and three residues in V_H spread out as part of a β-sheet, forming a "socket" (Figure 7). The three V_H and two C_{H1} residues are packed together and move relative to each other in a manner similar to a socket moving over a ball (Lesk & Chothia, 1988).

Unlike the shear motions discussed above, which are characterized by close-packed interfaces of interdigitating sidechains, the ball-and-socket joint has a "smooth" interface, in which the side chains do not interdigitate. This interface facilitates motion over a wide range of relative orientations. It also permits greater flexibility than is found in shear motions: the socket residues can move up to 4.5 Å relative to those in the ball, rather than the 1.5–2.0-Å displacement usually found at an interface undergoing shear motion.

THE STABILITY OF THE CLOSED AND OPEN STATES

The evidence currently available suggests that the open and closed states are only slightly different in energy and at room temperature are in dynamic equilibrium. This small energy difference between the open and closed states is most directly suggested by the discovery that relatively weak crystal packing forces can stabilize the unliganded closed forms of lactoferrin and the binding proteins (Baker *et al.*, 1991; Sharff *et al.*, 1992, and references therein). It is also suggested by simulations of loop closure (Wade *et al.*, 1993, 1994).

The relative stabilities of the open and closed states depend on the presence or absence of the substrate. A likely

progression is that the substrate first binds to one domain, then thermal fluctuations bring the second domain into contact with it, and the newly formed contacts stabilize the closed conformation. The ability of a ligand to bind to a single domain has, in fact, been observed in transferrin (Lindley *et al.*, 1993). Inspection of the structures of liganded closed states invariably shows that the ligand makes numerous interlocking salt bridges, hydrogen bonds, and packing interactions with both domains (references in Table 2), and these interactions account for the stability and specificity of the closed state. Catalytic transformation of the substrate destroys, at least in part, the interactions made with the protein and so makes the open form more stable. The rate of domain movements, consequently, is governed to a degree by the catalytic efficiency of the protein. This may be particularly relevant to domain movements involved in locomotion.

The main function of the open form is to allow access to the active site. By itself, this function does not require the open form to have a unique conformation, as opposed to a range of conformations. Experimental evidence for a unique open form is sketchy and mixed. On the one hand, there is clear evidence that the open form has a unique conformation in certain proteins. As discussed above [Examples of Hinged Domain Movements (C)], in lactoferrin the interdomain interface formed in the open form appears to uniquely fix its conformation. Likewise, within particular species, AAT has the same open conformation in different crystal forms, which have very different intermolecular contacts (McPhalen *et al.*, 1992). On the other hand, there is also evidence that the open form of other proteins can have a range of conformations. T4 lysozyme has been found to have a number of different "open" conformations in various crystal forms (Faber & Matthews, 1991; Dixon *et al.*, 1992). The leucine/isoleucine/valine-binding protein has been solved in a "more-opened" form (Sharff *et al.*, 1992, and references therein). A variety of different orientations have been found for the two domains of *Escherichia coli* $NADP^+$-dependent glutamate dehydrogenase; this hexameric protein has been solved in a crystal form where all six subunits are in the asymmetric unit (D. Rice, personal communication).

Note that the crystallographic evidence relating to the uniqueness of end states must be treated with care as there is a possibility that the intermolecular contacts in the crystal may fix domains in orientations not preferred in solution. Also, crystallography tends to make one think in terms of discrete, rigid conformational states, which may be an erroneous model for open and closed conformations.

CONCLUSIONS

We have shown how hinge and shear motions, which constitute the repertoire of low-energy conformation changes available to proteins, can be combined to describe most of the known instances of domain movements. We emphasize the importance of the architecture of the interdomain interface in determining the relative mix of hinge and shear motions. While our hinge and shear mechanisms do not describe domain motions precisely enough for accurate energy calculations, they provide a conceptual framework for understanding complicated structural transformations and can be used as a guide for more quantitative formulations. As more data become available, the descriptions of hinge and shear mechanisms should be refined and extended so that they can be applied to the complex large-scale motions that occur in structures such as myosin (Rayment *et al.*, 1993).

An expanded and routinely updated version of Table 2 (a listing of protein structures that undergo conformational change) will be available electronically in plain text and hypertext forms. Use (i) anonymous ftp or WWW with URL "file://cb-iris.stanford.edu/pub/ProteinMovements/ProteinMovements.html", (ii) anonymous ftp to "al.mrc-lmb.cam.ac.uk" for filename "pub/ProteinMovements/ProteinMovements.html", or (iii) electronic mail to mbg@cb-iris.stanford.edu.

REFERENCES

Anderson, B. F., Baker, H. M., Norris, G. E., Rumball, S. V., & Baker, E. N. (1990) *Nature 344*, 784.

Anderson, C. M., Zucker, F. H., & Steitz, T. (1979) *Science 204*, 375.

