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Optical Spectra and Electronic Structure of Flavine Mononucleotide in Flavodoxin Crystals†

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ABSTRACT: The polarized single-crystal absorption spectra of the oxidized and semiquinone forms of flavodoxin from *Clostridium MP* have been measured with a double beam recording microspectrophotometer. The spectra establish that the radical species in the crystal is the neutral (blue) flavine semiquinone. Combination of the spectra reported here with polarization data from previous fluorescence and stretched-film studies provides transition moment directions for the first two $\pi \rightarrow \pi^*$ transitions of the oxidized form. Predictions of molecular orbital theory are in good agreement with these experimental directions. The crystal spectra of the semiquinone indicate that the two lowest frequency transitions have the same detailed orbital origin as the corresponding transitions of the oxidized form; in the semiquinone these transitions appear at lower frequency, are closer together, and, as predicted from detailed considerations of transition probabilities, exhibit approximately half the absorption intensity. Our hypothesis of a common orbital origin suggests that semiquinone formation takes place by the addition of an electron to the lowest empty π orbital of the oxidized form without any gross electronic rearrangement.

The optical and magnetic resonance spectra of the flavine prosthetic group have been extensively utilized in studies of the reactions of flavoproteins (Ehrenberg and Hemmerich, 1968; Kamin, 1971). Comparisons between the spectra of flavoproteins and model systems have also been used to derive information about the nature of the flavine binding sites in the proteins (Harbury et al., 1959; Ghisla et al., 1974). Because the free radical forms generally dismute extensively to mixtures of the fully reduced and fully oxidized states, model flavine semiquinones have received little attention from optical spectroscopists or crystallographers (cf. Land and Swallow, 1969). Most of the information on their electronic structure has come from electron spin resonance studies (see, for example, Ehrenberg et al., 1971; Müller et al., 1970). In contrast, a number of flavoprotein radicals can be obtained in nearly quantitative yields. The recent determinations of the crystal structures of flavodoxins in both the oxidized and semiquinone forms (Andersen et al., 1972; Burnett et al., 1974; Watenpaugh et al., 1973) now permit optical experiments which give new insight into the electronic structure of flavoproteins. In this paper we present the polarized optical absorption spectra of single crystals of the oxidized and semiquinone forms of *Cl. MP* flavodoxin measured with a double beam recording microspectrophotometer. We combine the structural and polarization data to assign transition moment directions, and we propose a simple interpretation of the low frequency part of the semiquinone spectrum.[1]

Experimental Section

Oxidized flavodoxin from *Clostridium MP* was purified and crystallized as previously described (Ludwig et al., 1969). Single crystals suitable for optical measurements were hexagonal prisms. For single crystal spectroscopy, the semiquinone form was obtained by addition of solid sodium dithionite to the suspending buffer. In solution the semiquinone was produced by anaerobic photoreduction in the presence of EDTA (Massey and Palmer, 1966).

Solution absorption spectra were measured on a Cary 17 recording spectrophotometer. Polarized single-crystal absorption spectra were measured with a double-beam recording microspectrophotometer with optics similar to the single-beam instrument previously described (Eaton and Lewis, 1970). The double-beam instrument will be described in detail elsewhere (J. Hofrichter, to be published). It is constructed around a Leitz polarizing microscope and

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[1] For all but some extremely weak electronic transitions, the probability of absorption of plane polarized light by a space-fixed molecule is proportional to the quantity: $|\langle\psi_e|\mu|\psi_g\rangle \cdot \mathbf{E}|^2$, where ψ_g and ψ_e are the wave functions for the ground and excited electronic states, μ is the electric-dipole operator, and $\mathbf{E}$ is the electric field vector of the plane-polarized light (Eyring et al., 1954). The vector integral is the electric-dipole transition moment; the square of its magnitude is proportional to the area under an absorption band, while its direction defines the molecular direction in which plane polarized light is maximally absorbed. Since the flavine chromophore has only a single symmetry plane, the transition moment direction is either parallel to this plane, as in $\pi \rightarrow \pi^*$ excitations, or perpendicular to this plane, as in $n \rightarrow \pi^*$ excitations. For a $\pi \rightarrow \pi^*$ transition the transition moment direction may be parallel to *any* direction in the flavine plane. Thus, a critical and sensitive test of the accuracy of π-electron wave functions is their ability to predict the absolute direction of the transition moment in the flavine plane (Eaton and Lewis, 1970; Lewis and Eaton, 1971).

