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Spectral Studies of some Complexes of Riboflavin-2',3',4',5'-Tetraacetate

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The preparations are reported of complexes of riboflavin-2',3',4',5'-tetraacetate with salts of cobalt(II), nickel(II) and copper(II). Electronic spectra indicate that the flavin is bonded to the metal through O_4 and N_5, the M–N bond being probably rather weak. The C=O stretching vibrations are shifted to lower energy on coordination. The electronic spectra of enzyme–substrate complexes involving flavin groups are discussed in the light of results for the metal complexes.

Introduction

Although riboflavin-2',3',4',5'-tetraacetate (RTA) has been used as a model for riboflavin in a number of solution studies, the only report of the isolation of a solid RTA complex [1] concerns a molybdenum compound, originally formulated as $MoOCl_3$·(HRTA)HCl. The proposal that this is a coordination

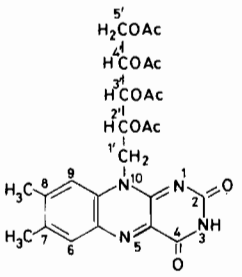

TABLE I. Analytical Data

Compound L = RTA	Colour	% Found			% Calculated		
		C	H	N	C	H	N
$CoL_2Cl_2 \cdot 2H_2O$	Red-Brown	48.19	4.79	8.95	47.85	4.82	8.93
$CoL_2Br_2 \cdot 2H_2O$	Red-Brown	44.33	4.54	8.61	44.69	4.50	8.34
$CoL_2I_2 \cdot 2H_2O$	Brown	42.13	4.60	8.05	41.77	4.21	7.80
$CoL_2(ClO_4)_2 \cdot 6H_2O$	Red	40.84	4.63	7.68	41.24	4.71	7.70
$CoL_2(NO_3)_2 \cdot H_2O$	Red	46.65	4.35	10.88	46.55	4.53	10.86
$NiL_2Cl_2 \cdot 2H_2O$	Red	48.34	4.54	9.03	47.86	4.82	8.93
$NiL_2Br_2 \cdot 6H_2O$	Red	42.41	4.19	7.99	42.40	4.84	7.91
$NiL_2I_2 \cdot 5H_2O$	Brown	40.18	3.90	7.47	40.26	4.46	7.51
$NiL_2(NO_3)_2$	Red	47.58	4.31	10.95	47.22	4.44	11.01
$CuL_2Cl_2 \cdot 6H_2O$	Orange	45.30	4.27	8.33	45.10	5.10	8.41
$CuL_2Br_2 \cdot 5H_2O$	Orange	43.39	3.92	7.90	42.82	4.74	7.99

TABLE II. Solution Electronic Spectra of Some Flavins.

Compound	Solvent	Absorption Bands (nm)				
RTA	solid	370sh		406vs	470sh	685w
RTA	water	373m		448vs		
RTA	ethanol	350m	424sh	446s	470sh	
RTA	acetone	342m	434sh	444s	470sh	
RTA	chloroform	352m	425sh	450s	475sh	
FAD[a]	water	375		448		
D-amino acid oxidase[a]	water	370		455		
D-amino acid oxidase + benzoate[a]	water	379		462	490sh	

[a]Data from reference 11.

TABLE III. Diffuse Reflectance Spectra of the Complexes (nm).

Compound	Band Maxima					
RTA	370sh,	406vs,	470sh,	685w		
$Co(RTA)_2Cl_2 \cdot 2H_2O$	390sbr,	475vs,	520sh,	625sh,	770sh,	1185w
$Co(RTA)_2Br_2 \cdot 2H_2O$	390sbr,	475vs,	510sh,	665sh,	800sh,	1220w
$Co(RTA)_2I_2 \cdot 2H_2O$	390sbr,	475vs,	510sh,	730sh,	920sh,	1250w
$Co(RTA)_2(ClO_4)_2 \cdot 6H_2O$	390sbr,	480vs,	520sh,	650sh,	1150w	
$Co(RTA)_2(NO_3)_2 \cdot H_2O$	380sh,	460vsbr,	637sh,	752sh,	1075w	
$Ni(RTA)_2Cl_2 \cdot 2H_2O$	390s,	485vs,	671sh,	755sh,	1170w	
$Ni(RTA)_2Br_2 \cdot 6H_2O$	400s,	490vs,	665sh,	760sh,	1180w	
$Ni(RTA)_2I_2 \cdot 5H_2O$	400s,	480vs,	540sh,	690sh,	1170w	
$Ni(RTA)_2(NO_3)_2$	400s,	485vs,	520sh,	675w,	810sh,	1210w
$Cu(RTA)_2Cl_2 \cdot 6H_2O$	390s,	485vs,	615w,	750sh,	930w	
$Cu(RTA)_2Br_2 \cdot 5H_2O$	390s,	460vs,	510sh,	620sh,	940w	