Babu, Y. S., Sack, J. S., Greenhough, T. J., Bugg, C. E., Means, A. R., & Cook, W. J. (1985) *Nature 315*, 37.

Baker, E. N., Rumball, S. V., & Anderson, B. F. (1987) *Trends Biochem. Sci. 12*, 350.

Bennett, W. S., & Huber, R. (1984) *Crit. Rev. Biochem. 15*, 291.

Bennett, W. S., Jr., & Steitz, T. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4848.

Bennett, W. S., Jr., & Steitz, T. A. (1980) *J. Mol. Biol. 140*, 211.

Berchtold, H., Reshetnikova, L., Reiser, C. O. A., Schirmer, N. K., Sprinzl, M., & Hilgenfeld, R. (1993) *Nature 365*, 126.

Birktoft, J. J., Rhodes, G., & Banaszak, L. J. (1989) *Biochemistry 28*, 6065.

Blake, C. C. F., Rice, D. W., & Cohen, F. E. (1986) *Int. J. Pept. Protein Res. 27*, 443.

Bystroff, C., & Kraut, J. (1991) *Biochemistry 30*, 2227.

Chasman, D. I., Flaherty, K. M., Sharp, P. A., & Kornberg, R. D. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 8174.

Chothia, C., Lesk, A. M., Dodson, G. G., & Hodgkin, D. C. (1983) *Nature 302*, 500.

Colonna-Cesari, F., Perahia, D., Karplus, M., Eklund, H., Brändén, C. I., & Tapia, O. (1986) *J. Biol. Chem. 261*, 15273.

Concha, N. O., Head, J. F., Kaetzel, M. A., Dedman, J. R., & Seaton, B. A. (1993) *Science 261*, 1321.

Derewenda, U., Brzozowski, A. M., Lawson, D. M., & Derewenda, Z. S. (1992) *Biochemistry 31*, 1532.

Diederichs, K., & Schulz, G. E. (1991) *J. Mol. Biol. 217*, 541.

Dixon, M. M., Nicholson, H., Shewchuk, L., Baase, W. A., & Matthews, B. W. (1992) *J. Mol. Biol. 227*, 917.

Eklund, H., Samaha, J. P., Wallen, L., Branden, C. I., Åkeson, Å., & Jones, T. A. (1981) *J. Mol. Biol. 146*, 561.

Elber, R., & Karplus, M. (1987) *Science 235*, 318.

Engh, R. A., Wright, H. T., & Huber, R. (1990) *Protein Eng. 3*, 469.

Faber, H. R., & Matthews, B. W. (1990) *Nature 348*, 263.

Fitzgerald, P. M. D., McKeever, B. M., VanMiddlesworth, J. F., Springer, J. P., Heimbach, J. C., Leu, C.-T., Herber, W. K., Dixon, R. A. F., & Darke, P. L. (1990) *J. Biol. Chem. 265*, 14209.

Flaherty, K. M., de Luca-Flaherty, C. R., & McKay, D. B. (1990) *Nature 346*, 623.

Flaherty, K. M., McKay, D. B., Kabsch, W., & Holmes, K. C. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 5041.

Frauenfelder, H., Sligar, S. G., & Wolynes, P. G. (1991) *Science 254*, 1598.

Gerstein, M., & Chothia, C. H. (1991) *J. Mol. Biol. 220*, 133.

Gerstein, M., Schulz, G., & Chothia, C. (1993a) *J. Mol. Biol. 229*, 494.

Gerstein, M., Lesk, A. M., Baker, E. N., Anderson, B., Norris, G., & Chothia, C. (1993b) *J. Mol. Biol. 234*, 357.

Gilliland, G. L., & Quiocho, F. A. (1981) *J. Mol. Biol. 146*, 341.

Harlos, K., Vas, M., & Blake, C. F. (1992) *Proteins: Struct., Funct., Genet. 12*, 133.

Holland, D. R., Tronrud, D. E., Pley, H. W., Flaherty, K. M., Stark, W., Jansonius, J. N., McKay, D. B., & Matthews, B. W. (1992) *Biochemistry 31*, 11310.

Ikura, M., Clore, G. M., Gronenborn, A. M., Zhu, G., Klee, C. B., & Bax, A. (1992) *Science 256*, 632.

Jacobo-Molina, A., Ding, J., Nanni, R. G., Clark, A. D. J., Lu, X., Tantillo, C., Williams, R. L., Kamer, G., Ferris, A. L., Clark, P., Hizi, A., Hughes, S. H., & Arnold, E. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 6320.

Janin, J., & Wodak, S. (1983) *Prog. Biophys. Mol. Biol. 42*, 21.

Joseph, D., Petsko, G. A., & Karplus, M. (1990) *Science 249*, 1425.

Kabsch, W., Mannherz, H. G., Suck, D., Pai, E. F., & Holmes, K. C. (1990) *Nature 347*, 37.

