SEMIEMPIRICAL CALCULATIONS OF MODEL DEOXYHEME

VARIATION OF CALCULATED ELECTROMAGNETIC PROPERTIES WITH ELECTRONIC CONFIGURATION AND DISTANCE OF IRON FROM THE PLANE

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ABSTRACT The electronic structure of deoxyheme units and the electric field gradient at the iron nucleus are calculated as a function of the displacement of the iron from the heme plane. The high spin state has a calculated minimum energy with the iron out of plane at a distance similar to that observed for metMb. This could correspond to a relaxed form of deoxyHb, while the greater displacement observed in the intact protein could be strained. The calculated electric field gradient is relatively insensitive to iron displacement from the heme plane. This insensitivity could account for the very similar values of quadrupole splittings observed in low-affinity deoxyhemoglobin A and its separate high-affinity α and β subunits, and still allow small differences in iron displacement consistent with the stereochemical trigger mechanism of cooperativity.

In normal deoxyhemoglobin A (deoxyHb A) the iron is in a five-coordinated high-spin ferrous state, axially bound to an imidazole ring of the proximal histidine residue (F8) and about 0.6 Å from an apparently planar porphyrin ring (1). By comparison, in a model deoxytetraphenyl porphyrin compound with a 2-methyl imidazole ligand the iron is 0.42 Å from the mean plane of the pyrrole nitrogens (2) and 0.55 Å from the mean plane of the porphyrin ring, which has an unusually large "doming" distortion.

When oxygen or any of a number of small molecules binds to deoxyHb A, the iron moves into the plane of the porphyrin ring and the complex becomes diamagnetic. The nearly planar iron position in a liganded form has been verified in a neutron diffraction study of carboxymyoglobin (3). The proximal histidine residue moves with the iron and the iron-imidazole nitrogen bond length is thought to remain at the same value (4) as in deoxyHb A (2.06 Å) or to be shortened by 0.1–0.2 Å (5).

The oxygen affinity of the fully combined oxyhemoglobin is about 70 times greater than that of deoxyHb (6,7). Free α and β subunits or high affinity mutants such as Hb Kempsey have an oxygen affinity equivalent to the fully combined oxyhemoglobin but retain a high-spin ground state (8). However, the structure of these high-affinity forms of deoxyHb is unknown. Odd electron Co(II) analogues of deoxyHb remain in a

$S = \frac{1}{2}$ spin state during oxygenation and yet demonstrate cooperativity. Recent X-ray analyses indicate a small movement of 0.25 to 0.36 Å by Co(II) into the heme plane (2). As communicated by Perutz, the ratio K_4/K_1 in the presence of organic phosphates is about 15 compared to 500 for ferrous deoxyHb A. Thus a concerted physical movement of the metal and the histidine residue into the plane upon oxygenation, but not a spin state change, appears to be a requirement for cooperativity.

The cause of this movement and its relationship to the observed increase in oxygen affinity as deoxyHb A subunits bind oxygen are long-standing questions still under active experimental and theoretical investigation. Building on a suggestion by Hoard (9), Perutz originally proposed a sequential process by which the concerted movement of the iron and F8 histidine residue towards the heme plane triggers a series of changes in the positions of key amino acids and in the interactions within and among the four heme subunits. He has shown how these changes could account for cooperativity of O_2 (ligand) binding, the Bohr effect, regulation of oxygen affinity by organic phosphates, and a change in the quaternary structure of normal hemoglobins from the deoxy (T) to the oxy (R) form (5,10–12). Perutz and TenEyck have suggested that the low oxygen affinity of the T structure may be related to increased tention (t) at the heme in each subunit, which constrains the iron atom to be further away from the plane of the porphyrin than its equilibrium position and thus opposes its movement into the ring upon reaction with oxygen. Oxygenation at one unit could cause a change in tertiary structure (r) at a neighboring unit which enhances its oxygen affinity and subsequently causes a change to the high-affinity quaternary structure (R).

