The Structure of a Metal-Flavin Complex

10-METHYLISOALLOXAZINE SILVER NITRATE*

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SUMMARY

A crystalline silver nitrate complex of 10-methylisoalloxazine, the aromatic moiety of flavocoenzymes, has been prepared and characterized by x-ray diffraction methods. The red crystals are monoclinic, with symmetry C2/c and unit cell constants a = 10.424 (5) (numbers in parentheses represent the standard deviation in the least significant figures quoted), b = 16.564 (8), c = 16.260 (8) A, \( \beta = 116.87^{\circ} \), \( Z = 8 \), and \( \rho_{\text{calc}} = 2.06 \) g per cm\(^3\). The structure has been refined to \( R = 6.5\% \), using 1432 counter-measured reflections.

Each flavin binds to two distinct silver ions. A centrosymmetric square planar silver ion binds 2 flavin molecules fairly strongly through O(4) and N(5); a six-coordinate silver ion lying on a 2-fold axis binds two nitrate groups and the O(2) oxygen atoms of two flavins, and also interacts more weakly with the N(1) nitrogen atoms of the same flavins. The N(3) proton is retained by the flavin, and is hydrogen-bonded to nitrate. These results suggest that at least two distinct sites exist for metal-flavin chelation in N(3)-protonated flavins and thus this compound may be a model for the metal-flavin interactions in xanthine oxidase as well as other metalloflavoproteins.

A number of flavoproteins contain metal atoms which are assumed to interact with the flavin at some point in the catalytic reaction. In most cases the metal is non-heme iron, although it is sometimes molybdenum and in xanthine oxidase (1-3), which contains both, EPR studies have shown that electrons flow in the order substrate \( \rightarrow \) molybdenum \( \rightarrow \) flavin \( \rightarrow \) iron. Spectroscopic studies have shown (4-7) that in general only the semiquinone form of flavin has significant affinity for metals, but that certain oxidation-reduction-active metals are exceptions and bind strongly to oxidized flavin. Among these are Fe(II), Mo(V), Cu(I), and Ag(I), with only Ag(I) complexes being air-stable. Bamberg and Hemmerich (7) attribute this anomalous stability to resonance of the form \( \text{M}^{n+} \text{F} \leftrightarrow \text{M}^{n+} \text{F}^- \), where F represents oxidized or quinone flavin and F\(^-\) semiquinone flavin. They also argue that flavin binds to most metals by chelation through O(4) and N(5). (See formula below for numbering.)

Several crystalline silver-flavin complexes have recently been synthesized in this laboratory to study further the interaction of flavins with metals.\(^1\)

EXPERIMENTAL PROCEDURES

A hot, nearly saturated solution of 10-methylisoalloxazine in formic acid was added to a warm methanolic solution of silver nitrate and cooled slowly. Most often, a mixture of orange microcrystalline needles and metallic silver resulted, but in one such mixture a few flat diamond to bladelike red crystals appeared. The best of these was mounted on the blade axis (c) and used for all further measurements. It was of flattened hexagonal cross-section, with small \{110\} and large \{010\} faces, truncated by \{001\}. Dimensions were approximately 0.16 mm parallel to a, 0.10 mm parallel to b, and 0.13 mm parallel to c. The crystal was shown by Weissenberg photography to have monoclinic symmetry and to exhibit systematic absences characteristic of space group C2/c. Unit cell constants, refined using \( \sin^2 \theta \) values determined with a Picker four-circle diffractometer assuming \( \lambda_{CuK\alpha} = 1.5418 \) A, are \( a = 10.424(5) \), \( b = 16.564(8) \), \( c = 16.260(8) \), \( \alpha = 116.87^{\circ} \). With \( Z = 8 \) and empirical formula \( C_{16}H_{23}N_4O_2 \cdot AgNO_3 \), \( \rho_{\text{calc}} \) is 2.06 g per cm\(^3\).