Karlsson, R., Zheng, J. H., Xuong, N. H., Taylor, S. S., & Sowadski, J. M. (1993) *Acta Crystallogr. D49*, 381.

Kim, J. L., Nikolov, D. B., & Burley, S. K. (1993) *Nature 365*, 520.

Kim, Y., Geiger, J. H., Hahn, S., & Sigler, P. B. (1993) *Nature 365*, 512.

Kjeldgaard, M., Nissan, P., Thirup, S., & Nyborg, J. (1993) *Structure 1*, 35.

Knighton, D. R., Zheng, J., Ten Eyck, L. F., Ashford, V. A., Xuong, N., Taylor, S. S., & Sowadski, J. M. (1991) *Science 253*, 407.

Knowles, J. R. (1991) *Philos. Trans. R. Soc. London B332*, 115.

Kohlstaedt, L. A., Wang, J., Friedman, J. M., Rice, P. A., & Steitz, T. A. (1992) *Science 256*, 1783.

Koshland, D. E., Jr., (1958) *Proc. Natl. Acad. Sci. U.S.A. 44*, 98.

Lawson, C. L., Zhang, R., Schevitz, R. W., Otwinowski, Z., Joachimiak, A., & Sigler, P. B. (1988) *Proteins 3*, 18.

Lebioda, L., & Stec, B. (1991) *Biochemistry 30*, 2817.

Lesk, A. M., & Chothia, C. (1984) *J. Mol. Biol. 174*, 175.

Lesk, A. M., & Chothia, C. (1988) *Nature 335*, 188.

Lindley, P. F., Bajaj, M., Evams, R. W., Garratt, R. C., Hasnain, S., Jhoti, H., Kuser, P., Neu, M., Patel, K., Sarra, R., Strange, R., & Walton, A. (1993) *Acta Crystallogr. D49*, 292.

Loebermann, H., Tokuoka, R., Deisenhofer, J., & Huber, R. (1984) *J. Mol. Biol. 177*, 531.

Lolis, E., & Petsko, G. A. (1990) *Biochemistry 29*, 6619.

Louie, G. V., Brownlie, P. D., Lambert, R., Cooper, J. B., Blundell, T. L., Wood, S. P., Warren, M. J., Woodcock, S. C., & Jordan, P. M. (1992) *Nature 359*, 33.

Luecke, H., & Quiocho, F. A. (1990) *Nature 347*, 402.

Mas, M. T., & Resplandor, Z. E. (1988) *Proteins 4*, 56.

Mas, M. T., Resplandor, Z. E., & Riggs, A. D. (1987) *Biochemistry 26*, 5369.

McPhalen, C. A., Vincent, M. G., Picot, D., Jansonius, J. N., Lesk, A. M., & Chothia, C. (1992) *J. Mol. Biol. 227*, 197.

Meador, W. E., Means, A. R., & Quiocho, F. A. (1992) *Science 257*, 1251.

Meador, W. E., Means, A. R., & Quiocho, F. A. (1993) *Science 262*, 1718.

Milburn, M. V., Tong, L., DeVos, A., Brünger, A., Yamaizumi, Z., Nishimura, S., & Kim, S.-H. (1990) *Science 247*, 939.

Miller, M., Schneider, J., Sathyanarayana, B. K., Toth, M. V., Marshall, G. R., Clawson, L., Selk, L., Kent, S. B. H., & Wlodawer, A. (1989) *Science 246*, 1149.

Montfort, W. R., Perry, K. M., Fauman, E. B., Finermoore, J. S., Maley, G. F., Hardy, L., Maley, F., & Stroud, R. M. (1990) *Biochemistry 29*, 6964.

Mottonen, J., Strand, A., Symersky, J., Sweet, R. M., Danley, D. E., Goeghegan, K. F., Gerard, R. D., & Goldsmith, E. J. (1992) *Nature 355*, 270.

Müller, C. W., & Schulz, G. E. (1988) *J. Mol. Biol. 202*, 909.

Müller, C. W., & Schulz, G. E. (1992) *J. Mol. Biol. 224*, 159.

Noel, J. P., Hamm, H. E., & Sigler, P. B. (1993) *Nature 366*, 654.

Oh, B.-H., Pandit, J., Kang, C.-H., Nikaido, K., Gokcen, S., Ames, G. F.-L., & Kim, S.-H. (1993) *J. Biol. Chem. 268*, 11348.

Olson, A. J., Bricogne, G., & Harrison, S. C. (1983) *J. Mol. Biol. 171*, 61.

Parry, N., Fox, G., Rowlands, D., Brown, F., Fry, E., Acharya, R., Logan, D., & Stuart, D. (1990) *Nature 347*, 569.

