>NH is methylamine, $K_I \sim 10^{-7.5}$,[72] $K_a'' = 10^{+0.46}$,[73] and K_a is calculated from eq 45 to be $10^{+7.6}$. Hence, K_T is 10^{-8} and K_T^+ is 10^{+7}.

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The equilibration of *O*-methylvalerolactim to *N*-methylvalerolactam at 130° in the liquid phase is accompanied by an enthalpy change of −17.4 kcal/mol or −14.1 ± 3.5 kcal/mol in the gas phase[74] in qualitative agreement with the above calculations.

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The Origin of the Heme Cotton Effects in Myoglobin and Hemoglobin[1-3]

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Abstract: The induced heme optical activity in heme proteins has been investigated theoretically. Myoglobin and hemoglobin were studied for their three-dimensional structures are known. The rotational strengths of the heme π–π^* transitions in these two proteins (Q, B (Soret), N, and L bands) were calculated according to Kirkwood's theory as extended by Tinoco, using atomic coordinates provided by Kendrew and Watson and by Perutz. We have examined several possible contributions, but the only one which can account for the observed Cotton effects is a coupled oscillator interaction between the heme transitions and allowed π–π^* transitions in nearby aromatic side chains. The calculated Soret rotational strengths are 0.3 and 0.1 DBM for myoglobin and hemoglobin, respectively, while the experimental values are 0.5 and 0.2 DBM. Calculations for other heme transitions—the Q, N, and L bands—lead to qualitative agreement with experiment in both proteins. The near degeneracy of the Soret transition has interesting consequences for the shape of the Soret CD band. The results indicate that the two Soret components are polarized toward the bridging methine carbons. Calculations on horse oxyhemoglobin show that the interactions of the heme in one subunit with aromatic groups in another subunit of the $\alpha_2\beta_2$ tetramer are not negligible. Heme Cotton effects of mutant hemoglobins and other heme proteins are discussed, based on the mechanism identified in myoglobin and hemoglobin.

One of the most interesting aspects of the optical activity of heme transitions in heme proteins is the induced nature of the Cotton effects. Heme proteins contain a prosthetic group which is a derivative of porphyrin. Because of its symmetry, the porphyrin alone is optically inactive. When it is bound to the protein, induced Cotton effects arise from the heme–protein interaction. In principle, the sign and magnitude of these effects should yield information about the nature of the heme binding site. The use of optical rotation to determine the helical content of proteins has been known for a long time. However, the study of protein conformation by the induced optical activity of prosthetic groups is a virtually unexplored field.[4]

Recently, Cotton effects associated with heme electronic transitions have been studied experimentally in a number of heme proteins, such as myoglobin,[5-9] hemoglobin,[5,10-13] cytochrome *c*,[11,14,15] and horseradish peroxidase.[4,8] A great variety in the signs, magnitudes, and shapes of the circular dichroism and optical rotatory dispersion curves has been observed for the heme transitions in these proteins. This indicates that the origin of the heme Cotton effects may be rather complicated. Several proposals have been made for the origin of these Cotton effects,[7,16] but no extensive study has been carried out.

In this paper we report theoretical calculations of the heme rotational strengths in myoglobin and hemoglobin. These molecules were chosen because their three-dimensional structures are known in considerable detail.[17,18] The origin of heme Cotton effects in other heme proteins will also be discussed based on the mechanism identified in hemoglobin and myoglobin.

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(1) Based in part on a thesis submitted by Ming-Chu Hsu in partial fulfillment of the requirements for the Degree of Doctor of Philosophy at the University of Illinois, 1970.
(2) Presented in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969.
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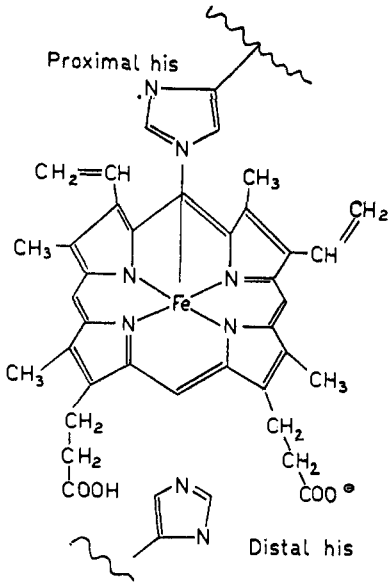

Figure 1. Structure of the heme group (protoheme IX) and its binding to the globin in myoglobin and hemoglobin.

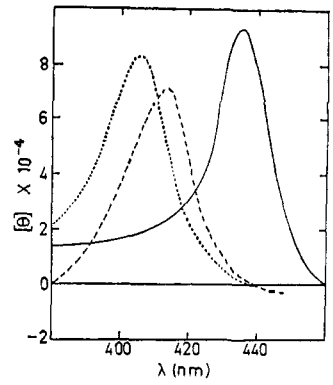

Figure 2. Circular dichroism of myoglobin in the Soret region. The heme concentrations were 13 μM in 0.1 M phosphate buffer, pH 7.5. Ellipticities, [θ], are on a molar heme basis: ---, oxymyoglobin; ——, deoxymyoglobin; · · ·, metmyoglobin. (Data taken from ref 24. Reproduced by permission of the authors and of the American Society of Biological Chemists, Inc.)

Experimental Aspects

Before we discuss the heme Cotton effects, a brief description of the absorption bands which are responsible for these Cotton effects is desirable. The hemes in myoglobin and hemoglobin are ferro- or ferriprotoporphyrin IX (Figure 1). One feature common to the absorption spectra of all heme proteins is the occurrence of an intense band in the region 400–430 nm ($\epsilon_{mM} \sim 120$–170). This band is known as the Soret band (or B band) and is assigned as a π–π^* transition of the porphyrin.[19] In the visible region there is a much weaker system between 500 and 600 nm. For most of the low-spin species, such as oxymyoglobin and oxyhemoglobin, only two bands are observed in this region (the Q band or the α and β bands). These two bands are assigned to different vibronic components of an electronic transition which is also assigned as a porphyrin π–π^* transition.[19] However, two extra bands are observed in the visible region for most of the high-spin species. These two extra bands have been assigned to charge-transfer bands,[20,21] but this assignment is not certain. The intensities of the Q bands are about 0.01–0.02 that of the Soret band.

In free-metal porphyrins two absorption bands, the N and L bands (or δ and ϵ bands), are observed in the ultraviolet,[22] but the L band is obscured in heme proteins because of the absorption of aromatic side chains. The N and L bands are also considered to arise from porphyrin π–π^* transitions.[23]

Sperm whale myoglobin, the three-dimensional structure of which is known in great detail[17] and on which we carried out the theoretical investigation, consists of a single polypeptide chain with one heme per molecule having a molecular weight of about 16,000. More than two-thirds of the 153 amino acids are involved in helical structures. There are eight helices (denoted by A, B, C... H) separated by nonhelical segments (AB, BC, CD...GH). The heme group is in a crevice surrounded by mostly hydrophobic side chains. Its iron is coordinated with a histidine residue (the proximal histidine) of the polypeptide chain (Figure 1). The sixth ligand of iron can be O_2, H_2O, CO, CN^-, OH^-, etc. On the side of the heme plane opposite that of the proximal histidine, another histidyl residue (the distal histidine) is located about 4 Å away from the iron.