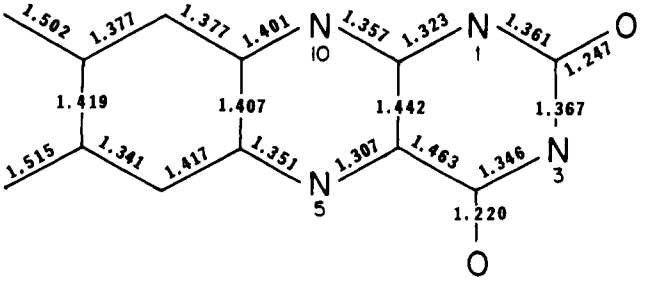

FIGURE 1: Bond distances in the planar isoalloxazine used for determination of the N(5)-N(10) axis. The interatomic distances in this isoalloxazine are very similar to those suggested by Fritchie (Wang and Fritchie, 1973) for an idealized flavine.

photometer attachment. Monochromatic light is obtained with either a 150-W xenon arc source or 40-W quartz-iodine lamp, and a double monochromator (0.25-m Ebert grating monochromators in tandem). A crystal, sealed between two cover slips in a solution similar to its mother liquor, is optically masked by imaging an adjustable field diaphragm onto the crystal plane with a Zeiss Ultrafluar Pol 32X objective (0.4 numerical aperture). The transmitted light is collected by another 32X Ultrafluar and projected onto the cathode of a photomultiplier tube (usually a Hamamatsu R446 or RCA 1P28) with a Zeiss 10X quartz-fluorite eyepiece, which has replaced the glass eyepiece in the Leitz photometric attachment. Light reflected from a fused silica plate, inserted between the field diaphragm and condenser, serves as a reference beam and is collected on a matched photomultiplier tube. The currents from the two tubes are amplified and fed into a Teledyne Philbrick 4361 log ratio module. The derived log ratio is RC filtered and plotted vs. wavelength on a Moseley "Autograf" x-y recorder. The wavelength axis is driven by a voltage signal which is derived from a precision potentiometer coupled to the monochromator drive.

Coordinates of the isoalloxazine atoms in flavodoxin were derived from the results of crystal structure analyses of the oxidized form at 1.9-Å resolution (Burnett et al., 1974) and of the semiquinone form at 2-Å resolution (Ludwig et al., 1975). Flavine orientations were determined using the real space refinement procedure of Diamond (1971) with a planar isoalloxazine model based on the structure of 10-methylisoalloxazine (Wang and Fritchie, 1973) (Figure 1). The angle between the flavine normal and the *c* crystal axis increases by about 3° in going from the oxidized to the semiquinone form, but the ring remains essentially planar (Andersen et al., 1972). As an alternative procedure, the atomic positions after three cycles of difference Fourier refinement (R = 0.289) were used to calculate the orientation of the oxidized flavine plane. We have used the difference between the two methods of positioning the ring to estimate an error of ±2° in the determination of the flavine orientation from the electron density map (see Results). We report transition moment directions calculated from the Diamond-refined coordinates of the appropriate oxidation state.

Results and Discussion

The flavodoxin crystal is optically uniaxial and belongs to the trigonal space group $P3_121$ (Andersen et al. 1972; Ludwig et al., 1969). In a uniaxial crystal there are only two principal spectra: one for the electric vector of the plane-polarized light parallel to the unique *c* crystal axis (a three-fold screw axis in this crystal), and a second for light polarized in the plane perpendicular to the *c* axis in which all directions are optically equivalent (Born and Wolf, 1959). We refer to these spectra as the ∥c and ⊥c spectra, respectively. They are shown in Figures 2 and 3 for the oxidized and semiquinone forms, together with the solution spectra. Also shown is the isotropic spectrum calculated from the crystal spectra using the relation

$$\epsilon(\text{iso}) = \frac{1}{3}[\epsilon(\parallel c) + 2\epsilon(\perp c)] \quad (1)$$