The structure was solved and refined in three stages. Crude intensity data from a set of Weissenberg photographs were used to solve the structure, with the aid of Patterson and Fourier techniques. A set of counter data limited to \( 2\theta = 90^{\circ} \) for CuK\(\alpha \) radiation was used to refine the structure to \( R = 7.6\% \), and then reflections in the \( 2\theta \) region from \( 90^{\circ} \) to the instrumental limit, \( 132^{\circ} \), were scanned if they had calculated structure magnitudes exceeding 25. The final refinement included 954 reflections having \( I > 2\sigma(I) \).

\( ^1 \) G. D. Sproul and C. J. Fritchie, Jr., American Crystallographic Association Winter Meeting, Albuquerque, New Mexico, 1972, Abstract E12; C. J. Fritchie, Jr., G. D. Sproul, and T. D. Wade, IX International Congress of Crystallography, Kyoto, Japan, 1972, Abstract III-45.

\( ^2 \) Throughout this paper, a number in parentheses immediately following a numerical value represents the standard deviation in the least significant figures quoted.
ing $2\theta \leq 90^\circ$ and 478 having $2\theta > 90^\circ$. All were measured by $2\theta$ scan on a card-controlled Picker four-circle diffractometer, using nickel-filtered CuKα radiation and a Na(Tl)I scintillation counter set to accept about 90% of the Kα pulses. Standard deviations were calculated with the formula $\sigma_f^2 = C + (t_c/2t_s)^2$ $(B_1 + B_2) + p\sigma_\varepsilon$, where $I$ = net intensity, $C$ = scan count, $B_1$ and $B_2$ are background counts at each end of the scan range, $t_c$ is the time of the scan, $t_s$ is the time of each background count (20 s), and $p = 0.02$. The scan rate was 1° per min, and the range varied from 2° to 2.7°. Only reflections having $I \geq 2\sigma I$ were included in the final refinement. Structure factors ($F_\alpha$) were derived from the intensities by application of Lorentz, polarization, and absorption corrections.$^{3,4}$ The latter ranged from 0.24 to 0.38 in $|F_\alpha|^2$.

$^3$ Major computer programs used were ORABS for absorption correction, by Busing, Martin, and Levy; FOUR for Fourier summation, by Fritsche; HUGHES, a local modification of UCLALSI, by Gantzel, Sparks, and Trueblood, for least squares refinement; and ORTEP, by Johnson, for graphics.

$^4$ One (810) face contained a large hollow. This makes the absorption correction somewhat inaccurate and contributes to final errors which are slightly greater than might otherwise be expected.

Table I
Atomic positional parameters

| Atom | x | y | z |
|------|---|---|---|
| Ag(1) | 0 | 0 | 0 |
| Ag(2) | 1/2 | 0 | 0 |
| N(1) | 0.4046(6) | 0.2000(4) | 0.6009(4) |
| C(2) | 0.3542(8) | 0.2446(8) | 0.6523(5) |
| N(3) | 0.3436(6) | 0.3207(4) | 0.6410(4) |
| C(4) | 0.3816(7) | 0.3275(7) | 0.5849(5) |
| N(5) | 0.4912(6) | 0.3621(4) | 0.4856(4) |
| C(6) | 0.6033(8) | 0.3389(5) | 0.3853(5) |
| C(7) | 0.6605(8) | 0.3140(6) | 0.3384(5) |
| C(8) | 0.6644(9) | 0.2313(6) | 0.3430(6) |
| C(9) | 0.6084(9) | 0.1885(5) | 0.3923(5) |
| N(10) | 0.4983(6) | 0.1929(4) | 0.4978(4) |
| C(10a) | 0.4472(7) | 0.2356(5) | 0.5464(5) |
| C(4a) | 0.4438(6) | 0.3228(5) | 0.5346(5) |
| C(5a) | 0.5409(7) | 0.3162(5) | 0.4376(5) |
| C(9a) | 0.5504(8) | 0.2323(5) | 0.4421(5) |

$a$ These parameters are fixed by symmetry.