A number of recent calculations have addressed the question of the origin of the conformation change and strain energy associated with ligand binding. Hopfield (13), using a simple model of two springs, suggests that the heme-heme interaction energy is distributed among many degrees of freedom in the protein instead of the heme unit itself. Gelin and Karplus (14), using an empirical energy function, describe oxygen binding to an isolated Hb A α chain. They find little strain on the unliganded hemoglobin but suggest 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. However, in these calculations crucial assumptions are made that the deoxy structure is domed and that the Fe-imidazole nitrogen distance is shortened to 1.835 Å in the liganded geometry. There is no experimental evidence for so short a bond in the oxy form nor for nonplanar heme units in intact deoxyHb A and steric constraints resulting from these changes may be overestimated. Warshel (15), using a combination of empirical and quantum mechanical techniques, suggests that it is steric interaction between the oxygen and heme nitrogens that causes the change in heme geometry and that relaxation occurs by an increase in the hole size of the porphyrin ring to accomodate the in-plane iron. Finally, *ab initio* generalized valence bond (GVB) calculations of Olafson and Goddard (16) on the force constant associated with the movement of the iron into the heme plane indicate that a very small displacement of 0.04 Å of the iron towards the plane is enough to account for the differences in bonding energy between low and high affinity deoxyHb forms.

Thus the possibility exists that strain energy is stored by the heme unit in a number of different modes, including the displacement of iron from the heme plane.

Definitive experimental verification of the presence or absence of a partial movement of the iron into the porphyrin plane in high affinity deoxy forms has been difficult to obtain. Electronic and nuclear magnetic resonance spectra of high and low affinity deoxyhemoglobins have differences clearly associated with changes at the heme unit. The interpretation of these combined results (17,18) is consistent with, but not proof of, an increased displacement of the iron atom from the plane of the porphyrin ring in going from the R to the T form.

X-ray absorption spectra of deoxyHb Kempsey, which has an abnormal R form, have been compared to that for normal deoxyHb A (19). Differences in backscattering patterns between the two proteins indicate changes in the distances of iron to all of its neighboring atoms. Analyses of the data indicate that the Fe-N distances are the same to within ±0.02 Å. These results seem to rule out the possibility that shortening of the $Fe\text{-}N_{imid}$ distance occurs in a high affinity form but does not rule out a small movement of the iron towards the heme plane. A breathing motion of the porphyrin ring could keep the $Fe\text{-}N_{pyr}$ distances nearly constant as the iron moves into the plane. Even if the porphyrin ring is rigid, a change in $Fe\text{-}N_{pyr}$ distance of 0.02 Å corresponds to a movement of the iron 0.1 – 0.2 Å closer to the heme plane.

The Mössbauer resonance of intact deoxyHb A and isolated high-affinity α and β chains have recently been measured (20,21). Identical spectra are obtained at $T >$ 100°K, with small differences found among the three systems below 100°K. The inference is that there is no major change in electronic structure around the iron in these high and low affinity species. However, the sensitivity of the quadrupole splitting (ΔE_Q) to the movement of the iron towards the heme plane has not been established. It remains a possibility that small movements of the iron would not appreciably affect the electric field gradient at the iron nucleus and therefore the measured values of the quadrupole splitting.

Despite a good deal of experimental effort, then, the question of the position of the iron in high affinity deoxyheme units is not resolved. In this study, the techniques of quantum chemistry are used to consider further whether high and low oxygen affinity forms of deoxyheme could involve different degrees of displacement of the iron from the porphyrin plane. To this end, the electronic structure, energy, spin state, and electric field gradients at the iron nucleus have been calculated as a function of extent of displacement of the iron from the porphyrin ring. The results indicate that a change from a low to high affinity form in deoxyheme could involve a partial movement of the iron towards the plane and that this movement need not cause significant changes in Mössbauer resonance spectra or alter the high-spin ground state of the normal deoxyHb unit.

