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Chemistry

Mechanism of tertiary structural change in hemoglobin

(cooperativity/protein dynamics/stereochemical trigger/conformational energy calculations)

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ABSTRACT **A reaction path is presented by which the effects of oxygen binding in hemoglobin are transmitted from a heme group to the surface of its subunit. Starting from the known deoxy geometry, it is shown by calculations with empirical energy functions and comparisons with available data how the change in heme geometry on ligation introduces a perturbation that leads to the tertiary structural alterations essential for cooperativity. It is found that there is little strain on the *unliganded* heme; instead, the reduced oxygen affinity of hemoglobin results from the strain on the liganded subunit in a tetramer with the deoxy quaternary structure.**

The cooperative nature of the binding of oxygen by hemoglobin is one of the most intensively studied phenomena in protein chemistry (1). From the classic x-ray work of Perutz and his collaborators (2, 3), supplemented by the physical and chemical studies of others (4, 5), the outlines of the cooperative mechanism have been determined. The essential elements are two quaternary structures (oxy and deoxy) for the hemoglobin tetramer (6), two tertiary structures (liganded and unliganded) for each subunit, and the presence of ionic, van der Waals, and hydrophobic interactions that couple the tertiary structural change of the subunits to the relative stabilities of the quaternary structures. These elements have been incorporated into a statistical mechanical model that describes ligand binding as a function of solution conditions and accounts for the effects of mutations and chemical modifications (7, 8).

To complete the description of the cooperative mechanism, the statistical-mechanical model must be supplemented by an understanding of the origin of the important structural changes and their associated energies. It is necessary to know at the atomic level how ligand binding alters the tertiary structure of an individual subunit and how these alterations in subunit geometry affect and can be affected by the quaternary structure of the hemoglobin tetramer. In this paper, we focus on the first of these two problems. From calculations based on empirical energy functions (9) and comparisons with the available data, we are able to determine the properties of the heme group and of the surrounding globin chain that lead to the essential tertiary structural changes. A localized reaction path that involves directly only a relatively small number of globin atoms is found to transmit information concerning ligand binding from the heme group to the surface of the subunit. It is shown that there is little strain on the heme in unliganded hemoglobin, but that the flattening of the heme and shortening of the iron-histidine bond induced by oxygen binding produce steric repulsions between the heme and the globin that alter the subunit geometry. Nonbonded contacts between the asymmetrically positioned His F8(87) and the heme appear to initiate a rotation of the latter. This in turn produces a large displacement of Val FG5(93), which is the key residue in transmitting structural changes to the $\alpha_1\alpha_2$ contacts and the $\alpha_1\beta_2$ interface. Details of the pathway and the supporting theoretical and experimental evidence are given in the following sections. Many of the data come from the work of Perutz and Ten Eyck (2, 3), Hoard (10, 11), Makinen and Eaton (12, 13), Anderson (14), and Ibers *et al.* (15), all of whom have made suggestions concerning some of the elements of the reaction path.

Abbreviations: XRG, x-ray geometry; ERG, energy-refined geometry; rms, root-mean square.

METHODOLOGY

The method used for evaluating the conformational energy of a protein has been outlined previously (9). Calculations of tertiary structural changes were made for an isolated human deoxyhemoglobin α chain whose initial x-ray geometry (XRG) was that determined by Fermi (16) at 2.5 Å nominal resolution. To obtain an energy-refined geometry (ERG) (9) for use in simulating ligand binding, the x-ray coordinates were subjected to 200 steepest-descent steps. The root-mean square (rms) atom shift was 0.186 ± 0.114 Å, with a distribution peaked near the origin and decreasing smoothly with increasing magnitude of shift; almost all of the larger motions involved side-chain atoms. The total energy decreased from 653 to -1270 kcal/mol and the rms Cartesian force component acting on an atom dropped from 61.8 to 0.5 kcal/mol-Å. Thus, the energy minimization produced a near-equilibrium structure with only small changes from the original x-ray coordinates.