Perry, K. M., Fauman, E. B., Finer-Moore, J. S., Montfort, W. R., Maley, G. F., Maley, F., & Stroud, R. M. (1990) *Proteins 8*, 315.

Perutz, M. (1989) *Q. Rev. Biophys. 22*, 139.

Pflugrath, J. W., & Quiocho, F. A. (1988) *J. Mol. Biol. 200*, 163.

Rayment, I., Rypiewski, W. R., Schmidt-Bäse, K., Smith, R., Tomchick, D. R., Benning, M. M., Winkelmann, D. A., Wesenberg, G., & Holden, H. M. (1993) *Science 261*, 50.

Remington, S., Wiegand, G., & Huber, R. (1982) *J. Mol. Biol. 158*, 111.

Rojewska, D., & Elber, R. (1990) *Proteins 7*, 265.

Sack, J. S., Saper, M. A., & Quiocho, F. A. (1989a) *J. Mol. Biol. 206*, 171.

Sack, J. S., Trakhanov, S. D., Tsigannik, I., & Quiocho, F. A. (1989b) *J. Mol. Biol. 206*, 193.

Sali, A., Veerapandian, B., Cooper, J. B., Foundling, S. I., Hoover, D. J., & Blundell, T. L. (1989) *EMBO J. 8*, 2179.

Sali, A., Veerapandian, B., Cooper, J. B., Moss, D. S., Hofmann, T., & Blundell, T. L. (1992) *Proteins: Struct., Funct., Genet. 12*, 158.

Sarra, R., Garratt, R., Gorinsky, B., Jhoti, H., & Lindlay, P. (1990) *Acta Crystallogr. B46*, 763.

Schlichting, I., Almo, S. C., Rapp, G., Wilson, K., Petratos, K., Lentfer, A., Wittinghofer, A., Kabsch, W., Pai, E. F., Petsko, G. A., & Goody, R. S. (1990) *Nature 345*, 309.

Schulz, G. E., Elzinga, M., Marx, F., & Schirmer, R. H. (1974) *Nature 250*, 120.

Schulz, G. E., Muller, C. W., & Diederichs, K. (1990) *J. Mol. Biol. 213*, 627.

Sharff, A. J., Rodseth, L., Spurlino, J. C., & Quiocho, F. A. (1992) *Biochemistry 31*, 10657.

Skarzynski, T., & Wonacott, A. J. (1988) *J. Mol. Biol. 203*, 1097.

Sopkova, J., Renouard, M., & Lewit-Bentley, A. (1993) *J. Mol. Biol. 234*, 816.

Spurlino, J. C., Lu, G. Y., & Quiocho, F. A. (1991) *J. Biol. Chem. 266*, 5202.

Stehle, T., & Schulz, G. E. (1990) *J. Mol. Biol. 211*, 249.

Stein, P., & Chothia, C. (1991) *J. Mol. Biol. 221*, 615.

Stillman, T. J., Baker, B. J., Britton, K. L., & Rice, D. W. (1993) *J. Mol. Biol. 234*, 1131.

Stoddard, B. L., & Koshland, D. E., Jr. (1993) *Biochemistry 32*, 9317.

Taylor, D. A., Sack, J. S., Maune, J. F., Beckingham, K., & Quiocho, F. A. (1991) *J. Biol. Chem. 266*, 21375.

Thayer, M. M., Flaherty, K. M., & McKay, D. B. (1991) *J. Biol. Chem. 266*, 2864.

Vyas, N. K., Vyas, M. N., & Quiocho, F. A. (1988) *Science 242*, 1290.

Vyas, N. K., Vyas, M. N., & Quiocho, F. A. (1991) *J. Biol. Chem. 266*, 5226.

Wade, R. C., Davis, M. E., Luty, B. A., Madura, J. D., & McCammon, J. A. (1993) *Biophys. J. 64*, 9.

Wade, R. C., Brock, A. L., Demchuk, E., Madura, J. D., Davis, M. E., Briggs, J. M., & McCammon, J. A. (1994) *Nature Struct. Biol. 1*, 65.

Weber, I. T., & Steitz, T. A. (1987) *J. Mol. Biol. 198*, 311.

White, J., Hackert, M. L., Buehner, M., Adams, M. J., Ford, G. C., Lentz, P. J. J., Smiley, I. E., Steindel, S. J., & Rossman, M. G. (1976) *J. Mol. Biol 102*, 759.

Wierenga, R. K., Noble, M. E. M., Postma, J. P. M., Groendijk, H., Kalk, K. H., Hol, W. G. J., & Opperdoes, F. R. (1991) *Proteins 10*, 93.

Wodak, S. J., & Janin, J. (1981) *Biochemistry 20*, 6544.

Zhang, R. G., Joachimiak, A., Lawson, C. L., Schevitz, R. W., Otwinowski, Z., & Sigler, P. B. (1987) *Nature 327*, 591.