METHODS

The molecular model used for this study of the deoxyheme unit is shown in Fig. 1. The geometry of the porphyrin ring and imidazole ligand are taken from the X-ray structure of a model

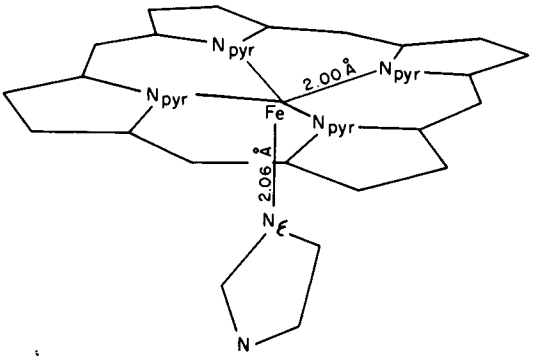

FIGURE 1 Model of deoxyheme unit. Iron atom displaced 0.0, 0.25, 0.42, and 0.75 Å from mean plane of pyrrole nitrogens (with iron-pyrrole nitrogen distance 1.95, 1.98, 2.00, 2.10 Å), assuming a constant iron-imidazole nitrogen bond length (2.06 Å). Coordinate axes defined with pyrrole nitrogen on $\pm x$ and $\pm y$ axes and imidazole nitrogen on $-z$ axis. The plane of the imidazole moiety is out of the xz plane, corresponding to a 22° rotation about the iron-imidazole nitrogen axis ($\tau_{CN_\epsilon FeN_{pyr(x)}} = 22°$).

oxyheme unit (22). In this structure, the porphyrin hole radius is 1.95 Å. In a sequence of calculations, the iron is moved to specific distances from the porphyrin plane without changing this radius or any other features of the geometry. The corresponding decrease of the Fe-N_{pyr} distance with the increasing extent of planarity of the iron is shown in Table I.

Electron distributions and orbital energies were calculated for a series of geometries with the iron 0.0, 0.25, 0.42, and 0.75 Å out of the plane of the porphyrin nitrogen atoms. For each of these geometries, calculations were made for the high spin (S = 2), intermediate (S = 1), and low spin (S = 0) configurations listed in Table II. A semiempirical molecular orbital program based on the iterative extended Hückel theory (IEHT) and parameterized for metal porphyrin complexes (23) was used for all the calculations. The IEHT method yields an electron distribution in terms of a set of occupied molecular orbitals and also a configuration energy as a sum of molecular orbital energies.

Variations of configuration energies with specific geometry changes often parallel the behavior of the total energy. Thus, the variation of configuration energies of high spin, intermediate

TABLE I

VARIATION IN Fe-N_{pyr} BOND LENGTH ($r_{Fe\text{-}N_{pyr}}$) AS A FUNCTION OF THE DEGREE OF NONPLANARITY OF THE IRON (z_{Fe})

z_{Fe}	$r_{Fe\text{-}N_{pyr}}$
Å	Å
0.00	1.95
0.25	1.98
0.42	2.00
0.50	2.02
0.60	2.05
0.75	2.10

TABLE II

DEFINITION OF ELECTRONIC CONFIGURATIONS INCLUDED IN PRESENT STUDY OF MODEL DEOXYHEME UNIT

	Configuration						
	I	II	III	IV	V	VI	VII
$\|d_{x^2-y^2}$	↑	↑	↑				
$\|d_{z^2}$	↑	↑	↑	↑	↑	↑	
$\|d_{xz}>$	↑ ↓	↑	↑	↑ ↓	↑ ↓	↑	↑ ↓
$\|d_{yz}>$	↑	↑ ↓	↑	↑ ↓	↑	↑ ↓	↑ ↓
$\|d_{xy}>$	↑	↑	↑ ↓	↑	↑ ↓	↑ ↓	↑ ↓
S	2	2	2	1	1	1	0

Definition given in terms of occupancy of the five molecular orbitals localized primarily on the *d*-type atomic orbitals of iron. All remaining filled ligand-based molecular orbitals are doubly occupied.