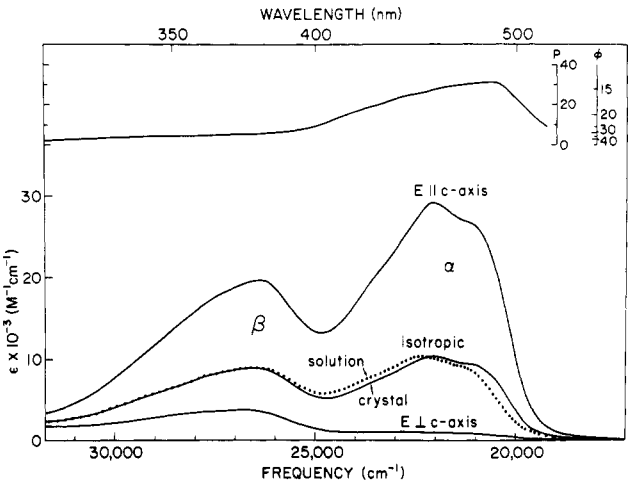

FIGURE 2: Single crystal, solution, and polarization ratio spectra of oxidized flavodoxin from *Clostridium MP*. The crystal spectra were measured with the electric vector of the plane-polarized light parallel and perpendicular to the *c* crystal axis. The polarization ratio, P, is defined at each wavelength by eq 2 and is related to ϕ, the angle that a transition moment for a linearly polarized transition makes with the *c* crystal axis, by eq 3. The crystal was immersed in a solution consisting of 2.8 M ammonium sulfate-0.1 M Tris buffer (pH 6.8) while the composition for the solution spectrum was 1.6 M ammonium sulfate-0.1 M Tris buffer (pH 6.8). The isotropic spectrum was calculated from the polarized crystal spectra using eq 1, assuming equal extinction coefficients of 10,400 M^{-1} cm^{-1} (Mayhew, 1971) for the peaks of the solution and crystal isotopic spectra near 22,000 cm^{-1}.

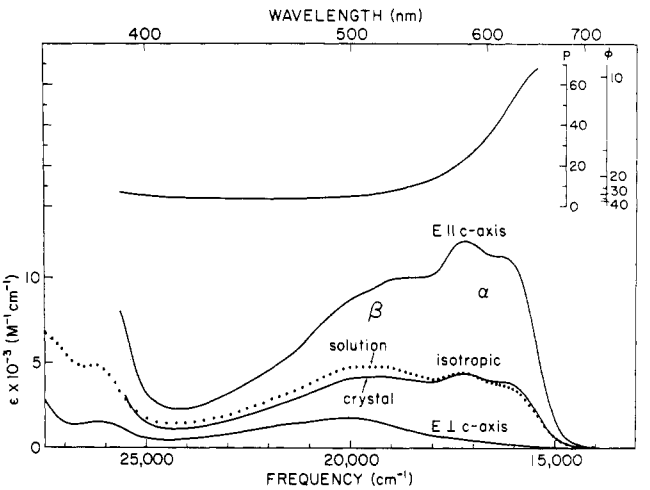

FIGURE 3: Single crystal, solution, and polarization ratio spectra of the semiquinone form of flavodoxin from *Clostridium MP*. The crystal was immersed in a solution consisting of 2.8 M ammonium sulfate-0.5 M potassium phosphate-0.1-0.5 M sodium dithionite (pH 5.5-6) while the composition for the solution spectrum was 1.6 M ammonium sulfate-0.5 M potassium phosphate-0.06 M EDTA (pH 6.2). The isotropic spectrum was calculated from the polarized crystal spectra using eq 1, assuming equal extinction coefficients of 4600 M^{-1} cm^{-1} (Mayhew, 1971) for the peaks of the solution and crystal isotropic spectra at 17,250 cm^{-1}.

