The Crystal Structure of a Riboflavin-Metal Complex

RIBOFLAVIN SILVER PERCHLORATE HEMIHYDRATE* (Received for publication, August 22, 1972)

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SUMMARY
A crystalline silver perchlorate complex of riboflavin, a possible model for enzymic metal-flavin interactions, has been prepared and characterized by x-ray techniques. The orange crystals are monoclinic, with symmetry C2. and unit cell constants a = 19.464(10) (figures in parentheses are standard deviations in the least significant figures cited), b = 7.886(4), c = 15.459(8)Å, β = 107.34(2)°, Z = 4, $\rho_{\text{obs}} = 1.79$ g/cm$^3$, and $\rho_{\text{calc}} = 1.77$ g per cm$^3$ for AgClO$_4$·C$_{17}$H$_{20}N$_4O$_6$(CO)$_0.45$·$\frac{1}{2}$H$_2$O. The crystal structure, refined using 2837 counter-measured reflections, gives an $R$ factor of 6.2% and revealed that about 45% of the riboflavin in the crystal used was formulated in the 5' position by the formic acid solvent.

Each riboflavin molecule bonds fairly strongly to two silver ions: one via N(1) (2.304(5)Å), O(2) (2.786(5)Å), and O(2') (2.559(6)Å), and the other via N(2) (2.295(5)Å) and O(4) (2.521(5)Å). These results confirm the existence of two separate chelate sites in the N(3)-protonated, quinoid isoalloxazine ring system, and together with earlier crystal structure studies suggest that both these chelate sites will nearly always be occupied by positive ions or dipoles (such as —OH). Two conformationally preferred forms of riboflavin seem both to have the ribityl chain extending above the isoalloxazine ring when viewed with the benzo ring to the right and N(10) at the top. In one, C(1')—C(4') and O(4') are nearly planar and fully extended; in the other, O(2') and C(2')—C(5').

A number of flavoproteins contain metals which serve in addition to the flavin as catalytic agents. Although the relationship of flavin and metal is generally unknown, the catalytic electron flow in xanthine oxidase (1-3) is known to occur in the order substrate $\rightarrow$ molybdenum $\rightarrow$ flavin $\rightarrow$ non-heme iron. The only metals found in model studies to form complexes with quinoid riboflavin (4-7) are Fe(II), Mo(V), Cu(I), Hg(I), Hg(II), and Ag(I), with the latter having the advantage of air stability. To study likely modes of metal-flavin interactions, whether stable or transitory, we have prepared a crystalline complex of riboflavin and silver perchlorate and we report here its crystal structure. This work has previously been reported in part† and confirms observations recently noted in similar crystal structures‡ regarding flavin-silver interactions. In addition, this crystal structure when compared with a series of others (8-18) serves to define the preferred hydrogen-bonding environment of the isoalloxazine nucleus and two preferred configurations of the ribityl side chain.

EXPERIMENTAL PROCEDURES
A warm aqueous solution of silver perchlorate was added to a concentrated solution of riboflavin in warm formic acid, to which a small amount of hydrogen peroxide had been added. Upon slow cooling, large orange plate-like crystals appeared. A silver-riboflavin molar ratio of 2.6 to 2.8 yielded best results. Preliminary rotation and Weissenberg photography showed the crystals to be monoclinic, with systematic absences characteristic of space group C2 (hkl with h + k = 2n + 1 absent). Accurate unit cell constants for the crystal examined (which contains about 45% O(5')-formylated riboflavin, vide infra) were determined by least squares fitting of $\sin \theta$ values measured with the aid of a Picker four-circle diffractometer. The density of crystals from the same preparation was measured by flotation in a dibromothene-carbon tetrachloride mixture. Unit cell constants, based on $\lambda_{\text{MoK}α} = 0.7107$ Å, are $a = 19.464(10)$ Å, $b = 7.886(4)$ Å, $c = 15.459(8)$ Å, $\beta = 107.34(2)$°. For four groups of formula AgClO$_4$·C$_{17}$H$_{20}N$_4O$_6$(CO)$_0.45$·$\frac{1}{2}$H$_2$O per unit cell, the calculated density is 1.77 g per cm$^3$, in fair agreement (vide infra) with the observed value of 1.79 g per cm$^3$.