The Soret CD of oxy-, deoxy-, and metmyoglobin from horse heart[24] are shown in Figure 2. The Soret CD of myoglobin from other species is essentially the same as that from horse heart. All the myoglobin derivatives studied so far exhibit a positive Cotton effect in the Soret region.[5–9] Its magnitude and position depend on the oxidation state of iron and the nature of the ligand. From the data of Beychok[7] and Willick, Schonbaum, and Kay[8] we estimate the Soret rotational strength in metmyoglobin to be 0.5 ± 0.05 DBM (1 DBM = 0.9273×10^{-38} cgs unit).

In the ultraviolet, myoglobin exhibits a positive Cotton effect at 260 nm and a weak, negative CD band between 300 and 350 nm[25] (Figure 3). The magnitude of the 260-nm CD band is comparable to that of the Soret band. So far only a few ORD and CD measurements on myoglobin in the visible region have been reported.[6,7] Oxymyoglobin shows a positive ORD Cotton effect between 500 and 600 nm.[6] Its magnitude is about 10% that of the Soret band. As is the case with absorption spectra, the visible and uv Cotton effects are very sensitive to changes in ligand and oxidation state of iron.

All mammalian hemoglobins have a molecular weight of approximately 67,000 and are essentially tetrameric, consisting of four peptide chains, to each of which is bound a heme group. The X-ray diffraction studies of Perutz, *et al.*,[18] have revealed the three-dimensional structure of hemoglobin in considerable detail. Human and horse hemoglobin molecules consist of two pairs of identical polypeptide chains, usually designated as $\alpha_2\beta_2$. The horse α and β chains are different from human α

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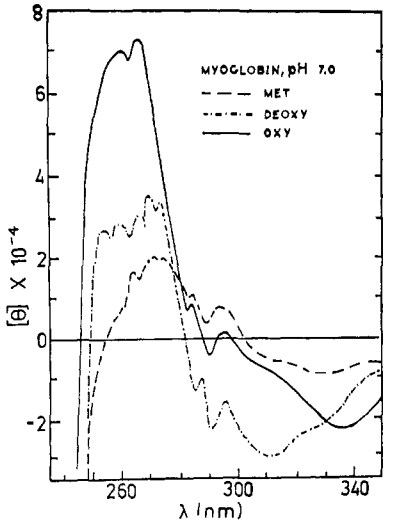

Figure 3. Circular dichroism of myoglobin in the ultraviolet. Ellipticities on a molar heme basis. (Data taken from ref 25. Reproduced by permission of the author and of the American Society of Biological Chemists, Inc.)

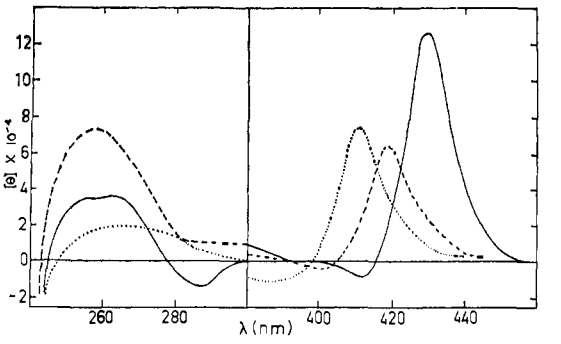

Figure 4. Circular dichroism of human hemoglobin. Conditions as for Figure 2. Ellipticities are on a molar heme basis: - - -, oxyhemoglobin; ——, deoxyhemoglobin; · · ·, methemoglobin. (Data taken from ref 24. Reproduced by permission of the authors and of the American Society of Biological Chemists, Inc.)

and β chains in amino acid composition. Each chain has 140–145 amino acids and a tertiary structure similar to that of myoglobin. The coordination of heme to the polypeptide chain is also through a histidyl residue. The four chains are so arranged that there is a twofold axis of symmetry[26] and the four hemes are separated by 25–35 Å.

Among hemoglobins of different species, human hemoglobin is the one on which optical activity studies have been carried out most extensively. We have performed theoretical calculations of heme rotational strengths by using atomic coordinates of horse oxyhemoglobin, but comparison will be made with the CD data on human hemoglobin. We expect only minor differences in the heme Cotton effects of these two kinds of hemoglobins.

Figure 4 shows the CD spectra of human hemoglobins in the Soret and uv region.[24] The spectra are very similar to those of myoglobin except that there is a negative trough in the Soret region. Recently Li and Johnson[13] reported that high-spin hemoglobin derivatives show four-band CD spectra in the visible region, while low-spin derivatives give only two-band spectra. The intensities of these visible CD bands are about 0.01 that of the Soret band.

Theoretical Background

The theory on which these calculations are based is Kirkwood's theory[27] as extended by Tinoco.[28] The theory has been described in detail[28] and only the final expressions used in this work are given here for convenience.

The underlying assumption of Kirkwood's theory[27] is that the molecule can be divided into N groups and that there is no charge transfer between them. We will be concerned with heme transitions which are electrically allowed. The rotational strength for an electrically allowed, magnetically forbidden transition, a, in the ith group of a molecule, is[28]

$$R_{ia} = -\left\{\frac{2\pi}{c}\sum_{j\neq i}\sum_{b\neq a}\frac{V_{ioa;job}\nu_a\nu_b\mathbf{R}_{ij}\cdot\mathfrak{y}_{job}\times\mathfrak{y}_{ioa}}{h(\nu_b^2-\nu_a^2)} + 2\sum_{j\neq i}\sum_{b\neq a}\frac{ImV_{ioa;job}\nu_a\mathfrak{y}_{ioa}\cdot\mathbf{m}_{jbo}}{h(\nu_b^2-\nu_a^2)}\right\} \quad (1)$$

where $V_{ioa;job}$ is the interaction potential between transition a in the ith group and transition b in the jth group. ν_a is the transition frequency, $\mathfrak{y}_{kon}$ and $\mathbf{m}_{kon}$ are, respectively, electric and magnetic dipole transition moments associated with the transition from the ground state o to the excited state n in the kth group, and $\mathbf{R}_{ij}$ is the distance between the ith and jth group in the molecule.

The point monopole approximation[28] is used to calculate the interaction potential $V_{ioa;job}$. The transition monopole is defined as

$$q_{itoa} = e\int\psi_{io}^*\psi_{ia}d\tau_t \quad (2)$$

Here ψ_{io} and ψ_{ia} are the ground-state and excited-state wave functions of group i. e is the charge of an electron. The integration is carried out over region τ_t in which the sign of the product $(\psi_{io}^*\ \psi_{ia})$ is the same throughout. The position of the monopole is given by

$$\mathbf{R}_{it} = (\int\psi_{io}^*\mathbf{R}\psi_{ia}d\tau_t)/(\int\psi_{io}^*\psi_{ia}d\tau_t) \quad (3)$$

Thus, the interaction potential can be written as

$$V_{ioa;job} = \sum_{i,s}\sum_{j,t}\frac{q_{isoa}q_{jtob}}{\mathbf{R}_{is,jt}} \quad (4)$$

where

$$\mathbf{R}_{is,jt} = |\mathbf{R}_{is,jt}| = |\mathbf{R}_{is} - \mathbf{R}_{jt}| \quad (5)$$

Tinoco's formalism has been applied frequently for the calculation of the optical rotation of biopolymers. The results have been useful in the assignment of polypeptide ORD bands and in the determination of the helical sense of synthetic polypeptides.[29-33] They

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have also given information about base stacking in oligonucleotides.[34,35]

Possible Mechanisms

Considering the porphyrin dianion and neglecting the side chains, the heme group is of D_{4h} symmetry. Thus, as pointed out by Schellman,[36] a given Coulombic potential is less effective in inducing optical rotation in a highly symmetrical chromophore, for only its higher multipole terms transform as a pseudoscalar of the symmetry group of the chromophore. Therefore, the one-electron term contribution to the heme rotational strength should be small. Furthermore, the heme is surrounded by a large number of charged and dipolar groups in the protein. Induced heme Cotton effects caused by these surrounding charges should largely cancel one another.