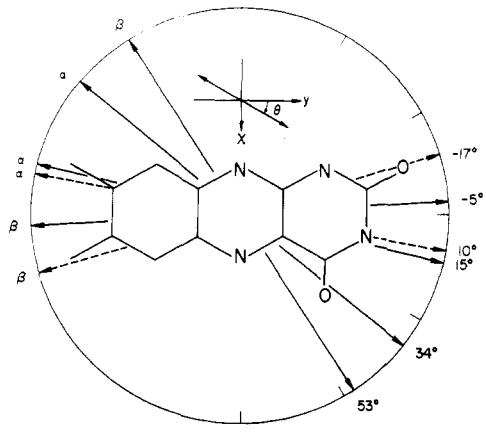

FIGURE 4: Transition moment directions for α and β transitions of the oxidized and semiquinone forms of *Clostridium MP* flavodoxin. The directions are defined relative to the y axis, which is parallel to the flavine plane and perpendicular to the line joining the two nitrogen atoms (N(5)-N(10)) of the central ring. Experimental transition moment directions are the solid double-headed arrows, while the dotted arrows are the directions for oxidized flavodoxin predicted from molecular orbital theory. The experimental angles given are for oxidized flavodoxin, but the corresponding experimental values for the semiquinone are the same to within 7°. For each transition there are two possible choices for the experimental transition moment directions; the choice is discussed in the text.

At the top of each figure is plotted the polarization ratio, P, defined at each wavelength by

$$P \equiv \epsilon(\parallel c)/\epsilon(\perp c) \quad (2)$$

and ϕ, the angle that the transition moment for a linearly polarized transition makes with the c crystal axis. P and ϕ are related by

$$\cos^2 \phi = P/(P + 2) \quad (3)$$

The solution and isotropic crystal spectra are very similar for both the oxidized and semiquinone forms. This is a particularly important result in the case of the semiquinone, since it proves that the chemical species in the crystal for which the X-ray structure has been determined is the neutral "blue" semiquinone and not the "red" semiquinone anion (Massey and Palmer, 1966). Hence crystallization has not altered the ionization state of the flavine semiquinone.[2]

There are, however, small differences between the isotropic crystal and solution spectra in both the oxidized and semiquinone forms. Flavine-flavine interactions are not expected to modify the spectra since in these flavodoxin crystals the nearest neighbor N(5)-to-N(5) distance (see Figure 1) is 24 Å. The difference could arise from *intra*molecular structural changes attending crystallization, or from the introduction of *inter*molecular interactions between the flavine and amino acid residues on adjacent molecules in the crystal lattice. One close intermolecular contact is observed in the electron density maps; the carboxylate of Glu-25 of a neighboring molecule is within 4 Å of the dimethylbenzene end of the flavine ring.

[2] The previously reported single crystal spectrum which appeared to be the spectrum of a "red" semiquinone was measured in *un*polarized light (Ludwig et al., 1971). This spectrum cannot be compared to a solution spectrum since Lambert's law is not obeyed in a crystal for *un*polarized light. The reported spectrum appears similar to our $\perp c$ spectrum, presumably because the crystal was sufficiently thick that essentially all the transmitted light was polarized perpendicular to the c axis, the $\parallel c$ component being totally absorbed.

The optical measurements, in conjunction with the flavine coordinates obtained from the X-ray analysis, permit the calculation of possible transition moment directions. In this discussion we shall neglect intermolecular interactions entirely. The use of this so-called "oriented gas" model (Craig and Walmsley, 1968; Makinen and Eaton, 1973) is a tremendously simplifying feature in interpreting the crystal spectra and should lead to errors of no more than a few degrees in our determination of transition moment directions. To relate transition moment directions to molecular directions we define an orthogonal axis system in the flavine, x,y,z, where x coincides with the line joining the two nitrogen atoms of the central ring, y is perpendicular to this direction and contained in the flavine plane, and z is perpendicular to the flavine plane. This axis system is shown in Figure 4. The direction cosines of the c crystal axis of oxidized flavodoxin in this system are: $n_x = 0.4027$; $n_y = 0.8958$; $n_z = 0.1884$. For transitions polarized parallel to the flavine plane, it can easily be shown that the angle between the transition moment and the y molecular axis, θ, is given by

$$(n_x \sin \theta + n_y \cos \theta)^2 = \cos^2 \phi = P/(P + 2) \quad (4)$$

Notice that for each value of P there are generally two values of θ, and, therefore, two possible in-plane directions for the transition moment.