spin, and low spin states as a function of the out-of-planarity of the iron atom provides a qualitative idea of how the total energy of each spin state would vary. However, the results cannot be used without exchange corrections to compare energies of different spin states, i.e. to determine whether a high or low spin state is the ground state as the iron moves into the porphyrin plane. For such a comparison, previously estimated exchange energy corrections for

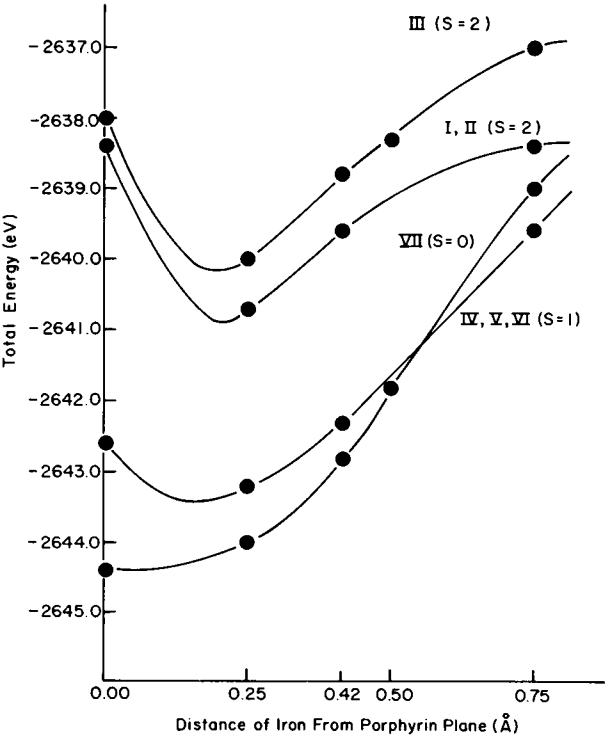

FIGURE 2 Variation of configuration energy with extent of nonplanarity of the iron atom for each configuration of deoxyheme.

quintet and triplet states relative to singlet states for iron(II) were added to their configuration energies as an estimate of total energy variation. Another indication of a spin state change is the relative energies of the five *d*-type orbitals. The larger the energy differences among these orbitals, the lower the probable multiplicity of the ground state.

Electron distributions obtained from IEHT results for each configuration and geometry were used to calculate the electric field gradient at the iron nucleus and the corresponding value of quadrupole splitting. By a procedure described elsewhere (24), all nine components of the field gradient were calculated and the 3 × 3 matrix diagonalized to find the principal axis value used to calculate the quadrupole splitting. The effect of contributions from several low-lying quintet states on the observed quadrupole splitting was also calculated. The main object of this part of the study was to determine the sensitivity of the field gradient and quadrupole splitting to the movement of the iron into the plane.

RESULTS

The variation of configuration energy with the degree of nonplanarity of the iron atom is given in Fig. 2 for high, intermediate, and low spin states. From this figure, it is observed that the low spin configuration has a minimum energy with the iron in the porphyrin plane, whereas the intermediate and high spin configurations have a mini-

