12

Conformational Changes

INTRODUCTION

In Chapters 9 to 11 we examined in great detail the conformation of a protein—from its secondary to its tertiary to its quaternary structure. The forces involved in maintaining the structure and its dynamic nature have been emphasized. In the remainder of this book the emphasis is concerned more with functional properties of proteins and the experimental approaches used to examine them. Throughout Chaps. 12 to 21, the concept that a protein can change its conformation in response to ligand binding or to its environment is implicitly assumed. Proteins may change conformation as ambient conditions such as temperature and pH change, thus affecting the forces that hold the protein in a particular conformation and allowing other conformations to become predominant. When ligands (either small molecule or macromolecule) interact with a protein at a specific binding site, part of the free-energy change of binding involves a free energy for the conformational adjustment of the protein. Such conformational changes include induced-fit conformational changes (which may be necessary to allow a second ligand molecule to bind to a protein or to induce an active conformation of the protein) and conformational changes associated with the regulation of the protein's activity.

As we discuss in Chap. 21, allosteric regulation is an important property of some oligomeric proteins, and a number of models have been proposed to explain allosteric properties. The term "allosteric" indicates that a response in one part of a protein conformation is elicited by a ligand binding at a distant site. In monomeric proteins, or in oligomeric proteins that do not exhibit subunit–subunit communication, such changes involve the secondary and tertiary structures. Where the protein consists of

several domains within the tertiary structure, conformational changes may occur within a single domain or may involve interaction between domains. In the context of regulation of oligomeric protein activity, two types of "allosteric" phenomena can be distinguished. Conformational changes may be induced within a subunit in much the same way as can be considered for a monomeric protein; such changes are involved in heterotropic regulation. Many oligomeric proteins display homotropic effects, and this usually implies that ligand binding to one subunit in an oligomer induces a conformational change in a separate subunit. Such conformational changes may involve the secondary, tertiary, and quaternary structures of a protein.

In this context a very important point concerning conformational changes must be raised. As we shall discuss in Chap. 21, the principal burden of "proof" for one allosteric model over another lies in detecting the conformational changes associated with a particular model. As is obvious from the nature of allosteric interactions in oligomeric proteins, this involves detecting conformational changes occurring in subunits other than the one to which the initial ligand is bound. Unfortunately, this is made more difficult by the fact that in an oligomeric protein, anything that affects the conformation of one subunit might be expected to affect the conformation of adjacent ones. Just as when a ligand binds to a monomeric protein there is a free energy of conformational adjustment, so too when a subunit "binds" to another subunit in an oligomer there is an effect from the interaction on the conformation of both subunits. Anything affecting the conformation of one subunit may then affect the subunit–subunit interface and hence the conformation of the adjacent subunit. As a result, we must distinguish between induced conformational changes that might be expected to occur as a natural consequence of ligand binding to one subunit in an oligomer, and those involved in allosteric interactions. In allosteric interactions, the induced conformational change must affect not just an adjacent subunit conformation but specifically the conformation of the appropriate binding site on the adjacent subunit. Rather than simply detecting a conformational change in a specific subunit, we must be able to detect conformational changes at specific points within the subunit.

Since many of the techniques for following conformational changes in proteins involve the methods discussed in Chap. 9 to 11 for examining their structure, in this chapter we discuss a number of examples of the application of these techniques to the problem of following conformational changes. The main emphasis is the type of conformational change that it is possible to follow by the various experimental approaches. For convenience we examine the various methods in three categories: (1) spectroscopic approaches, (2) protein chemistry approaches, and (3) overall shape approaches.

METHODS OF STUDYING CONFORMATIONAL CHANGES

Spectroscopic Approaches

Spectroscopic techniques for following conformational changes in proteins can be grouped as giving information either about overall conformation or specific sites on a protein. Into the first group fall approaches that reflect the overall conforma-

tion rather than specific regions of the protein, and involve protein fluorescence, polypeptide backbone circular dichroism (CD), and absorbance changes either in the form of difference spectra or the use of solvent perturbation. Into the second group fall the spectral properties of ligands with specific binding sites, approaches with covalently attached spectral probes, or those that give information on the spectral properties of specific, individual side chains within a protein. Both groups depend on the fact that spectral properties in some way reflect the environment of the spectrally active moiety, and thus changes in that environment affect the characteristic parameters of the signal being monitored. In this section we look at a number of examples that have been used to give information about conformational changes using spectroscopic studies.

Protein Fluorescence. The fluorescence properties of proteins arise from the aromatic residues tryptophan, tyrosine, and phenylalanine. Because of the degeneracy of the protein fluorescence signal it is usually not possible, on the basis of steady-state fluorescence measurements, to determine which particular tryptophan or other residue in a protein is involved in changes in the fluorescence properties, and hence measurements of protein fluorescence changes usually follow conformational changes on a gross scale. It is sometimes possible to resolve steady-state fluorescence measurements by using three-dimensional spectra to give more defined information on the spectral properties of contributing aromatic residues in the protein. In some proteins there is a single tyrosine or tryptophan residue and the fluorescence properties reflect those specific residues.

A particularly sensitive way of using protein fluorescence to detect conformational differences is illustrated by the experiment shown in Fig. 12-1. Here the excitation wavelength dependence of the quenching of protein fluorescence for glyceraldehyde-3-phosphate dehydrogenase was followed as a function of NAD bound per tetramer. If the four subunits of the enzyme behave as identical subunits with no interactions between them, ligand binding to each would be expected to produce the same fluorescence quenching characteristics. As is clear in Fig. 12-1, this is

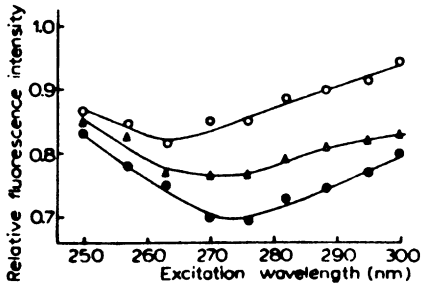

Figure 12-1 Fluorescence quenching spectrum obtained when NAD binds to glyceraldehyde-3-phosphate dehydrogenase. Data show the wavelength dependence of quenching with one molecule of NAD bound per tetramer (○–○), with two molecules bound (△–△), and with three molecules bound (●–●). [Reprinted with permission from: J. E. Bell and K. Dalziel, *Biochim. Biophys. Acta, 410*, 243–251 (1975).]