Table II
Anisotropic thermal parameters

The thermal expression is exp.$-(B_1x^2 + B_2y^2 + B_3z^2 + B_4xy + B_5yz + B_6xz + B_7xt)$.

| Atom | $B_{11}$ | $B_{22}$ | $B_{33}$ | $B_{12}$ | $B_{13}$ | $B_{23}$ |
|------|----------|----------|----------|----------|----------|----------|
| Ag(1) | 0.02810(10) | 0.00229(3) | 0.01081(4) | 0.000293(11) | 0.002949(7) | 0.000055(7) |
| Ag(2) | 0.01566(15) | 0.00242(3) | 0.00961(7) | 0.00017(7) | 0.0083(6) | 0.00035(5) |
| N(1) | 0.0093(6) | 0.0023(2) | 0.0050(3) | -0.0009(9) | 0.0050(7) | 0.0013(5) |
| C(2) | 0.0067(7) | 0.0036(3) | 0.0040(4) | -0.0006(8) | 0.0071(6) | 0.00056(6) |
| C(3) | 0.0110(6) | 0.0025(2) | 0.0039(3) | -0.0003(7) | 0.0056(6) | 0.0010(4) |
| C(4) | 0.0084(7) | 0.0032(3) | 0.0027(3) | -0.0012(10) | 0.0097(6) | -0.0005(6) |
| N(5) | 0.0010(7) | 0.0019(2) | 0.0030(3) | -0.0010(11) | 0.0076(8) | -0.0020(7) |
| C(6) | 0.0114(7) | 0.0032(3) | 0.0033(3) | -0.0013(10) | 0.0060(7) | -0.0027(6) |
| C(7) | 0.0106(9) | 0.0045(4) | 0.0035(3) | -0.0001(11) | 0.0076(8) | -0.0020(7) |
| C(8) | 0.0120(10) | 0.0048(4) | 0.0040(4) | -0.0022(9) | 0.0082(8) | -0.0016(8) |
| C(9) | 0.0130(9) | 0.0031(3) | 0.0039(4) | 0.0020(7) | 0.0041(5) | -0.0007(5) |
| N(10) | 0.0001(6) | 0.0021(2) | 0.0033(3) | -0.0022(8) | 0.0069(7) | -0.0006(5) |
| C(10a) | 0.0076(7) | 0.0027(3) | 0.0023(3) | -0.0002(8) | 0.0026(7) | -0.0010(5) |
| C(4a) | 0.0061(7) | 0.0027(3) | 0.0028(3) | -0.0010(9) | 0.0017(4) | -0.0009(9) |
| C(5a) | 0.0055(7) | 0.0027(3) | 0.0024(4) | -0.0008(9) | 0.0038(8) | -0.0003(6) |
| C(9a) | 0.0076(8) | 0.0034(3) | 0.0032(4) | 0.0022(7) | 0.0167(9) | 0.0039(4) |
| O(2) | 0.0141(6) | 0.0020(2) | 0.0066(3) | 0.0010(6) | 0.0137(5) | 0.0004(4) |
| O(4) | 0.0102(9) | 0.0021(3) | 0.0054(6) | 0.0023(4) | 0.0054(9) | 0.0003(7) |
| C(10) | 0.0146(9) | 0.0031(3) | 0.0040(3) | -0.0011(9) | 0.0066(8) | 0.0002(6) |
| Oa | 0.0185(8) | 0.0049(3) | 0.0068(3) | 0.0047(8) | 0.0141(7) | 0.0005(6) |
| Ob | 0.0260(13) | 0.0072(4) | 0.0045(4) | 0.0014(12) | 0.0052(11) | 0.0010(7) |
| Oc | 0.0244(11) | 0.0068(4) | 0.0064(4) | 0.0043(10) | 0.0104(9) | -0.0065(5) |

$a$ These parameters are fixed by symmetry.
The final refinement resulted in \( R(= \sum |F_o - F_e|/2|F_o|) = 6.5 \), and \( R_e(= \sum w |F_o - F_e|^2/\sum w |F_o|^2)^{1/2} = 6.5 \), where \( w = 1/\sigma^2 \). The goodness of fit parameter, \( S \), is 3.6. Atomic scattering factors (8, 9) were corrected for complex dispersion effects where necessary (10). All atoms except hydrogen were given ellipsoidal temperature factors. Hydrogen atoms, after location in a series of difference maps, were placed in calculated positions, 0.95 Å from the appropriate bonded atom. The orientation of the methyl group was determined by a difference Fourier map in the \( H_2 \) plane. There were three fairly sharply resolved peaks in this map. The isotropic thermal parameter, \( B \), was fixed at 3.2 for all hydrogen atoms. The final atomic parameters appear in Tables I and II.