Intensity data were measured on a blade-shaped crystal having large [001] and smaller [100] and [110] faces, about 0.6 mm || b, 0.26 mm || a, and 0.12 mm || c and mounted on b. The instrument used was a Picker four-circle, card-controlled diffractometer, equipped with a molybdenum target, zirconium filter, and Na(Tl)I scintillation counter with pulse-height analyzer set to accept 90% of the MoKα pulse distribution. Reflections were scanned over a 2θ range varying from 2.5 to 2.9° at a rate

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§ Throughout this paper, figures in parentheses immediately following a numerical value give the standard deviation in the least significant digits quoted.

2337
of 1° per min, and a background count of 20 s was collected at each end of the scan.

All independent data in the sin θ/λ range from 0 to 0.632 were collected and after the structure was refined to approximately R = 8%, the 476 reflections of a possible 4707 in the range 0.632 < (sin θ/λ) < 0.9 having |F>| > 2σ(F) were also scanned. The final data set included 2331 observed and 242 unobserved reflections having sin θ/λ ≤ 0.632 and 475 observed and 1 unobserved in the extended range. Reflections are classed as observed or unobserved according to whether the net intensity exceeds twice its standard deviation as defined by σ = \sqrt{C + (t_c/2w)^2 (B_1 + B_2) + p^2 I^2}, where C = total scan count, t_c = time of scan, t_b = time of each background, B_1 and B_2 are background counts, p = 0.02 for low order data and 0.03 for the extended set, and I = net intensity. Unobserved reflections were assigned threshold values of I = 2σ. All data were corrected for Lorentz, polarization, and absorption effects, the latter factor ranging from 0.87 to 0.94 in F, and values of σ(F) were extracted from σ/λ by the propagation-of-error equation.

The structure was solved by Patterson and Fourier techniques and refined by the method of least squares to a final R = Σ |F_0| - |F_cal|/Σ |F_0| of 6.2, R = Σ |F_0| - |F_cal|/Σ |F_0| of 6.0, and S(goodness of fit): S = Σ |F_0| - |F_cal|/|F_cal| (observations-parameters)^1/2 = 3.6. Heavy atoms were given ellipsoidal thermal parameters. All hydrogen atoms except the alcoholic ones on the ribityl chain and those on the water molecule were located in a series of difference maps but input at idealized positions, 0.95 Å from the appropriate bonded atom, and not refined. At a late stage of refinement, two persistent peaks near 0(5') were identified as a formyl group attached to O(5'). The occupancy factor of 0.45 was chosen to make the anisotropic thermal parameters of C, V approximately the size of those of O(5'). The occupancy parameter of 0.9 for the water molecule, W, attached to Ag(2)+ was similarly chosen.

The final difference map reveals, in addition to expected peaks in the silver and perchlorate regions, a small peak on the 2-fold axis about 2.5Å from hg(l)+, on the side opposite O(2'). It is estimated that this site may be about 25 to 40% occupied by water, but difficulties with refining an atom here, and also with the perchlorate group, led to omission of this water and fixing of the perchlorate oxygen parameters at the values they had at the end of the sin θ/λ ≤ 0.632 refinement (R = 6.6%). In fact no good model could be found for the perchlorate group, and the parameters used are regarded only as a reasonable approximation with little detailed physical significance.

Structure factors (19, 20) were corrected for the complex effects of dispersion where necessary (21). The dextro enantiomer was assumed. Final atomic parameters appear in Tables I and II; observed and final structure magnitudes are given in the Appendix.

RESULTS AND DISCUSSION

The numbering scheme and flavin geometry are shown in Fig. 1. As in 10-methylisoalloxazine silver nitrate (8), the bond distances seem to show that little if any charge is donated into the isoalloxazine system by Ag+. C(4)+C(10)+ is 1.442(8)Å in this structure, 1.406(12)Å in the 10-methylisoalloxazine complex, an average of 1.444(8)Å in quinoid flavins (8), and probably 1.42(3)Å in semiquinoid flavins (8). Corresponding values for C(4)+C(10)+ are 1.444(8)Å in quinoid flavins (8), and probably 1.444(8)Å in semiquinoid flavins (8). Corresponding values for N(1) are 1.327(8)Å, 1.321(10)Å, 1.327(8)Å, and 1.342(8)Å; for C(4)+C(10)+ are 1.444(8)Å in quinoid flavins (8), and probably 1.444(8)Å in semiquinoid flavins (8). Corresponding values for N(1) are 1.327(8)Å, 1.321(10)Å, 1.327(8)Å, and 1.342(8)Å; and for C(4)+C(10)+ are 1.444(8)Å, 1.466(8)Å, 1.500(11)Å, 1.466(8)Å, and 1.427(10)Å.