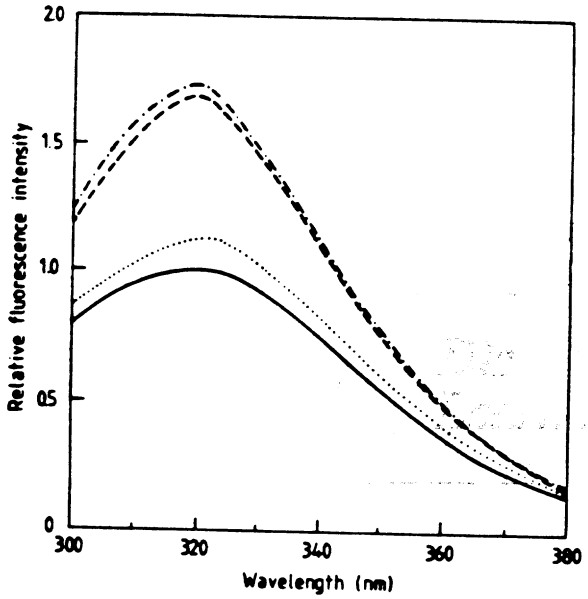

Figure 12-2 Protein fluorescence emission spectra of α_2-macroglobulin (α_2M) alone (——), activated with methylamine (· · ·), interacting with trypsin (– – –), or activated by methylamine and interacting with trypsin (–·–). (Reprinted with permission from: I. Bjork, T. Lindblom, and P. Lindahl, *Biochemistry*, *24*, 2653–2660. Copyright 1985 American Chemical Society, Washington, D. C.)

not the case, indicating either that the premise that the subunits are initially identical is not correct, or that a conformational change is occurring in the protein as the saturation is increased, resulting in different quenching spectra for the different NAD ligand molecules bound.

Protein fluorescence emission spectra have also been used to yield information on conformational differences in proteins. Figure 12-2 illustrates the protein emission spectra of the protease inhibitor bovine α_2 macroglobulin in several different states, showing conformational differences between the protein as it is activated or interacts with a target protease.

Noncovalent Ligand Fluorescence. Two types of noncovalent ligands can be envisaged. In the first, a fluorescent molecule such as TNS [6,-(4-toluidino)-2-naphthalenesulfonic acid] interacts with the protein at some site or sites unknown, causing some change in the fluorescence properties of the ligand. When a second ligand interacts with the protein, various situations can occur: (1) the bound TNS fluorescence changes as the result of the environment of the bound TNS changing, (2) the overall TNS fluorescence (bound plus free TNS) changes as a result of a change in the distribution of bound and free TNS, or (3) there is no change in the overall TNS fluorescence. In the first case it is clear that a conformational change is occurring which affects the TNS fluorescence. In the second case, if there is an increase in the

amount of bound TNS it is clear that a conformational change in the protein must have occurred, resulting in either an increased number of sites or an increased affinity of the existing sites. If, however, the fluorescence change indicates that there is a decreased number of sites or lower affinity, it is possible that direct ligand competition is responsible, in which case conclusions about induced conformational changes are hard to draw. In both cases it is necessary to determine either the fluorescence properties of the bound ligand or the affinity of the protein for the ligand. These matters are discussed in detail in Chap. 17 and it is sufficient for the present discussion to say that the properties of the bound fluorophore can be established by titrating a fixed concentration of fluorophore with increasing concentrations of protein until no further changes in ligand fluorescence are detected; at this point *all* of the fluorophore is bound and the fluorescence properties reflect those of the bound fluorophore only.

In the third case, that where no change in overall TNS fluorescence occurs, the only definable conclusion that can be drawn is that if a conformational change in the protein is occurring, TNS fluorescence cannot be used to detect it. It is possible that there is no overall conformational change, but it is also possible that any conformational change is not detected by the particular fluorescent ligand used.

Proteins in general do not have specific binding sites for fluorophores such as TNS, and as a result such fluorophores are of the most use in detecting overall conformational changes. Figure 12-3 shows the fluorescence emission spectra of TNS bound to α_2 macroglobulin under circumstances similar to those in Fig. 12-2. Obviously, very similar conclusions can be drawn from this experiment as were drawn from the protein fluorescence measurements shown in Fig. 12-2.

The second type of ligand we consider is one that has a specific binding site on the protein. In many proteins a naturally occurring ligand is fluorescent and can be used as a probe of its own binding site if its fluorescence properties change upon binding. Figure 12-4 illustrates experiments using NADH fluorescence to follow conformational changes induced across subunit interfaces by NAD binding to the hexameric enzyme glutamate dehydrogenase.

In these experiments the fluorescence enhancement of the bound NADH is determined by titrating NADH with enzyme (see Chap. 17) in the presence of various amounts of NAD. The results clearly indicate that at half-saturation of the hexamer with NAD, the fluorescence enhancement of the bound NADH shows an abrupt decrease, suggesting that NAD induces a conformational change in the hexamer that is detectable by NADH fluorescence. In this particular instance, however, a more far-reaching conclusion can be drawn. Since NAD and NADH compete for the same site *on each subunit*, the NAD that is causing the conformational change in the hexamer (as detected by the trace amount of NADH used) *cannot* be bound to the same subunit as the fluorescent reporter NADH. The conformational change that is occurring must be induced across subunit interfaces. In addition, since the reporter fluorophore group (the NADH) is bound at the active site of the enzyme and the NAD also binds there (but to a different subunit under experimental conditions used), it is possible to reach the conclusion that ligand binding to one active site affects the conformation, as detected by the altered fluorescence properties of NADH, of a separate active site within the oligomer.

