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STRUCTURAL PROPERTIES OF PROTEIN β-SHEETS

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CONTENTS

I. INTRODUCTION

X-ray crystallographic studies of proteins have revealed a rich variety of structural architectures. Despite this diversity, many proteins share common features of structural organization. The extent to which proteins can be described as being structurally similar depends upon their incorporation of regular secondary structures. The observation that many proteins are predominantly composed of α-helices, antiparallel β-sheet, or a combination of α-helices and parallel β-sheet, has provided a basis for the structural classification of proteins (Levitt and Chothia, 1976; Richardson, 1981). Such classification schemes have been an important first step in understanding protein structural organization. More importantly, these studies make evident that many structural motifs recur among proteins otherwise bearing little similarity in amino acid sequence or function. The recurrence of similar structural motifs among evolutionarily unrelated proteins presumably reflects underlying physical factors which depend only in a general way on protein sequence, but nevertheless are effective determinants of protein structural organization.

The present work examines the structural properties of β-sheets in proteins. Crystallographically observed β-sheets in globular proteins exhibit an extraordinary diversity of structural forms. In contrast to the classical flat β-sheet arrangements first described by Pauling and Corey (1951), globular protein β-sheets conform to a variety of twisted and curved surfaces. A basic objective of this review is to describe the operative forces and constraints which produce different twisted β-sheet geometries. The factors involved are most readily understood by considering the properties of a classical flat structure (Fig. 1). A β-sheet is basically an aligned, planar array of conformationally regular polypeptide chains which are interconnected by hydrogen bonds. The flat sheet can be viewed as a regular, two-dimensional lattice stabilized by covalent bonds along the direction of the polypeptide chains, and by hydrogen-bonds (i.e. dipole interactions) between or across the chains. The minimum energy configuration of the lattice will generally reflect the simultaneous

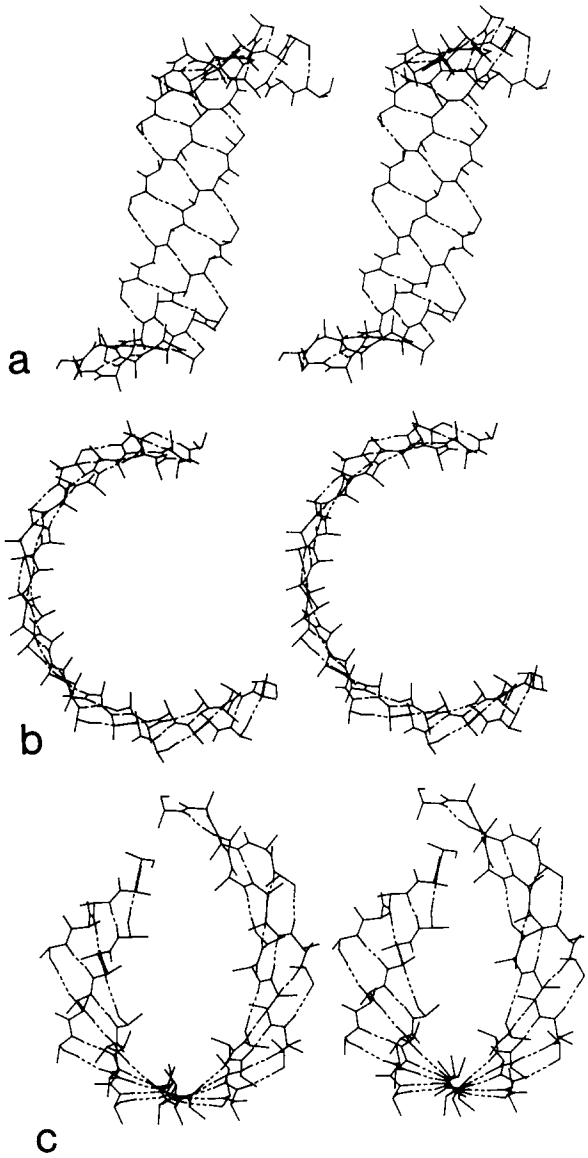

FIG. 12. Stereoviews showing the results of optimizing interchain hydrogen-bonds between straight helical chains arranged in a staggered plan. Owing to the combination of interchain twist and rise, a cylindrical surface results.

conformations, all lying on the $n=2$ line of the ϕ, ψ plot. These alternative conformations reflect continuous variation of the chain dipeptide repeat period and an associated isoenergetic bending of the hydrogen-bonds at the carbonyl oxygens (Hagler *et al.*, 1974). As described below, this ability of the antiparallel structure to alter its extension while still preserving its hydrogen bonds is a principal factor leading to geometrical diversity in twisted antiparallel sheets.

