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FULL PAPER

Complexes of cobalt(III) with phenolate-containing polydentate ligands and bovine serum apo-transferrin: towards creating spectroscopic models for cobalt(III)–tyrosinate interactions

Musa S. Shongwe,*[a,b,c] Said K. M. Al-Hatmi,[a] Helder M. Marques,[b] Rachel Smith,[b] Ryoji Nukada[c] and Masahiro Mikuriya[c]

[a] *Department of Chemistry, College of Science, Sultan Qaboos University, P. O. Box 36, Al Khod 123, Muscat, Sultanate of Oman. E-mail: musa@squ.edu.om*
[b] *School of Chemistry, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa*
[c] *Department of Chemistry, School of Science and Technology, Kwansei Gakuin University, Gakuen 2-1, Sanda 669-1337, Japan*

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Reaction of each member of a series of variously substituted *N*-(2-hydroxybenzyl)glycinate (hbg^{2-}) ligands [5-bromo- (5-Br-hbg^{2-}); 5-chloro- (5-Cl-hbg^{2-}); 3,5-dichloro- (3,5-Cl_2-hbg^{2-}); 5-chloro-3-methyl- (5,3-Cl,Me-hbg^{2-}); and 3,5-dimethyl- (3,5-Me_2-hbg^{2-})] with cobalt(II) in stoichiometric amounts under ambient conditions afforded the Co^{III} complex anions of the general formula *trans*,*trans*,*trans*-$[Co(hbg)_2]^-$. Single-crystal X-ray analyses of $[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-[Co(3,5-Cl_2-hbg)$_2$] **3** and $[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-[Co(3,5-Me_2-hbg)$_2$] **5** {$[C_5H_{10}NH_2]$ = piperidinium counter ion} have revealed similar structural motifs of these compounds. Each of the crystal structures shows two non-equivalent complex anions in the unit cell connected through hydrogen bonding *via* the piperidinium cation. The two hbg^{2-} ligands in each complex ion are arranged facially around the cobalt(III) ion and each ligand employs a phenolate oxygen, an amine nitrogen and a carboxylate oxygen to form a distorted octahedral geometry with C_i symmetry. These compounds (**1**–**5**) serve as spectroscopic models for the interaction of cobalt(III) with tyrosinate ligands. The electronic spectrum of the compound $[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-[Co(5,3-Cl,Me-hbg)$_2$] **4** bears a striking resemblance to that of the complex of cobalt(III) with bovine serum transferrin, [Co_2BTf]. In contrast to previous studies on the spectroscopy of the transferrins, one of the two ligand-field transitions expected to occur in [Co_2BTf] is evident in the electronic spectrum of this derivative of bovine serum transferrin *in vitro*. One of the fascinating characteristics of the *trans*,*trans*,*trans*-$[Co(hbg)_2]^-$ model system is the solvent dependence of the optical spectra.

Introduction

The past decade has seen remarkable strides in the crystallographic characterisation of the transferrin family of proteins, *viz.* human lactoferrin,[1a] bovine lactoferrin,[1b] rabbit serum transferrin,[1c] chicken and duck ovotransferrin,[1d] mare transferrin[1e] and derivatives thereof.[1f,g] Although the transferrins are primarily iron-binding proteins, they have been demonstrated in several studies *in vitro* to bind other metal ions such as VO^{2+},[2a] Cr^{3+},[2b,c] Mn^{3+},[2b,c] Co^{3+},[2b–d] Cu^{2+},[1f,2b–d] Al^{3+},[2e] Bi^{3+},[2f] Tl^{3+},[2g] Gd^{3+}[2h] and Ce^{n+} (n = 3 or 4)[2i] in place of the physiological high-spin Fe^{3+} at their specific binding sites. As a step towards gaining insight into the mechanism by which the transferrins bind and release cobalt(III), we are examining closely the interactions of the donor atoms with the cobalt(III) ion in both the natural and model systems, paying particular attention to the metal–phenolate/tyrosinate co-ordination. Herein we present a series of mononuclear complexes of Co(III) with variously substituted *N*-(2-hydroxybenzyl)glycinate ligands [Fig. 1(a)] and one complex of Co(III) with bovine serum apo-transferrin (apo-BTf), [Co_2BTf].