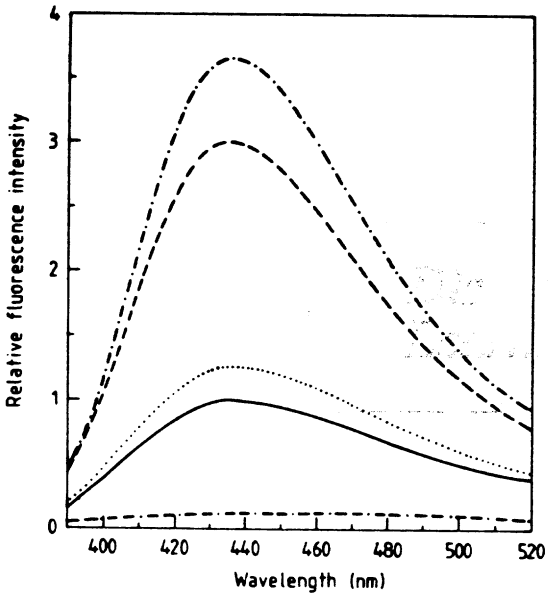

Figure 12-3 TNS fluorescence emission spectra for TNS bound to α_2M alone (—), activated with methylamine (· · ·), interacting with trypsin (– – –), or activated by methylamine and interacting with trypsin (–·–). Also shown is the fluorescence of free TNS in the presence of trypsin (– – ·– –). (Reprinted with permission from: I. Bjork, T. Lindblom, and P. Lindahl, *Biochemistry*, *24*, 2653–2660. Copyright 1985 American Chemical Society, Washington, D. C.)

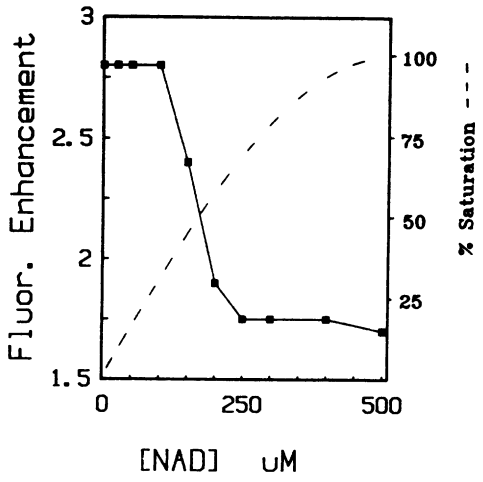

Figure 12-4 Fluorescence enhancement of NADH bound to glutamate dehydrogenase as a function of the saturation of the enzyme by NAD.

Even when a natural ligand of a protein is not fluorescent, it is often possible to use a ligand analog that is. In such instances it is important that control experiments be performed to show that the analog behaves the same way as the natural ligand; otherwise, it is possible that the interaction of the ligand analog with the protein will generate a different response in the protein conformation than in the natural ligand.

Covalent Fluorescent Probes. The most obvious potential problem with noncovalent fluorescent probes is the inability in some cases to experimentally determine the properties of the *bound* fluorophore. This is circumvented by using a covalently introduced fluorophore, making use for example of a reactive sulfhydryl group to introduce a fluorescent probe, as discussed in Chap. 7. Although this approach removes the problem of changes in the amount of fluorophore appearing to indicate conformational changes, it is important that the chemical modification used to introduce the fluorophore not result in changes in the normal properties of the protein that could alter its sensitivity to induced conformational changes.

Resonance Energy Transfer Measurements. In the discussion of fluorescence used to detect conformational changes we have concerned ourselves primarily with spectral measurements or intensities. We could equally as well have considered parameters such as polarization, lifetime, or ability to be quenched by collisional quenching. Somewhat different in approach, however, is the use of resonance energy transfer measurements to detect conformational changes. Resonance energy transfer between a donor and an acceptor depends on both the relative orientation of the donor and the acceptor, and the separation distance of the donor and acceptor. As such, it is exquisitely sensitive to conformational changes in a protein. If a suitable donor and acceptor are available, resonance energy transfer measurements between the two as a function of the concentration of a ligand thought to induce a conformational change in the protein can be used to study such a change. These measurements often involve resonance energy transfer from the protein aromatic residues to a covalent fluorophore acting as the transfer acceptor. Changes in energy transfer are easily interpreted. It is also possible to use either a noncovalent donor or acceptor, but changes in energy transfer must then be shown not to be due to changes in the binding of the noncovalent partner.

Circular Dichroism Measurements. Backbone CD measurements have been used to show that a protein changes its amounts of secondary structure in response to a ligand binding. As shown in Fig. 12-5, measurements of the far-UV CD of ATPase have been used to show that there is an increase in the α-helix content from 35% to 42% when MgATP binds to the enzyme in the presence of phosphate. In similar experiments (Fig. 12-6) it has been shown that calmodulin undergoes a conformational change, as detected by CD measurements at 222 nm, when calcium binds to the protein.

In an approach exactly analogous to that described earlier using NADH as a conformational probe with glutamate dehydrogenase, CD measurements of blue

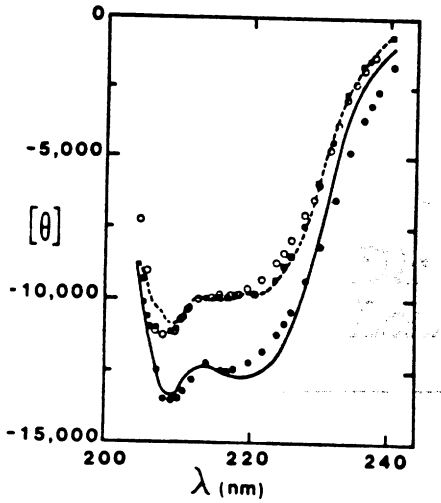

Figure 12-5 CD spectra of ATPase alone (○–○), in the presence of phosphate (■–■), or with phosphate plus MgATP (●–●). (Reprinted with permission from: B. Roux, G. Fellous, and C. Godinot, *Biochemistry*, *23*, 534–537. Copyright 1984 American Chemical Society, Washington, D. C.)