compound of RTA has, however, been questioned [2]. We report here the preparation of complexes of RTA with cobalt(II), nickel(II) and copper(II). Attempts to prepare complexes with manganese(II), iron(II), zinc, and cadmium were unsuccessful. All of the complexes prepared contained two mol of RTA per metal ion, and, except for the nitrates, were hydrated with at least two mol of water (Table I).

Results

Mode of Bonding of the RTA

The electronic spectrum of RTA contains two strong bands in the visible and near ultraviolet regions, whose exact positions and shapes are very dependent on the environment (Table II), as found for other flavin derivatives. Since the metal complexes are either insoluble in, or are decomposed by, all the solvents tried, spectra were compared for the solid state. In the reflectance spectrum, RTA itself also shows a weak, rather broad band at about 14,600 cm^{-1}, which is most probably due to a spin-forbidden electronic transition. Its intensity is comparable with that of the ν(C–H) overtone at 5800 cm^{-1}. It is possible that this band persists in the complexes, since most of them show a weak shoulder near this energy, but the possibility of d–d bands in this region precludes definite assignment.

In all the complexes, both strong ligand bands are shifted to lower energy, with partial resolution of the lower energy band (Table III). This type of behaviour has been observed in solution for complexes in which metal ions are believed, on n.m.r. evidence, to be bound at the O_4–N_5 chelation site [3]. Quite different behaviour is found for complexes which are believed to bind to N_5 or O_2 of the flavin. These give rise to spectra similar to those of protonated flavins and the normal protonation site is thought [4] to be N_1.

Addition of an electron to the lowest unoccupied molecular orbital of riboflavin puts the highest spin density on N_5 [5], so binding of an electrophile at this position would tend to stabilise the π^* state, and cause a bathochromic shift of both π–π^* transitions. Moreover, it would make N_1 less available for hydrogen bonding, so the n–π^* transition involving the pyrimidine nitrogen lone pair would also shift to lower energy, accounting for the shoulder observed near 500 nm.

It seems reasonable, therefore, to assume that in our complexes the metal ions are chelated by O_4 and N_5, the "primary chelation site" suggested for riboflavin itself.

The d–d spectra suggested 6-coordination in all cases, with low-intensity bands in the near infrared region. The structures of the complexes are therefore thought to involve a *trans*-arrangement of two chelating ligands, with anions or water molecules completing the coordination sphere. The ligand field, however, is rather weak for $MN_2O_2X_2$ chromophores, and it is likely that the metal–nitrogen bond is rather long, as found for the copper complex of riboflavin itself [6], where Cu–N is about 2.4 Å.

Infrared Spectra

The spectrum of RTA in the carbonyl stretching region is complicated. For riboflavin itself, bands at 1725 and 1645 cm^{-1} were assigned [7] as due to $\nu(C_4{=}O)$ and $\nu(C_2{=}O)$ respectively. This is in agreement with the work of Hemmerich *et al.* [8] who, for a series of isoalloxazines, found $\nu(C_4{=}O)$ at 1701–1721 cm^{-1} and $\nu(C_2{=}O)$ at 1660 to 1686 cm^{-1}. The latter might be expected to be lowered by hydrogen bonding when the ribityl group is present. For RTA, Selbin *et al.* [1] reported bands at 1730 and 1670 cm^{-1}, and preferred the reverse assignment. We have been unable to resolve the higher energy band from that at 1744 cm^{-1} which is presumably due to the acetate groups, though this band is

TABLE IV. Infrared Spectra in the Carbonyl Stretching Region.