The structure contains two independent silver ions, each lying on a 2-fold axis and coordinated to riboflavin. As in 10-methylisoalloxazine silver nitrate (8) and as originally predicted by Bamberg and Hemmerich (6), one silver, Ag(2)+, is coordinated moderately strongly to the primary chelate site, N(5)—O(4), with distances of 2.28(5)Å and 2.52(5)Å, respectively. The corresponding distances in the 10-methylisoalloxazine complex are 2.29(6)Å and 2.52(6)Å. In a silver complex with the strong chelating ligand 8-hydroxyquinoline, bis (8-hydroxy-

FIG. 2. A stereoscopic illustration of the binding of Ag(1)+ and Ag(2)+ to riboflavin and of the riboflavin configuration.

quinoine)-silver(I) pyridine solvate (22). Ag...N distances are 2.145(4) and 2.155(4)Å and Ag...O values are 2.451(4) and 2.505(4)Å. Ag(2)+ in this structure is not four-, but five-coordinate, being bonded to a water molecule, W, on the 2-fold axis at a distance of 2.48(2)Å in addition to the two flavin molecules. The configuration of the basically square pyramidal coordination polyhedron can be measured by displacements parallel with the 2-fold axis relative to Ag(2)+: W(2) and N(5) lie, respectively, 2.48(2)Å and 0.02(1)Å to one side and O(4) is displaced 0.56(1)Å to the other. The isoalloxazine rings are nearly planar (see Table III) but Ag(2)+ lies 0.13Å from this plane; the dihedral angle between two isoalloxazines related by either 2-fold axis is 23.3°.

The model complex with 10-methylisoalloxazine did not illuminate directly the effect of possible steric hindrance by the ribityl group on binding of a metal to the secondary chelate site, N(1)—O(2). This study shows that the ribityl group, despite the weakness of alcoholic oxygen atoms as ligands, actually assists in coordination at this site, through O(2'). Parallel to the 2-fold axis, the N(1) atoms are −0.61Å from Ag(1)+, O(2) are −1.03Å, and O(2') are 1.93Å. (The partial water is about −2.5Å away.) Coordination distances are Ag(1)—N(1) = 2.304(6), Ag(1)—O(2) = 2.786(5), and Ag(1)—O(2') = 2.559(6)Å. In fact, the “coordination” to O(2) is quite weak and it is primarily N(1) and O(2') which bind the silver. The relationship of the metal to flavin is seen with particular clarity in stereoscopic Fig. 2. The minor water site (25 to 40%) is not shown.

From both these studies of flavin-silver complexes, it is clear that a quinoine riboflavin molecule can bind appropriate metals with at least six ligands (roughly octahedral coordination) at either of two sites. In the primary site, N(5)—O(4), the flavin supplies two coordinating atoms, permitting an octahedral metal to bind four other atoms, and making possible such complexes as that suggested (8) between an Fe₃S₄ cluster and riboflavin. In the secondary site, N(1)—O(2)—O(2'), the riboflavin is likely always to supply three coordinating atoms or to block the equivalent of three bonding sites, leaving only three for an octahedral metal such as iron, or possibly more for a metal of higher ligancy such as molybdenum.
It has been suggested (9) that the primary site is likely always to be filled by either a metal ion or a dipole (such as —OH) and that in flavins (11) which are not protonated at N(1), a similar situation pertains in the secondary site at least with regard to dipoles. This structure, particularly when compared with two other riboflavin structures (13, 14), reinforces this view and suggests that riboflavin coenzymes may adopt as a "resting mode" a loose association with ligands (or metals) in one or both of these sites which then becomes true bonding during the catalytic process. In virtually every isoalloxazine complex studied (8–16), such associations exist. Fig. 3, which shows the three riboflavin complexes studied to date, illustrates these associations and in particular the effect of O(2') on coordination in the secondary site.