Complexation of Co(III) with the *apo* forms of human serum transferrin,[2b] human lactoferrin[2c] and ovotransferrin[2d] has been reported, but to our knowledge reaction of Co(III) with *bovine* serum apo-transferrin has not been investigated. Interestingly, our present work shows manifestation of one of the expected d–d transitions in [Co_2BTf] which was not observed in previous studies of the other members of the transferrin family. A schematic diagram of the metal-binding site of the transferrins based on the structural characterisation of human lactoferrin is given in Fig. 1(b).[1a] There are two such specific metal-binding sites per molecule of transferrin. The sites are very similar with very subtle differences. As can be seen from the diagram, the ligands provided by the protein are two tyrosinates, one histidine and one aspartate. Additionally, a carbonate ion is co-ordinated under physiological conditions to complete the preferred geometry. So far the few known cobalt(III) derivatives of the transferrins have not been elucidated by single-crystal X-ray crystallography, hence the need for model systems to illuminate the nature of the cobalt(III)–tyrosinate interactions.

In this study, we have drawn spectroscopic analogies between the *trans*,*trans*,*trans*-$[Co(hbg)_2]^-$ and [Co_2BTf] complexes and have highlighted the similarities between the cobalt(III)–phenolate and the cobalt(III)–tyrosinate interactions. Whilst exploring the chemistry of the *trans*,*trans*,*trans*-$[Co(hbg)_2]^-$ system, we have observed a remarkable dependence of the electronic absorption spectra on the nature of the solvent. Furthermore, the electronic effects on the energy of the phenolate-to-cobalt(III) charge transfer transition brought about by the nature of the substituent groups on the ligand (hbg^{2-}) framework – especially in the *para* position – have been noted. Based on the solvent dependence of, and ligand effect on, the UV-Visible spectroscopy of the model system, we have

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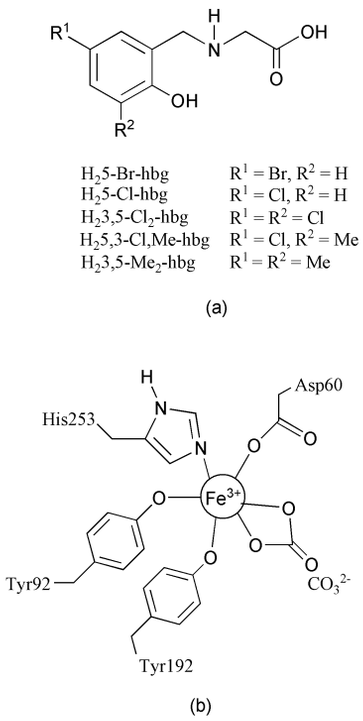

Fig. 1 (a) Variously substituted *N*-(2-hydroxybenzyl)glycines (hbg); (b) schematic representation of one of the two specific metal-binding sites in native human lactoferrin.[1a]

produced the complex ion *trans*,*trans*,*trans*-[Co(5,3-Cl,Me-hbg)$_2$]$^-$ which gives the best spectroscopic match with [Co$_2$BTf].

Results and discussion

Synthetic route to [Co(hbg)$_2$]$^-$ complex ions and physical characterisation

Reaction of each ligand, H$_2$hbg, with 2 molar equivalents of piperidine in MeOH afforded the deprotonated ligand hbg^{2-} which upon treatment with 0.5 equivalent of cobalt(II) gave immediately a yellow-orange solution which darkened progressively with time indicative of further ligand complexation and metal oxidation. The oxidation of Co^{2+} to Co^{3+} by O_2(g) under ambient conditions was relatively facile, reaching completion within minutes. The compounds **1**–**5** {[$C_5H_{10}NH_2$][Co(hbg)$_2$]: hbg^{2-} = 5-Br-hbg^{2-} (**1**); 5-Cl-hbg^{2-} (**2**); 3,5-Cl$_2$-hbg^{2-} (**3**); 5,3-Cl,Me-hbg^{2-} (**4**); 3,5-Me$_2$-hbg^{2-} (**5**)} were isolated as red-orange crystals using various crystallisation techniques. Elemental analyses (C, H and N) are consistent with the chemical composition [$C_5H_{10}NH_2$][Co(hbg)$_2$]. Further support for this formulation is provided by the molar conductivities ranging from 86 to 91 S cm^2 mol^{-1} (measured in MeOH at a concentration of *ca.* 10^{-3} mol dm^{-3}) which point to a 1:1 electrolyte type.[3] Room temperature magnetic susceptibility measurements confirmed the diamagnetism of these compounds. The vast majority of octahedral Co(III) complexes have diamagnetic ground states. In the Tanabe–Sugano diagram for a d^6 configuration, the $^1A_{1g}$ state for Co^{3+} crosses the $^5T_{2g}$ state at a very low value of Δ_o resulting in almost all cobalt(III) complexes being low spin – the exception being complexes possessing weak field ligands, such as [CoF_6]$^{3-}$.