To investigate the heme-globin interaction in the unliganded and liganded α chain, two types of calculations were made. In the first, the heme was displaced relative to a fixed globin geometry (XRG or ERG) to determine the origins and magnitudes of the resulting interaction energies. In the second, the ERG structure was perturbed by displacing the heme, which was then fixed while the globin energy was reminimized (9). This permits a determination of the changes induced in the globin by alterations in the heme group that simulate ligation.

RESULTS AND DISCUSSION

In this section, we consider the elements of the reaction path for tertiary structural change on ligand binding and relate them to the results of the energy calculations and the available experimental data.

The Heme Group. Calculations indicate that in the absence of a sixth ligand the heme group has a domed form and is unstrained inside the globin. Although the x-ray structure (XRG) has a planar porphyrin with the iron 0.6 Å out of the plane, the energy minimized geometry (ERG) was found to have the heme pyrrole groups tilted *toward* the iron atom. This domed geometry is close to that of a high-spin five-coordinate model compound, 2-methylimidazole $\alpha,\beta,\gamma,\delta$-tetraphenylporphinatoiron (II) (17, 18) (Table 1). Doming is calculated to be present independently of the globin and is a consequence primarily of the terms in the force field that tend to maintain the pyrrole nitrogens as planar centers. However, the energy difference between the domed and planar structures is very small, so that

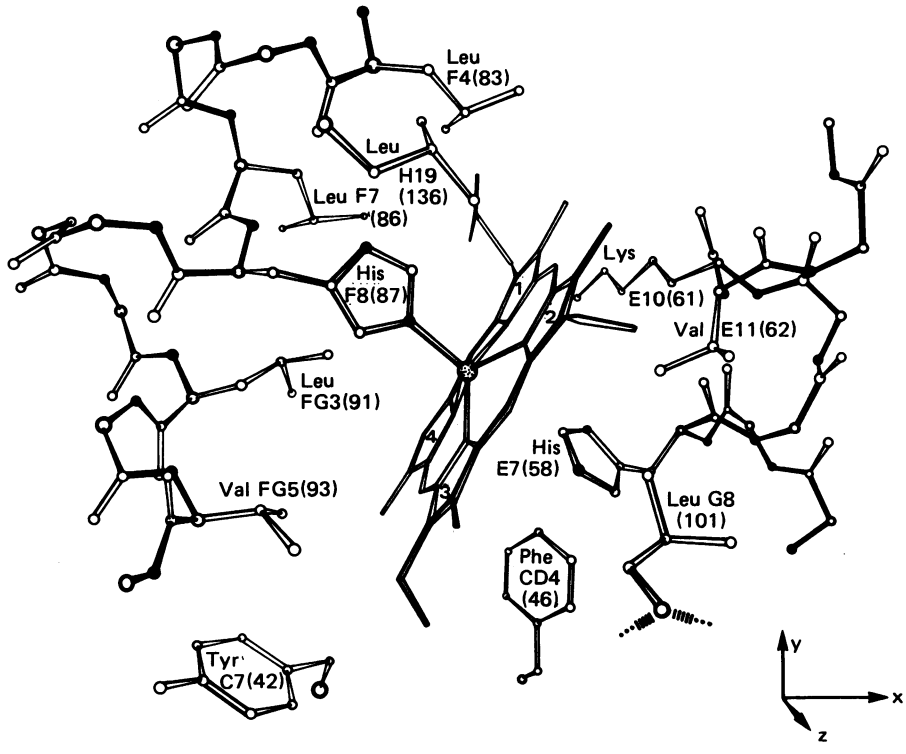

FIG. 1. Heme group and its environment in the unliganded XRG α-chain. Solid bonds denote polypeptide backbone. Only selected side chains are shown; the heme 4-propionate is omitted for clarity.