In order to apply eq 4 a transition must be linearly polarized parallel to the flavine plane. We can test this assumption in the case of oxidized flavodoxin by using the crystal spectra together with the results of fluorescence polarization experiments on model flavines to predict the orientation of the flavine plane relative to the c crystal axis. The spectrum between 20,000 and 30,000 cm^{-1} has been ascribed to two $\pi \rightarrow \pi^*$ transitions (Palmer and Massey, 1968; Sun et al., 1972; Weber, 1966). The first $\pi \rightarrow \pi^*$ transition produces the 20,000–25,000-cm^{-1} absorption system, while the second transition gives the broad band centered at 26,400 cm^{-1}. We label these transitions α and β, respectively. Using the polarization ratios from the crystal spectra and the angle between the α and β transition moments, $\theta_{\alpha\beta}$, from fluorescence polarization experiments, we can calculate the angle between the normal to the flavine plane and the c crystal axis, θ_{zc} ($\cos \theta_{zc} \equiv n_z$). The relevant expression is

$$n_z^2 = \csc^2 \theta_{\alpha\beta}[(1 - n_\alpha^2)(1 - n_\beta^2) - (\cos \theta_{\alpha\beta} - n_\alpha n_\beta)^2] \quad (5)$$

where

$$n_\alpha^2 \equiv \cos^2 \phi_{\alpha c} = P_\alpha/(P_\alpha + 2)$$

and

$$n_\beta^2 \equiv \cos^2 \phi_{\beta c} = P_\beta/(P_\beta + 2)$$

For calculating θ_{zc} we use $P_\alpha = 30 \pm 3$, the value at the center of the 0,0 band at 21,000 cm^{-1}, where we expect the smallest contribution from vibronic components of differing polarization. For P_β we choose the value at the β band maximum at 26,400 cm^{-1}, with a relatively larger uncertainty to take into account the variation of P_β through the peak, i.e., $P_\beta = 5.6 \pm 1.0$. There is considerable variation in the values reported for $\theta_{\alpha\beta}$ (Sun et al., 1972; Kurtin and Song, 1968; Lhoste, 1971; Siodmiak and Frackowiak, 1972; Gordon-Walker et al., 1970). We use the most recently deter-

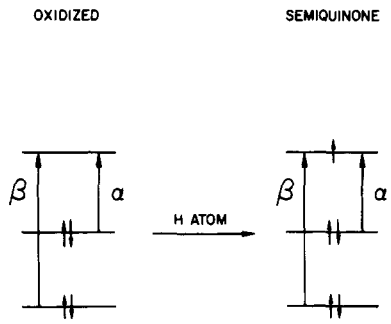

FIGURE 5: Schematic molecular orbital energy level diagram depicting the origin of the two lowest frequency transitions of the oxidized and semiquinone forms of *Clostridium MP* flavodoxin.

mined value of $\theta_{\alpha\beta} = 20 \pm 5°$, reported by Sun et al. (1972) for riboflavine in ethanol at 77°K. From eq 5 θ_{zc} is calculated to be $\pm 79°$ ($\pm 11°$, $\pm 3°$). The optical results thus predict an orientation for the flavine plane which is in excellent agreement with that determined from the X-ray structure ($\theta_{zc} = 79 \pm 2°$), and thereby strongly support the assumption that the α and β transitions are linear and in-plane polarized.