In contrast to parallel β-sheets which typically form an internal structural backbone in proteins, antiparallel sheets are generally found to form exterior surfaces of domains or globular proteins. When antiparallel sheets are situated internally, it is usually the consequence of the association of antiparallel β-domains within a globular protein, or a result of subunit packing within an oligomeric protein structure (Salemme and Weatherford, 1981b). In situations where rectangular-plan antiparallel sheets do occur at internal interfaces in proteins (Fig. 18), they may approximate saddle-shaped surfaces composed of straight helical chains (Fig. 19). Alternatively, antiparallel strands may be incorporated into

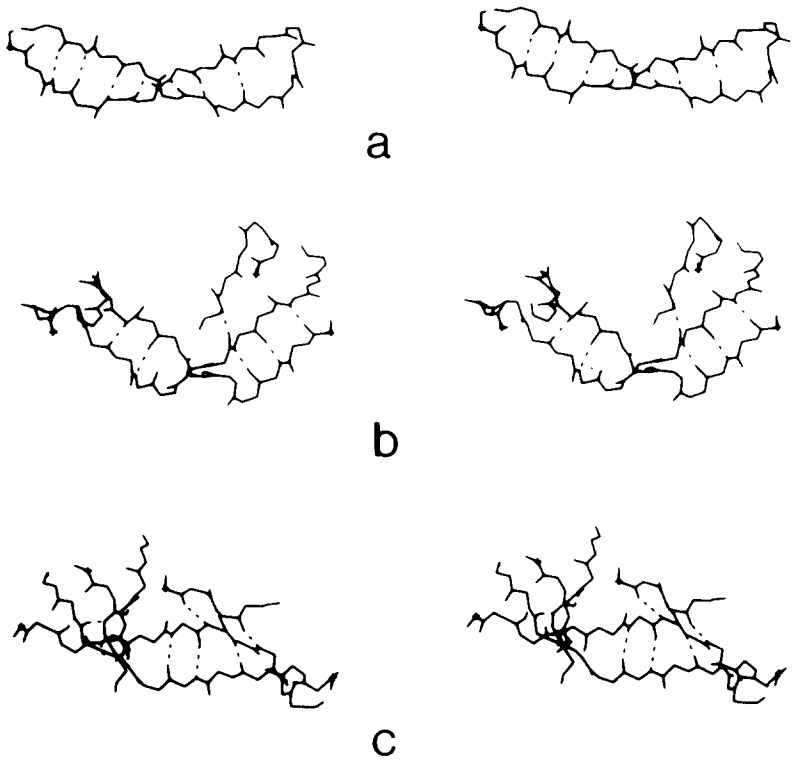

FIG. 22. Examples of double-strand antiparallel coils in proteins. (a) Pancreatic trypsin inhibitor, (b) lactate dehydrogenase, (c) thermolysin (Feldmann, 1976).