Infrared spectroscopy of [$C_5H_{10}NH_2$]-*trans*,*trans*,*trans*-[Co(hbg)$_2$] 1–5

Of structural interest in the infrared spectra of the compounds **1**–**5** are the symmetric and asymmetric stretching frequencies of the carboxylate groups (Table 1). The absorption band corresponding to ν(C–O) appears in the range 1368–1379 cm^{-1} whereas that corresponding to ν(C=O) is observed in the range 1602–1620 cm^{-1}. The average difference between these two absorptions, $\Delta\nu$(COO), is *ca.* 240 cm^{-1} implying unidentate co-ordination of the carboxylate groups.[4a,b] This mode of co-ordination has been confirmed subsequently by single-crystal X-ray analyses of two members of the [Co(hbg)$_2$]$^-$ series [hbg^{2-} = 3,5-Cl$_2$-hbg^{2-} (**3**) and 3,5-Me$_2$-hbg^{2-} (**5**)] even though the crystal structures show the carbonyl oxygen (C=O) or the carboxyl oxygen (C–O$^-$) to be hydrogen-bonded to the piperidinium counter cation. The stretching frequencies of the amine group, ν(N–H), occur between 3066 and 3252 cm^{-1} inclusive. Both carboxylate and amine stretching frequencies are shifted to lower energies on co-ordination to the cobalt(III) ion. The presence of the piperidinium cation, [$C_5H_{10}NH_2$]$^+$, is verified by the characteristic absorption bands of this ion in the region 2400–3000 cm^{-1}, especially those bands associated with the NH_2^+ fragment.

Single-crystal X-ray crystallography

Single crystals of [$C_5H_{10}NH_2$]-*trans*,*trans*,*trans*-[Co(3,5-Cl$_2$-hbg)$_2$] **3** suitable for X-ray structure determination were grown from a solution of this compound in MeCN by controlled solvent evaporation. However, in the case of [$C_5H_{10}NH_2$]-*trans*,*trans*,*trans*-[Co(3,5-Me$_2$-hbg)$_2$] **5**, X-ray-quality crystals were obtained by diffusion of a vapour of Et$_2$O into the solution of this compound in MeOH. In both cases, red-orange rectangular blocks of crystals were formed. Parameters for data collection as well as structure solution and refinement are listed in Table 2. These two compounds (**3** and **5**) are isostructural implying that the substituent groups on the phenolic ring do not have a significant effect on the overall geometry at the metal centre. The unit cell contains two discrete *trans*,*trans*,*trans*-[Co(hbg)$_2$]$^-$ complex ions (designated complex anions A and B in the ORTEP diagrams in Figs. 2 and 3), hydrogen-bonded to a piperidinium cation, N2 ··· O3B [2.773(4) Å] and N2 ··· O2A′ [2.874(4) Å] for **3** and N2 ··· O3A [2.814(5) Å] and N2 ··· O3B [2.887(5) Å] for **5**.