We may now proceed to calculate transition moment directions using eq 4, and find (Figure 4): $\theta_{\alpha y} = 15 \pm 4°$ *or* $33.5 \pm 4°$ and $\theta_{\beta y} = -5 \pm 4°$ *or* $53 \pm 4°$. Studies on flavines in stretched films provide sufficient qualitative information to permit a choice between the two possible values for each transition. Lhoste has measured the polarized absorption of several neutral lumiflavine derivatives, partially oriented in stretched films of poly(vinyl alcohol) (Lhoste, 1971) and has found that both the α and β transitions appear to be polarized more nearly parallel to the y than the x molecular axis. We therefore tentatively accept $\theta_{\beta y} = -5 \pm 4°$ as the correct value. This choice of $\theta_{\beta y}$ suggests that we select $\theta_{\alpha y} = 15 \pm 4°$ to give agreement with the fluorescence polarization result of $\theta_{\alpha\beta} = 20 \pm 5°$ (Sun et al., 1972) ($\theta_{\alpha y} - \theta_{\beta y} = \theta_{\alpha\beta} = 20 \pm 8°$). To compare these transition moment directions with theory we use the most recent molecular orbital calculations of Song et al. (1972) who find $\theta_{\alpha y} = 7$–$12°$ and $\theta_{\beta y} = -14$ to $-21°$. In view of the heteroatomic structure of the flavine ring, we consider this agreement between theory and experiment to be very good.

Comparable fluorescence polarization measurements or theoretical predictions are lacking for model flavine semiquinones. Nevertheless, considerable insight can be obtained from the crystal spectra alone. Upon comparing the 15,000–25,000-cm^{-1} region of the semiquinone spectrum with the 20,000–30,000-cm^{-1} region of the oxidized spectrum we see a striking similarity in the polarization data. If we divide the semiquinone spectrum into contributions from two electronic transitions, which we again call α and β, then the polarization ratios are almost identical with those found for the oxidized form. Using $P_\alpha = 50 \pm 10$ from the 0,0 band at 16,200 cm^{-1} and $P_\beta = 5.0 \pm 1.0$ from the band centered at about 20,000 cm^{-1}, we may then use the direction cosines of the c crystal axis with respect to the coordinate system of the flavine plane in the flavodoxin semiquinone which are: $n_x = 0.4053$; $n_y = 0.9031$; $n_z = 0.1415$, to calculate transition moment directions

$$\theta_{\alpha y} = 16^{+6°}_{-4°} \text{ or } 32^{+4°}_{-6°}$$

$$\theta_{\beta y} = -7 + 4° \text{ or } 56 \pm 4°$$

The estimated errors include worst-case estimates of the error in the polarization ratio as well as a rotation of the molecular axes within $\pm 2°$ from those specified by the direction cosines reported.[3] Including experimental errors, the calculated values for the semiquinone are all within only 7° of the corresponding directions of the oxidized form. This result strongly suggests that the α and β transitions of the semiquinone have the same detailed orbital origin as the α and β transitions of the oxidized form. In the semiquinone form, however, the α and β transitions are at lower frequencies and are closer together. Figure 5 is a schematic molecular orbital energy level diagram depicting the orbitals involved in these transitions. Only the three π orbitals that make the major contribution to the transitions are shown. In both the oxidized and the semiquinone forms electrons are promoted from doubly occupied orbitals. In the semiquinone form the promotion from the half-empty orbital to the lowest empty orbital, which might be expected to be the lowest frequency π electron transition, is either at higher frequencies, or produces much weaker absorptions bands that are obscured by the α,β system.

A comparison of several other parameters of the two spectra supports the hypothesis for a common origin. The approximately 1000-cm^{-1} separation between the 0,0 and 0,1 bands of the semiquinone α transition at 16,200 and 17,200 cm^{-1} is the same frequency interval observed for the α transition of oxidized flavodoxin; it is a typical frequency for a ring breathing mode which usually dominates allowed $\pi \rightarrow \pi^*$ spectra of planar aromatic molecules. The relative intensities and band shapes of the 0,0 and 0,1 bands for the α transition of both forms are also very similar. Furthermore, the band shape of the β transition in the semiquinone form, centered at about 20,000 cm^{-1}, is essentially identical with the β transition of the oxidized form. This can be seen most readily by comparing the $\perp c$ spectra. When properly normalized the absolute intensities of the oxidized and semiquinone spectra are also nearly the same. The ratio of the extinction coefficients in the solution spectra is 2.4 for the α (0,0) band and, after subtracting a small contribution in the semiquinone spectrum from the α transition, is 2.2–2.4 for the β transition. To compare intensities which are proportional to the squared transition moment, these factors must be multiplied by the ratios of the frequencies (Sandorfy, 1964) (16,200/21,000 for the α band, and 20,000/26,400 for the β band), after which they become 1.9 for the α band and 1.7–1.8 for the β band. We must also consider that, unlike the oxidized form, the semiquinone has spin-degenerate ground and excited states. From detailed considerations of transition probabilities it can be readily shown that, if the *orbital* part of the wave functions for the ground and excited states of the oxidized and semiquinone forms are identical, a factor of approximately 2 is expected for the ratio of

