Inorganica Chimica Acta, 56 (1981) L71–L73

The Origin of the Visible Absorption in Metal Transferrins

MARIANNE G. PATCH and CARL J. CARRANO

Department of Chemistry, University of Vermont, Burlington, Vt. 05405, U.S.A.

Received June 26, 1981

Introduction

The transferrins are a series of iron binding globulins isolated from various biological sources. Serum transferrin (plasma), ovotransferrin (egg white) and lactoferrin (milk), all are capable of binding two mol of ferric ions tightly but reversibly [1]. Although an iron transport role for serum transferrin is well established, the function of the other transferrins is more obscure. It is thought that they may play a bacteriostatic role by denying iron to bacteria [2, 3].

The visible absorption spectrum of diferrie transferrin is dominated by a rather intense absorption band with a λ_{max} of ≈470 nm and ϵ = 2500/iron. This gives rise to the characteristic salmon pink color of the iron containing protein. Recent investigations show that this band is likely to be a ligand to metal charge transfer (LMCT) transition. The principle evidence for this comes from resonance enhanced Raman spectroscopy [4, 5]. Chemical and physical studies on the protein have implicated histidine, tyrosine, carbonate and water as Fe^{3+} ligands [1]. Laser excitation within the visible band of the protein reveals resonance enhanced Raman bands which are assigned to imidazole and/or phenolate vibrational modes. The most recent of these studies seems to indicate that all the Raman bands are explicable in terms of phenolate vibrations and thus assign the visible band as a phenolate to iron charge transfer.

The copper(II), chromium(III), cobalt(III) and manganese(III) complexes of transferrin have also been prepared. All of these except the chromium also display a moderately intense band in the 400–470 nm region of the spectra. These complexes also show the characteristic tyrosinate vibrations in their resonance Raman spectra [9, 10]. However, there have been no further attempts thus far to explain the optical properties of these metal substituted transferrins in terms of the assumed LMCT scheme or more significantly, to explain the absence of a charge transfer band in the Cr complex.

In this report we analyze the intense bands in the metal substituted transferrins in terms of optical electronegativities, and put their assignment as phenolate to metal charge transfer transitions on a firmer basis.

Experimental

The preparations of the Fe(III), Cr(III) and Co(III) complexes of ethylene bis-[*o*-hydroxyphenylglycine] (EHPG) have been described [5, 11]. The Cu(II) and Mn(III) complexes will be reported in a separate publication. Visible spectra were recorded on a Perkin-Elmer 552 spectrophotometer.

Calculations

The details of the calculations can be found in references 12–14, however, a *brief* account is given below. Equation (1) has been found to correlate the transition energies of LMCT bands for a wide variety of transition metal complexes.

$$\nu_{corr} = 30(X_L - X_M) \quad (1)$$

where X_L and X_M are the optical electronegativities of the ligand and metal respectively. The quantity ν_{corr} is the transition energy of the first allowed charge transfer band, corrected for certain interelectronic repulsion effects and ligand field effects. These interelectronic effects are determined from differences in the spin pairing energy (SPE) between the [core]-$\sigma_L^2 d^n$ and [core]$\sigma_L^1 d^{n+1}$ states.

$$SPE = [3/4\ n(1 - \frac{n-1}{9} - S(S+1)]D \quad (2)$$

where D ≈ 7B and B is the effective Racah parameter of the system. The difference in the SPE, ΔSPE, is added to the frequency of the observed transition to give the ν_{corr}:

$$\nu_{corr} = \nu_{obs} + \Delta(SPE) \quad (3)$$

No further corrections need be applied if the transition terminates in the t_{2g} level of the metal d orbitals. However, ligand field splittings must be considered if the LMCT transition terminates in an e_g set of orbitals in an octahedral complex.

$$\nu_{corr} = \nu_{obs} + \Delta(SPE) - 10D_q \quad (4)$$

The calculation of these optical electronegativities is complicated by the fact that the interelectronic repulsion parameter, B, and ligand field splittings are often difficult to estimate with a high degree of accuracy [15]. Fortunately, the spectra of the metal transferrin complexes are sufficiently rich to allow estimates of these quantities with reasonable accuracy. In addition, we have also examined the well defined metal EHPG complexes as an aid to the analysis of the transferrin results. EHPG contains the same

donar groups previously implicated in metal binding by transferrin and has been recognized as a reasonable model compound. The data used in the calculations are shown in Tables I and II.

