DONALD VOET
University of Pennsylvania

JUDITH G. VOET
Swarthmore College

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INTRODUCTION

This is the third annual Supplement to *Biochemistry* (2nd ed.) by Donald Voet and Judith G. Voet. The biochemical literature covered in this Supplement extends from around April, 1997 to around April, 1998. During this ~12 month time period, the biochemical literature has continued its explosive growth. It is therefore increasingly important for the student and teacher alike to keep up with the literature. The annual Supplements to *Biochemistry* should be taken as a guide for doing so.

As with previous supplements to *Biochemistry,* this Supplement is keyed to the textbook in that we refer to new advances in terms of the textbook sections in which they would logically fit. Since space limitations permit only the most cursory discussions of these topics, the interested reader should consult the pertinent references provided at the end of each discussion. References that are not preceded by a discussion or which are placed under the subheading "Additional References" provide up-to-date coverage of subjects discussed in the corresponding section of the textbook. Note that many of these references are followed by a capsule synopsis.

The cover illustration of this Supplement is a monochrome version of the front cover illustration of *Biochemistry* (2nd ed.), a painting by Irving Geis of horse heart cytochrome *c* illuminated by its iron atom.

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Readers should note that John Wiley & Sons has recently published *Fundamentals of Biochemistry* by Donald Voet, Judith G. Voet, and Charlotte Pratt, a newly written textbook that is less detailed than is *Biochemistry* (2nd ed.) but maintains its chemical focus. *Fundamentals of Biochemistry* is packaged with a CD-ROM, entitled *Biochemical Interactions,* that contains a series of interactive computer graphics exercises and computerized animations that will also be of interest to readers of *Biochemistry* (2nd ed.).

We expect to publish *Biochemistry* (3rd ed.) in the summer of 2000.

Donald Voet
Judith G. Voet

Chapter 7

THREE-DIMENSIONAL STRUCTURES OF PROTEINS

1. SECONDARY STRUCTURE

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Chapter 8

PROTEIN FOLDING, DYNAMICS, AND STRUCTURAL EVOLUTION

1. PROTEIN FOLDING: THEORY AND EXPERIMENT

(a) A Disulfide Intermediate Is Required for the Folding of a Protein that Lacks Native Disulfide Bonds

The trimeric tailspike protein of bacteriophage P22 contains 8 Cys residues per subunit but lacks disulfide bonds in its native state as indicated by both X-ray crystallography and Raman spectroscopy. Nevertheless, the presence of the disulfide reducing agent dithiothreitol strongly inhibits the folding of this protein. The isolation, through nondenaturing gel electrophoresis, of a precursor of the fully folded trimer in both the *in vitro* and the *in vivo* folding pathways revealed that this so called prototrimeric intermediate contains interchain disulfide bonds. The treatment with reducing agent of the isolated prototrimeric intermediate yielded the native trimer. Evidently, the formation of intersubunit disulfide bonds facilitates the folding of the non-disulfide bonded native protein. It remains to be determined whether this folding process requires external sources of reducing and oxidizing equivalents or if there is some sort of internal redox shuffle that permits the trimer to undergo self-oxidation and self -reduction.

Robinson, A.S. and King, J., Disulfide-bonded intermediate on the folding and assembly pathway of a non-disulfide bonded protein, *Nature Struct. Biol.* **4,** 450–455 (1997).

(b) The X-Ray Structure of GroEL–GroES–$(ADP)_7$ Reveals its Allosteric Mechanism

The chaperonins are large cagelike protein complexes that facilitate the proper folding of improperly folded proteins. They do so through the ATP-driven binding and release of an improperly folded substrate protein's exposed hydrophobic groups and by providing this protein with a protected microenvironment that prevents it from nonspecifically aggregating with other improperly folded proteins. The chaperonins are composed of two types of subunits, Hsp60 and Hsp10 (GroEL and GroES in *E. coli*). The Hsp60 (GroEL) subunits form two back-to-back heptameric barrel-like rings, each surrounding a large cavity that encapsulates the substrate protein. The Hsp10 (GroES) subunits form a heptameric cap that seals the open end of a substrate protein-containing Hsp60 barrel. Previously determined X-ray structures [Section 8-1(a) of the 1996 Supplement] reveal that each GroEL subunit consists of three domains: a large equatorial domain that provides the contacts between the GroEL rings, an apical domain that forms the mouth of the barrel, and a small intermediate domain.