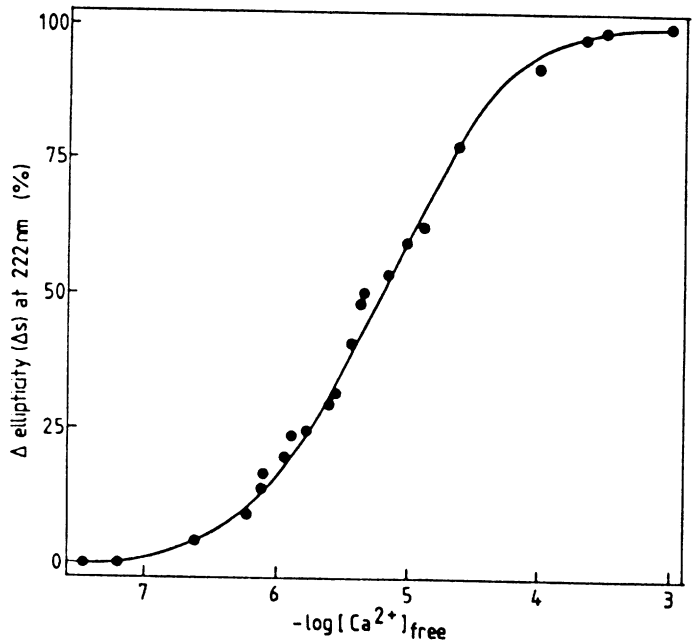

Figure 12-6 Titration of the Δ ellipticity at 222 nm of calmodulin as a function of the concentration of free calcium. (Reprinted with permission from: D. Burger, J. A. Cox, M. Comte, and E. A. Stein, *Biochemistry*, *23*, 1966–1971. Copyright 1984 American Chemical Society, Washington, D. C.)

dextran–Sepharose bound to the catalytic subunit of protein kinase have been employed to show that a variety of bound substrates induce similar conformational changes in the phosphoryl donor site. This enzyme, which catalyzes the cAMP-dependent phosphorylation of a variety of substrate proteins, consists of two regulatory and two catalytic subunits. The catalytic subunits are active in the absence of the regulatory subunits and phosphorylate a number of suitable peptide and protein substrates containing an exposed serine or threonine with one or more arginine residues to the N-terminal side. Kemptide is a synthetic substrate heptapeptide, with the sequence Leu-Arg-Arg-Ala-Ser-Leu-Gly.

Blue dextran has been shown to interact with a number of proteins containing nucleotide binding sites, and binds at the ATP site of the catalytic subunit. It has an absorbance maximum at 612 nm, and the low-energy transitions giving rise to this peak are extremely sensitive to alterations in the conformation of the chromophore. Blue dextran is used covalently linked to Sepharose to allow the removal of unbound enzyme or other ligands prior to the CD spectral measurements. Figure

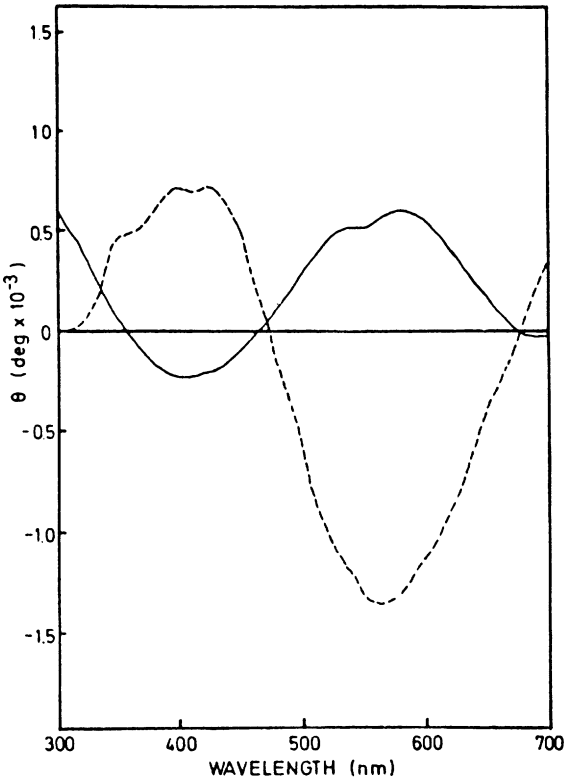

Figure 12-7 CD spectra of protein kinase catalytic subunit complexed with blue dextran Sepharose in the absence of substrate (——) or in the presence of a saturating amount of histone IIa (– – –). (Reprinted with permission from: J. Reed and V. Kinzel, *Biochemistry*, *23*, 968–973. Copyright 1984 American Chemical Society, Washington, D. C.)

12-7 illustrates the CD spectrum of blue dextran bound to the catalytic subunit in the presence and absence of histone IIa.

The induced positive peak at approximately 400 nm and the large negative spectrum at about 560 nm are also seen when a variety of other substrates, such as glycogen synthase or kemptide, are bound to the kinase. Since the blue dextran is bound to the ATP site of the catalytic subunit, the induced CD spectral changes indicate that substrate binding induces a conformational change in the ATP site. When kemptide-induced changes are compared with those of the synthetic peptide Arg-Gly-Tyr-Ala-Leu-Gly, which has no phosphorylatable serine, different changes in the blue dextran are observed. These differences indicate that the substrate must contain the phosphorylatable serine as well as the arginine to induce conformational changes in the ATP site.