Compound	ν(C=O)(acetate)	$\nu(C_4=O)$	$\nu(C_2=O)$[a]
RTA	1744vs	b	1665vs
$Co(RTA)_2Cl_2 \cdot 2H_2O$	1755vs	1687	1656vs
$Co(RTA)_2Br_2 \cdot 2H_2O$	1745vs	1681	1641vs
$Co(RTA)_2I_2 \cdot 2H_2O$	1752vs	1684	1648vs
$Co(RTA)_2(ClO_4)_2 \cdot 6H_2O$	1746vs br	c	1651vs br
$Co(RTA)_2(NO_3)_2 \cdot H_2O$	1749s, 1738s	1686	1653vs
$Ni(RTA)_2Cl_2 \cdot 2H_2O$	1754s, 1744s	1685	1647vs
$Ni(RTA)_2Br_2 \cdot 6H_2O$	1745vs	1685	1645vs
$Ni(RTA)_2I_2 \cdot 5H_2O$	1748s, 1734s	1684	1642vs
$Ni(RTA)_2(NO_3)_2$	1747s, 1735s	1684	1649vs
$Cu(RTA)_2Cl_2 \cdot 6H_2O$	1750vs	1705	1663vs
$Cu(RTA)_2Br_2 \cdot 5H_2O$	1755s, 1745s	1700	1655vs

[a]For assignments see text. [b]Obscured by 1744 band. See text. [c]Not resolved.

TABLE V. Infrared Bands 440–200 cm^{-1}.

Compound	ν(M–L)		Ligand			
RTA			426sh,	386vs,	355sh,	341sh
$CoL_2Cl_2 \cdot 2H_2O$	277vs,	233s	399vsbr,	330mbr		
$CoL_2Br_2 \cdot 2H_2O$	272w,	234vs	396vsbr,	344s		
$CoL_2I_2 \cdot 2H_2O$	267w,	233s	420sbr,	329m		
$CoL_2(ClO_4)_2 \cdot 6H_2O$	258sh,	234ms	408vsbr,	345sbr		
$CoL_2(NO_3)_2 \cdot H_2O$	276w,	252m	406vs,	384vs,	350sh,	318sh
$NiL_2Cl_2 \cdot 2H_2O$	279s,	240s	390vsbr,	327vsbr		
$NiL_2Br_2 \cdot 6H_2O$	244s		437vs,	342s		
$NiL_2I_2 \cdot 5H_2O$	267sh,	239s	390sh,	315vsbr		
$NiL_2(NO_3)_2$	268m,	240w	393vsbr,	348s		
$CuL_2Cl_2 \cdot 6H_2O$	273sh,	243w	405sh,	323vsbr		
$CuL_2Br_2 \cdot 5H_2O$	261vs		385vsbr,	348sh,	325sh	

broadened to low energy, and could conceal another component at 1720–1730 cm^{-1}.

On complex formation, all the bands in this region are normally sharpened, and the three components are clearly resolved. The 1744 cm^{-1} band shifts to slightly higher energy (Table IV), and that at 1665 cm^{-1} to lower energy, while a new band appears at 1680–1705 cm^{-1}, presumably shifted from near 1725 cm^{-1}. The shift to higher energy reported by Selbin *et al.* [1] is probably due to protonation of the ligand in their complexes.

Since both $\nu(C_2=O)$ and $\nu(C_4=O)$ move to lower energy on complex formation and since, moreover, there is some disagreement over assignments, it is difficult to draw conclusions from this about the structure of the complexes. However, if the evidence from electronic spectra is accepted, that the metal is bound at the $C_4=O$, N_5 site, then these infrared spectra give some support to the original assignment of $\nu(C_4=O) > \nu(C_2=O)$, since the higher energy band is always shifted much more than the lower.