The two hbg^{2-} ligands in each complex ion are co-ordinated to the cobalt(III) ion in a *facial* mode with this metal ion lying on a crystallographic centre of inversion. The coordination sphere around the Co(III) ion is *pseudo*-octahedral with deviations from the right angle ranging from 2.0 to 4.7° for

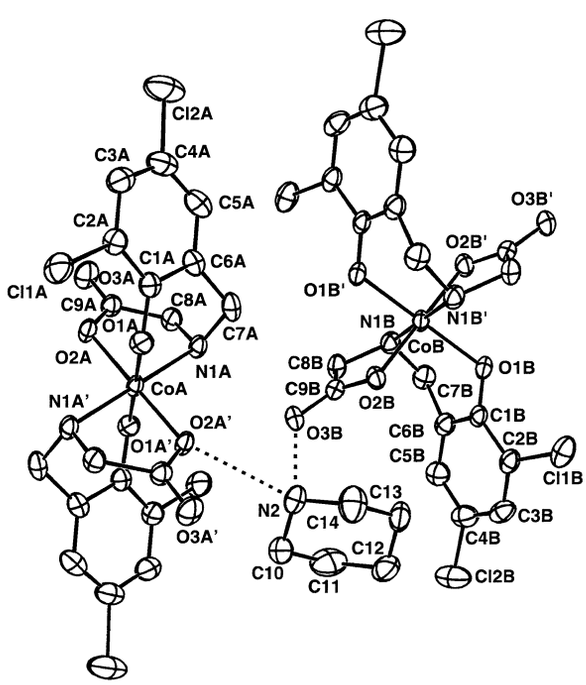

Fig. 2 X-Ray crystal structure of [$C_5H_{10}NH_2$][Co(3,5-Cl$_2$-hbg)$_2$] **3**.

Table 1 Electronic and vibrational spectroscopic data for $[C_5H_{10}NH_2][Co(hbg)_2]$ **1**–**5**

Compound	λ_{max}/nm (ε/M^{-1} cm^{-1})[a]	ν(N–H)/cm^{-1}	ν(C=O)/cm^{-1}	ν(C–O)/cm^{-1}	$\Delta\nu$[b](COO)/cm^{-1}
1	383 (4060), 669 (140)	3090	1609	1370	239
2	383 (3720), 668 (130)	3066	1616	1368	248
3	384 (4150), 669 (190)	3194	1610	1368	242
4	396 (3690), 690 (160)	3252	1620	1379	241
5	414 (4430), 682 (135)	3200	1602	1373	229

[a] Electronic spectra were recorded in MeOH. [b] $\Delta\nu$(COO) = ν(C=O) − ν(C–O).

Table 2 Crystallographic data for $[C_5H_{10}NH_2][Co(3,5\text{-}Cl_2\text{-}hbg)_2]$ **3** and $[C_5H_{10}NH_2][Co(3,5\text{-}Me_2\text{-}hbg)_2]$ **5**

	3	**5**
Empirical formula	$C_{23}H_{26}Cl_4CoN_3O_6$	$C_{27}H_{38}CoN_3O_6$
M	641.23	559.55
Crystal system	Triclinic	Triclinic
Space group	$P\bar{1}$	$P\bar{1}$
a/Å	11.440(3)	11.826(3)
b/Å	12.608(3)	12.940(4)
c/Å	9.745(2)	9.423(2)
α/°	98.75(1)	102.00(1)
β/°	95.45(1)	95.71(2)
γ/°	100.36(1)	76.44(2)
U/Å^3	1355.6(6)	1369.2(6)
Z	2	2
D_c/g cm^{-3}	1.57	1.36
T/K	298	298
μ/cm^{-1}	10.70	6.67
Measured reflections	5555	5434
Independent reflections	5051	4762
Observed reflections	3797	3357
R [$I > 3\sigma(I)$]	0.0337	0.0400
wR (all data)	0.0630	0.0871