Therefore, the most probable dominant mechanism is the two-electron or coupled-oscillator mechanism. The transitions which may be coupled with the heme $\pi-\pi^*$ transitions are the $\pi-\pi^*$ transitions of globin aromatic side chains, $\pi-\pi^*$ and $n-\pi^*$ transitions in the polypeptide backbone, and the $\sigma-\sigma^*$ transitions in alkyl side chains. The details of the calculation based on this mechanism will be presented in the following section.

There are two other possible mechanisms which could lead to rotational strength for heme transitions. (a) Nonplanarity of the porphyrin in myoglobin and hemoglobin would make heme an inherently dissymmetric chromophore.[37] This occurs in a chromophore related to heme–vitamin B_{12} coenzyme.[38] Puckering of the corrin ring leads to Cotton effects in the coenzyme. It is known that the iron is out of the heme plane in myoglobin[17] but nonplanarity within the porphyrin has not been demonstrated in heme proteins. Therefore, we neglect this possibility for the present. (b) Mixing of the heme $\pi-\pi^*$ transition with d–d transitions of the iron could lead to magnetic dipole character in the Soret, Q, N, and L bands. The absence of large changes in rotational strength upon reduction or on changing the ligand argues against this. Recently, Ruckpaul, *et al.*,[39] have reported that complexes between globin and metal-free porphyrins have CD spectra qualitatively resembling those of native hemoglobin. This clearly demonstrates that mixing of porphyrin π orbitals with metal d orbitals is not a crucial factor in determining the rotational strengths of heme transitions.

Calculation of Rotational Strengths

The calculation of the rotational strengths of the heme transitions requires a knowledge of the geometrical structure of the heme protein. Myoglobin atomic coordinates from Kendrew and Watson's X-ray diffraction study at 1.5 Å resolution[40] were used in our calculation. For hemoglobin, the atomic coordinates were obtained from Perutz, *et al.*,[41] from their most recent X-ray diffraction study on horse oxyhemoglobin at 2.8-Å resolution.

Coupling of Heme Transitions in Aromatic Side Chains of the Globin. There are 23, 21, and 22 aromatic residues (phenylalanine, tyrosine, tryptophan, and histidine) in sperm whale myoglobin, the α chain, and the β chain of horse hemoglobin, respectively. Nearly half of these aromatic side chains are within 12 Å of the heme iron. The size of the porphyrin ring is about 9 $\times$ 9 Å^2. Therefore, the interactions of the $\pi-\pi^*$ transitions of these aromatic side chains with those of the heme group could give significant rotational strength to heme absorption bands.

The electronic structures of imidazole and indole are discussed in the Appendix. The transitions which were considered as coupling with heme transitions are the first four low-energy bands in both groups. Calculated energies and transition dipole moments were used in the calculation of the rotational strengths. In benzene only the doubly degenerate $A_{1g} \rightarrow E_{1u}$ transition at 185 nm was incorporated in the calculation. We neglected the 260- (f = 0.001) and 200-nm (f = 0.126) bands because of their vibronically allowed character. No serious error should arise from this for the intensities of these two bands are much less than that of the 185-nm band. The two components of the benzene 185-nm band are polarized in the molecular plane and perpendicular to each other. The assignment of phenol transitions follows Kimura and Nagakura.[42] The phenol transition monopoles were calculated from the wave functions of Nishimoto and Fujishiro.[43] Parameters for the side-chain transitions considered are given in Table I.

Table I. Transition Parameters for $\pi-\pi^*$ Transitions in Porphyrin and Aromatic Side Chains

Group	Excited state	λ, nm	Oscillator strength	Polarization[a]
Porphyrin[b]	Q	550	0.01	x,y
	B	400	1.40	x,y
	N	320	0.14	x,y
	L	265	0.17	x,y
Phenyl[c]	E_{1u}	185	1.03	x,y
Phenol[d]	B_2	270	0.02	x
	A_1'	213	0.13	y
	B_2'	185	0.64	x
	A_1''	178	0.47	y
Imidazole[e]	I	243	0.12	−34.5
	II	206	0.03	−21.2
	III	164	0.41	53.5
	IV	155	0.07	13.4
Indole[e]	I	265	0.02	58.4
	II	245	0.18	−25.7
	III	210	0.36	50.9
	IV	204	0.29	−22.5

[a] x or y indicates polarization along the corresponding axes in Figure 5. x,y indicate doubly degenerate transition with perpendicular transition moments. Numerical values for groups of low symmetry indicate the angle of the transition moment measured counterclockwise from the positive x axis in Figure 5. [b] For sources of data, see text. [c] S. Nagakura, *Bull. Chem. Soc. Jap.*, 37, 1336 (1964). [d] Reference 42. [e] Calculated energies and oscillator strengths. See Appendix.

Neglecting the porphyrin side chains, the Soret band is doubly degenerate. This leads to ambiguity in the

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Table II. Calculated Myoglobin Soret Rotational Strength Arising from Interaction with Allowed π–π^* Transitions in Aromatic Side Chains[a,b]

		Porphyrin transition				
		Soret		Q	N	L
Amino acid	Distance,[c] Å	R_x	R_y	$R_x + R_y$	$R_x + R_y$	$R_x + R_y$
His (36,1C)[d]	14.3	−0.080	0.060	0.0003	−0.0055	0.0123
His (64,7F)	4.7	0.248	−0.426	−0.0018	−0.0056	0.0044
His (93,8F)	3.6	0.417	−0.393	0.0001	0.0047	−0.0023
His (97,2FG)	6.5	0.068	−0.169	0.0017	0.0044	0.0122
Phe (33,14B)	6.7	0.106	0.160	0.0015	0.0121	0.0258
Phe (43,1CD)	5.9	0.261	−0.217	0.0120	−0.0411	0.0788
Phe (46,4CD)	9.9	−0.093	0.142	0.0001	0.0070	0.0009
Phe (106, 7G)	14.3	−0.089	0.033	0.0003	−0.0094	−0.0032
Phe (138,15H)	9.6	0.033	0.045	0.0008	0.0049	0.0128
Tyr (103,4G)	11.3	0.252	−0.004	−0.0005	0.0328	0.0360
Tyr (146,23H)	11.8	0.135	−0.234	0.0001	−0.0123	−0.0188
Tyr (151,2HC)	15.3	0.037	0.014	0.0000	0.0030	0.0030
Subtotal		1.295	−0.989			
Total		0.306		0.0146	−0.0050	0.1619

[a] All rotational strengths are in Debye–Bohr magnetons (DBM). [b] For a more detailed listing indicating the contribution of each aromatic π–π^* transition, see M.-C. Hsu, Ph.D. Thesis, University of Illinois, Urbana, Ill., 1970. [c] Distance between the centers of the aromatic side chain and the porphyrin. [d] A, B, C, etc., designate helices A, B, C, etc., and AB, BC, CD, etc., designate the nonhelical regions between helices A and B, B and C, and C and D, etc.

choice of excited state wave functions and correspondingly in the orientation of the transition moments. A given choice of excited-state wave functions defines the orientation of two perpendicular transition moments. However, any linear combination of these two wave functions is also a valid representation with a different orientation of transition moments. In the presence of a perturbation, the linear combination chosen should be the one which diagonalizes the perturbation matrix. It can be shown that rotation of the pair of transition moments can affect the *distribution* of rotational strength among the two components but leaves the *net* rotational strength unchanged. Therefore, we arbitrarily carried out our initial calculations using wave functions which lead to polarization along the x and y axes shown in Figure 5.