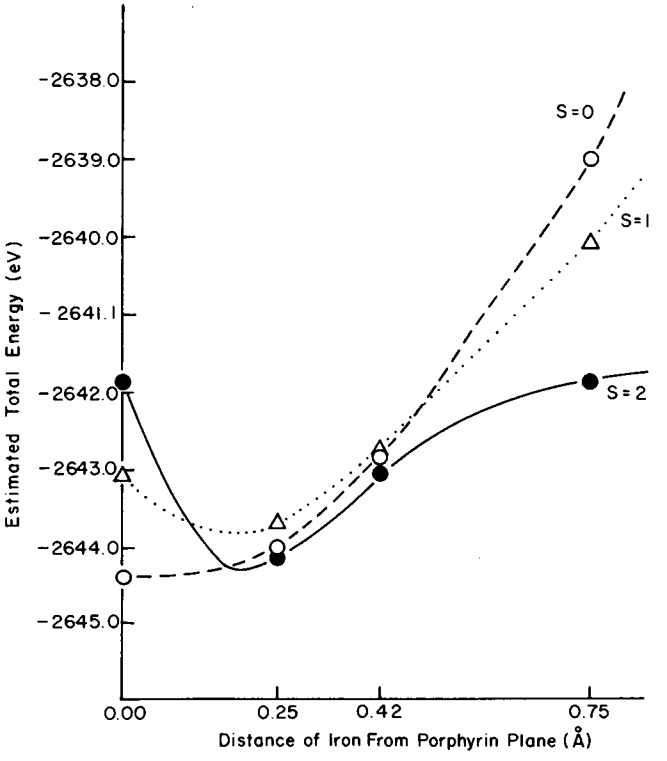

FIGURE 3 Variation of estimated total energy (configuration energy plus exchange corrections) with extent of nonplanarity of the iron atom for the most stable singlet, triplet, and quintet states.

mum energy with the iron out of the plane. If an exchange correction similar in magnitude to that previously suggested (23) is added to the configuration energy calculated for the quintet and triplet states, approximate total energy curves as shown in Fig. 3 are obtained. This figure provides a qualitative idea of how the ground state spin might change as the iron moves into the plane.

Fig. 4 shows the variation in orbital energy splittings of the five *d*-type delocalized molecular orbitals which can contain unpaired spins with the degree of nonplanarity of the iron. As the iron moves into the plane, the orbital energy separations increase, indicating greater preference for a low or intermediate spin ground state.

Calculated quadrupole splittings for each configuration as a function of the distance of the iron from the plane of the porphyrin nitrogens are summarized in Table III.

DISCUSSION

The energy curve for the $S = 2$ configuration has a minimum at $z_{Fe} \simeq 0.2$ Å with a porphyrin radius of 1.95 Å. This result compares very favorably with a generalized valence bond calculation on a simpler model for a deoxyheme unit. It is significantly smaller than the extent of nonplanarity determined for intact protein and model deoxy systems (0.42–0.60 Å). Thus, it is possible that protein and crystal constraints can keep

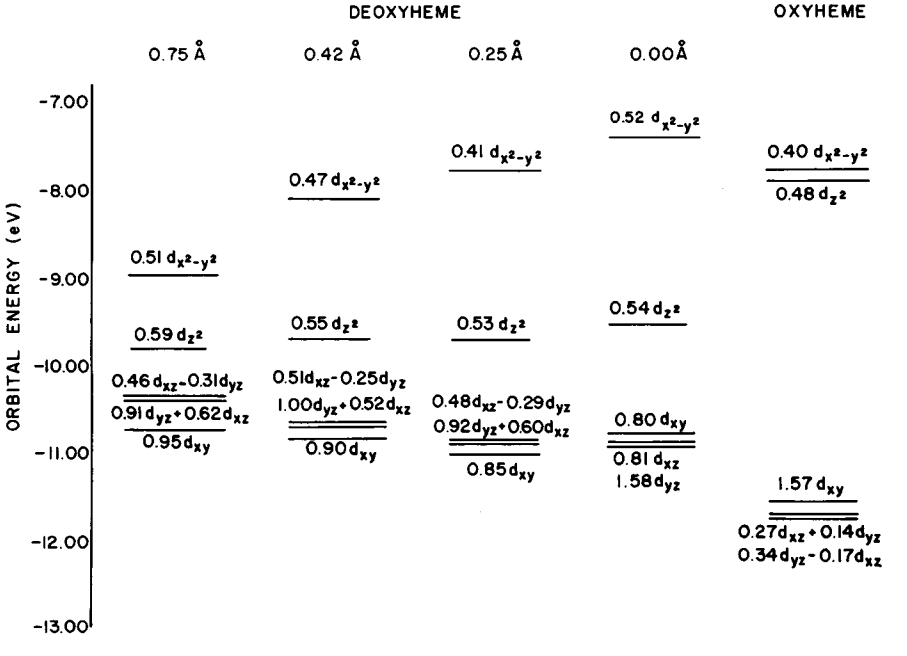

FIGURE 4 Variation of orbital energy and degree of delocalization of the five highest occupied molecular orbitals of deoxyheme (configuration II) with extent of nonplanarity of the iron atom. For comparison the same orbitals are shown for oxyheme. The d_{xz} and d_{yz} atomic orbitals mix by symmetry considerations. The x and y axes are nearly but not exactly equivalent.