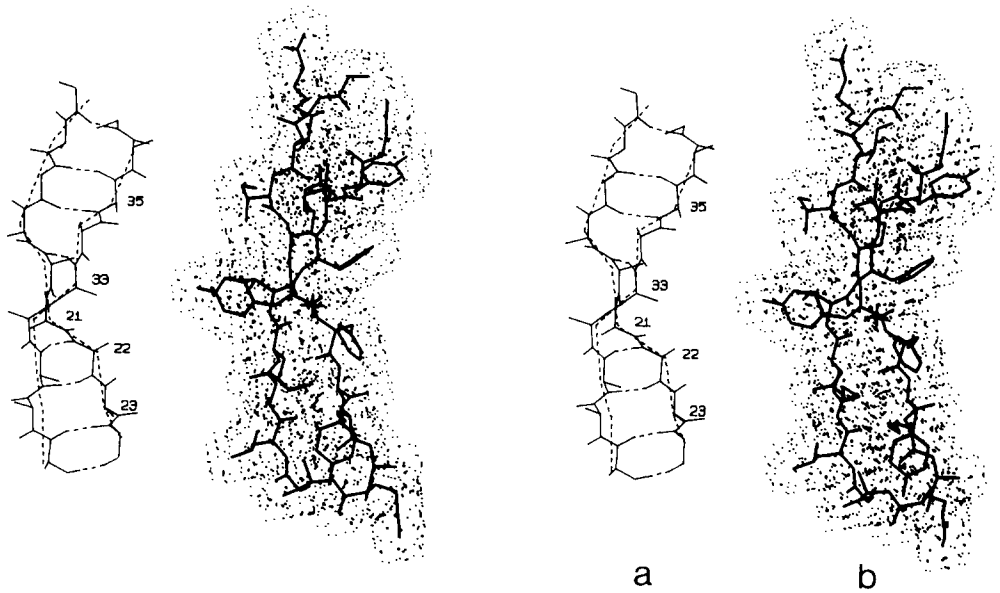

FIG. 23. (a) A least squares superposition of a regular antiparallel coiled-coil with $\phi_s = -114°$, $\psi_s = 150°$, $\phi_1 = -92°$, $\psi_1 = 114°$, $\delta = 163°$ (unbroken lines) with the α-carbon positions of the double-strand β-sheet (broken lines) in pancreatic trypsin inhibitor. The average superposition error in model vs observed C_α coordinates is 0.52 Å. (b) A pseudo-space-filling representation of the trypsin inhibitor β-coil illustrating the intimate side-chain packing attained in this strongly twisted conformation.