The magnitude of the Soret transition dipole moment ($|\mu|$ = 7.22 D for each Soret component) was derived from the total Soret oscillator strength of 1.4, a value in the middle of the range observed for metalloporphyrins.[44] The transition frequency was taken as 25,000 cm^{-1}. We placed the transition monopoles at the center of each atom, both for the heme group and the aromatic side chains. The actual calculations of rotational strengths were carried out on an IBM 1800. The aromatic groups considered, the distances (center to center) between the heme group and aromatic side chains, and the calculated rotational strengths are listed in Table II, for myoglobin. The results for hemoglobin are given in less detail in Table III.

For myoglobin we considered 12 out of 23 aromatic side chains which are near the heme group. Assuming a definite relative orientation between the heme group and an aromatic group, the rotational strength arising from the coupling of the electrically allowed transitions is roughly inversely proportional to the square of the distance between these two groups.

The net calculated Soret rotational strength (sum of R_x and R_y) arising from coupling with aromatic transitions is 0.3 DBM. This is to be compared with

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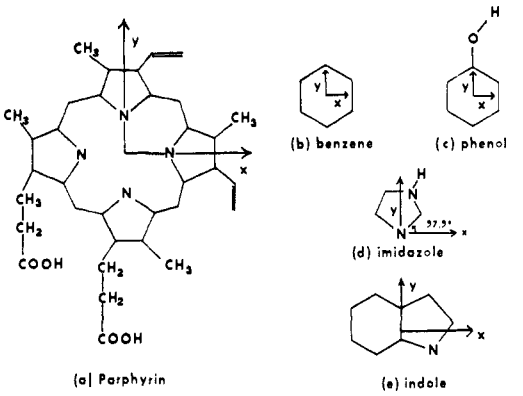

Figure 5. Coordinate axes for porphyrin, benzene, phenol, imidazole, and indole.

Table III. Calculated Soret Rotational Strength of Hemoglobin[a,b]

		Side chains			
Heme		α_1	α_2	β_1	β_2
α_1[c]	R_x	0.143	−0.011	0.152	0.349
	R_y	0.488	−0.028	−0.006	−0.223
β_1	R_x	−0.158	0.191	−1.525	−0.060
	R_y	0.069	−0.137	0.891	0.064
Av/heme		R_x = −0.455		R_y = 0.563	

[a] In DBM. [b] For a more detailed listing of rotational strength contributions of various amino acid side chains see Table II, footnote *b*. [c] For the designation of α_1, α_2, β_1, and β_2 see ref 18.

the estimated experimental value of 0.5 ± 0.05 DBM. Among the aromatic residues, His (64, 7E), His (97, 2FG), Phe (33, 14B), Tyr (103, 4G), and Tyr (146, 23H) give the major contributions (see footnote *d* of Table II for the notation of amino acid sequence).

His (64, 7E) is the distal histidine which is close to the sixth ligand of the iron. The fifth ligand, His (93, 8F), gives only a small effect for its imidazole group sits in a rather symmetrical position with respect to the heme. The imidazole plane is nearly perpendicular to the heme plane and the two planes intersect along a line which is ~30° away from the x axis

shown in Figure 5. Because of the closeness of the proximal imidazole and the heme, one might expect that the resultant Soret rotational strength would be very sensitive to the relative position of these two groups. If so, highly accurate atomic coordinates of the proximal imidazole would be needed for the calculation. Calculations were carried out with the imidazole plane rotated by 0–90° and tilted by 1–10° with respect to the heme plane. In all cases the calculated R_x and R_y are nearly equal in magnitude and opposite in sign. Hence, the proximal histidine is probably not to be expected to play a major role in the induced Cotton effects in the heme proteins.

From the data of Nagai, Sugita, and Yoneyama,[24] we estimated the Soret rotational strength in human oxy-, deoxy-, and methemoglobin to be 0.23, 0.38, and 0.30 DBM, respectively. Our calculations, based on the atomic coordinates of horse oxyhemoglobin, show that the α chain and β chain have contributions to the average Soret rotational strength which are nearly equal in magnitude and opposite in sign (α chain, 0.631 DBM; β chain, −0.634 DBM). Among the aromatic residues in the α chain, His-58, Phe-33, Phe-43, Phe-98, Tyr-42, Tyr-140, and Trp-14 make the major contribution to the rotational strength. For the β chain the major contribution comes from His-63, His-69, Phe-42, Phe-41, Phe-71, Phe-85, Phe-103, Tyr-130, and Trp-15. Again the proximal histidines, His-87 of the α chain and His-92 of the β chain, give only small net contributions to the Soret rotational strength. Because of the strongly allowed transitions in the far-uv region, tyrosine and tryptophan at 10–15 Å distance from the heme group interact with the heme group rather strongly and give significant rotational strength to the heme transitions. This remote interaction might be useful for the study of quaternary structure.

We have also calculated the induced Soret rotational strength due to intersubunit interactions by generating the α_2- and β_2-chain atomic coordinates through a 180° rotation about the twofold symmetry axis. (For the designations of α_1, α_2, β_1, and β_2 chains, see ref 18.) In Table III the calculated Soret rotational strengths for tetrameric hemoglobin are listed. The calculated average Soret rotational strength is 0.1 DBM as compared to the experimental value, 0.23 DBM, for human oxyhemoglobin.

Because of the large distances (30–35 Å) between the heme group and the aromatic side chains of the other like chain (heme (α_1) and aromatic groups (α_2); heme (β_1) and aromatic groups (β_2)), their interactions give only very small induced Soret rotational strengths. On the other hand Soret rotational strength arising from the interactions of heme with aromatic side chains of unlike chains (heme (α_1) and aromatic groups (β_1 or β_2); heme (β_1) and aromatic groups (α_1 or α_2)) are significant, for the distances between some of these interacting groups are as small as 15 Å. Such groups include heme (α_1)–Phe (β_2 41), heme (α_1)–Trp (β_2 37), heme (β_1)–His (α_1 103), heme (β_1)–His (α_1 122), and heme (β_1)–Tyr (α_2 42). We report here only the two hemes on the α_1 and β_1 chains. The hemes on α_2 and β_2 chains are related to those of the α_1 and β_1 chains by the twofold symmetry axis mentioned previously.