TABLE III

QUADRUPOLE SPLITTINGS AND ANISOTROPY CALCULATED* AS A FUNCTION OF ELECTRON CONFIGURATION AND DISTANCE OF IRON FROM PORPHYRIN PLANE IN MODEL DEOXYHEME

Configuration§	z_{Fe}(Å)	ΔE_Q‡ (η) 0.75	0.42	0.25	0.00	$V_{ii\,max}$‖
I		2.44 (0.17)	2.23 (0.17)	2.06 (0.26)	2.25 (0.47)	V_{yy}
II		2.69 (0.05)	2.62 (0.02)	2.71 (0.07)	2.12 (0.48)	V_{xx}
III		2.61 (0.08)	2.56 (0.09)	2.32 (0.09)	2.98 (0.07)	V_{zz}
IV		−4.39 (0.05)	−4.19 (0.10)	−4.10 (0.11)	−4.01 (0.13)	V_{zz}
V		2.37 (0.68)	2.33 (0.81)	¶	2.33 (0.92)	V_{yy}
VI		2.10 (0.66)	1.95 (0.82)	¶	1.97 (0.95)	V_{xx}
VII		0.77 (0.60)	0.93 (0.63)	0.94 (0.59)	0.93 (0.50)	V_{zz}
I + II		−1.44 (0.29)	−1.40 (0.73)	−1.36 (0.67)	−0.74 (0.60)	V_{zz}
V + VI		−0.40 (0.70)	0.26 (0.42)	¶	0.20 (0.34)	V_{yy}**

*ΔE_Q in millimeters per second calculated from $\Delta E_Q = 8(1-R)\langle r^{-3}\rangle Qq[1+\eta^2/3]^{1/2}$ where $(1-R)$ = Sternheimer shielding factor = 0.68, Q = 0.187 Barns, $\langle r^{-3}\rangle$ = 5.00 AU, and the principal axis values of the field gradient ordered $|V_{ii}| > |V_{jj}| > |V_{kk}|$ such that $q \equiv$ largest magnitude V_{ii} and $\eta \equiv (V_{kk} - V_{jj})/V_{ii}$ $(0 \leq \eta \leq 1)$. Sign of ΔE_Q is sign of largest magnitude V_{ii} (V_{ii} max). Anisotropy (η) in parentheses.

‡Experimental values of ΔE_Q are 2.40 ± 0.02 mm/s at 4.2°K and 1.93 mm/s at 200°K for intact Hb A; 2.41 and 2.47 for isolated α and β subunits at 4.2°K. (Ref. 20, 28).

§Electronic configurations defined in Table I. For the energetically degenerate states I and II, an additional configuration designated as I + II is considered. The quadrupole splittings for this mixed configuration are calculated assuming an equal contribution to the field gradient from the two pure configurations. In the same manner, V + VI is formed from the degenerate pure configurations V and VI.

¶Not calculated.

‖Direction of largest magnitude V_{ii}. V_{xx} and V_{yy} are in the plane of the porphyrin ring. V_{zz} is perpendicular to this plane.

**For a distance of 0.75 Å, direction of largest magnitude V_{ii} is V_{zz}.

the iron in a tense conformation, i.e., prevent it from moving to its equilibrium position in a high spin configuration of normal deoxyheme.

Experimental measurements of the extent of the nonplanarity of the iron have not yet been obtained for any high affinity form such as isolated α and β subunits of deoxyHb A or an abnormal deoxyhemoglobin which crystalizes in the R form (6). However, in methemoglobin (25) which also crystalizes in the R form, the iron is about 0.40 Å nearer to the porphyrin plane than in deoxyHb units (1). In metmyoglobin (26) the iron is about 0.15 Å nearer to the heme and nitrogen planes than in deoxyMb (27). These combined results suggest that the relaxed deoxy form could correspond to a movement of the iron about halfway towards the porphyrin plane from a conformation of higher energy to one closer to the minimum energy for high spin complex.