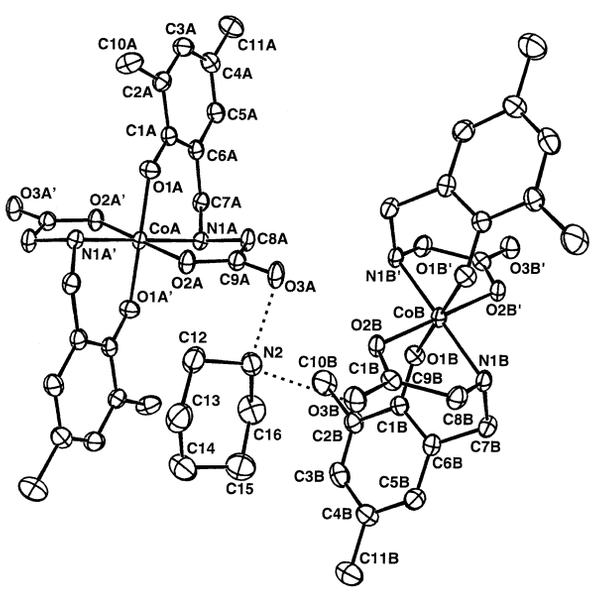

Fig. 3 X-Ray crystal structure of $[C_5H_{10}NH_2][Co(3,5\text{-}Me_2\text{-}hbg)_2]$ **5**.

trans,*trans*,*trans*-$[Co(3,5\text{-}Cl_2\text{-}hbg)_2]^-$ and from 0.8 to 4.6° for *trans*,*trans*,*trans*-$[Co(3,5\text{-}Me_2\text{-}hbg)_2]^-$ (Table 3). The average cobalt(III)-donor atom bond distances for the two complex ions of each compound are comparable to those of similar compounds of cobalt(III).[5] The average bond distances for *trans*,*trans*,*trans*-$[Co(3,5\text{-}Cl_2\text{-}hbg)_2]^-$ are: Co^{III}–O(phenolate) ≈ 1.907 Å, Co^{III}–N(amine) ≈ 1.950 Å and Co^{III}–O(carboxylate) ≈ 1.895 Å; whereas those for *trans*,*trans*,*trans*-$[Co(3,5\text{-}Me_2\text{-}hbg)_2]^-$ are: Co^{III}–O(phenolate) ≈ 1.890 Å, Co^{III}–N(amine) ≈ 1.945 Å and Co^{III}–O(carboxylate) ≈ 1.903 Å.

Table 3 Selected bond lengths (Å) and angles (°) for $[C_5H_{10}NH_2][Co(3,5\text{-}Cl_2\text{-}hbg)_2]$ **3** and $[C_5H_{10}NH_2][Co(3,5\text{-}Me_2\text{-}hbg)_2]$ **5**

	3	**5**
CoA–O1A	1.902(2)	1.872(3)
CoA–O2A	1.895(2)	1.916(3)
CoA–N1A	1.953(2)	1.936(3)
CoB–O1B	1.911(2)	1.907(2)
CoB–O2B	1.895(2)	1.890(2)
CoB–N1B	1.946(3)	1.954(4)
O1A–CoA–O1A′	180	180
O1A–CoA–O2A	92.4(1)	90.8(1)
O1A–CoA–O2A′	87.6(1)	89.2(1)
O1A–CoA–N1A	94.3(1)	91.6(1)
O1A–CoA–N1A′	85.7(1)	88.4(1)
O2A–CoA–O2A′	180	180
O2A–CoA–N1A	85.6(1)	86.2(1)
O2A–CoA–N1A′	94.4(1)	93.8(1)
N1A–CoA–N1A′	180	180
O1B–CoB–O1B′	180	180
O1B–CoB–O2B	92.0(1)	91.9(1)
O1B–CoB–O2B′	88.0(1)	88.1(1)
O1B–CoB–N1B	94.7(1)	94.6(1)
O1B–CoB–N1B′	85.3(1)	85.4(1)
O2B–CoB–O2B′	180	180
O2B–CoB–N1B	86.2(1)	86.0(1)
O2B–CoB–N1B′	93.9(1)	94.0(1)
N1B–CoB–N1B′	180	180