the heme is not suited for storing significant strain energy. The atoms of the globin surrounding the heme (Fig. 1) are arranged to accommodate the domed structure. With respect to its nonbonded interactions with the rigid globin, the domed heme is located in a broad potential well for translation perpendicular to its mean plane (Fig. 2). The calculated heme position, 0.15 Å on the near side of the potential minimum, corresponds to a strain energy of less than 1 kcal/mol. Because the net force on the heme is smaller on a per atom basis than for the globin as a whole, the apparent strain is not significant. In terms of the "distributed energy" model of Hopfield (19), it could be argued that the nonbonded heme–globin interaction represents a small fraction of the total strain energy stored in displacements throughout the globin. This seems unlikely from independent studies of protein energetics. In a detailed examination of tyrosine sidechain rotations in the basic pancreatic trypsin inhibitor (9) it has been shown that much larger rigid protein nonbonded interactions can be greatly reduced by small atomic displacements whose energy cost is negligible; further, most of the residual strain energy present after allowing the protein to relax was found to remain in the local nonbonded interactions.

The above conclusion concerning the lack of strain on the unliganded heme group in the globin is in agreement with a number of experimental results. The recent refinement of deoxyhemoglobin by Fermi (16) indicates that the iron is not significantly farther from the mean plane of the heme than in five-coordinate model compounds (17, 18). A detailed interpretation (B. H. Huynh and M. Karplus, unpublished data) of the Mössbauer data of Huynh *et al.* (20) and a comparison between their results for free chains and the deoxy tetramer indicate that the iron energy levels, and therefore, the iron position, are not changed significantly upon incorporation of the chains into the tetramer. Correspondingly, the recent synchrotron radiation study of deoxyhemoglobin by Eisenberger

Table 1. Selected coordination parameters of ferrous porphyrins[a]

Parameter	2-MeImFe TPP[b]	Heme group from ERG[c]	$(Pip)_2$ Fe TPP[d]
N_{axial}-Fe	2.16[e]	1.987	2.127[f]
$N_{pyrrole}$-Fe	2.086	2.092	2.004
Fe···P_N[g]	0.42	0.32	0
Fe···P_C[g]	0.55	0.48	0
N_{axial}···P_C[g]	2.67 (Domed)	2.467 (Domed)	2.127 (Planar)

[a] All distances in Å (1 Å = 0.1 nm).
[b] 2-Methylimidazole $\alpha,\beta,\gamma,\delta$-tetraphenylporphinatoiron(II); see ref. 17.
[c] Calculated; see *text*.
[d] Bis(piperidine) $\alpha,\beta,\gamma,\delta$-tetraphenylporphinatoiron(II); see ref. 23.
[e] Bond inclined 10° from normal to heme plane; perpendicular distance is 2.12 Å.
[f] Argued to be stretched by up to 0.18 Å; see *text*.
[g] P_N is mean plane of pyrrole nitrogens; P_C is mean plane of porphin carbons.

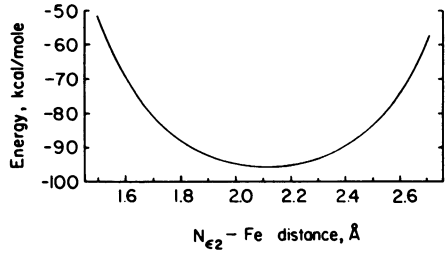

FIG. 2. Potential due to heme-globin nonbonded interactions for translation of heme in unliganded XRG α-chain; the zero of energy is at infinite separation and the Fe-N_ϵ bond energy is not included.

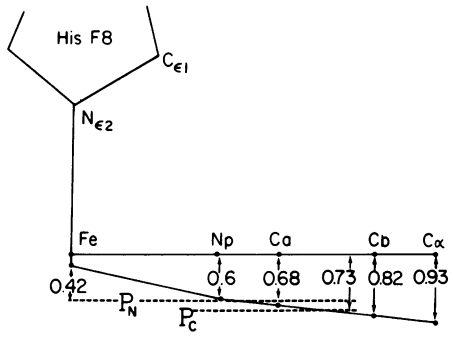

FIG. 3. Changes in heme conformation and significant distances on ligation (relative to fixed axial histidine). Unliganded nonplanar heme is from 2-MeImFeTPP (Table 1). P_N and P_C are defined in footnote g of Table 1; N_p, C_a, and C_b are the pyrrole nitrogen and carbons in standard order (see ref. 11); C_α is the first carbon of a substituent on C_b.

et al. (21) implies that the Fe-N distances are not altered when the unliganded tetramer is shifted from the deoxy to the oxy quaternary structure. Also, the resonance Raman studies of Spiro and his coworkers (22) suggest that the five-coordinate heme is essentially unchanged upon incorporation into the globin.