The X-ray structure of the GroEL–GroES–$(ADP)_7$ complex provides considerable insight into how this chaperonin carries out its function. In this complex, the GroES cap and the 7 ADP are bound to the same GroEL ring (the cis ring; the opposing GroEL ring is known as the trans ring) to form an asymmetric complex. The apical and intermediate domains of the cis ring have undergone large *en bloc* movements relative to their positions in the structure of unliganded GroEL so as to widen and elongate the cis cavity in a way that more than doubles its volume (from 85,000 $Å^3$ to 175,000 $Å^3$). The cis ring cavity can thereby enclose a partially folded substrate protein of at least 70 kD. In contrast, the trans ring subunits have conformations that closely resemble those in unliganded GroEL.

The hydrophobic groups that line the inner surface of the trans barrel presumably bind to the improperly exposed hydrophobic groups of substrate proteins. In the cis ring, however, these hydrophobic groups participate either in binding GroES or in stabilizing the newly formed interface between the rotated and elevated apical domains. Consequently, these hydrophobic groups are no

longer exposed on the inner surface of the cis cavity, thereby depriving a substrate protein of its binding sites.

A mutant form of GroEL, D398A, binds but cannot hydrolyze ATP. In the presence of ATP, D398A GroEL binds GroES as well as substrate protein. However, it does not release GroES or the protein when the trans ring is exposed to ATP, as is the case when the cis ring can hydrolyze ATP. Evidently, ATP's γ phosphate group provides strong contacts that stabilize the GroEL–GroES interaction. When the ATP in the cis ring is hydrolyzed, the resulting P_i is released and these interactions are lost.

The binding of ATP and GroES to one GroEL ring strongly inhibits their binding to the second ring – a negative allosteric effect. The X-ray structure of the GroEL–GroES–$(ADP)_7$ complex suggests that this occurs through small conformational shifts in the GroEL equatorial domains that apparently prevent the trans ring from assuming the conformation of the cis ring. However, once the cis ring has hydrolyzed its bound ATP, the trans ring can bind ATP and the resulting allosteric shifts release GroES from the cis ring. This explains why a mutant form of GroEL that has only one ring can bind substrate protein and GroES but does not release them after it hydrolyzes its bound ATP.

The foregoing information suggests how the GroEL–GroES system cycles: One GroEL ring binds substrate protein to its cavity walls. ATP binding then induces GroES to bind to this ring and releases the substrate protein to fold within the resulting enlarged closed cavity. The ATP is hydrolyzed with a measured half-time of ~15 s (the time the substrate protein has to fold), thereby weakening the interactions that bind GroES to GroEL. ATP binding (but not hydrolysis) to the trans ring then induces the conformational changes that release the GroES and permit the now better-folded substrate protein to rebind to the hydrophobic groups that are again exposed on the walls of the GroEL cavity (if the substrate protein has achieved the native fold, it no longer binds to these groups and hence is released from the chaperonin). The cis and trans rings then exchange roles and the cycle repeats. This explains why the proper functioning of GroEL requires two rings even though their cavities are unconnected. Note that this mechanism differs from that indicated in Fig. 8-11.

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(c) The *De Novo* Design of a Protein through Automated Sequence Selection

Although we are, as yet, unable to predict the native structure of a protein that is unrelated to proteins of known structure, considerable effort and ingenuity has been expended solving the inverse problem: generating polypeptide sequences that assume specific 3-dimensional structures, that is, protein design. This is because it is likely that one can "overengineer" a protein to take up a desired conformation. This idea has now been confirmed by the *de novo* design of a 28-residue $\beta\beta\alpha$ motif to have a backbone conformation closely similar to that of the zinc finger motif of Zif268 (Fig. 33-53*a*), but which contains no metal ions (zinc fingers are largely held together by their liganding of zinc ions).