NMR Measurements. In some proteins it has been possible to uniquely assign proton NMR signals to individual side chains. In such instances NMR measurements can give detailed information on the conformation of those side chains and can detect conformational changes involving them. Four-hundred-megahertz proton NMR

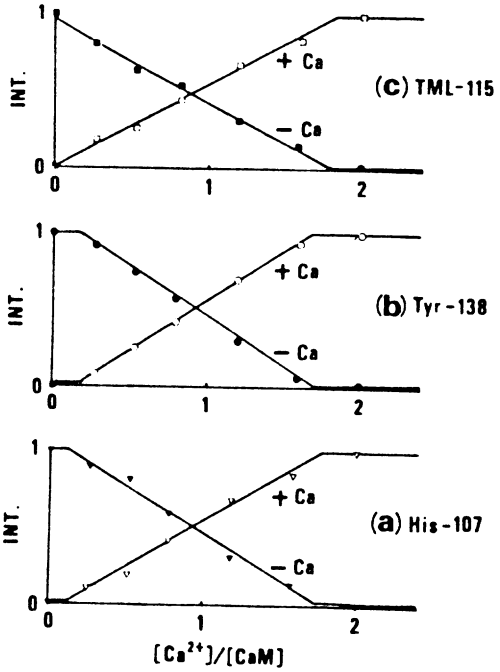

Figure 12-8 Relative intensities of proton resonances of His-107, Tyr-138, and Tml-115 as a function of the calcium concentration. −Ca indicates the Ca-free state; +Ca indicates the Ca-bound state. (Reprinted with permission from: M. Ikura, T. Hiraoki, K. Hikichi, M. Yazawa, and K. Yagi, *Biochemistry*, *22*, 2573–2579. Copyright 1983 American Chemical Society, Washington, D. C.)

studies of calmodulin have shown that side-chain resonances fall into three categories: (1) those perturbed by calcium binding to its high-affinity site, which includes Tyr-138, trimethyllysine-115, His-107, and Tyr-99; (2) those associated with calcium binding to its low-affinity site; and (3) those associated with calcium binding at both sites. In the absence of calcium there is a hydrophobic region containing three phenylalanine residues (Phe-89, 92, and 141), a valine, and an isoleucine, all in the vicinity of the calcium binding sites. Titration of calmodulin with calcium and its effect on three of the group 1 resonances is shown in Fig. 12-8.

Nuclear Overhauser enhancement (NOE) techniques can be used to measure distances between atoms that are close enough to each other to provide a relaxation pathway. The NOE is obtained by irradiating one of the two coupled nuclei and measuring the resonance of the other. NOE difference spectra measurements suggest that upon calcium binding to the high-affinity site, a conformational change occurs in which one of the phenylalanine residues and the valine from the calcium-binding-site region approach Tyr-138, which is located away from the binding site.

Protein Chemistry Approaches

Any protein chemistry technique that can give information concerning the exposure of regions of the polypeptide chain to the general solvent environment can also be used to indicate whether the protein conformation has changed in such a way as to change the exposure. Where defined information concerning which region of the protein is so exposed is available, detailed information about regions of the protein involved in conformational changes can be obtained. Usually, however, such information is not sought and the experiment is limited to giving information only at the level of overall conformational changes.

In this section we consider side-chain reactivity to modification reagents, susceptability to proteolysis, the use of specific antibodies to detect conformation, and hydrogen-exchange studies.

Side-Chain Reactivity. In Chap. 7 a great many chemical modification reagents were discussed together with ways of quantitating their reactions with a protein. The exposure of a particular side chain together with any particular environmental effects on its reactivity are the major contributors when one considers how the side chain reacts toward a particular modification reagent. If a certain number of a particular type of residue reacts with a reagent (such as, for example, iodoacetic acid) when the protein is in a given conformation, changes in the protein conformation may affect the reactivity in several ways.

1. The protein conformation may change such that the number of groups modifiable does not change but the kinetics of modification does.
2. The conformational change may allow an altered number of side chains to react but not affect the rate of those side chains reactive in the original conformation.
3. Some combination of the first two possibilities may occur as a result of the conformational change.

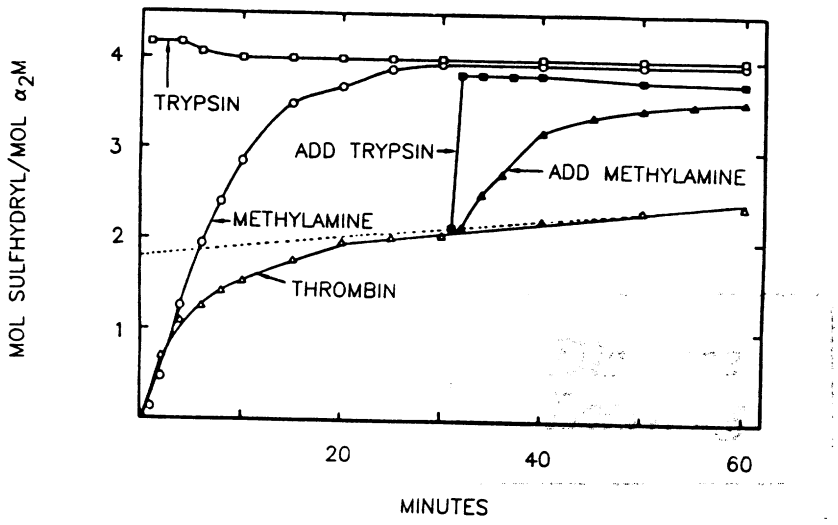

Figure 12-9 Time course of sulfhydryl generation when α_2M interacts with methylamine, thrombin, or trypsin. SH reactivity determined by DTNB titration. (Reprinted with permission from: J. P. Steiner, P. Bhattacharya, and K. Strickland, *Biochemistry*, *24*, 2993–3001. Copyright 1985 American Chemical Society, Washington, D. C.)