The changes expected in an isolated heme on ligation are well documented from model compound studies (Table 1) (17, 18, 23); the important geometric parameters are illustrated in Fig. 3. For a six-coordinate low-spin iron, the porphyrin is essentially planar, with the iron in the heme plane. Further, the axial Fe-$N_{\epsilon 2}$ bond length is expected to decrease by 0.15–0.18 Å (23), though steric repulsions prevent this shortening in the model compound of Table 1. Fig. 3 shows that the transition from a domed to a planar heme can produce large changes in the relative positions of the porphyrin atoms; if all the pyrrole atoms are planar centers, the peripheral and substituent carbons move nearly 1 Å. Thus a small change in the effective Fe-N bond lengths (about 0.18 Å for Fe-$N_{\epsilon 2}$ and about 0.08 Å for Fe-$N_{pyrrole}$) is greatly amplified by the requirement of a planar, six-coordinate heme (15).

An essential consequence of the bond shortening and "undoming" is the interaction between the axial histidine and certain porphyrin atoms. From the XRG coordinates, His F8(87) is asymmetrically oriented with respect to the heme. In addition to its intrinsic asymmetry (i.e., the $N_{\epsilon 2}$-$C_{\epsilon 1}$ bond is shorter than the $C_{\delta 2}$-$N_{\epsilon 2}$ bond), the imidazole ring has a projection on the heme that forms an angle of 13° with the Fe-1N bond, the Fe-$N_{\epsilon 2}$ bond is not perpendicular to the heme plane (the angles $N_{\epsilon 2}$-Fe-1N and $N_{\epsilon 2}$-Fe-3N are 100.4° and 113°, respectively), and the imidazole ring is not symmetric with respect to the Fe-$N_{\epsilon 2}$ bond (the angles $C_{\delta 2}$-$N_{\epsilon 2}$-Fe and $C_{\epsilon 1}$-$N_{\epsilon 2}$-Fe are 129° and 120°, respectively). Although the individual angles change slightly on energy refinement, the basic asymmetry remains. Further, displacements of the imidazole ring that reduce this asymmetry increase the nonbonded interaction energy between the histidine and the rest of the globin; the major contributions to this increase come from the F-helix and the "hydrophobic cage" (see below) that surrounds the histidine.

Fig. 4 *left* presents a potential energy map for the nonbonded interaction of a liganded heme group with the imidazole ring of His F8(87) as a function of heme orientation. The tilt angle τ and rotation axis angle α are defined in the figure caption. To simulate the liganded geometry, the calculation of this figure is made with Fe-$N_{\epsilon 2}$ shortened to 1.835 Å (relative to 1.985 Å in XRG) and with the heme coplanar with the iron atom; the Fe-$N_{\epsilon 2}$ value is about 0.1 Å shorter than the estimates for model compounds. The map shows that the untilted heme ($\tau = 0°$) is in an unstable position, due primarily to the short, repulsive 1N-$C_{\epsilon 1}$F8 (2.34 Å relative to 2.87 Å for 3N-$C_{\delta 2}$F8) and 1C4-$C_{\epsilon 1}$F8 contacts, which also produce the high energy contours in the left-hand part of the figure. A tilt of $\tau = 8°$ about the axis $\alpha = -25°$ lowers the energy by 8 kcal/mol; such a tilt moves pyrrole 1 away from and pyrrole 3 towards the F-helix. In addition, there is a weaker tendency for the heme to rotate about the Fe-$N_{\epsilon 2}$ bond. The existence of these forces, tending to reorient the heme in the liganded geometry, contrasts sharply with the unliganded situation, where the untilted heme ($\tau = 0°$) is essentially at an energy minimum.