RESULTS AND DISCUSSION

The numbering system and immediate surroundings of a flavin molecule are shown in Fig. 1. The flavin in fact does coordinate to O(4) and N(5), which will be described as the primary chelate site.6 It should also be noted that the flavin in this structure is protonated at N(3), whereas the spectroscopic model studies (5-7) dealt with a flavin ion formed by loss of this proton.

5 The complete list of observed and calculated structure factors is given in the “Appendix.”

6 “Chelation” to the secondary site might be considered inappropriate for this structure in view of the weak bonding to N(1), but it is part of a pattern seen also in bis-(10-methylisoalloxazine)sesqui(silver perchlorate)2 where the bonding is stronger.

### TABLE III

Interatomic distances and angles

| Distances | Angles |
|-----------|--------|
| **Atoms** | **Dist. (Å)** | **Atoms** | **Dist. (Å)** | **Atoms** | **Dist. (Å)** | **Atoms** | **Dist. (Å)** |
| Ag+ (1) - O(4) | 2.484(6) | N(1) - C(1a) | 1.321(10) | C(7) - C(8) | 1.372(14) | Ag+ (1) - N(5) | 2.294(6) | N(1) - C(2) | 1.386(10) | C(8) - C(9) | 1.382(12) | Ag+ (2) - O(2) | 2.026(7) | C(2) - N(3) | 1.398(12) | C(9) - C(9a) | 1.409(11) | Ag+ (2) - O(2) | 2.629(6) | N(3) - C(4) | 1.370(10) | C(9a) - N(10) | 1.409(10) | Ag+ (2) - O(2) | 2.532(9) | C(4a) - C(4a) | 1.500(11) | N(10) - C(10a) | 1.366(10) | N(10) - C(10a) | 1.243(10) | N(5) - C(5a) | 1.390(11) | C(2) - C(2) | 1.241(11) | N(10) - C(10a) | 1.466(11) | N(10) - C(10a) | 1.245(11) | C(6) - C(7) | 1.245(11) | C(2) - O(2) | 1.377(11) |