From these three studies, it is also possible to predict preferred configurations for a major portion of the riboflavin moiety of flavo coenzymes. Kim and Jeffrey (23) have noted after examining the crystal structures of a number of open chain polyols that the carbon backbone is invariably nearly planar and extended, except where this configuration would lead to parallel —OH substituents on second-neighbor carbon atoms such as O(2')—O(4') in the ribitol molecule shown in Fig. 4. In this case, a unique rotation about one of two bonds will substitute a hydrogen atom for the bulkier —OH and will give a preferred configuration. (They also note that exceptions sometimes exist, which permit more favorable intermolecular or intramolecular hydrogen bonding and that, for example in Fig. 4, a hydrogen bond between O(2') and O(4'), although rare, is permitted and leads to formation of a stable six-membered ring.) The end OH groups are often extended but do not obey such rigorous rules.

As applied to ribitol, this argument predicts one of the two rotations shown in Fig. 4. In ribitol itself, one is the mirror image of the other and both exist in the crystal (17). In the parabromophenylhydrazone of ribose (18), C(1)—C(4) are planar, and there is a rotation about C(3)—C(4), but of the opposite sense from that predicted, which leads to distortion of some bond angles and is partly stabilized by formation of an

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**Fig. 4.** The fully extended, planar configuration of ribitol, showing an unfavorable O(2)—O(4) interaction and two preferred rotations for relief of this contact.

**Table IV**

Possible hydrogen bonds

<table>
<thead>
<tr>
<th>Distance</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(3)...O(3')</td>
<td>2.08A</td>
</tr>
<tr>
<td>H(3)...O(5')</td>
<td>2.32</td>
</tr>
<tr>
<td>O(4')...O(2)</td>
<td>2.87</td>
</tr>
<tr>
<td>O(3')...O(5')</td>
<td>2.89</td>
</tr>
<tr>
<td>O(4')...O(3')</td>
<td>2.80</td>
</tr>
<tr>
<td>C(3')...O(4')</td>
<td>2.80</td>
</tr>
<tr>
<td>N(3)...H(3)...O(3')</td>
<td>152°</td>
</tr>
<tr>
<td>N(3)...H(3)...O(5')</td>
<td>122°</td>
</tr>
<tr>
<td>C(4')...O(4')...O(2)</td>
<td>120°</td>
</tr>
<tr>
<td>C(4')...O(4')...O(3')</td>
<td>122°</td>
</tr>
<tr>
<td>C(3')...O(3')...O(4')</td>
<td>93°</td>
</tr>
</tbody>
</table>
O(2')—O(5') hydrogen bond. In a complex between riboflavin and 5'-deoxy-5'-bromoadenosine (14), Fig. 3C, the "ideal" rotation about C(2)—C(3) is found and even O(5') is extended. In riboflavin hydrobromide monohydrate (13), Fig. 3B, one again finds the ideal C(2)—C(3) rotation, but O(5') forms an internal hydrogen bond to O(3'). The present structure, Fig. 1A, contains the alternate ideal configuration with rotation about C(3')—C(4'), but again with O(5') evidently forming a hydrogen bond with O(3') (and also serving with O(3') as double receptors in a possible bifurcated hydrogen bond from N(3)—H(3)). It thus seems likely that the ribityl side chain will often adopt one of the two basic configurations shown in Fig. 3. Furthermore, the relationship of the ribityl and isoalloxazine moieties is likely to be as shown, with O(2') extending toward the pyrimidine region, since all three structures display this form and since it enhances the polar nature of the secondary binding site of isoalloxazine.

Although none of the hydroxyl protons were located, a number of plausible hydrogen bonds can be recognized and are listed in Table IV.

**APPENDIX**

Observed and final calculated structure factors. Groups of \(10 | F_o | \) and \(10 | F_c | \) are tabulated, with each group headed by values of \(h\) and \(k\) common to the group.
REFERENCES


The Crystal Structure of a Riboflavin-Metal Complex: RIBOFLAVIN SILVER PERCHLORATE HEMIHYDRATE
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