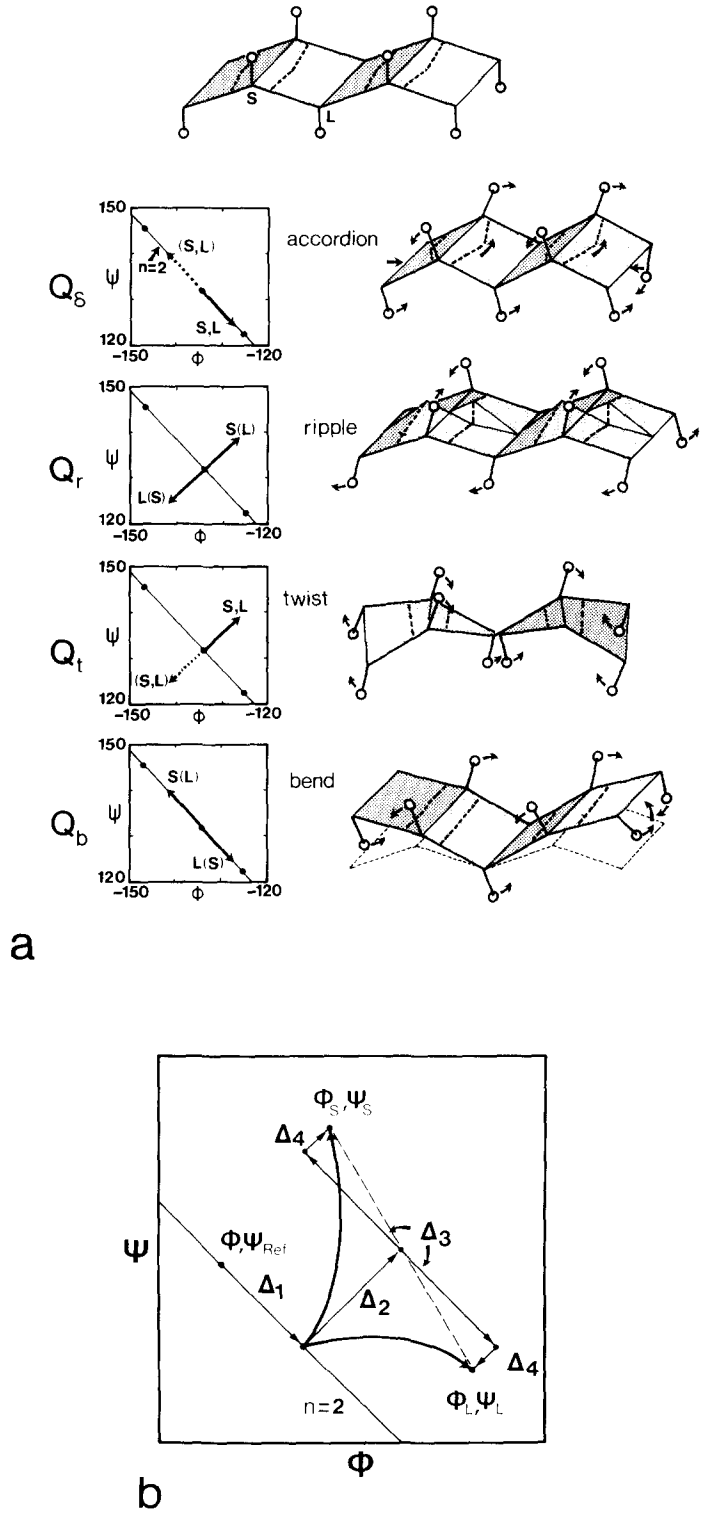

FIG. 28. Part (a) illustrates how cooperative ϕ, ψ displacements in a flat antiparallel sheet deform the structure as a whole. In each case, the ϕ's and ψ's situated in large (L) and small (S) hydrogen-bonded rings move along trajectories either coincident with or perpendicular to the $n=2$ line of the ϕ, ψ plot. For example, the accordion motion involves a correlated displacement of both large and small ring ϕ, ψ values along the $n=2$ line, while bend involves the conformations of S and L residues moving in opposite directions on the $n=2$ line, relative to an initial flat conformation. Twist and ripple are similarly related. Part (b) illustrates how the ϕ, ψ trajectories followed by the residues of a coiling β-sheet can be resolved as a superposition of the cooperative deformations shown in part (a).

motions that cooperatively return the structure to its potential minimum. Double-strand antiparallel β-sheets, and other antiparallel sheets to the extent that their local conformational minimum are governed by "soft" potential functions, consequently appear stable owing to their flexibility. They are structures that may accommodate transient loads by deforming elastically.

2. *β-Sheet Folding*

Essential to the rapidity of protein folding is the transient formation of a succession of intermediates, each of lower free energy and more completely resembling the final folded state. Aspects of both the temporal organization and cooperativity associated with folding are suggested by the foregoing description of β-sheet architecture. For example, it is presumably some statistical preference, related to the tendency for extended polypeptide chains to locally form left-helical conformations, which favors the right-handed coiling of polypeptide chains during initial stages of protein folding (but, see below). This could favor the assembly of β-α-β domains with "right-handed crossover" connections, as is almost invariably observed in known protein structures (Richardson, 1976, 1977, 1981; Sternberg and Thornton, 1976, 1977) (Fig. 36). Once an initial chain contact is made, one might readily

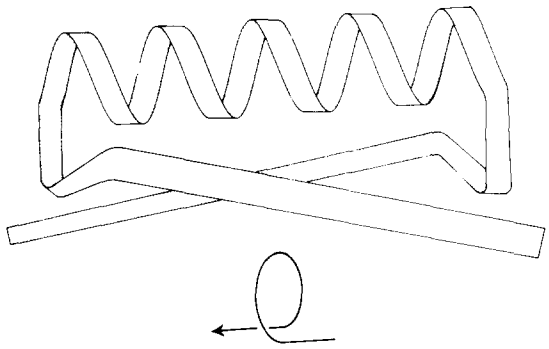

FIG. 36. Schematic of a local β α β domain typically seen in extended parallel sheets (Fig. 5). β α β domains have sheet strands interconnected by α-helices arranged with an overall right-handed supercoil sense.