There is no experimental evidence concerning the role of the His F8(87) in producing the heme tilt, though the existence of short nonbonded contacts between an imidazole and a planar heme has been pointed out (24). That tilting of the heme does occur on ligation is indicated by two sets of results. The first is the polarized ultraviolet absorption measurement of single crystals of hemoglobin made by Makinen and Eaton (12, 13). Because the observed Soret band is in-plane polarized, the change in the polarization ratios along the different crystal axes can be interpreted as a tilting of the heme group. As a result of comparisons of deoxy, oxy, and methemoglobin, Makinen and

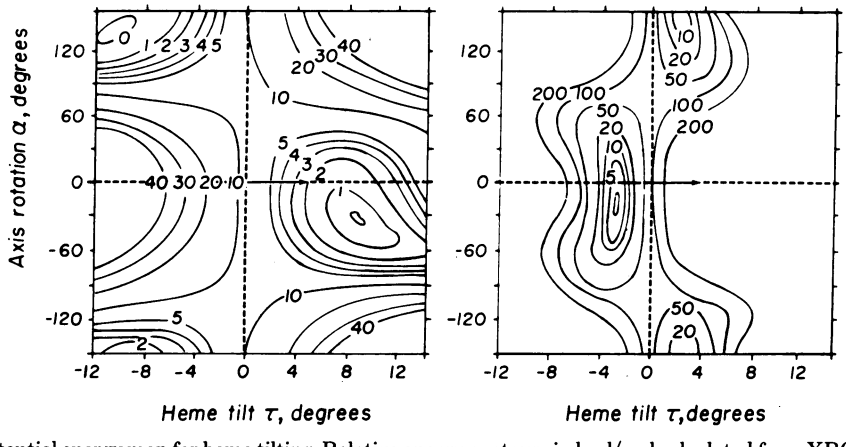

FIG. 4. Nonbonded potential energy map for heme tilting. Relative energy contours in kcal/mol calculated from XRG with porphyrin translated 0.75 Å toward proximal side; iron atom is coplanar with porphyrin and Fe-N_ϵ bond length is 1.835 Å. Vertical scale: axis about which heme tilt occurs; reference axis 0° is the line 2N-4N and 45° is 2CH-4CH. Horizontal scale: tilt magnitude; positive tilt about reference axis swings pyrrole 3 toward FG-region and pyrrole 1 toward upper E-helix. *Left.* Interaction with His F8(87) only. *Right.* Interaction with rigid globin.

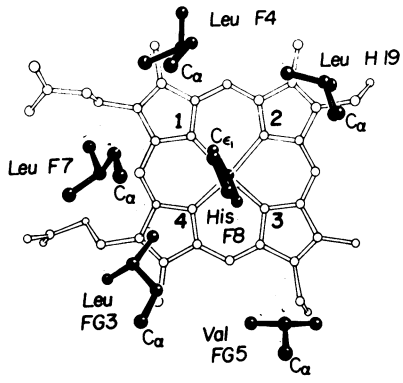

FIG. 5. View along N_ϵ-Fe bond showing hydrophobic residue cage about proximal His F8(87).

Eaton have suggested that there is a correlation between the magnitude of the tilt and the iron displacement from the heme plane; the tilt difference between deoxy and oxy is shown to be greater than 4°, though the actual magnitude and direction of the tilt were not determined. Anderson's (14) met-minus-deoxy x-ray difference map of an acrylamide gel strengthened crystal also shows a heme tilt on oxidation. Although the magnitude was not determined due to the limited resolution (3.5 Å), its direction is close to that suggested by the calculation of Fig. 4 *left*, i.e., a positive tilt ($\tau > 0°$) about an axis near the 2N-4N line (α about 0°). Both Makinen and Eaton (12, 13) and Anderson (14) have suggested that the heme tilt plays a role in initiating tertiary structural changes.

Heme–Globin Interaction. The previous section described the nature of the relative heme/histidine motion expected on ligation. For this to be able to affect the globin, there must be a mechanism that fixes His F8(87) so that it is primarily the heme that moves relative to the globin. One constraining factor is the interaction between His F8(87) and the F-helix of which it is a part. Also important is the "cage" of hydrophobic residues (Fig. 5); that they are well-positioned to hold the histidine in place is evident from Fig. 5 and energy calculations (see previous section and below). In what follows, we consider the effect on the globin of the heme tilt induced by the histidine.