| **Atoms** | **Angle (deg.)** | **Atoms** | **Angle (deg.)** | **Atoms** | **Angle (deg.)** |
|-----------|------------------|-----------|------------------|-----------|------------------|
| C(2) - N(1) | 110.6(6) | C(6) - C(7) | 121.2(7) | C(2) - N(1) | 118.9(7) | C(7) - C(8) | 122.2(8) |
| N(3) - C(2) | 118.9(7) | N(1) - C(2) | 121.8(8) | N(3) - C(2) | 119.3(6) | C(8) - C(9) | 118.2(7) |
| N(1) - C(2) | 119.3(6) | N(1) - C(3) | 121.4(7) | C(9) - C(9a) | 119.4(7) |
| C(2) - N(3) | 125.4(6) | N(3) - C(4) | 113.8(6) | C(9a) - C(9a) | 119.4(7) |
| C(3) - C(4) | 113.8(6) | C(5a) - C(9a) | 119.4(7) | C(5a) - C(9a) | 119.2(6) |
| N(3) - C(4) | 120.1(7) | C(9a) - N(10) | 118.7(7) | N(10) - C(10a) | 119.8(6) |
| C(4a) - C(4a) | 126.0(7) | C(2) - C(2) | 118.9(7) | C(10) - N(10) | 121.5(6) |
| C(4) - C(4a) | 116.7(7) | C(9a) - N(10) | 117.2(7) | C(10) - C(10a) | 119.8(6) |
| C(4) - C(4a) | 117.5(6) | C(9a) - N(10) | 117.2(7) | C(10) - C(10a) | 121.5(6) |
| C(10a) - C(4a) | 118.9(7) | C(9a) - N(10) | 117.2(7) | C(10a) - C(10a) | 119.8(6) |
| C(4a) - N(5) | 116.5(7) | C(2) - C(2) | 118.9(7) | C(10a) - C(10a) | 121.5(6) |
| C(5a) - C(5a) | 117.1(7) | C(2) - C(2) | 118.9(7) | C(10a) - C(10a) | 119.8(6) |
| C(6) - C(5a) | 121.3(7) | C(2) - C(2) | 118.9(7) | C(10a) - C(10a) | 121.5(6) |
| C(5a) - C(6) | 117.5(7) | C(2) - C(2) | 118.9(7) | C(10a) - C(10a) | 119.8(6) |
In this structure, Ag⁺(1) is bound quite strongly to the primary chelate site. It is only 0.26 Å from the least squares plane of the flavin and as Table III reveals, is bound only slightly weaker than Ag⁺ in bis(8-hydroxyquinoline) silver(I)pyridine solvate (11), a salt of what is generally considered a strongly coordinating ligand. In the latter salt, Ag⁺ ... N is 2.145(4) and 2.155(4) Å, and Ag⁺ ... O is 2.451(4) and 2.505(4) Å, whereas corresponding values in this structure are 2.294(6) and 2.484(6) Å. Other comparable Ag ... N distances are 2.21(1) Å in pyrazine silver nitrate (12) and 2.24 Å in catena-μ-thiocyanato-bis(thiosemicarbazide silver(I) (13). The shortest known, rather strongly covalent, Ag⁺ ... N distance is 2.115(8) Å in silver thiocyanate (14). Any evaluation of the strength of the Ag⁺ ... N(5) bond must give due regard to the fact that a short C(6)H(6)·O(4)' contact of 2.36 Å involving the second flavin coordinated to Ag⁺(1) must surely lengthen Ag⁺(1) ... N(5) beyond the distance it would assume were the benzo group absent. The sum of hydrogen and oxygen van der Waals radii is 2.6 Å. The Ag⁺(1) ... O(4) distance is quite comparable with values found in other, presumably at least moderately strongly coordinated ligands. Thus, the two nitrate oxygen atoms in this structure are 2.532(8) and 2.613(8) Å from Ag⁺(2); those in AgN0₃ (15), 2.48, 2.55 Å, and further; in cyclooctatetraene silver(1) nitrate (16), 2.36(2) and 2.43(2) Å.

That square-planar coordination can occur in the primary site of a planar flavin molecule (one of the two independent flavins in bis(10-methylisoalloxazine)sesqui-(silver perchlorate) is similarly coordinated') suggests that octahedral coordination is also possible for metals at this site. A conceivable model for interaction of flavin with the type of Fe&S₈ cluster found in ferredoxin? is il

Fig. 2. A postulated complex between riboflavin and an Fe₅S₈ cluster of the type found in Micrococcus aerogenes ferredoxin. The R groups attached to the iron-sulfur cluster are sites of attachment to the protein chain.

TABLE IV
Bond lengths in oxidized and reduced flavins

| Bond     | I          | II         | III        | IV         |
|----------|------------|------------|------------|------------|
| N(1)-C(10a) | 1.330(8)   | 1.324(12)  | 1.36(3)    | 1.357(10)  |
| C(10a)-C(4a) | 1.443(8)   | 1.445(12)  | 1.37(3)    | 1.384(12)  |
| C(4a)-N(5)  | 1.311(8)   | 1.297(12)  | 1.43(2)    | 1.484(10)  |
| N(1)-C(2)   | 1.358(8)   | 1.387(12)  | 1.37(3)    | 1.329(12)  |
| C(2)-O(2)   | 1.236(8)   | 1.231(12)  | 1.20(3)    | 1.277(11)  |
| C(2)-N(3)   | 1.408(8)   | 1.389(12)  | 1.43(3)    | 1.376(12)  |
| N(3)-C(4)   | 1.347(8)   | 1.349(12)  | 1.37(4)    | 1.409(11)  |
| C(4)-O(4)   | 1.230(8)   | 1.212(12)  | 1.25(3)    | 1.224(12)  |
| C(4)-C(4a)  | 1.447(8)   | 1.459(12)  | 1.42(3)    | 1.427(13)  |
| N(5)-C(5a)  | 1.364(8)   | 1.376(12)  | 1.44(2)    | 1.491(11)  |
| C(5a)-C(9a) | 1.406(8)   | 1.420(12)  | 1.39(3)    | 1.375(12)  |
| C(9a)-N(10) | 1.371(8)   | 1.418(12)  | 1.40(2)    | 1.431(10)  |
| N(10)-C(10a) | 1.361(8)  | 1.356(12)  | 1.37(3)    | 1.353(11)  |