TABLE I. Values Used to Calculate X_L for Metal EHPG Complexes[a].

Complex	ν_{obs}	B	ΔSPE	10Dq	ν_{corr}	X_M[b]
CuEHPG	26.7	0	0	15.4	11.3	2.4
FeEHPG	21.1	0.667[c]	−12.46	0	8.6	2.47
CoEHPG	28.6	0.533	3.73	21.8[d]	10.5	2.4
MnEHPG	23.4	0.630	12.0	17.3[e]	18.1	2.15
CrEHPG	–	0.642	8.99	19.7[e]	–	1.8

[a]All values are in units of kK except those of X_M.
[b]Data from ref. [14] except that for Fe(III) which is from ref. [13].
[c]The effective Racah parameter, B, was calculated from the visible absorbtion spectra of CrEHPG via the procedure described by Lever [16]. Using this value and the empirical procedures of Jörgenson (see 'Absorption Spectra and Chemical Bonding in Complexes', Pergamon Press, London, 1962) the other values can be estimated.
[d]Determined from the optical spectra via the analysis described by Wentworth and Piper [17].
[e]Determined from the optical spectrum via standard methods.

TABLE II. Values Used to Calculate X_L for Metal Transferrin Complexes.[a,b]

Complex	ν_{obs}	B	ΔSPE	10Dq	ν_{corr}	X_M
CuTr	22.8	0	0	14.8	8.0	2.4
FeTr	21.25	0.67	−12.52	0	8.73	2.47
CoTr	24.7	0.540	3.78	18.0[c]	10.48	2.4
MnTr	23.2	0.63	11.7	15.6	19.3	2.15
CrTr	–	0.645	9.03	16.26	29.4	1.8

[a]All values are in units of kK except those of X_M.
[b]All values in Table II have been determined as described in Table I using the appropriate metal transferrin spectra.
[c]Estimated from the difference in 10Dq between CrEHPG and CrTr.

Results and Discussion

One way to ascertain if a particular band in the spectra of a transition metal complex is a LMCT is to observe the shift in the band as a function of metal substitution while keeping the ligand environment constant. Jörgenson has been able to show that one can predict these shifts by associating an optical electronegativity with both the ligand and each of the metals [13].

Our approach was to first determine what an appropriate optical electronegativity would be for a phenolate ligand, since this is the suspected source of the LMCT band. We have utilized in this regard the metal complexes of EHPG. The iron complex of this ligand has already been recognized as a good model for the optical spectrum of ferric transferrin [5]. We have extended its use as a model to a variety of other metals [18]. This complex was chosen as there is little doubt that the bands observed in most of these compounds must arise from phenolate to metal CT, as there are no other donor groups which could be expected to give rise to intense visible absorptions. In addition, we have determined that the phenolate groups are coordinated to the metal in these complexes by X-ray crystal structures [18].

Using the metal substituted EHPG complexes, we have determined a self consistent optical electronegativity for the phenolate ligand of $X_L = 2.76$ (data in Table I).

Utilizing the data in Table II and the appropriate equations, we were able to calculate values of X_L for transferrin itself. The results are presented in Table III. The $X_{L\ calc}$ values are seen to be consistent among the metal substituted derivatives, with the possible exception of the Cu(II) complex. The close agreement between the values calculated for the transferrins and the EHPG complexes strongly suggests that the bands observed are LMCT which arise from one or more phenolate groups as ligands. They are also consistent with the contention that all of the metal ions bind at a common site in transferrin. The Cr(III) complex of transferrin has been somewhat anomolous in that it does not display a CT band in the visible region of the spectrum. Using the data presented herein we calculate the CT band of this complex should occur at about 240 nm which is well into the UV where it will be obscured by the intense $\pi-\pi^*$ transitions of the protein itself. Thus there is no need to postulate a different ligand environment for the Cr(III) in order to explain the absence of a LMCT in the visible region.