The compound $[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(3,5\text{-}Cl_2\text{-}hbg)_2]$ is isostructural with the Mn^{III} analogue,[6] but in the latter high-spin d^4 compound the geometry about the metal ion was tetragonally distorted in accordance with the Jahn–Teller effect. The general solid-state structure of the *trans*,*trans*,*trans*-$[Co(hbg)_2]^-$ complex ions differs markedly from that of the Co(III) complex with the corresponding Schiff-base ligand, *N*-salicylideneglycinate (sal-gly^{2-}). In this Schiff-base complex, $[Co(sal\text{-}gly)_2]^-$,[7] the more rigid ligands are arranged around the cobalt(III) ion almost perpendicularly to each other and are somewhat buckled. In sharp contrast to the *trans*,*trans*,*trans*-$[Co(hbg)_2]^-$ system, two axes of the octahedral geometry of $[Co(sal\text{-}gly)_2]^-$ contain donor atoms from the same ligand, *i.e.* $[O_{phenolate}–Co^{III}–O_{carboxylate}]$. Moreover, in this Schiff-base compound, there are significant deviations from linearity for a given pair of axial bonds [*e.g.* $N_{imine}–Co^{III}–N_{imine}$: 173.6(3)°]. As expected, the Co–N(imine) bond (average ≈ 1.885 Å) in $[Co(sal\text{-}gly)_2]^-$ is shorter than the Co–N(amine) bond (average ≈ 1.947 Å) in *trans*,*trans*,*trans*-$[Co(hbg)_2]^-$. The relatively more flexible hbg^{2-} ligands impose less constraints on the geometry around the Co(III) ion than does the *N*-salicylideneglycinate ligand.

Synthesis and electronic spectrum of $[Co^{III}_2BTf]$

Initially, the purity of the bovine serum apo-transferrin (apo-BTf = metal-free protein) was ascertained by titrating this protein against Fe^{III}NTA (NTA = nitrilotriacetate) spectrophotometrically. The reason for using Fe^{3+} in the titration is that this metal ion binds to the transferrins much more readily than does

Co^{3+} under ambient conditions. Extrapolating the titration curve of molar absorptivity *versus* $n\,Fe^{3+}/n\,BTf$ ($n\,Fe^{3+}/n\,BTf$ = ratio of moles of Fe^{3+} to moles of BTf) gave $n\,Fe^{3+}/n\,BTf = 1.8$ (expected ratio = 2, since the protein has two metal-binding sites) pointing to 90% purity of the protein. The synthesis of $[Co^{III}_2BTf]$ was performed in 0.050 M HEPES buffer containing HCO_3^-(aq) (0.10 M) and NaCl(aq) (0.10 M) at pH ≈ 8. Considering the hydrolysis of high-valent metal ions in aqueous medium, the divalent cobalt ion was the obvious choice for this reaction. The apoprotein (*ca.* 20 mg mL^{-1}) was reacted with 1.8 molar equivalents of Co^{2+} and incubated for approximately one week. As observed in the case of the $[Co(hbg)_2]^-$ model system, the colour of the solution began as pale yellow, but intensified progressively.

The visible spectrum of $[Co^{III}_2BTf]$ was recorded in HEPES buffer (Fig. 4). It displays an intense band at 405 nm and a relatively weaker one centred around 725 nm. This spectrum resembles those of $[Co^{III}_2HTf]$,[2b] $[Co^{III}_2HLf]$[2c] and $[Co^{III}_2OTf]$[2d] (HTf = human serum transferrin; HLf = human lactoferrin and OTf = ovotransferrin) *except* that – for no obvious reason – the lower-energy band was not observed in the spectra of the latter three complexes. Assuming an octahedral geometry around the cobalt(III) ion (two tyrosinates, one aspartate, one histidine and a bidentate carbonate synergistic ion)[1] and occurrence of diamagnetism in $[Co^{III}_2BTf]$, the higher-energy band (λ_{max} = 405 nm; ε_{max} = 8800 $dm^3\ mol^{-1}\ cm^{-1}$) is assigned to a tyrosinate (O^-) $p_\pi \rightarrow$ cobalt(III) d_{σ^*} charge transfer transition whereas the lower-energy band (λ_{max} = 725 nm; ε_{max} = 100 $dm^3\ mol^{-1}\ cm^{-1}$) is attributed to the $^1A_{1g} \rightarrow {}^1T_{1g}$ ligand-field transition. The other d–d band ($^1A_{1g} \rightarrow {}^1T_{2g}$)[8] for low-spin Co^{III} not observed in the electronic spectrum of $[Co^{III}_2BTf]$ is probably obscured by the charge transfer band.

