

Metal Complexes of Flavin Mononucleotide

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In recent years there has been considerable interest in the interaction of metal ions with riboflavin [1, 2]. However, in spite of its probably greater relevance to the question of flavin-metal interaction in enzyme systems, study of the metal-binding properties of riboflavin-5'-phosphate (flavin mononucleotide, FMN) has been almost totally neglected. Taqui Khan and Mohan reported [3] the formation of precipitates, possibly metal complexes, during potentiometric studies on aqueous FMN-metal ion systems, but no attempt was made to characterise them. Similarly, Hemmerich, *et al.*, [4] mention dark red-brown precipitates formed on adding aqueous solutions of metal salts to aqueous semiquinoid FMN, but have not investigated the compounds further.

In this work, we have prepared a series of complexes of the type $M(\text{FMN}) \cdot n\text{H}_2\text{O}$ (Table I), where the FMN is present in the anionic form. Only these 'inner complexes' were obtained, despite the use of a wide variety of metal salts.

Results and Discussion

Infrared Spectra

Riboflavin itself shows strong bands at 1725 and 1645 cm^{-1} , assigned [5] as due to $\nu(\text{C}_4=\text{O})$ and $\nu(\text{C}_2=\text{O})$ respectively. In complexes of riboflavin tetraacetate [6], which are considered to have the metal ions bound at the $\text{N}_5\text{-O}_4$ chelation site, both

$\nu(\text{C}=\text{O})$ bands move to lower energy. FMN has a very similar pair of bands, at 1726 and 1644 cm^{-1} , but these are not significantly changed in the complexes, in either position or intensity, indicating that there is no strong interaction between the metal ion and either of the carbonyl oxygens.

In contrast, there is some evidence for perturbation of the vibrations due to the phosphate group. In most of the complexes, the strong $\nu_{\text{as}}(\text{P-O})$, which occurs as a broad band at about 1050 cm^{-1} in the sodium salt of FMN, shows some resolution into at least two components, centred on about 1010 and 1070 cm^{-1} . Moreover, for complexes of pyrimidine and purine mononucleotides, it has been suggested [7] that the appearance of $\nu_{\text{s}}(\text{P-O})$ at about 980 cm^{-1} indicates binding of the metal ion to the phosphate group. Its absence indicates bonding of the metal ion to some other part of the molecule. All of the FMN complexes, with the exception of the zinc compound, show a band or shoulder at 975–990 cm^{-1} , which is not present in the spectrum of riboflavin. In the zinc complex, the 1010 band is broadened to low energy. It is therefore suggested that in the FMN complexes, metal-binding takes place at the phosphate site.

Electronic Spectra

The d-d bands of the complexes (Table II) are consistent, in both energy and intensity, with octahedral stereochemistry about the metal ion. The ligand fields are in each case rather weak.

The strong intra-ligand bands at 370–500 nm, which are very similar to those of riboflavin, are scarcely shifted in the complexes, and those small changes which do occur are such as to shift the main components to slightly higher energy. This contrasts strongly with the observation for complexes of riboflavin tetra-acetate, for which all the bands show a low-frequency shift on coordination [6]. Again, the

TABLE I. Analytical Results.

Compound	% Found			% Calculated		
	C	H	N	C	H	N
Mn(FMN)4H ₂ O	34.98	4.65	9.34	35.12	4.68	9.64
Fe(FMN)(OH)3H ₂ O ^a	34.73	4.99		35.07	4.56	
Co(FMN)4H ₂ O	34.43	4.75	9.59	34.88	4.65	9.57
Ni(FMN)5H ₂ O ^a	33.14	4.92		33.85	4.85	
Cu(FMN)3H ₂ O	35.90	4.23	9.94	35.70	4.41	9.80
Zn(FMN)2H ₂ O ^a	36.86	4.04		36.74	4.17	
Cd(FMN)2H ₂ O ^a	34.56	4.17		33.87	3.85	

^a Pregl analysis (C, H only).

TABLE II. Diffuse Reflectance Spectra of the Complexes.

Compound		Band Maxima (nm)
Na(FMN)		490s,br, 440vs,br, 380sh
Mn(FMN)4H ₂ O		485sh, 440vs,br, 385s
Fe(FMN)(OH)3H ₂ O		485sh, 440vs,br, 385s
CO(FMN)4H ₂ O	1350w,	490sh, 430vs,br, 385s
Ni(FMN)5H ₂ O	1200w, 760w, 665sh	490sh, 440vs,br, 380sh
Cu(FMN)3H ₂ O	800w	490s, 430vs,br, 375s
Zn(FMN)2H ₂ O		485s, 420vs,br, 375sh
Cd(FMN)2H ₂ O		490s, 425vs,br, 370sh

evidence is against coordination of the metal ion at the N₅-O₄ chelation site of the flavin in these FMN complexes.

E.S.R. Spectra

The iron complex gives a strong signal at $g = 2.01$, indicating that the iron is present in the +3 oxidation state. This signal, and those from the manganese(II) ($g = 2.03$) and copper(II) ($g = 2.19$) complexes, are all broad and featureless, indicating magnetic interaction in the solid complexes. The stoichiometry of most of the compounds requires the FMN to be at least bidentate if 6-coordination is to be achieved. If this occurs by means of bridging phosphate groups, strong magnetic interaction between neighbouring metal ions would be expected.

A sample of the zinc complex containing manganese(II) (nominal doping 1%) shows resolution of the manganese hyperfine structure, with $A = 80$ gauss, $g = 2.026$. The spin is clearly localised largely on the metal ion. Only one transition is seen, unlike the case for complexes with a MnNO₅ chromophore, which show distinct zero-field splitting. However, the central hyperfine components show incipient splitting, indicating a very slight distortion from regular cubic symmetry.

Experimental

The complexes were all prepared by the following method:

A solution of the metal salt (10^{-4} mol) in water (5 cm^3) was added, with stirring, to a warm solution of the sodium salt of FMN (0.051 g, 10^{-4} mol) in water (5 cm^3). After a few minutes heating and stirring, a dense yellow precipitate formed. This was filtered off, washed with water and dried *in vacuo* over silica gel.

Spectroscopic measurements were made as described previously [6].

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