With each of these possibilities it is necessary to experimentally determine both the number of groups reacting and their rate (or rates).

Within each of these parameters there can be an increase or a decrease as a result of a ligand-induced conformational change of the protein. The conclusion is clear with increased rates of reactivity or numbers of modifiable groups; the conformation of the protein has changed. However, with decreased rates of reactivity or numbers of groups modifiable, it is possible that the ligand is sterically blocking access of the reagent rather than causing a conformational change.

If, in addition to these parameters the location of the groups involved (in terms of the primary sequence of the protein) can be ascertained, quite detailed information concerning conformational changes can be obtained.

The generation of reactive sulfhydryls in α_2 macroglobulin resulting from induced conformational changes has been followed using the DTNB reactivity of the exposed sulfhydryls. Figure 12-9 shows that the interaction of a variety of substrate ligands produces a conformational change resulting in the exposure of either two or four sulfhydryl groups.

Susceptibility to Proteolysis. This has been used in a number of systems to demonstrate ligand-induced conformational changes. The acid-induced unfolding of lactate dehydrogenase has been examined by limited proteolysis with pepsin and analysis of the resultant peptides by SDS-PAGE. The native enzyme is transferred to low pH and at various time intervals is pulsed with pepsin. The digestion products are separated on SDS-PAGE and undigested monomers and a variety of fragments

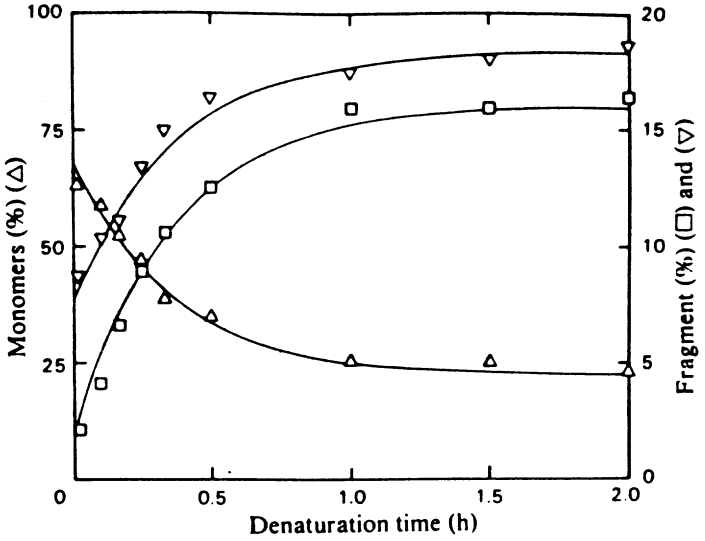

Figure 12-10 Correlation of loss of monomer and appearance of major fragments as a function of time of denaturation for lactate dehydrogenase. (Reprinted with permission from: R. Rudolf, *Biochem. Soc. Trans.*, *13*, 308–311. Copyright 1985 The Biochemical Society, London.)

ranging from 5 to 31 kDa are found. The decrease in the undigested monomer parallels the formation of two major fragments, as shown in Fig. 12-10.

This demonstrates that during the acid-induced unfolding of the lactate dehydrogenase monomer a conformational rearrangement results in the exposure of a specific interdomain cleavage site. From the molecular weights of the two major fragments (25 and 10.5 kDa) it would appear that cleavage occurs between Trp-225 and Lys-226.

Limited tryptic cleavage has been used to examine nucleotide- or phosphate-induced conformational changes in ATPase. Incubation with trypsin causes a rapid but slight activation, followed by a slower inactivation. Analysis of the proteolytic fragments by SDS-PAGE suggests that the initial rapid pulse is due to clipping of the α subunit, while the slower inactivation is due to subsequent proteolysis of α as well as β and γ subunits. The presence of ADP and ATP has no effect on the rapid phase but prevents the slower inactivation phase. Inorganic phosphate, on the other hand, slows both the initial phase and the subsequent inactivation phase, suggesting that it induces a quite different conformation in the protein than either ADP or ATP.

The Use of Specific Antibodies to Detect Conformation. Antibodies may recognize specific sequences of a polypeptide chain or a specific conformation of a region of polypeptide chain. In either case it is possible that the interaction of such an antibody with a protein can be used to detect conformational changes. In Chap. 9 we discussed the use of antibodies to detect folded structures of a protein, and emphasized the