Recently, several experimentalists[24,45,46] have pointed out that the CD spectra of reconstituted human hemoglobin, which are essentially identical with those of native hemoglobin, are not simply the sum of the CD spectra of the isolated subunits. This nonaddivity could be explained either by conformational changes on going from monomer to tetramer or by the nonnegligible subunit interaction mentioned above. Both α and β chains are known to have higher oxygen affinities[47] than tetrameric hemoglobin. One might expect that oxygen affinity is linked to the molecular conformation. Nevertheless, unless the three-dimensional structure of a single-chain hemoglobin is known, we cannot determine which factor, change of conformation or subunit interaction, is predominant in accounting for the change of CD spectra on going from monomeric to tetrameric hemoglobin.

CD measurements[24,45,46] on single α chains have shown a positive Soret CD band with a rotational strength of 0.3 ± 0.25 DBM as compared to our calculated value of 0.63 DBM. While experimental results on β chains are also quite variable, they all show a rather pronounced negative trough in the Soret region and a smaller net positive rotational strength as compared to the α chain. Thus our calculations on the separated chains of hemoglobin, though qualitatively correct insofar as the difference in rotational strengths of two chains is concerned, appear to agree rather poorly with experiment. However, this may simply reflect conformational changes on going from the intact tetramer to isolated α and β chains.

From the results presented above, one sees that coupling of the Soret band with π–π^* transitions of nearby aromatic side chains can account for the observed Soret rotational strength.

Coupling of Heme Transitions with Transitions in the Polypeptide Backbone and Alkyl Side Chains. For the polypeptide backbone only the 220- (n–π^*) and the 190-nm (π–π^*) bands of amides were considered. The parameters used were those employed by Woody and Tinoco.[30] Except for helices A and D which are rather far from the heme group, all the other amide groups in myoglobin (about 120 in all) were included in the calculation of couplings between the Soret and n–π^* or π–π^* transitions of amide groups. The net contribution to the Soret rotational strength from these two types of coupling is negligibly small: 0.003 DBM (Table IV).

For the alkyl side chains of the globin, we shall be concerned with the σ–σ^* transitions of the C–C bonds. In their calculations of optical rotation of helical polypeptides, Woody and Tinoco[30] treated the coupling of these σ–σ^* transitions with transitions in the amide by Kirkwood's polarizability method.[27] However, there is a large uncertainty in their estimation of the anisotropy of bond polarizability. Recently, the vacuum ultraviolet absorption of alkanes has been studied by Raymonda and Simpson[48] and by Pearson and Innes.[49] These two studies gave different directions

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(48) J. W. Raymonda and W. T. Simpson, *J. Chem. Phys.*, 47, 430 (1967).
(49) E. F. Pearson and K. K. Innes, *J. Mol. Spectrosc.*, 30, 232 (1969).

Table IV. Soret Rotational Strength[a] of Myoglobin Arising from Interactions with n–π^* and π–π^* Transitions in the Amide Groups of the Peptide Backbone

Backbone region[b]	n–π^*		π–π^*	
	R_x	R_y	R_x	R_y
B	0.0004	0.0001	−0.0143	−0.0319
C	0.0011	0.0009	0.0224	−0.0527
CD	0.0001	0	−0.0224	−0.0548
E	0.0023	−0.0009	−0.1046	0.1128
EF	0.0003	−0.0003	0.0227	−0.0012
F	0.0001	0.0023	−0.0891	0.0814
FG	−0.0013	0.0005	−0.0776	0.1762
G	−0.0006	0.0017	0.0133	0.0352
GH	0	−0.0001	0.0040	0.0078
H	0.0001	0.0002	0.0823	−0.1133
Total	0.0025	0.0043	−0.1632	0.1595

[a] In DBM. [b] A, B, C, etc. designate helices A, B, C, etc.; CD, EF, FG, etc., the nonhelical segment CD, EF, FG, etc. See also text.

of polarization of the σ–σ^* transition in the C–C bond. For simplicity, we adopted Simpson and Raymonda's assignment—the direction of transition dipole moment was assumed to be along the C–C bond. The natural frequency and dipole length were calculated to be 75.5 kK and 0.435 Å.[48] We treated all C–C bonds in the myoglobin alkyl side chains as being equivalent and neglected the σ electrons of the C–H, C–N, and C–O bonds. Neglecting C–H bonds is partially justified by the generally assumed isotropy of polarizability of the C–H bond.[50]

Sixty-five alkyl side chains in myoglobin were included in the calculation. This excludes aspartic acid, glutamic acid, lysine, and arginine residues which are on the surface of the molecule and far away from the heme group. The interaction of the Soret transition with the σ–σ^* transition of each C–C bond was considered separately, placing the transition monoples at the two carbon atoms. The intensity of the σ–σ^* transition in the C–C bond is rather large, but the induced Soret rotational strength through coupling with these transitions in the alkyl side chains is at most a few hundredths of a DBM. This is partly because of the energy separation between the Soret and σ–σ^* transitions (~280 nm). The total contribution to the myoglobin Soret rotational strength from the σ–σ^* transitions of the 65 alkyl side chains considered is only 0.04 DBM.

Because of the defects in our treatment discussed above, this result should be accepted with caution. Furthermore, possible contributions from transitions at shorter wavelength than the 75.5-kK band are not considered here. However, this calculation indicated that although coupling with far-ultraviolet transitions in nearby alkyl side chains may contribute to the Soret rotational strength, the contribution is likely to be small and is not the dominant factor.

Other Heme Transitions. We have also considered the induced rotational strength for the Q, N, and L bands of myoglobin and hemoglobin. Since the nature of the Q band of vibronic origin is not clearly understood, only the 0–0 band will be discussed here. Its oscillator strength is taken as 0.01. For the L and N bands, we estimated the transition dipole moments from the absorption spectra of cobalt deuteroporphyrin dimethyl ester[22] (Table I). As in the case of the Soret band the two transition dipole moments of each degenerate band are in the porphyrin plane and perpendicular to each other.

The calculated myoglobin Q-, N-, and L-band rotational strengths are shown in Table II. Only the coupling of these heme bands with the allowed π–π^* transitions of aromatic side chains were included in the calculation. This should be the dominant mechanism as it is for the Soret Cotton effect. The calculated total rotational strengths are 0.015, −0.008, and 0.188 DBM for the Q, N, and L, bands, respectively. They are qualitatively in agreement with experimental results. Namely, the Q band rotational strength is positive and about one order of magnitude smaller than that of the Soret band. The L band exhibits a CD band which is positive and with magnitude comparable to that of the Soret band. The N band rotational strength is negative and much smaller than that of the L band.

The hemoglobin N and L bands were also investigated. Because these two bands are much weaker than the Soret band, only the coupling with the aromatic residues within each single chain are considered. The results are shown in Table V.