Rigid Globin. From Fig. 2 it follows that ligation of the heme and the consequent undoming (Fig. 3) will lead to repulsive interaction with the globin. To determine the distribution of the forces exerted on the globin by tilting of the ligated heme, a potential energy map was calculated for heme reorientation in the presence of the rigid globin (see Fig. 4 *right*). The energy contours shown in the figure arise from the nonbonded interactions (van der Waals plus electrostatic) between the heme and the globin atoms. The largest repulsion (i.e., the steepest rise in the potential) occurs for a positive tilt about the reference axis. It is just this direction of tilt that is engendered by the heme/histidine interaction shown in Fig. 4 *left*. If the globin were rigid, no such tilt would occur because the heme interactions with the globin are larger than those with the histidine; however, the globin can relax in response to the forces resulting from the heme tilt. It is this relaxation that leads to the tertiary structural change of the subunit.

Globin Relaxation. To investigate the relaxation of the globin, a model calculation was performed in which the ERG *deoxy*hemoglobin α chain (see *Methodology*) was perturbed by introducing a heme group simulating the liganded structure; that is, a planar heme with an Fe-$N_{\epsilon 2}$ distance of 1.85 Å and a $\tau = 20°$ tilt about the reference axis ($\alpha = 0°$) was held fixed and the protein was allowed to relax in the presence of this perturbation. Although the forces in the perturbed system are initially very high, energy minimization yields a final rms force-component of 0.6, as compared with 0.5 kcal/mol-Å for ERG. The resulting rms coordinate shift is 0.061 Å, only one-third of that obtained in the XRG → ERG calculation (*Methodology*). However, in contrast to the latter, the distribution of shifts is highly skewed, with 90% of the globin atoms moving less than the rms shift and 5% more than twice its value. This implies, as is confirmed by the specific atom displacements, that the heme perturbation is propagated through the globin along a narrow and well-defined pathway.

In the heme pocket, the most affected residue is Val FG5(93), which is pushed away by pyrrole 3 with an average atom shift of nearly 1 Å. This valine, as already suggested by Anderson (14), is a key residue in the propagation of tertiary structural change. Other residues in the heme pocket that undergo significant motion are those adjacent to and interacting with Val FG5(93); they are Arg FG4(92), Leu FG3(91), and Asp G1(94). Phe G5(98), one turn of the G-helix from Asp G1(94), also moves slightly. These residue shifts can be summarized as involving a movement of the FG corner. Comparison of the deoxy and met x-ray structures shows corresponding displacements (14). On the distal side of the heme, less movement occurs in the model calculation, partly because it was done without a ligand. Lys E10(61) is pushed away by the heme, but the side chain is sufficiently flexible to damp the motion before it reaches the E-helix. Leu G8(101) moves slightly forward due to its attraction by pyrrole 3.

An essential contact between Val FG5(93) and the heme group involves pyrrole 3 and its vinyl group. The absence of the vinyl group in deuteroporphyrin may provide an explanation of its reduced cooperativity. Also, Asakura and Sono (25) have demonstrated that substitution of the vinyl by a formyl group on pyrrole 3 greatly reduces cooperativity while the corresponding substitution on pyrrole 2 has no effect. The absence of cooperativity in hemoglobin Köln, which has the β chain Val FG5(98) replaced by Met, is of interest though it has been suggested that the heme is lost from the same chain (1). It would be of importance to have structural data to permit a more detailed analysis of these results.