The method used to design the polypeptide was entirely computationally based. The side chain selection algorithm used considered, in a quantitative manner, the interactions between side chains and between side chains and backbone, as well as the solvation of the protein. It screened

all possible amino acid sequences and, in order to take into account side chain flexibility, considered all sets of energetically allowed torsion angles for each side chain (each of which is known as a **rotamer**). By restricting core residues to be only hydrophobic and surface residues to be only hydrophilic, but permitting residues at the interface between the core and surface to be hydrophobic or hydrophilic, the number of amino acid sequences that was considered was limited to $1.9 \times 10^{2.7}$, which collectively had 1.1×10^{62} possible rotamers. The search therefore required a particularly computationally efficient algorithm and a fast computer.

The optimal sequence, called FSD-1, has only 6 of its 28 residues (21%) identical to those of Zif268 and an additional 5 residues (18%) similar. FSD-1's 8 core and boundary positions are predicted to form a well-packed cluster with Phe residues replacing the two zinc-liganding His residues in Zif268. FSD-1 was synthesized and its NMR structure was found to closely resemble its predicted structure and to have a backbone conformation nearly superimposable on that of Zif268. FSD-1's small size makes it but marginally stable. However, it is the smallest known polypeptide that is capable of folding into a unique structure without the aid of disulfide bonds, metal ions, or other subunits and thereby demonstrates the power of this protein design algorithm.

Dahiyat, B. and Mayo, S.L., De novo protein design: Fully automated sequence selection, *Science* **278,** 82–87 (1997).

(d) Changing the Fold of a Protein: Meeting the Paracelsus Challenge

Proteins that share as little as ~20% sequence identity may be structurally similar. What, then, is the smallest fractional change in the residue identity of a globular protein that will convert its fold to that of another globular protein? In order to encourage the exploration of this problem, George Rose and Trevor Creamer issued the "Paracelsus Challenge": A $1000 award for the first instance of transforming the conformation of one globular protein into that of another by changing no more than half the sequence (Paracelsus was the 16th century Swiss physician and alchemist who essentially invented pharmacology).

The authors of the following paper have met the Paracelsus Challenge: They induced the 56-residue B1 domain of *Streptococcal* IgG-binding protein G to assume the fold of Rop protein by changing exactly half of its residues. The B1 domain consists of a 4-stranded mixed β sheet, curled around a single α helix, whereas Rop protein (Rop for repressor of primer; a transcriptional regulator) is a homodimer whose 63-residue subunits form an αα motif (Fig. 7-48*c*) that dimerizes with its 2-fold axis perpendicular to the helices to form a 4-helix bundle. Rop's 7-residue C-terminal tail is disordered and hence does not appear to be part of the 4-helix bundle. Half of the residues of the B1 domain were changed based largely on a secondary structure prediction algorithm, energy minimization, and visual modeling to yield a new polypeptide named Janus (after the 2-faced Roman god of new beginnings) that is 41% identical to Rop. In this manner, B1 domain residues with high helix-forming propensities were retained, whereas in regions required to be α helical, a number of residues with high β sheet forming propensities were replaced; hydrophobic residues were incorporated at the appropriate *a* and *d* positions of a heptad repeat (Fig. 7-27) to form the core of Rop's 4-helix bundle; and residue changes were made to mimic Rop's distribution of surface charges. Fluorescence and NMR measurements indicated that Janus assumes a stable Rop-like conformation. These studies thereby indicate that not all residues play an equally important role in specifying a particular fold. Indeed, the Janus sequence is more closely related to that of the B1 domain (50% identity) than to that of Rop (41% identity), even though Janus structurally resembles Rop but not the B1 domain.