[3] The maximum measurable polarization ratio consistent with a given n_z is calculated to be $P_{max} = 2(1 - n_z^2)/n_z^2$. While the values for P_{max} calculated from the Diamond-refined coordinates are 54 for the oxidized flavodoxin, and 98 for the semiquinone, the introduction of $a \pm 2°$ uncertainty in the determination of the flavine plane from the X-ray data decreases these values considerably. Convergence effects and other possible experimental errors in the determination of large polarization ratios give values which are lower than the true value for the crystal. Furthermore a small out-of-plane component arising either from interactions between the flavine and the protein or from a very weak out-of-plane transition could also lower the observed polarization ratios of the α transitions. If we allow for these effects, then, we cannot rigorously exclude the range $20° < \theta_{\alpha y} < 30°$ for the oxidized form and $22° < \theta_{\alpha y} < 26°$ for the semiquinone. The estimated confidence limit for the exclusion of these regions is about 80%.

the absolute intensities.[4] Thus the "normalized" intensities differ by only 5% for the α transition and less than 15% for the β transition.

Another sensitive comparison of the oxidized and semiquinone spectra may be made by examining the natural circular dichroism spectra of the flavodoxins measured by Edmondson and Tollin (1971). It can be seen in their spectra that the α and β transitions carry the same signs in both oxidized and semiquinone forms. Furthermore, for *Clostridium MP* flavodoxin, the anisotropy factors, g (defined as $(\epsilon_l - \epsilon_r)/\epsilon$ at the band extrema), are (Edmondson, 1970): g_α (ox) $= +3 \times 10^{-4}$, g_α(semi) $= +7 \times 10^{-4}$; and g_β (ox) $= -10 \times 10^{-4}$, g_β(semi) $= -5 \times 10^{-4}$. Considering that the circular dichroism may be affected by very small structural changes of the flavine and nearby environment, the agreements of these anisotropy factors are quite good.

Thus, the comparison of the polarizations, band shapes, intensities, and anisotropy factors all indicate that both the α and β transitions of the oxidized and semiquinone forms arise from orbitals with very similar electron distribution. This hypothesis suggests that semiquinone formation takes place by the addition of an electron to the lowest empty π orbital of the oxidized form without any gross electronic rearrangement. At present we know of no other fused, heterocyclic aromatic ring system for which a similar distribution of the π-orbital density has been demonstrated for both oxidized and radical species. Our conclusion, however, is supported by electron paramagnetic resonance and electron-nuclear double resonance investigations. These studies indicate a qualitatively similar distribution of spin density for the triplet excited state of oxidized flavines and the doublet ground state of the corresponding semiquinones (Erickson and Ehrenberg, 1964; Ehrenberg et al., 1967, 1971; Lhoste et al., 1966).

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[4] Consider a molecular system that has two orbitals, described by the wave functions ϕ_a and ϕ_b, and contains three electrons. This system possesses only two orbitally distinct states, one in which ϕ_b contains one electron and ϕ_a is doubly occupied, and another in which ϕ_a contains one electron and ϕ_b is doubly occupied. Each state has a two-fold spin degeneracy. The wave functions for the pair of doubly degenerate states, written as Slater determinants, are

$$^2\Psi_A(S_z = +1/2) = \frac{1}{\sqrt{6}} |\phi_a\bar{\phi}_a\phi_b|$$

$$^2\Psi_A(S_z = -1/2) = \frac{1}{\sqrt{6}} |\phi_a\bar{\phi}_a\bar{\phi}_b|$$

and

$$^2\Psi_B(S_z = +1/2) = \frac{1}{\sqrt{6}} |\phi_a\phi_b\bar{\phi}_b|$$

$$^2\Psi_B(S_z = -1/2) = \frac{1}{\sqrt{6}} |\bar{\phi}_a\phi_b\bar{\phi}_b| \quad \text{(i)}$$