In addition to its essential role in the motion of the FG corner, Val FG5(93) is involved in a feedback loop stabilizing the proximal histidine and, thus, contributing to the tilting of the heme. The side chain of Leu FG3(91) supports the imidazole ring and prevents it from tilting backward or rotating to diminish its interaction with pyrrole 1. When Val FG5(93) is displaced by the heme, it pushes Leu FG3(91) toward His F8(87), thereby completing the loop. Further, as is shown by calculations of the nonbonded interactions between other globin residues and the imidazole ring of the histidine, leucines F4(83), F7(86), and H19(136) form a cage with Leu FG3(91) to help fix His F8(87) in its special position (see Fig. 5). Leu FG3(91) and Leu H19(136) also act to anchor the tilt axis of the heme, while Leu F4(83) and Leu Fa7(86) make short contacts with pyrrole 1 after ligation and help to tilt the heme.

That there is strain on the *liganded* heme when it is in a subunit that is part of a tetramer with the deoxy quaternary structure is demonstrated by a number of experimental studies (26, 27). The most striking evidence is perhaps that obtained from nitrosylhemoglobin, in which the Fe-$N_{\epsilon 2}$ bond appears to be broken or at least greatly stretched when the quaternary structure is shifted from the oxy to the deoxy form by the addition of inositol hexaphosphate (28, 29).

The region of the α subunit that is involved in the $\alpha_1\beta_2$ interface, identified by Perutz and Ten Eyck (2, 3) as an essential component of the cooperative mechanism, is significantly al-

tered in the model calculation. In addition to the displacement of the FG-region, residues in the C-helix are affected. As Val FG5(93) is repelled by pyrrole 3, it rotates the aromatic ring of Tyr C7(42) by 20° about the ring axis and displaces the hydroxyl oxygen, whose hydrogen bond with Asp G1(99) β could thus be weakened. Further, Thr C3(38) and Thr C6(41) come in contact with Tyr C7(42) and are slightly shifted.

The importance of Tyr HC2(140) in breaking the interchain salt bridges has been stressed by Perutz and Ten Eyck (2, 3). The tyrosine itself moves only slightly in the model calculation, but the motion of Val FG5(93) stretches and bends the hydrogen bond between its carbonyl oxygen and the hydroxyl group of Tyr HC2(140). This strengthens the view (8, 14) that the tertiary changes resulting from ligation do not eject the tyrosine in a tetramer that has the deoxy quaternary structure, but do weaken the $\alpha_1\alpha_2$ salt bridges by decreasing the stabilizing effect of the tyrosine-valine hydrogen bond.

Many of the residue shifts that result from the heme tilt perturbation are in accord with those observed by Perutz and his coworkers. Of particular importance in testing the validity of the protein relaxation calculation has been a detailed comparison with the already mentioned (met minus deoxy) difference map determined by Anderson (14). The complete results of this comparison will be given in a separate publication.

CONCLUSIONS

A complete reaction path is presented for the tertiary structural change that occurs on ligand binding by hemoglobin. Starting from the known deoxy geometry, it is shown by calculations with empirical energy functions and comparisons with available data how the change in heme geometry on ligation introduces a perturbation that alters the subunit structure. The initial step is the "undoming" of the heme, which results in a repulsive histidine–porphyrin contact. In the presence of the globin residues that support the histidine (including a feedback loop), this contact leads to tilting of the heme in a direction calculated to produce the maximum interaction with the globin. The relaxation of the globin in the presence of the forces engendered by the heme tilt is transmitted to the surface of the subunit by a well-localized reaction path involving only a small number of residues and yields the experimentally observed changes at the intersubunit contacts. A key residue in the reaction path is Val FG5(93), which forms part of the feedback loop and whose displacement by the heme leads in turn to other residue shifts.

The present study shows that there is little strain on the *unliganded* heme. Instead, it appears that the reduced oxygen affinity of deoxyhemoglobin results from the strain on the *liganded* subunit in a tetramer with the deoxy quaternary structure; that is, the tertiary structural changes induced by ligation require more energy if the subunit is in the deoxy than in the oxy tetramer.

The use of an isolated subunit in the calculation of the globin relaxation is an approximation that should be valid for finding the reaction path. To determine the effect of quaternary constraints the calculations would have to be extended to include more than the one subunit or be modified to hold fixed appropriate surface residues. Also, it would be of interest to supplement the static evaluation of the reaction path by a dynamical study in order to obtain a more complete picture of the motions involved.