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2. PROTEIN DYNAMICS

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Chapter 9

HEMOGLOBIN: PROTEIN FUNCTION IN MICROCOSM

2. STRUCTURE AND MECHANISM

(a) Tension in the Fe–His F8 Bond Is Demonstrated by its Rupture in the X-Ray Structure of Cyanomet T-State Hemoglobin

Ligand binding to deoxy hemoglobin induces the heme Fe to shift from its position out of the plane of the heme group on the side of His F8 to a position in the plane of the heme. In the Perutz mechanism, this movement of the Fe pulls its liganded His F8 after it, thereby building tension in the Fe—His F8 bond that, when a sufficient number of subunits have bound ligand, triggers Hb's T to R conformational shift. The existence of this tension has now been directly demonstrated in the X-ray structure of the fully ligated T-state cyanide complex of metHb.

Although liganded Hb in solution assumes the R state, it can be trapped in the T state by its interactions in a crystal lattice [e.g., see Section 9-2(b) of the *1997 Supplement to Biochemistry*].

Crystals of T-state deoxyHb were oxidized to the met [(Fe(III)] state by reaction with ferricyanide and then soaked in NaCN solution to yield crystals of cyanomet T-state Hb. The X-ray structure of these crystals reveals that the bond between the heme Fe and the N_ε atom of His F8 (the proximal His) is ruptured in the α subunits but not in the β subunits. The Fe···N_ε(His F8) distance in the α subunits is ~3.0 Å (too long for an Fe—N bond), whereas in the β subunits it is ~2.2 Å (a typical Fe—N bond distance). This is because the Fe atom in the α subunits of cyanomet Hb has moved 0.8 Å to the distal side of the heme plane (toward its CN^- ligand) from its position in T-state deoxyHb on proximal side of the heme plane, whereas in the β subunits of cyanomet T-state Hb, the Fe has moved into the plane of the heme where it is liganded by both the N_ε of His F8 and by cyanide.

In the β subunits, cyanide binding causes a series of structural adjustments in the direction of the R state that, for the most part, are smaller than those seen on O_2 binding to T state Hb. Evidently, the Fe—N_ε bond in the α subunits cannot support the tension caused by such conformational shifts and ruptures so as to permit the α subunits to maintain their t-state conformation. This rupture uncouples the structural changes at the α heme groups from those at the β heme and in the globin, thereby unequivocally demonstrating the existence of tension in the Fe—N_ε bond and its role in conveying changes in the α heme to the β subunits.

Paoli, M., Dodson, G., Liddington, R.C., and Wilkinson, A.J., Tension in haemoglobin revealed by the Fe-His(F8) bond rupture in the fully liganded T-state, *J. Mol. Biol.* **271,** 161–167 (1997).

(b) Blood Flow Is Regulated by the Formation and Breakdown of *S*-Nitrosohemoglobin

NO stimulates the relaxation of blood vessels, thereby increasing blood flow (Section 34-4B). This compound reacts with hemoglobin's highly conserved Cys 93β to form ***S*-nitrosohemoglobin.** The affinity of R-state Hb for NO is higher than that of T-state Hb. Consequently, at low pO_2, NO is released from S-nitrosohemoglobin, thereby stimulating increased blood flow to the hypoxic tissues. Conversely, Hb takes up NO in hyperoxic tissues, thereby causing a reduction in blood flow. Thus, the T to R transition in Hb also functions physiologically as an oxygen sensor that coordinates local blood flow with oxygen requirements.

The X-ray structures of T- and R-state hemoglobins and the modeling of their *S*-nitroso adducts with Cys 93β rationalizes their differential affinity for NO. In T-state Hb, the modeled NO group points above the external His 146···Asp 94 salt bridge (Fig. 9-18*b*), where it is exposed to solvent and where the proximity of Glu 90 and Asp 94 to the S atom would appear to maintain it in its protonated form. This presumably stimulates NO release. In the R state, however, the His 146···Asp 94 salt bridge is ruptured, which presumably causes the *S*-nitroso group to take up a buried position out of contact with solvent, where the Cys 93β S atom is brought into proximity with His 92β. This is expected to facilitate the S atom's deprotonation and hence enhance its reactivity toward *S*-nitrosylation. Thus it appears that NO release from Hb is facilitated in the T state and its *S*-nitrosylation is in the R state.