imagine the successive formation of hydrogen-bonds to propogate a double strand sheet, and further chain coiling to extend the number of sheet chains. Whatever the nature of the structures' excursions during the process, it is clear that as more sheet hydrogen-bonds are formed, the sheets final conformation becomes progressively more well defined. Consequently, the constraints on chain conformation accompanying successive steps in interchain hydrogen bond formation determine how the structure can deform as a whole. The extended interactions in the sheet can therefore define both a cooperative folding pathway for the structure and the geometry of its final state (Salemme, 1982). The determinative step in β-α-β structural organization then becomes the initial formation of the interstrand hydrogen-bonds. According to whether the strands initially associate in aligned or staggered plan, the sheet will cooperatively fold to form either a saddle-surface or a barrel (Fig. 5).

Antiparallel sheets present a more complicated picture owing both to diversity in their hydrogen-bonding arrangements and final geometries relative to parallel sheets. However, many antiparallel sheets are folded in ways that suggest the involvement of hairpin-like double-strand structures as folding intermediates (Richardson, 1981). It is of consequent interest to consider how the properties of this sheet may relate both the stabilization of folding intermediates and the chiral folding of the final structure. Consider first the process of folding a double strand sheet into a double-helical coiled conformation. Figures 21 and 29 illustrate that there potentially exist a multitude of highly cooperative and similarly low-energy pathways for folding the structure from a variety of flat sheet conformations. While this would obviously provide a facile path for domain folding, it is useful to ask why this

situation might occur, rather than some arbitrarily large number of alternatives. Basically this is a result of the coupled nature of the hydrogen-bonded sheet. As illustrated by analogy in Fig. 37, a random polypeptide chain can be viewed as an ensemble of oscillators which behave essentially independently. However, formation of an extended β-sheet couples these oscillators so that they are no longer independent, and there emerges a new set of vibrational modes characteristic of the structure as a whole. The modes of lowest energy (and frequency) are those which correspond to collective motions in the structure (i.e. have high effective mass) that encounter weak restoring forces. Peticolas (1979) has pointed out that such soft collective modes in proteins are likely to be highly vibrationally excited at ambient temperature. Thus, a sheet undergoing cooperative oscillations achieves some transient free energy stabilization owing to the structure's librational entropy. The net effect is to drive the cooperative modes of the structure to large amplitude excursions, which indeed, correspond to highly cooperative folding trajectories for the double-helical domain as a whole. As outlined above in the context of structural stability, these entropic stabilization effects may persist even after the structure is folded, and so contribute to the stabilization of the folded domain structure (Sturtevant, 1977).

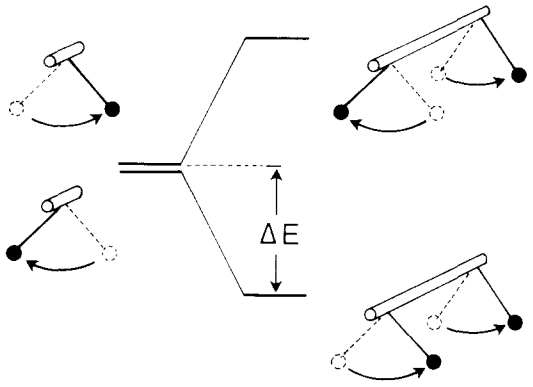

FIG. 37. A simple example of the effects of coupling oscillators. When initially independent oscillators are coupled together, there emerges a new set of vibrational modes characteristic of the structure as a whole. As a result, kinetic energy is partitioned differently in the coupled structure.