The case of copper(II) transferrin must be considered as unresolved. The calculated X_L from copper transferrin is significantly different from that determined with the other metals. This could arise from a number of causes: (1) The copper in transferrin is known to interact with a variable number of nitrogen ligands as a function of pH. Thus, the binding site may be different for the copper com-

TABLE III. Calculated Optical Electronegativities for the Transferrin Ligand.

Complex	$X_{L\ calc}$
CuTr	2.67
FeTr	2.76
CoTr	2.75
MnTr	2.79
CrTr	–

plexes with respect to the other metals. (2) Copper(II) is a d^9 system and hence subject to a severe Jahn–Teller distortion which usually takes the form of an extreme lengthening of the axial bonds. This effect could be significant. Indeed, the copper EHPG complex is severely distorted so it is not unreasonable to assume that the transferrin complex should be so. However, the manganese(III) derivatives of EHPG and transferrin, which can also be expected to be Jahn–Teller distorted, show unexceptional behavior. The final possibility is that the visible absorption band in copper transferrin is M → L rather than L → M in character. This possibility has been raised [5]. Similar bands in a series of related copper–phenol complexes have been given this M → L assignment on the basis of thermochemical data [19]. One final point; the LMCT band of diferric transferrin has been found to shift when carbonate is replaced by alternative anions. Since the spin pairing energy itself should be unaffected by this change and since the crystal field splitting, 10Dq does not directly affect the band position in the case of Fe(III), any changes must be due to a variation in the Racah parameter, B. The reduction in the Racah parameter needed to explain the shift from 465 nm with carbonate as the anion to 488 nm with glycine and 505 nm with thioglycolate, is consistent with the increased covalency expected in going from O → N → S donors.

In summary, we conclude that the strong band in the visible spectra of diferric transferrin and most of the metal substituted derivatives is a LMCT transition which originates on a phenolate oxygen and that all of the metal ions probably bind to a common site on the protein.

Acknowledgement

This work was supported by the Research Corporation.

References

1 N. D. Chasteen, *Coord. Chem. Rev., 22,* 1 (1977).
2 E. D. Weinberg, *Science, 184,* 952 (1974).
3 M. Sussman, *'Iron in Biochemistry and Medicine'* A. Jacobs and M. Worwood, eds. Academic Press, New York, 649–679 (1974).
4 P. Carey and N. M. Young, *Can. J. Biochem., 52,* 273 (1974).
5 B. P. Gaber, V. Miskowski, and T. G. Spiro, *J. Am. Chem. Soc., 96,* 6868 (1974).
6 P. Aisen, R. Pasa and A. G. Redfield, *J. Biol. Chem., 244,* 4628 (1969).
7 R. Prados, R. K. Boggess, R. B. Martin, and R. C. Woodworth, *Bioinorg. Chem., 4,* 135 (1975).
8 E. W. Ainscough, A. M. Brodie and J. E. Plowman, *Inorg. Chim. Acta, 33,* 149 (1979).
9 E. W. Ainscough, A. M. Brodie, J. E. Plowman, S. J. Bloor, J. S. Loehr and T. M. Loehr, *Biochemistry,* 4072 (1980).
10 Y. Tominatsu, S. Kint and J. R. Scherer, *Biochemistry, 15,* 4918 (1976).
11 K. Sugiura and K. Yamasaki, *Nippon Kaguka Zasshi, 89,* 853 (1968).
12 D. R. McMillin, *Bioinorg. Chem., 8,* 179 (1978).
13 C. K. Jörgensen, *Prog. Inorg. Chem., 12,* 101 (1970).
14 C. K. Jörgensen, *Mol. Phys., 6,* 43 (1963).
15 J. C. Barnes and P. Day, *J. Chem. Soc.,* 3886 (1964).
16 A. B. P. Lever, *J. Chem. Ed., 11,* 711 (1968).
17 R. A. D. Wentworth and T. S. Piper, *Inorg. Chem., 4,* 709 (1965).
18 C. J. Carrano, P. A. Riley, V. L. Pecorano and K. N. Raymond, manuscript in preparation (1981).
19 J. F. Harrod, *Can. J. Chem., 47,* 637 (1969).