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3. ABNORMAL HEMOGLOBINS

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Chapter 10

SUGARS AND POLYSACCHARIDES

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Chapter 11

LIPIDS AND MEMBRANES

3. BIOLOGICAL MEMBRANES

(a) Structure of Bacteriorhodopsin

The electron crystallography-based structure of bacteriorhodopsin, reported in 1975, provided the first glimpse into the structural organization of a membrane protein. Over the past 23 years, advances in electron crystallography have led to steady improvements in the resolution of this 7-transmembrane helix protein. Indeed, the most recently reported electron crystallography structure of bacteriorhodopsin has a resolution of 2.8 Å, comparable to that of a medium resolution X-ray structure. This structure is largely in agreement with previous studies but, in addition, reveals the positions of charged residues on both sides of the membrane surface and has thereby provided insight into the function of this light-driven proton pump.

Although there had been numerous attempts over the years to crystallize bacteriorhodopsin, none had yielded diffraction-quality crystals. Now, however, such crystals have been formed by dissolving bacteriorhodopsin in lipidic cubic phases. The latter are mixtures of lipid and water that display cubic symmetry. The particular lipidic cubic phase from which bacteriorhodopsin crystallizes is of the so-called bicontinuous class, which consists of a higly convoluted but continuous bilayer surface that is interpenetrated by a connected series of aqueous channels. Using this technique yielded hexagonally-shaped microcrystals that were 20 to 40 μm across and 5 μm thick. X-ray diffraction data from these extremely small crystals (diffraction-quality protein crystals are typically 300 μm across) were obtained using a very intense 10-μm in diameter beam of synchrotron radiation. This technique has great promise for the determination of the X-ray crystal structures of membrane proteins, relatively few of which have heretofore been crystallized.

The X-ray crystal structure of bacteriorhodopsin, which has a resolution of 2.5 Å, not surprisingly, closely resembles the corresponding electron crystallography-based structure. However, there are several significant differences between these structures, particularly changes in the conformations of the AB loop (the loop connecting transmembrane helices A and B) and the BC loop. Curiously, some of the loops that are clearly defined in the electron crystallography-based structure are not visible in the X-ray crystal structure. The molecular basis for these differences is unclear.

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4. MEMBRANE ASSEMBLY AND PROTEIN TARGETING

(a) Image of the Sec61 in Complex with a Ribosome

The Sec61 heterotrimeric complex is a strong candidate for the protein-conducting channel through which the ribosome extrudes nascent membrane-bound proteins and proteins destined for secretion into the endoplasmic reticulum (ER). Cryoelectron microscopy of detergent-solublized canine Sec61p revealed an ~85 Å in diameter toroidal particle whose central pore is ~20 Å across [Section 11-4(a) of the *1997 Supplement to Biochemistry*]. Cryoelectron microscopy has now been used to visualize yeast Sec61 in complex with a yeast ribosome at 26 Å resolution. Sec 61 appears as a somewhat pentagonally-shaped toroid that has an outer diameter of 95 Å and a thickness of 40 Å. It is tenuously linked to the ribosome via a single stem attached to the base of the large ribosomal subunit. Sec61 is positioned and oriented such that its pore is essentially coaxial with the ribosome tunnel through which the nascent polypeptide chain is thought to exit the ribosome. The central pore of Sec 61 is funnel-shaped with a diameter of 15 Å on the luminal side of the ER and 35 Å on the side facing the ribosome, thereby forming a small vestibule. The tenuous connection between Sec61 and the ribosome does not appear to form the tight seal between the two that has been experimentally demonstrated. Perhaps seal-forming proteins are missing or, more likely, a signal sequence may be required to promote seal formation.