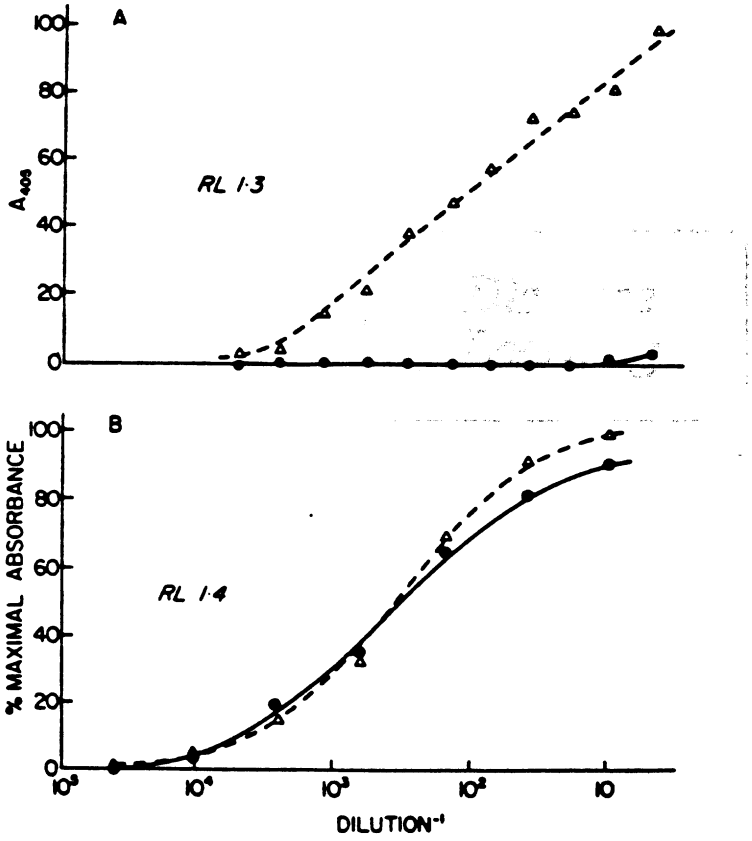

Figure 12-11 Effects of calcium on anti-prothrombin antibody interaction with prothrombin. Two different monoclonal antibodies, RL 1-3 and RL 1-4, were used in an ELISA direct binding system. The amount of antibody bound, detected by absorbance measurements at 450 nm, is plotted as a function of the dilution of the antibody in the presence of calcium chloride (△–△) or EDTA (●–●). (Reprinted with permission from: R. M. Lewis, B. C. Furie, and B. Furie, *Biochemistry, 22*, 948–954. Copyright 1983 American Chemical Society, Washington, D. C.)

care that had to be taken in the interpretation of such experiments. Similar conditions hold with the use of antibodies to detect conformational changes. The experiments shown in Fig. 12-11 indicate that it is possible to prepare monoclonal antibodies to prothrombin that are sensitive to conformational changes induced by calcium binding.

With the monoclonal antibody RL 1-3, the affinity of the interaction with prothrombin is clearly dependent on the presence of calcium. On the other hand, the monoclonal antibody RL 1-4 binds to prothrombin in an essentially calcium-independent manner. Monoclonal antibodies have also been produced which can discriminate between the various forms of the chromoprotein phytochrome.

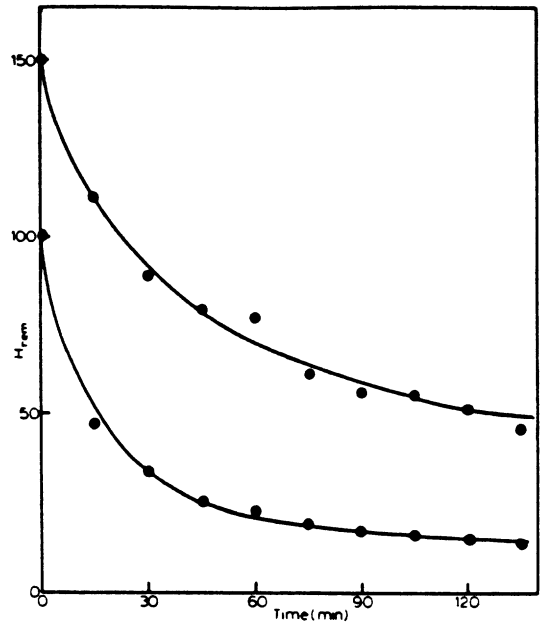

Figure 12-12 Exchange-out of tritium from labeled rhodanese in the *E* form (lower curve) and the E_s form (upper curve). [Reprinted with permission from: P. Horowitz and K. Falksen, *Biochim. Biophys. Acta*, *747*, 37–41 (1983).]

Hydrogen-Exchange Kinetics. The accessibility of protons to solvent in many instances governs their behavior in hydrogen exchange with tritiated water. Clearly, conformational changes that alter this accessibility change the hydrogen-exchange kinetics of particular protons. Ligand-induced changes in hydrogen exchange have been used to follow conformational changes in many proteins. Allosteric regulators have been shown to increase the rates of hydrogen exchange in various proteins, including glutamate dehydrogenase and glutamine synthase. With the enzyme rhodanese, exchange out of tritium from pre-tritiated enzyme has been used to examine different conformational states of the protein. The experiments shown in Fig. 12-12 involve the free enzyme and a substrate-induced conformation of the enzyme.

In these studies the enzyme was prelabeled by incubation in tritiated water and the amount of tritium remaining with the protein determined after varying time intervals. The free enzyme, *E*, shows a faster overall back exchange of tritium than the substrate-induced conformation, E_s. Further analysis shows that the exchangeable protons fall into two classes and the major difference between the two conformations involves the number of protons in the faster class as well as the rate of exchange.

Where hydrogen-exchange kinetics can be followed by NMR measurements, it is possible to follow the exchange reactions of individual amino acids in a structure

and obtain quite precise information on conformation. In bovine pancreatic trypsin inhibitor (BPTI), the β-sheet amide protons exchange extremely slowly. The effects of complex formation with trypsin on the hydrogen–deuterium exchange of these protons has been followed and it has been shown that whereas the NH proton of Tyr-35 is slowed by a factor of more than 1000, other NH protons are slowed only by a factor of 3 to 15. In a related system, streptomyces subtilisin inhibitor, the molecule exists as a dimer with extensive β-sheet regions at the interface. As in BPTI the exchange rates of NH groups in the sheet regions are extremely slow. This is in contrast to the two highly exposed regions of the molecule, one of which carries the recognition site for proteases, which are highly mobile. In this instance it has been suggested that the extremely slow rates of exchange of the β sheet protons result from an unusually rigid structure which compensates for the flexibility and mobility of other regions of the molecule.

Overall Shape Approaches

Various of the hydrodynamic techniques that were discussed in Chap. 4 in the context of molecular weight determination can also be used to obtain information about the overall shape of a macromolecule. In general, such approaches give no detail of any conformational change but in a variety of cases are used to indicate that a conformational change has occurred. In this section we briefly discuss the basis for using such methods.