Many multiple-strand antiparallel sheets observed in proteins are organized in ways which suggest their folding from double-strand intermediates (Richardson, 1982). Perhaps the simplest examples are the "jelly-roll" β-sheets. This is an arrangement where a double strand hairpin sheet is coiled around a cylindrical surface to produce an extended hydrogen-bonded sheet (Fig. 38). The sense of the coiling is always observed to be "right-handed", i.e. the hairpin follows a right-handed helical path on the cylinder surface. The invariance of this chiral property in these structures (as well as the "right-handed" $\beta\ \alpha\ \beta$ crossover connections described above (Fig. 36)) is of some interest because there exists no *a priori* relationship between the sense of the local polypeptide chain helix and its supercoiling sense (Nishikawa and Scheraga, 1976). However, a more careful analysis of double-strand coiling suggests how this situation might arise. Consider first the cooperative coiling of a simple double-helical structure from a flat sheet. The salient point is that the two surfaces of a *double-stranded antiparallel* sheet are different, one surface being populated by residues situated in "small" hydrogen-bonded rings, and the other by residues in "large" rings (Fig. 2). In principle, two alternative double helical arrangements could be imagined, both of which are "right-handed", but which differ according to the situation of the small and large ring substituents relative to the structures coil axis (Fig. 39). The fact that only one of these alternatives is actually observed, with the small ring residues on the coil interior, is a consequence of the structural properties of the double-strand sheet. Specifically, coiling the sheet involves the cooperative superposition of both components of sheet twist and bend (Fig. 28). Both of these deformation components are asymmetric in the production of a

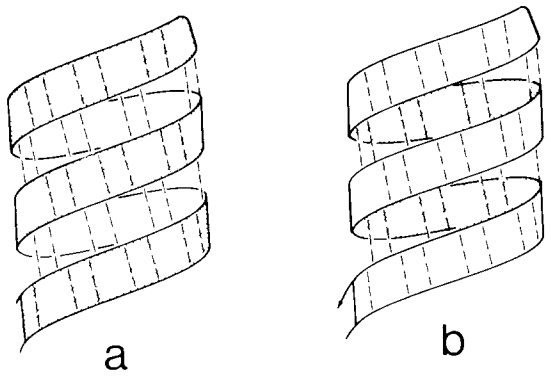

FIG. 38. Right-supercoiled β-sheets form "jelly-roll" structures which suggest their folding from double-strand hairpin intermediates. However, a double-strand sheet has surfaces which differ (Fig. 2), so that two alternative coiled arrangements ((a) and (b)) can potentially be envisioned. Studies of the potential energy surfaces governing cooperative coiling of antiparallel sheets suggests facile folding pathways only for arrangement (b). This folds from a double-strand intermediate with "small-ring" residues situated on sheets concave surface (Fig. 39).

double helical coil which preserves the interchain hydrogen-bonds; the chains locally twists in a left-handed sense, and the sheet bends as a whole, with the small ring residues on the concave surface (Fig. 39) (in fact, "left-handed" coils exhibit the same bend asymmetry). The chiral coiling of the structure therefore fundamentally reflects a coupling between asymmetric sheet twist and an asymmetric bend that results from geometrical constraints imposed by the interchain hydrogen-bonds.

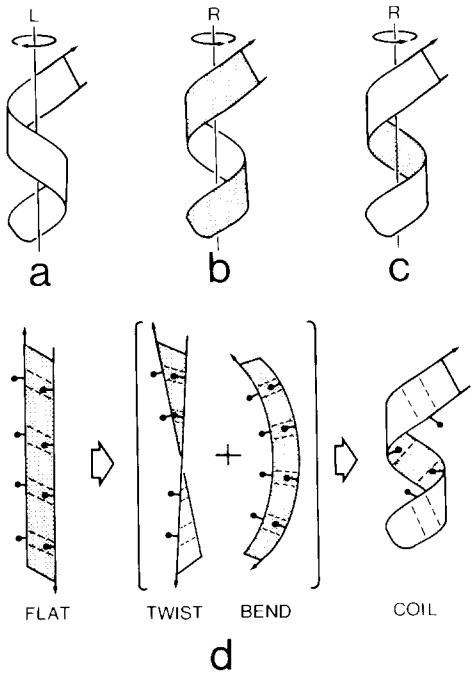

FIG. 39. Chiral coiling in antiparallel hairpin sheets. Part (a) illustrates a left-hand coiled sheet (unobserved in protein structures), while (b) and (c) illustrate alternative right-coiled structures where the small ring substituent surface (Fig. 2) (shaded) is oriented alternatively toward or away from the supercoil axis. In isolated β-coils (as in pancreatic trypsin inhibitor, Fig. 23) the small ring surface is always on the structure interior (c). Part (d) illustrates that this is a consequence of a coupling between sheet twist and bend, both of which are asymmetric (Fig. 26). These effects act in concert to produce the observed chiral supercoil geometry, both in this and more extended structures (Fig. 38). These results suggest that the observed chiral supercoiled arrangements must result from cooperative folding of a double-strand intermediate, since the asymmetry in bend required to produce the structures is a unique property of the double-strand sheet. The local chain twist, in contrast, is a property of all extended polypeptides composed of L-amino acids.