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5. LIPID-LINKED PROTEINS AND LIPOPROTEINS

(a) X-ray structure of an LDL receptor module reveals a molecular basis for familial hypercholesterolemia

The LDL receptor (**LDLR**) is responsible for the uptake of cholesterol-containing LDL particles by cells. In familial hypercholesterolemia (FH), a defect in the LDL receptor prevents this uptake, resulting in a greatly increased level of cholesterol in the blood which, in turn, causes severe cardiovascular disease. The N-terminal region of the LDL receptor, which consists of 7 tandemly repeated, ~40-residue Cys-rich modules known as **LDL-A modules,** mediates the binding of lipoproteins. The X-ray structure of the LDLR's fifth LDL-A module, LR5, reveals that it coordinates a Ca^{2+} ion via conserved acidic residues near its C-terminal end. The structure explains the effects of FH mutations that occur in LR5. Some of these eliminate or misplace Ca^{2+}-binding ligands, whereas others delete hydrogen bonds or disulfide bonds that stabilize the LR5 backbone structure. The structure calls into question models of the LDLR–LDL interaction in which LDLR's conserved acidic residues bind to positively charged residues on LDL. Since apolipoproteins must be associated with lipids in order to bind to LDLR with high affinity, the LR5 structure suggests that its hydrophobic concave face provides the binding site for LDL.

Fass, D., Blacklow, S., Kim, P.S., and Berger, J.M., Molecular basis of familial hypercholesterolaemia from structure of LDL receptor module, *Nature* **388,** 691–693 (1993).

(b) X-Ray Structure of truncated human apolipoprotein A-I suggests how it binds lipids

Apolipoprotein A-I (Apo A-I) is the major protein of HDL. Sequence analysis indicates that apo A-I and other apolipoproteins consist of repeated amphipathic α helices that are 11 or 22 residues long and which provide the lipid-binding regions of these proteins. The truncation mutant of the human apo A-I, apo Δ(1-43)A-I, which contains residues 44 to 243 of native apo A-I, has lipid-binding properties that closely resemble those of the native protein. A variety of criteria indicate that the conformation of native apo A-I in HDL differs from that of lipid-free apo A-I. However, apo Δ(1-43)A-I in both its lipid-bound and lipid-free states appears to have a conformation closely similar to that of native apo A-I in HDL. Lipid-free apo Δ(1-43)A-I may therefore provide a valid structural model for lipid-bound apo A-I.

The X-ray structure of apo Δ(1-43)A-I reveals that it consists primarily of a pseudo-continuous amphipathic α helix that is punctuated by kinks at regularly spaced Pro residues to form 10 helical segments arranged in the shape of a horseshoe. Two such monomers associate in an antiparallel fashion to form a dimer. Two such dimers, in turn, associate via their hydrophobic surfaces to form a tetramer with pseudo-D_2 symmetry that has the shape of an elliptical ring with outer dimensions of 135 × 90 Å and with an inner hole of 95 × 50 Å. The exterior of this tetrameric ring, which consists of an up-down-up-down 4-helix bundle over about three-fourths of its circumference, is hydrophilic with a uniform electrostatic potential, whereas its interior consists mainly of Val and Leu side chains. Since in this state these hydrophobic residues are unavailable for binding to lipid, it is postulated that they associate in the lipid-free crystal so as to shelter the lipid-binding face of apo Δ(1-43)A-I dimers from contact with water.

The sizes and shapes of the apo Δ(1-43)A-I dimer and tetramer seem ideal for wrapping around HDL particles, which have diameters in the range 80 to 120 Å. Since HDL particles often contain two or four apo A-I monomers, it is proposed that when pairs of apo A-I monomers bind to HDL, they do so as the antiparallel dimer seen in the structure of apo Δ(1-43)A-I. Two such dimers can associate on the surface of the HDL particle to form a tetramer although, it seems likely, in a different manner than is seen in the structure of apo Δ(1-43)A-I.