The absolute absorption intensity for the transition between states A and B, given by the dipole strength, D, is

$$D = \frac{1}{2e^2} \sum_{A,B} \left[\langle {}^2\Psi_B \left| \sum_i \mu_i \right| {}^2\Psi_A \rangle \right]^2 \quad \text{(ii)}$$

where e is the charge on an electron, and the factor of two arises from the twofold degeneracy of the ground state A (Sandorfy, 1964). The summations extend over the degenerate sublevels of both states and over all electrons ($i = 1, 2, 3$) in the system. Substituting the wave functions (eq i) into eq ii, recognizing that the electric dipole operator, μ, is a one-electron operator and that only transitions between sublevels with the same value for the spin quantum number, S_z, are allowed, we get

$$D = \frac{1}{e^2} [\langle \phi_b | \mu | \phi_a \rangle]^2 \quad \text{(iii)}$$

If we now consider a system composed of the same two orbitals, ϕ_a and ϕ_b (not necessarily at the same energy), that contains two electrons, there is only a single spin-allowed transition for electric dipole radiation. The transition is between the two singlet states, $^1\Psi_A$ and $^1\Psi_B$, whose wave functions are

$$^1\Psi_A = \frac{1}{\sqrt{2}} |\phi_a\bar{\phi}_a|$$

and

$$^1\Psi_B = \frac{1}{2} |\phi_a\bar{\phi}_b| - \frac{1}{2} |\bar{\phi}_a\phi_b| \quad \text{(iv)}$$

The dipole strength for the transition between these two nondegenerate states contains only a single squared transition moment, and is given by

$$D = \frac{2}{e^2} [\langle \phi_b | \mu | \phi_a \rangle]^2 \quad \text{(v)}$$

Thus, the absorption intensity for the two-electron system is exactly twice that for the three-electron system. In both the oxidized and semiquinone forms of flavodoxin, analogous to our two-electron and three-electron systems, respectively, many more orbitals and electrons must be considered in any detailed calculation of spectral intensities. Inclusion of these will add more configurations to the state wave functions in eq i and iv, and will modify the ratio of intensities from that predicted on the basis of a two-orbital system. Nevertheless, this oversimplified model does suggest that a factor of approximately two is expected for transitions between corresponding orbitals, if they are not substantially different in the oxidized and semiquinone forms.

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Cell Contact-Dependent Ganglioside Changes in Mouse 3T3 Fibroblasts and a Suppressed Sialidase Activity on Cell Contact†

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ABSTRACT: Certain enzyme activities for synthesis and degradation of gangliosides and the chemical quantity and incorporation of radioactivity from [^{14}C]galactose into gangliosides have been studied in 3T3 cells and their transformed counterparts at various cell population densities. The chemical quantity of and the incorporation of radioactivity into GD1a ganglioside increased at the early stage of cell contact ("contact response" of ganglioside), whereas this response was not detectable in transformed 3T3 cells at any stage of cell contact. These phenomena were reproduced in five separate qualitative analyses and two quantitative determinations of gangliosides. As the basis of these phenomena, a membrane-bound sialidase activity which acted on gangliosides was suppressed in 3T3 cells at the "touching" stage of cell-to-cell contact. Transformed cells did not display the change of sialidase activity at any stage of cell contact.

Nontransformed cells in culture reduce their growth rate significantly when their cell population density increases ("contact inhibition", "topoinhibition", or "density-dependent inhibition") (Abercrombie and Ambrose, 1962; Todaro and Green, 1963; Stoker and Rubin, 1967; Dulbecco, 1970). Because transformed cells do not significantly reduce their growth rate at higher cell population densities, the phenomenon is regarded as the basis for loss of growth control in transformed cells, although the essential biological difference between normal and transformed cells could be a difference in membrane susceptibility to "serum factor" and other nutrients (Holley, 1972).

The biochemical basis of cell surface changes associated with cell contact is important not only for understanding

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