The emergence of chiral folding patterns in multiple-strand antiparallel β-sheets (e.g. Fig. 38) therefore appears as a consequence of the coupling between sheet twist and bend that is necessarily associated with the transient formation of double-strand intermediates in folding. This model predicts that observed "jelly-roll" structures should conform to the sheet orientation pattern shown in Fig. 33(b), where the structure is folded by supercoiling a double-strand intermediate which bends with its "small-ring" surface oriented towards the structure's interior (Fig. 39). Otherwise, in the final folded state where all the hydrogen bonds are formed, the structures diagrammed in Fig. 33(a) and 33(b) would be expected to be energetically equivalent.

3. *Functional Implications*

A recurrent theme in this discussion has been the distributed nature of forces determining β-sheet structural geometry, folding pathways, and response to fluctuations in free energy. However, the spatial and temporal cooperativity implicit in these structures suggests correlations with protein function that go beyond the structural and physicochemical questions addressed previously.

Allostery represents one aspect of protein function where ligand–protein interactions at sites remote from the catalytic site affect protein function. At least two different sorts of effects might be envisioned which reflect the cooperative properties of β-sheets. Effector ligand binding to the protein could alter the way in which the sheet strands were coupled together, so causing the sheet to cooperatively deform to a new minimum energy configuration. This could result in the propagation of a mechanical displacement through the sheet, that could in turn alter the spatial arrangements of catalytic residues in the protein's active site. Owing to the cooperative nature of β-sheet twisting, a very small alteration in chain conformation at one edge of the sheet could be amplified into a relatively large spatial displacement at the other. Conformational energy studies suggest that such alterations in sheet twist are energetically reasonable, because while the energies of homopolymer sheets vary slowly with the extent of twist, changes in residue type (or anything else that alters interchain interactions) can result in significantly different twisted conformations (Chou and Scheraga, 1982).

Sturtevant (1977) has suggested that one source of free energy differences on ligand binding results from alterations in the protein's librational entropy. A second ligand binding effect might therefore involve alteration of the potential function governing thermally excited cooperative oscillations in the β-sheets (Salemme, 1977; Watt and Sturtevant, 1969). Such effects would appear most relevant to structures composed of antiparallel sheets, as they are relatively more flexible than the usually stressed parallel sheets. Few allosteric proteins incorporating β-sheets are currently known in sufficient structural detail to verify these proposals. However, it is possible that the hinge bending motions occurring in hexokinase (MacDonald *et al.*, 1979) and arabinose binding protein (Mao *et al.*, 1982) could reflect cooperative alterations in the twist of the sheets interconnecting structural domains in these molecules. On the other hand, inelastic neutron scattering studies on hexokinase, in both liganded and unliganded forms, suggest alterations in the molecule's vibrational properties on glucose binding (Jacrot *et al.*, 1982). Thus it may be that both static and dynamical alterations in structural architecture result as a consequence of ligand binding. In the current context, "static" variations in β-sheet twist might be expected to simultaneously modify sheet vibrational properties, since altering the sheet minimum energy geometry is similarly likely to alter the potential surface governing displacements in the vicinity of the minimum.

Rate-limiting steps in enzyme catalysis typically involve processes where activation energy barriers must be overcome in order to convert a substrate into an intermediate or product. The reaction rate is thus a measure of the frequency with which *particular* fluctuations occur of sufficient free energy to surmount the activation energy barrier. A major motivation for investigating protein dynamics (McCammon *et al.*, 1977) stems from the possibility that structural fluctuations *characteristic of a particular enzyme* assist in the catalytic process (Careri *et al.*, 1979). More specifically, it is possible that thermally-driven cooperative