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Chapter 14

ENZYMATIC CATALYSIS

1. CATALYTIC MECHANISMS

(a) Experimental demonstration of the significance of orbital steering

Molecules react most readily when they have the proper relative orientation. Thus, it has been postulated that a major factor in the catalytic power of an enzyme is that it arranges the trajectories of its reacting substrates so as to optimize the balance between maximizing the overlaps of the attracting (bonding) orbitals and minimizing those of repulsive (nonbonding) orbitals, a phenomenon dubbed **orbital steering.** The concept of orbital steering in enzymes was tested in the enzyme isocitrate dehydrogenase **(IDH),** which catalyzes the $NADP^+$-dependent oxidative decarboxylation of isocitrate to yield α-ketoglutarate (Section 19-3C).

The trajectory of the reaction catalyzed by IDH was modified in either of two ways: (1) by replacing the adenine residue of its $NADP^+$ cofactor with hypoxanthine (replacing the 6-NH_2 group with OH; IDH-bound hypoxanthine is in its enol form) to yield **nicotinamide hypoxanthine dinucleotide ($NHDP^+$);** or (2) by replacing IDH's bound Mg^{2+}, which prefers 6 ligands, with Ca^{2+}, which prefers 8 ligands.

Kinetic studies on the IDH–Mg^{2+}–$NHDP^+$ complex indicate its turnover number, k_{cat}, is reduced by a factor of 3.9×10^{-5} relative to the IDH–Mg^{2+}–$NADP^+$ complex and its second order rate constant, k_{cat}/K_M, is reduced by a factor of 8×10^{-7}. The comparison of the X-ray structures of the IDH–Mg^{2+}–$NHDP^+$ and IDH–Mg^{2+}–$NADP^+$ complexes reveal that the hypoxanthine ring in $NHDP^+$ has rotated relative to the adenine ring in $NADP^+$ such that hypoxanthine O6 is displaced by 1.4 Å from the position of adenine N6. This displacement is propagated throughout the $NHDP^+$ molecule such that the distance of the hydride donor–acceptor pair increases by 1.55 Å and their angle of approach deviates between 10° and 20° from the more in-line geometry observed in the $NADP^+$ structure.

IDH's bound Mg^{2+} ion is situated near the trajectory of the reacting groups but not on the path of hydride transfer. The k_{cat} of Ca^{2+}–IDH is reduced relative to that of Mg^{2+}–IDH by a factor of 2.5×10^{-3} although its K_M is not significantly altered. The X-ray structure of the IDH–Ca^{2+}–$NADP^+$ complex reveals that the Ca^{2+} ion is 8-coordinated and has moved 1.4 Å relative to the position of the 6-coordinated Mg^{2+} in the IDH–Mg^{2+}–$NADP^+$ complex. This displacement results in adjustments of both the isocitrate and the $NADP^+$ such that the distance beween the hydride donor-acceptor pair is reduced by 0.55 Å and the attacking angle (nicotinamide N–C4–isocitrate C2) is reduced from 159° in the Mg^{2+} complex to 131° in the Ca^{2+} complex, that is, the attack trajectory becomes less linear.

If the reduced activity of the IDH–Mg^{2+}–$NHDP^+$ complex is due to the longer distance between the hydride donor and acceptor, then the IDH–Ca^{2+}–$NAPD^+$ complex should have a greater activity due to its lesser value of this distance. Thus it seems clear that both distance and orientation play major roles in both examples, thereby supporting the orbital steering hypothesis.

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2. LYSOZYME

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3. SERINE PROTEASES

(a) Low-Barrier Hydrogen Bonds: The Debate Goes On

When the p*K*s of a hydrogen bonding donor (D—H) and acceptor (A) become nearly equal, the distinction between them, at least in the gas phase, breaks down such that the hydrogen atom becomes more or less equally shared between them (D···H···A). In the gas phase, such low-barrier hydrogen bonds (**LBHBs;** also called **short, strong hydrogen bonds**) have unusually large free energies of association (>80 kJ/mol *vs* ~30 kJ/mol for normal hydrogen bonds). LBHBs are unlikely to exist in dilute aqueous solution because water molecules, which are excellent hydrogen bonding donors and acceptors, effectively compete with D—H and A for hydrogen bonding sites. However, LBHBs may exist in nonaqueous solution and in the active sites of enzymes that exclude bulk solvent water. This has led to the hypothesis that, in some enzymes, a normal hydrogen bond in the Michaelis complex is converted, through transient p*K* shifts, to an LBHB in the transition state. This would facilitate proton transfer while applying the difference in the free energy between the normal hydrogen bond and the LBHB to preferentially binding the catalyzed reaction's transition state. Indeed, the formation of such LBHBs would explain how, in such widely accepted enzyme mechanisms as those of serine proteases, a conjugate base of an acidic group can abstract a proton from a far more basic group.