IEHT as well as GVB results predict that at a distance greater than 0.15 Å from the heme plane, the iron would remain in a high-spin ground state. However, in contrast to GVB, we predict from estimated total energies (Fig. 3) and from calculated orbital energies (Fig. 4) that with the iron closer to the heme plane an intermediate or low spin state would result.

As summarized in Table III, a small decrease in the extent of nonplanarity of the iron atom does not correspond to a significant change in the observed quadrupole splitting. The calculated change in ΔE_Q as a function of the extent of nonplanarity of the iron atom (z_{Fe}) is very small. For example, a variation of z_{Fe} from ~0.42 to 0.25 Å, a larger movement than need occur, causes a decrease of about 3–10% in ΔE_Q calculated for two of the quintet configurations (I and III) and a 3% increase for the third (II) compared to a similar increase in measured values of ΔE_Q between intact and separate α or β subunits of deoxyHb A. Even with the uncertainties inherent in the calculations, such small variations are indicative of the lack of sensitivity of measured values of ΔE_Q to the degree of nonplanarity of the iron as long as the system remains in a high-spin state. As seen in Table III, low-spin states would give a totally different value of ΔE_Q.

Some indication of the reliability of the results is that the lowest energy quintet configurations (I and II) both give good agreement with the measured low temperature value of ΔE_Q (2.38 mm/s) (20,28) and the known direction of the principal field gradient (in the heme plane) (28). The third quintent state of higher energy has a comparable magnitude of ΔE_Q but a principal field gradient perpendicular to the heme plane. Any mixing of the three quintet states with a pair of electrons in the d_{xy}, d_{xz}, or d_{yz} orbital, respectively, necessarily increases the symmetry of the electron distribution at the iron nucleus and consequently lowers the calculated value of ΔE_Q. This mixing can occur through a number of mechanisms, including thermal averaging of states or conformations and spin-orbit coupling. Regardless of the origin of the mixing, the qualitative effect will always be a lowering of the field gradient relative to any one configuration alone. For example, a contribution of configurations I, II, and III to each field gradient component in the ratio 40:2:1 lowers ΔE_Q from a value of 2.23 to 2.01 mm/s at z_{Fe} = 0.42 Å. This value is close to the observed high temperature quadrupole splitting of 1.93 mm/s and illustrates, as have previous extensive calculations (20,28), that such mixing can account for the observed lowering of ΔE_Q for deoxyHb A at high temperatures. This mixing also provides a plausible mechanism for further reducing the already small effect on ΔE_Q of a movement of the iron atom towards the heme plane. As shown in Fig. 4, both the energy separations of these three configurations as well as the nature of the molecular orbitals vary somewhat as a function of z_{Fe}. It is possible that these variations have compensatory effects on ΔE_Q. Since the IEHT method does not allow accurate state energy determinations, it cannot be used to investigate whether the small sensitivity of ΔE_Q to variations of z_{Fe} would be further reduced by this mechanism.

CONCLUSION

The results presented here lend support to the stereochemical trigger mechanism for cooperativity, i.e., that a movement of the iron towards the plane of the heme unit could play a crucial role in the observed cooperativity of oxygen (ligand) binding to hemoglobins. This movement can release enough energy to account for the high

affinity of the abnormal *r* form while still maintaining a high spin ground state. The postulated difference between the *t* and *r* forms of deoxyheme units is a relatively small movement (≤ 0.2 Å) of the iron towards the heme plane. X-ray absorption spectra of normal (Hb A) and high-affinity (Hb Kemsey) deoxy proteins are not inconsistent with such a displacement of the iron. Further, calculated values of the electric field gradient at the iron nucleus indicate a lack of sensitivity to such movement, making this hypothesis also consistent with Mössbauer resonance results.

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