Sedimentation Methods. If we consider a macromolecule as a prolate ellipsoid, we can consider two frictional coefficients: f_a, for rotation about the long a semiaxis, and f_b, for rotation about the short b semiaxis. The Perrin F factor is related to these frictional coefficients by

$$F_a = \frac{f_a}{f_{\text{rot}}} \tag{12-1}$$

$$F_b = \frac{f_b}{f_{\text{rot}}} \tag{12-2}$$

where f_{rot} is the rotational frictional coefficient and is related to the volume of the particle, V, and the viscosity of the solvent, η, by

$$f_{\text{rot}} = 6\eta V \tag{12-3}$$

As indicated in Chap. 4, the sedimentation properties of a macromolecule are related to the frictional coefficient, and as a result the sedimentation coefficient depends on the shape of the molecule. If we consider a monomer(m)–dimer(d) equilibrium, the sedimentation coefficients are related by

$$\frac{s_{\text{d}}}{s_{\text{m}}} = \frac{M_{\text{d}}/M_{\text{m}}}{f_{\text{d}}/f_{\text{m}}} = \frac{2f_{\text{m}}}{f_{\text{d}}} \tag{12-4}$$

When shape alone is the contributing factor to sedimentation coefficient changes, much smaller effects are observed. The theoretical ratio of sedimentation coefficients for a linear tetramer versus a tetrahedral tetramer is 0.944, indicating that even with such a drastic change in quaternary structure only small effects on the sedimentation coefficient are found. With such small changes it is vital that any molecular weight increase as a result of bound ligand be accounted for in assessing whether altered sedimentation properties are indeed the result of a conformational change.

Viscosity Methods. The intrinsic viscosity $[\eta]$ of a solute molecule is given in

$$[\eta] = \frac{\upsilon V_h N_0}{M} \tag{12-5}$$

where υ is the so-called Simha factor, N_0 the number of solute particles, M the molecular weight of the solute particles, and V_h the hydrated volume. The frictional coefficient, f, is related to the viscosity, η, by

$$f = 6\pi\eta\left(\frac{3}{4}\pi\right)^{1/3} V_h^{1/3} F \tag{12-6}$$

Since as with sedimentation methods the frictional coefficient depends on the shape of the molecule, viscosity measurements can be used to assess shape changes, provided that the molecular weight is constant. Fluorescence polarization measurements are a sensitive way of determining viscosity and are useful in assessing shape changes of a fluorescently labeled macromolecule.

Gel Filtration Methods. In Chap. 4 we made use of the relationship $K_{av} = -A \log M + B$ to determine the molecular weight of a protein by gel filtration methods, and pointed out in Eq. (4-52) that in principle the molecular weight should be replaced by the effective hydrated radius, R_h: It is related to the frictional coefficient of a molecule by

$$f = 6\pi\eta R_h \tag{12-7}$$

and as a result the elution behavior of a macromolecule in a gel filtration experiment is related to its shape. As before, changes in the elution behavior of a protein can be interpreted in terms of conformational changes only if molecular weight changes can be ruled out or taken into account.

Light-Scattering Methods. The radius of gyration of a macromolecule is obtained from light-scattering data (Chap. 4), and since it is defined as the root-mean-square distance of an array of atoms or groups from their common center of gravity, it is clear that light-scattering measurements can be used to detect conformational changes in a macromolecule if they are of sufficient magnitude to be reflected in the radius of gyration.

Summary

All the various methods discussed in this section *can* give indications of conformational changes in a macromolecule. The fact that in a particular system one or the other of these approaches fails to detect a conformational change does not mean that one is not occurring; it simply means that the experimental approach may not be sensitive enough to see the altered conformational state. With the approaches that give overall shape information, this is a particularly appropriate proviso which must be taken into account.

MECHANISMS OF CONFORMATIONAL CHANGES

Although we have discussed many experimental approaches that have been used to monitor conformational changes in proteins, not much attention has been paid to how such changes are transmitted through the protein. Except in a limited number of instances, little specific is known about how conformational changes occur. The most interesting exception is hemoglobin, where x-ray crystallographic studies of the protein in a number of conformations have allowed the mechanism of the cooperative interactions in oxygen binding to be understood in some detail. This particular example is discussed in Chap. 21. In the absence of such detailed information the general basis for the mechanism of conformational changes can be discussed.

Local changes such as might be involved in conformational adjustment of a binding site to optimize interaction with a substrate may involve only the reorientation of side chains of amino acid residues within the binding site. Depending on the stability of the local secondary structure, such reorientations may have no significant effect on the formal secondary structure. In Chap. 9 we examined the various forces involved in a particular secondary conformation: Small changes in one component (side-chain orientation) may not be sufficient to affect the overall conformational preference of a particular region of secondary structure.

Where the local secondary structure is poised delicately between two different conformations (e.g., a particular amino acid sequence may have almost equal preference for an α helix or a β sheet), apparently minor changes in side-chain orientation can cause a switch from one preference to the other. In this case the basis for the change in formal secondary structure can reside in the contribution of the side-chain orientation to the energy of a particular structure in terms solely of short-range interactions. However, long-range interactions that the side chain may make could well be altered, and changes in long-range interactions might trigger the conformational switch from one structure to another.

From this basis it is easy to understand how a conformational change in the protein can be triggered over long ranges. Because of the interplay of long- and short-range interactions in determining local structure of the protein, changes that affect either can be transmitted through the protein and result in conformational changes at points distant from the initial triggering point. Similarly, conformational

changes can be transmitted across subunit interfaces, since interactions between polypeptide chains at these interfaces are simply long-range interactions.

The considerations discussed here are also relevant to examining the effects of chemical modification on protein conformation. In Chap. 7 we looked at the use of side-chain chemical modification and stressed that the observation that a protein changes activity as the result of modification of a single defined residue does not indicate that the residue is directly present in the functional site of the protein. Conformational changes triggered by modification can occur across large distances and lead to alterations in the function of the protein.