Table V. Calculated Hemoglobin N- and L-Band Rotational Strengths

Chain	N Band		L Band	
	R_x (DBM)	R_y (DBM)	R_x (DBM)	R_y (DBM)
α	−0.067	−0.122	0.078	0.064
β	−0.052	−0.014	−0.082	0.070
Av/heme	−0.128		0.065	

From the reported CD data in the N-band region[24,25] the CD band at 320 nm is expected to be broad and weak. Its magnitude and even its sign depend on the oxidation state of the iron and the nature of the sixth ligand. The calculated N-band rotational strength is much larger than expected. The L-band rotational strength is calculated to be positive and comparable to that of the Soret band (0.07 DBM for the L band and 0.10 DBM for the Soret band). Human oxyhemoglobin exhibits a rotational strength around 260 nm which is about twice that of the Soret band.[24] For met- and deoxyhemoglobin the Soret rotational strengths are larger than those of the 260-nm band. Again it should be noted here that our calculations were based on the atomic coordinates of horse oxyhemoglobin.

Rotational Strengths of Aromatic Transitions Due to Coupling with Porphyrin. From the sum rule for rotational strengths, aromatic group transitions which couple with heme transitions should exhibit rotational strengths which are equal in magnitude but opposite in sign to those of the heme bands. Therefore, one might expect that benzene, phenol, and indole side chains should contribute circular dichroism in the L-band region (260 nm). In Table VI we give the rotational strengths for each of the aromatic transitions which result from coupling with the heme transitions.

The 280-nm bands of the three tyrosines considered in myoglobin (Table II) have a total rotational strength

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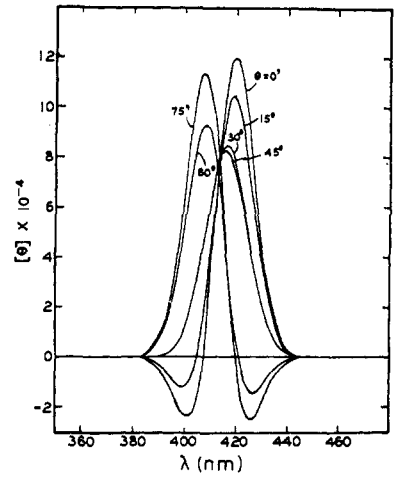

Figure 6. Theoretical Soret CD band of myoglobin as a function of orientation of Soret transition moments.

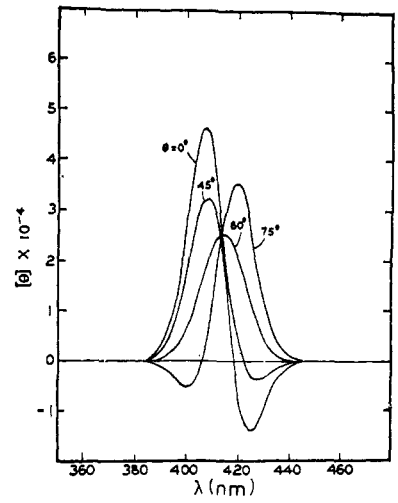

Figure 7. Theoretical Soret CD band of hemoglobin as a function of orientation of Soret transition moments.

Table VI. Contributions to the Rotational Strength[a] of Aromatic Transitions Arising from Coupling with Heme Transitions in Myoglobin

Amino acid	Transition[b]	Soret	Others[c]	Total
His	I	−0.302	−0.0356	−0.338
	II	−0.043	−0.0075	−0.051
	III	+0.691	+0.0151	+0.706
	IV	−0.071	+0.0031	−0.068
Phe	I	−0.021	−0.0520	−0.073
	II	−0.360	−0.0513	−0.411
Tyr	I	−0.011	−0.0099	−0.021
	II	−0.097	−0.0215	−0.119
	III	−0.075	−0.0025	−0.078
	IV	−0.017	−0.0035	−0.021

[a] In DBM. [b] Transitions are numbered starting from the longest wavelength band. [c] Q, N, and L bands.

of −0.0106 DBM, or about −0.003 DBM per tyrosine, through coupling with the Soret transition. This is small compared to the rotational strength of the 280-nm band in several other proteins. A rough calculation for the case of ribonuclease[7] indicates that assuming the 280-nm band is due to tyrosine, this has a rotational strength of about −0.05 DBM or −0.025 DBM per residue, depending on whether one considers three or six tyrosines to be contributing. Thus there is little hope of verifying the mechanism proposed here through a study of the CD in the 280-nm region.

The Shapes of the Soret CD Bands in Myoglobin and Hemoglobin. In myoglobin the two components of the Soret band have rotational strengths of opposite sign (Table II). This raises the question of why only a single positive band is observed in oxy-, deoxy-, and metmyoglobin. If the two components are completely degenerate, this is certainly what would be observed. However, the two components are not completely degenerate as shown by Eaton and Hochstrasser.[21] From their single-crystal polarization measurement on myoglobin a splitting of several hundred reciprocal centimeters^{-1} can be inferred. Such a splitting would lead to two distinct CD bands of opposite sign if the intensities were comparable in magnitude, but even a 2:1 disparity in magnitude can obscure the presence of two bands.[51]

(51) K. M. Wellman, P. H. A. Laur, W. S. Briggs, A. Moscowitz, and C. Djerassi, *J. Amer. Chem. Soc.*, 87, 66 (1965).

The calculations on which Table II is based assume that the two Soret components are polarized parallel to the opposing N–N lines (Figure 5). The ratio of the two rotational strengths thus calculated is 5:6. Various perturbations can rotate the two directions of polarization in the heme plane, keeping them perpendicular to each other. It can be shown that the magnitudes of the rotational strength of the two individual Soret components depend on the orientation of the transition dipole moments in the heme plane, but the total Soret rotational strength is independent of the directions of polarization. We have synthesized a theoretical myoglobin Soret CD curve (Figure 6) by adding together two Gaussian curves associated with two Soret components. The band maxima are taken as 415 and 412 nm for transitions associated with R_x and R_y, respectively. The half-widths are set equal to 12 nm for each band.

At θ = 30–45° the Soret CD curve is calculated to be a single positive band. However, with the Soret components polarized along directions different from these angles, we obtain a two-band CD curve with a small negative trough at shorter or longer wavelengths than the band maximum. Thus the shape of the Soret CD band may be rather sensitive to substituents and conformational changes which lead to variation in the directions of polarization of the Soret components. By comparison with the experimental myoglobin CD curve (a single positive band), we conclude that the two Soret components are polarized toward the bridging methine carbons.

A similar calculation was carried out on horse oxyhemoglobin. Figure 7 shows the theoretical Soret CD curve of hemoglobin. Between θ = 0 and 30°, two-band CD curves were obtained, with the magnitude of the negative band one-third that of the positive band. Around θ = 45° the negative band decreases to only one-sixth that of the positive band. A single positive band was obtained at θ = 60°. As θ increases, the negative band switches from the longer wavelength side of the positive band to the shorter wavelength side. The data so far reported on the Soret CD of human oxyhemoglobin are not consistent about the presence and magnitude of the negative trough.[10–12,24,25] However,

all reports show a very small negative trough (about one-eighth the magnitude of the positive band) or a single positive band. Thus, we tentatively conclude that in hemoglobin the two Soret components are polarized between $\theta = 45$ and 75°. Since the maximum of the Soret spectrum is observed at a longer wavelength than the absorption maximum, the lower energy component of the Soret band must have a positive rotational strength.

Discussion and Conclusions

The qualitative agreement which we find between theory and experiment for the rotational strengths of four heme transitions in two heme proteins suggests that we have identified the principal interaction which leads to induced Cotton effects in these systems. The dominant factor is a coupled-oscillator interaction between the heme chromophore and the substantial number of aromatic groups which surround the heme.