The hallmark of an LBHB is a particularly short D···A length of <2.45 Å *vs* 2.8 to 3.1 Å for normal hydrogen bonds. In most cases, however, the appropriate X-ray structure has not been determined or is of too low a resolution to provide a sufficiently accurate distance. However, the ^{1}H-NMR spectrum of an LBHB exhibits a particularly large downfield chemical shift of the hydrogen bonded proton as a consequence of its deshielding. Such ^{1}H-NMR evidence of LBHBs has been observed, for example, in the His···Asp interaction of the catalytic triads of chymotrypsin and subtilisin. Nevertheless, there has been a considerable debate as to the catalytic significance of LBHBs [e.g., see Section 14-3(b) of the *1997 Supplement to Biochemistry*].

New experimental measurements have led to a modification of the theory of how the LBHB between chymotrypsin's His 57 and Asp 102 is generated. **Peptidyl trifluormethyl ketones (TFKs)** form stable adducts with chymotrypsin in which Ser 195 forms a C—O bond with the carbonyl group of the TFK and hence provide good models for the tetrahedral intermediate in the enzyme's catalytic reaction sequence (Fig. 14-23). The ^{1}H-NMR spectra of the complexes of chymotrypsin with the TFKs *N*-acetyl-L-Leu-DL-Phe-CF_3 and *N*-acetyl-DL-Phe-CF_3 indicate that the removal of the Leu residue from the first complex decreases the basicity of chymotrypsin's His 57 in the His 57–Asp 102 diad (from a p*K* of 12.0 to 10.8) and weakens the LBHB between these side chains. Consideration of the structural data on chymotrypsin and other serine proteases suggests that substrate-induced conformational shifts induce the formation of the LBHB between His 57 and Asp 102 by compressing the distance between them. Since the N···O distance between His 57 and Asp 102 is shorter in an LBHB than in a normal hydrogen bond (the N···O distance in an LBHB is significantly less than the N···O van der Waals distance of 2.65 Å), the strain between these residues is relieved by the formation of the LBHB. However this LBHB can only form if the p*K*s of His 57 $N_{\delta 1}$ and Asp 102 are closely matched. This is the case when His 57 is protonated (in its imidazolium form) but not when it is unprotonated. Thus, the formation of the LBHB greatly increases the basicity of His 57 N_{e2}, thereby making it capable of abstracting a proton from Ser 195 (which has a p*K* of ~14) so as to become protonated. Evidently, the enzyme's binding of parts of the substrate that are remote from the active site provides the driving force to compress the His 57–Asp 102 hydrogen bond, which induces the formation of the LBHB, thereby increasing the basicity of His 57 $N_{\delta 1}$ to the point that it can abstract the proton from Ser 195.

cis-Urocanic acid (deaminated histidine with a C_α=C_β double bond; Fig. 24-13) forms an internal hydrogen bond between its carboxyl oxygen and imidazole $N_{\delta 1}$ atoms and hence structurally mimics the His 57–Asp 102 diad in chymotrypsin. Indeed, a low field ^{1}H-NMR signal is observed for *cis*-urocanic acid in organic solvents but not in water, consistent with the formation of an LBHB. However, the ^{15}N chemical shift behavior of *cis*-urocanic acid in 100% water closely resembles that of **α-lytic protease** (a bacterial serine protease) over the entire pH range in which this enzyme is stable. This suggests that the inability to detect *cis*-urocanic acid's internal hydrogen