It is hoped that the details of the reaction path suggested in this paper will serve as a stimulus for experimental work to check its validity. Of particular importance is the role of proximal histidine and its hydrophobic cage, for which improved x-ray data and mutant studies would be of great interest.

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1. Antonini, E. & Brunori, M. (1971) *Hemoglobin and Myoglobin in their Reactions with Ligands* (North-Holland, Amsterdam).
2. Perutz, N. F. (1970) *Nature* **228**, 726–734.
3. Perutz, M. F. & Ten Eyck, L. F. (1972) *Cold Spring Harbor Symp. Quant. Biol.* **36**, 295–310.
4. Edelstein, S. J. (1975) *Annu. Rev. Biochem.* **44**, 209–232.
5. Shulman, R. G., Hopfield, J. J. & Ogawa, S. (1975) *Quart. Rev. Biophys.* **8**, 325–420.
6. Monod, J., Wyman J. & Changeux, J. P. (1965) *J. Mol. Biol.* **12**, 88–118.
7. Szabo, A. & Karplus, M. (1972) *J. Mol. Biol.* **72**, 163–197.
8. Szabo, A. & Karplus, M. (1976) *Biochemistry* **15**, 2869–2877.
9. Gelin, B. R. & Karplus, M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2002–2006.
10. Hoard, J. L. (1968) in *Structural Chemistry and Molecular Biology*, eds. Rich, A. & Davidson, N. (W. H. Freeman, San Francisco, Calif.), pp. 573–594.
11. Hoard, J. L. (1971) *Science* **174**, 1295–1302.
12. Makinen, M. W. & Eaton, W. A. (1974) *Nature* **247**, 62–64.
13. Makinen, M. W. (1975) in *Techniques and Topics in Bio-Inorganic Chemistry*, ed. McAuliffe, C. A. (The MacMillan Press, London), pp. 39–43.
14. Anderson, L. (1973) *J. Mol. Biol.* **79**, 495–506.
15. Ibers, J. A., Lauher, J. W. & Little, R. G. (1974) *Acta Crystallogr. Sect. B* **30**, 268–272.
16. Fermi, G. (1975) *J. Mol. Biol.* **97**, 237–256.
17. Hoard, J. L. & Scheidt, W. R. (1972) *Proc. Natl. Acad. Sci. USA* **70**, 3919–3922.
18. Collman, J. P., Kim, N., Hoard, J. L., Lang, G., Radonovich, L. J. & Reed, C. A. (1974) in *Abstracts of the 167th Meeting of the American Chemical Society* (Los Angeles), INOR 29.
19. Hopfield, J. J. (1973) *J. Mol. Biol.* **77**, 207–222.
20. Huynh, B. H., Papaefthymion, B. C., Yen, C. S., Groves, J. L. & Wu, C. S. (1974) *J. Chem. Phys.* **61**, 3750–3758.
21. Eisenberger, P., Schulman, R. G., Brown, G. S. & Ogawa, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 491–495.
22. Spiro, T. G. & Burke, J. M. (1976) *J. Am. Chem. Soc.* **98**, 5482–5489.
23. Radonovich, L. J., Bloom, A. & Hoard, J. L. (1972) *J. Am. Chem. Soc.* **94**, 2073–2078.
24. Little, R. G. & Ibers, J. A. (1974) *J. Am. Chem. Soc.* **96**, 4452–4463.
25. Asakura, T. & Sono, M. (1974) *J. Biol. Chem.* **249**, 7087–7093.
26. Perutz, M. F., Heidner, E. J., Ladner, J. E., Beetlestone, J. G., Ho, C. & Slade, E. F. (1974) *Biochemistry* **13**, 2187–2200.
27. Ogawa, S. & Schulman, R. G. (1972) *J. Mol. Biol.* **70**, 315–336.
28. Perutz, M. F., Kilmartin, J. V., Nagai, K., Szabo, A. & Simon, S. R. (1976) *Biochemistry* **15**, 378–387.
29. Maxwell, J. C. & Caughey, W. S. (1976) *Biochemistry* **15**, 388–396.