If this mechanism is correct, one would expect significant differences in the CD spectra of mutant hemoglobins which have substitutions involving aromatic groups near the heme. There are a number of such mutants known,[52] but CD data are not available yet for most of them. A further difficulty is that a mutation at one point in the polypeptide chain may affect not only that residue but also its whole neighborhood and perhaps even the whole protein.

Li and Johnson[53] showed that hemoglobins F, S, and C exhibit visible and Soret ORD spectra indistinguishable from those of normal human hemoglobin (HbA). On the other hand, $MetHbM_{Boston}$ gives a Soret ORD spectrum with similar shape but much larger amplitude (~1.6 times) compared to that of HbA. In HbS and HbC[53] the amino acid replacements are at position 6 of the β chain (glutamic acid is replaced by valine and lysine, respectively) and by analogy to horse hemoglobin, they are about 20 Å away from the iron of the heme. Therefore the altered amino acids are too far away from the heme to affect the heme Cotton effects. HbF is composed of two α chains and two γ chains. The γ chain has 37 amino acids different from those of the β chain in HbA. Among these 37 amino acid replacements, two are close to the heme group: serine in place of alanine at position 70 and leucine in place of phenylalanine at position 71. If the conformation of HbF were the same as that of HbA, one would expect an increase of the Soret rotational strength in HbF, for the Phe 71 of the β chain makes a rather large negative contribution to the Soret rotational strength of the β chain. However, with one-fourth of the amino acids replaced it is very likely that the conformation of the γ chain is quite different from that of the β chain. This suggests that the similarity of the Soret ORD curves of HbF and HbA is accidental, and we anticipate that a more careful study using CD will uncover some differences between HbA and F.

The only abnormal amino acid in HbM_{Boston} is a tyrosine at position 58 of the α chain instead of the distal histidine. A calculation was carried out to examine the effect of this replacement on the Soret rotational strength, keeping the structure of the rest of the molecule the same as in HbA. We assumed that the carbonyl groups, C_α, C_β, and C_γ of the two amino acids occupy the same positions in HbA and HbM_{Boston}. When the phenol ring of a tyrosine is put in the place of the distal imidazole, a 5% decrease of the Soret rotational strength was calculated. However, as the benzene plane is turned along the C_β–C_γ bond from 0 to 45°, a 100% increase of the Soret rotational strength was obtained. Therefore the proposed mechanism for the origin of heme Cotton effects could account for the large increase of Soret rotational strength in HbM_{Boston}.

In HbM_{Yakima}, the β99 aspartic acid is replaced by a histidine. A slight change of Soret CD spectra was observed as compared to HbA.[46] Because of the rather large distance between the heme and the abnormal amino acid (>10 Å from the iron of the heme), a large change is not to be expected.

Our calculations also allow us to conclude that the Soret transition moments in myoglobin and hemoglobin are oriented toward the methine carbons rather than the pyrrole nitrogens. This conclusion follows from our study of the predicted shape of the Soret CD curve as a function of transition moment direction. Although the factors which dictate this orientation are not presently known, we may conjecture that the orientations of the fifth and sixth ligands are important.

In any event, we suggest that dramatic changes in the *shape* of the Soret CD band may occur on binding certain ligands without implying any major conformational change in the protein, if ligand binding produces a significant reorientation of porphyrin transition moments. Possible examples of this have been reported by Willick, *et al.*,[8] for horseradish peroxidase.

Recently, Volkenstein, Metlyaev, and Milevskaya[54] reported a theoretical investigation of induced Soret and Q band Cotton effects in iron–porphyrin complexes. They considered a single point charge or dipole as a perturbant and calculated the rotational strength due to the one-electron mechanism. Although the resultant values are comparable to the observed rotational strength in myoglobin, the contributions from many possible nearby point charges and dipoles should largely cancel each other as mentioned previously. These authors used Ohno, Tanabe, and Sasaki's[55] molecular orbitals for an iron–porphyrin complex. These molecular orbitals were calculated by a simple Hückel method. They indicate a large amount of metal d → d character in the Soret transition, which is crucial to obtaining significant rotational strengths by the one-electron mechanism. This strong mixing of Soret and metal d → d transitions is not borne out by the more extensive calculations of Zerner, *et al.*[56] There is strong experimental evidence that this mechanism is not a major factor in the observation[39] that globin complexes of metal-free porphyrins have Soret rotational strengths as large as or larger than those of native hemoglobin.

Summary

A coupled-oscillator interaction between the porphyrin π–π^* transitions and π–π^* transitions in nearby

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aromatic groups is responsible for most of the induced rotational strength of heme transitions in myoglobin, hemoglobin, and probably in other heme proteins. Contributions from coupling with peptide transitions are negligible. The number of groups which make significant contributions to the induced Cotton effects is substantial and no one group is dominant. Therefore, it will be difficult to interpret such Cotton effects in terms of stereochemistry. Such may not be the case, however, for d–d or ligand–metal charge-transfer transitions.

The shape of heme π–π^* Cotton effects depends on the orientation of the transition moments in the plane, because this determines the distribution of rotational strength among two nearly degenerate components. Comparison of the observed shape of the Soret Cotton effects in myoglobin and hemoglobin with calculated CD curves indicates that the Soret transition moments are directed approximately along axes defined by the methine bridges in both proteins.

Extrapolations of the results of this study are consistent qualitatively with CD studies of mutant human hemoglobins and horseradish peroxidase. However, detailed comparison in these systems must await further information concerning their three-dimensional structure.

Appendix

To calculate the heme rotational strengths, we need the wave functions of the heme and those groups in the globin which interact with the heme. We reproduced Weiss, Kobayashi, and Gouterman's[23] Pariser–Parr–Pople SCF–MO calculation on porphyrin to obtain the heme wave functions. Then, an analogous set of parameters was used to calculate wave functions for imidazole and indole. The PPP–SCF method is extensively used for the π-electron system of aromatic hydrocarbons and heterocyclic rings. The details have been described elsewhere.[23,57,58] Following Weiss, *et al.*,[23] standard parameters for γ_{ii} and w_i were used in the calculation.

The actual SCF–MO calculations were done with a program written for an IBM 7094, followed by configuration interaction. The bond lengths and bond angles of porphyrin, imidazole, and indole used in this work were taken from the known geometries of these compounds or their derivatives.[59–61] The assignment of coordinate axes is given in Figure 5. All three molecules were assumed to be planar.

The results for porphyrin, essentially the same as those obtained by Weiss, *et al.*,[23] explain the general features of the absorption spectra. The lowest excited configurations, $a_{1u}e_g$ and $a_{2u}e_g$, are nearly degenerate and they interact strongly. Their nearly equal dipoles are out of phase for the Q state and in phase for the Soret. By including the next higher energy configurations ($b_{2u}e_g$ and $a_{2u}'e_g$) in the six-orbital model, one also accounts for the N and L bands in the near-ultraviolet. Extensive CI calculations of Weiss, *et al.*,[23] showed that higher energy configurations do not mix to any appreciable extent with these four lowest configurations. The wave functions and monopole charges are given in detail in the thesis of M.-C. Hsu.[62]

Few experimental data on the ultraviolet absorption spectra of five-membered rings containing two heteroatoms have been reported. Therefore, we calculated the transition dipole moments and oscillator strengths of imidazole. The transition dipole moment is calculated using a dipole velocity operator which has been shown[63] to give more accurate results with wave functions obtained in a zero-differential overlap approximation such as the PPP method.