I = lumiflavin bis(naphthaene)-2,3-diol(18); II = 9-Bromo-3,7,8,10-tetramethylisoalloxazine monohydrate(19); III = 5-acetyl-9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisoalloxazine(20); IV = 5-diethyl-3,7,8,10-tetramethyl-1,5-dihydroisoalloxazine trihydrate(21).

TABLE V
Displacements from the least-squares plane of the isoalloxazine molecule

All atoms used were given equal weight. Distances in parentheses indicate atoms not used in calculating the plane. The equation of the plane is 0.62644m + 0.05665n + 0.77741p = 6.8067, where m, n, and p are orthogonalized coordinates in an axial system M, N, P, having N || b, and P || c.*

| Atom     | Distance (Å) | Atom     | Distance (Å) |
|----------|--------------|----------|--------------|
| Ag⁺(1)   | 0.264        | C(9)     | 0.039        |
| Ag⁺(2)   | 1.548        | N(10)    | 0.050        |
| N(1)     | 0.033        | C(10a)   | 0.016        |
| C(2)     | 0.089        | C(4a)    | 0.038        |
| N(3)     | 0.028        | C(5a)    | 0.019        |
| C(4)     | -0.062       | C(9a)    | 0.045        |
| N(5)     | -0.019       | O(2)     | 0.226        |
| C(6)     | 0.041        | O(4)     | -0.191       |
| C(7)     | 0.059        | C(10)    | -0.049       |
| C(8)     | 0.038        |          |              |
illustrated in Fig. 2. Comparatively little distortion of the Fe₈₆ cluster is required.

Coordination of the flavin through its secondary chelate site N(1)-O(2) is weaker and less regular. Only O(2) at a distance of 2.629(s) Å can be considered bound to Ag⁺(2), although if there were no interaction with N(1), one might expect less overlap of the two flavin molecules shown in Fig. 1 and a greater Ag⁺ ... N(1) distance. Strong coordination to both N(1) and O(2) is certainly possible, illustrating that flavin can coordinate in two distinct chelate sites, which can then serve as electron channels. Simultaneous or sequential transfer of electrons through the τ system is, of course, also possible.

Because of the anomalous stability of the silver salts of flavins, it is instructive to attempt determination of the oxidation state of the ligand in this structure by an examination of its detailed geometry. It has been shown, for example (17), that addition of a single electron to a molecule as complex as tetracyanoquinodimethane, C₄₂N₄, can result in bond length variations as large as 0.01 to 0.02 Å even with no configurational change. As anticipated (see Table IV), two studies of reduced flavins (20,21) show lengthening of bonds N(1)-C(10a) and C(4a)-N(5), and a contraction of C(4a)-C(10a) relative to oxidized flavin. The bond C(4)-C(4a) also appears to be shorter. One might expect bonds in the semiquinone to approximate the average of those in the oxidized and reduced flavins.

Examination of these "diagnostic" bonds in the silver salt leads to the conclusion that it is geometrically more similar to oxidized than to reduced, or even semiquinone, flavin. There is probably a significant shortening of C(4a)-C(10a), but C(3)-C(4a) is actually longer than in oxidized flavin. Thus, although there may be some transfer of charge from silver to the flavin, the extent of this transfer seems limited.

As Table V shows, the flavin molecule, excluding O(2) and O(4), is nearly planar but shows a slight twist around its long axis. O(2) is probably bent out of the plane by its attraction toward Ag⁺(2), and O(4) may bend out of the plane to reduce its contact distance with H(6'). The O ... H distance is approximately 0.06 Å greater than it would be if O(4) were in the least squares plane.

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