At low energy, RTA shows a series of bands at 340–430 cm^{-1}, but is clear from 340 to 200 cm^{-1}. In the complexes there are usually two extra bands (Table V) near 240 and 270 cm^{-1}, the lower being normally the more intense. The energy of this lower band increases slightly from the cobalt to the nickel complexes, and it is considered to have some metal–ligand stretching character, though in the presence of a chelate ring considerable coupling with other modes must occur

In the chlorides, and in the nickel nitrate complex, the higher energy band is also strong, and may be due to a metal–anion stretching mode. This is at rather high energy in the chlorides, though similar figures have been reported for some complexes of 1-methylpyrimidine-2-thione [9] which also gives an unsymmetrical chelate with one donor atom rather weakly bonded.

Discussion

The electronic spectra of flavins in different media have occasioned much interest on account of the differences in spectrum between, for example, FAD, holoenzymes containing this unit, and the complexes formed between enzyme and substrate. In general, for simple flavins, a change from aqueous to non-aqueous solution results in a blue shift of the 375 nm peak, and resolution of the 450 nm peak into two or three components, with little change in position or a small red shift [10]. Similar spectral changes are found when the flavin is bound to an enzyme, and have been interpreted as implying a hydrophobic environment for the flavin [11].

With the enzyme substrate complex, however, the picture is less simple. Addition of carboxylic acids to D-aminoacid oxidase results in a red shift, and marked resolution, of the 450 nm peak, combined with a red shift of the 375 nm peak. It has been suggested that in this complex the chromophore which absorbs near 450 nm is in a hydrophobic region, while that which absorbs near 370 nm is in a hydrophilic region [12, 13].

The metal complexes described in this work give electronic spectra very similar to those of the D-aminoacid oxidase-carboxylic acid complexes, with a red shift of both bands, and resolution of a low energy shoulder (Tables II and III). Quite apart from the difficulty of designating two different chromophores in a molecule with the conjugation possibilities of RTA, our compounds throw considerable doubt on the postulate of Kotaki *et al.* [12, 13] since no part of the molecule can be in a strongly hydrophobic environment in the metal complexes. This type of spectrum would seem, then, to be characteristic of the binding of an electrophile at the N_5–O_4 position of the flavin.

We therefore suggest that the reaction of D-aminoacid oxidase with carboxylic acids involves binding of an electrophile at N_5–C_4=O. This would make N_1 less nucleophilic, and may result in the rupture of any hydrogen bond at this position, as suggested by Massey and Ganther [11].

Experimental

Preparation of Complexes

$Co(RTA)_2X_2 \cdot 2H_2O$ (X = Cl, Br, I)

A warm solution of the cobalt halide (10^{-4} mol) in acetone (10 cm^3) was added, with stirring, to a warm solution of RTA (0.054 g, 10^{-4} mol) in acetone (10 cm^3). A dense brown precipitate formed almost immediately. This was filtered off, washed with acetone, and dried over silica gel.

$Co(RTA)_2(ClO_4)_2 \cdot 6H_2O$ and $Co(RTA)_2(NO_3)_2 \cdot H_2O$

A solution of the metal salt (10^{-4} mol) in ethanol (10 cm^3) was warmed, and added, with stirring, to a hot solution of RTA (0.109 g, 2×10^{-4} mol) in ethyl acetate (10 cm^3). A bright red solution formed, which was evaporated to a low bulk. In the case of the nitrate, a dense red precipitate formed, but for the perchlorate, it was necessary to precipitate the product by the addition of an equal volume of absolute ether.

$Ni(RTA)_2X_2 \cdot nH_2O$ (X = Cl, n = 2; X = Br, n = 6)

A warm solution of the nickel halide (2×10^{-4} mol) in ethanol (5 cm^3) was added, with stirring, to a warm solution of the ligand (0.109 g, 2×10^{-4} mol) in acetone (10 cm^3). On heating for a few minutes, an orange precipitate formed, which was isolated by the usual method.

$Ni(RTA)_2(NO_3)_2$ and $Cu(RTA)_2X_2 \cdot nH_2O$ (X = Cl, n = 6; X = Br, n = 5)

These complexes were prepared in an analogous manner to $Co(RTA)_2(NO_3)_2$.

Spectral Measurements

Infrared spectra were obtained with Perkin-Elmer 325 and 457 spectrometers, reflectance spectra with a Beckman DK2A and solution electronic spectra with a Perkin-Elmer 402 spectrometer.

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