McDiarmid[64] reported that in aqueous solution at neutral pH, histidine has a band maximum at 211 nm ($\epsilon_{mM} \sim 6$) and a barely observable shoulder at 205 nm. Furthermore, the integrated oscillator strength up to 190 nm is 0.178. The sum of the first two calculated oscillator strengths at longer wavelength is 0.145. The calculated next higher absorption band (at 160 nm) is about four times stronger than the lowest energy band. This is consistent with the observed spectra given by McDiarmid[64] which shows a rapid increase of absorption at wavelengths below 190 nm. Recently Del Bene and Jaffé[65] performed a CNDO (complete neglect of differential overlap) molecular orbital calculation on imidazole. This method treats explicitly all σ and π valence electrons in the molecule. They predicted a π–π^* absorption spectrum similar to that of this work.

We carried out four-, six-, and eight-orbital configuration interaction calculations on indole. The results indicate that because of the large energy separations the four lower energy excited states mix only to a small extent with the higher energy states. In neutral aqueous solution, tryptophan shows a strong absorption band at 224 nm ($\epsilon_{mM} \sim 35$) and a weak band at 284 nm ($\epsilon_{mM} \sim 2.8$) with a distinct shoulder at 294 nm.[66] If we assign the three calculated low-energy π–π^* transitions (bands I, II, III in Table I) to the three absorption bands observed, then the calculation gives too small a ratio (2 *vs.* ~6) of intensity between the 224-nm band and the 283-nm band. However, the predicted separations of these three bands are in good agreement with experiment. Our work differs from some other recent MO calculations on indole[67,68] in the directions of polarization of the transition. Mani and Lombardi[69] reported a high-resolution uv spectrum of indole together with a vibrational analysis of the absorption bands. They showed that the 284-nm band is polarized along a direction which is 23 ± 5° away from the x axis (the long molecular axis). Our calculation gives a value of 25°. Thus, we believe that the wave functions reported here should suffice for the calculation of rotational strengths in which the direction of polarization is a determining factor.

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Details of the wave functions and monopole charges for benzene, phenol, imidazole, and indole are given in ref 62.

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Communications to the Editor

Photochemistry of Cobalt(III) Complexes. I. A Complication Due to Thermal Reduction of $Co(NH_3)_5OCOCH_3^{2+}$ by Ketyl Radicals

Sir:

We wish to report our results on the study of the photolysis of $Co(NH_3)_5OCOCH_3^{2+}$ (**1**) in aqueous alcoholic solutions. The photoreduction of Co(III) salts is one of the most intensively studied areas of inorganic photochemistry,[1] yet the nature of the excited state(s) involved has not been resolved. It is clear that there are radical products of the photolysis, and both radical-pair[2] and "excited-state"[1d,3] mechanisms have been invoked. The situation is further complicated by the fact that even proponents of the excited-state mechanism are forced to discuss the details of the reaction in terms of radicals and charge-transfer states.[4,5]

A recent communication[5] describes the photolysis of **1** in aqueous alcohol and interprets the results by invoking a complicated mechanism involving two excited states. The second excited state was postulated to explain the increase of $\varphi_{Co^{2+}}$ with [*i*-PrOH], and the authors claimed to have demonstrated a solvent effect on the primary quantum yield.

Our conclusions, based on data qualitatively in agreement with those presented by Kantrowitz, *et al.*,[5] are as follows. (1) The increase in $\varphi_{Co^{2+}}$ is due to a secondary *thermal* reduction reaction of **1**. (2) [*i*-PrOH] has no effect on the *primary* quantum yield. (3) There is no need to postulate a second excited state.

The compound $[Co(NH_3)_5OCOCH_3](ClO_4)_2$ was prepared and purified by previously described procedures.[6] The absorption agreed with that in the literature,[7] and a satisfactory chemical analysis was obtained.

The photolyses were carried out with a Bausch and Lomb high-intensity SP200 mercury arc and high-intensity monochromator by using conventional techniques. Irradiations were performed at 250 ± 10 nm. Quantum yields were determined using ferrioxalate actinometry and modifications of previously described methods for determination of Co(II)[8] and acetone.[9] Degassing was accomplished by three freeze–thaw cycles. Our results are presented in Table I.

Table I. Quantum Yields of Products[a]

System	—Nondegassed— $\varphi_{Co^{2+}}$[b]	$\varphi_{acetone}$[c]	——Degassed—— $\varphi_{Co^{2+}}$[b]	$\varphi_{acetone}$[c]
H_2O	0.29		0.29	
5 *M* MeOH–H_2O	0.30		0.31	
5 *M* *i*-PrOH–H_2O	0.29	0.3	0.55	0.3

[a] $[Co(NH_3)_5O_2CCH_3]^{2+}$ = 10^{-2} *M*; $HClO_4$ = 10^{-2} *M*; temperature = 23°; $I_a \simeq 10^{-7}$ einstein/min. Values for $\varphi_{Co^{2+}}$ listed are the averages of two or more determinations and agree within ±5%. Kantrowitz,[5] *et al.*, obtained $\varphi_{Co^{2+}}(H_2O)$ = 0.19; $\varphi_{Co^{2+}}$ (5 *M* MeOH) = 0.21; $\varphi_{Co^{2+}}$(5 *M* *i*-PrOH) = 0.40. These values are substantially different from ours; however, we believe that both sets of values are internally consistent. [b] Zero-order kinetics were observed for 0.03–30% photodecomposition of the complex. [c] Quantum yield determinations at $I_a \simeq 10^{-5}$ einstein/min.

We find that $\varphi_{Co^{2+}}$ is 0.29 in both pure water and 5 *M* aqueous methanol. Degassing these systems has little effect. In 5 *M* aqueous isopropyl alcohol, undegassed, $\varphi_{Co^{2+}}$ is also 0.29. However, degassing this system causes a twofold increase in $\varphi_{Co^{2+}}$ with the concurrent formation of acetone; $\varphi_{acetone} = {}^1/_2\varphi_{Co^{2+}}$ (degassed) = $\varphi_{Co^{2+}}$(nondegassed).

These data can readily be accommodated by the following scheme.

$$\mathbf{1} \xrightarrow[250\text{ nm}]{h\nu} \mathbf{1}^* \quad (1)$$

$$\mathbf{1}^* \longrightarrow Co(NH_3)_5^{2+} + CH_3CO_2\cdot \quad (2)$$

$$CH_3CO_2\cdot \longrightarrow CH_3\cdot + CO_2 \quad (3)$$

$$2CH_3\cdot \longrightarrow CH_3CH_3 \quad (4)$$

$$CH_3\cdot + i\text{-PrOH} \longrightarrow CH_4 + CH_3\overset{\displaystyle OH}{\overset{|}{C}}CH_3 \quad (5)$$

$$CH_3\overset{\displaystyle OH}{\overset{|}{C}}CH_3 + \mathbf{1} \xrightarrow{\text{dark}} CH_3\overset{\displaystyle O}{\overset{\|}{C}}CH_3 + Co^{2+} + 5NH_3 + CH_3CO_2^- + H^+ \quad (6)$$

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