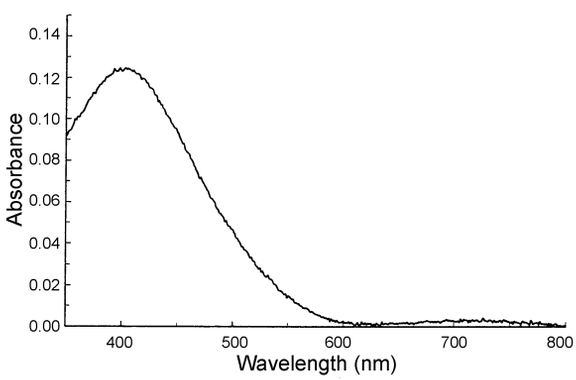

Fig. 4 Electronic spectrum of the ternary complex of bovine serum transferrin with cobalt(III) and carbonate, $[Co^{III}_2BTf]$ (1.4×10^{-5} mol dm^{-3}), in HEPES buffer.

Electronic spectra of *trans,trans,trans*-$[Co(hbg)_2]^-$ complex ions

Each of the visible spectra of the compounds **1**–**5** is very similar to that observed for the Co^{III}–transferrin complex $[Co^{III}_2BTf]$ and is characterised by two absorption bands [see, for instance, Fig. 5(a)]: the dominant higher-energy band is attributed to a phenolate (O^-) $p_\pi \rightarrow$ cobalt(III) d_{σ^*} charge transfer transition whereas the weaker, lower-energy band is associated with a d–d transition, more precisely $^1A_{1g} \rightarrow {}^1T_{1g}$ in nature. Justification for the assignment of these two bands derives from the diamagnetism of these compounds which is consistent with a low-spin d^6 configuration and $^1S_{1g}$ ground state for the Co(III) ion. The second ligand-field band corresponding to the $^1A_{1g} \rightarrow {}^1T_{2g}$ transition is absent from the electronic spectra of the $[Co(hbg)_2]^-$ ions recorded in MeOH or H_2O. However, the occurrence of this latter electronic transition in this series of mononuclear complex ions has been verified by recording the spectra in DMSO [see, for example, Fig. 5(b)].

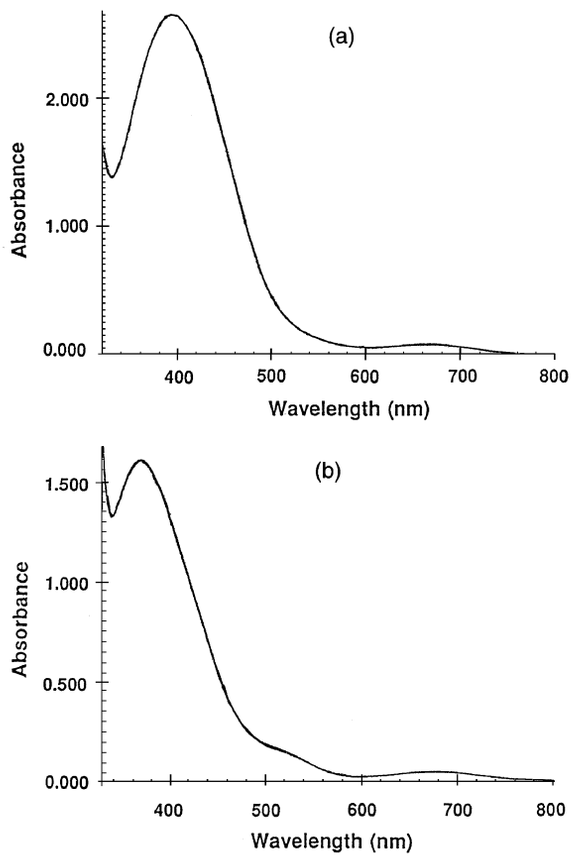

Fig. 5 Electronic spectrum of $[C_5H_{10}NH_2][Co(5\text{-Cl-hbg})_2]$ **2** (5.0×10^{-4} mol dm^{-3}) in (a) MeOH and (b) DMSO.

The dependence of the electronic absorption spectra of these compounds on the nature of the solvent is remarkable. Sensitivity of charge transfer bands to the nature of a given solvent is a well-known phenomenon, but the extent to which this phenomenon is experienced in the $[Co(hbg)_2]^-$ system is beyond expectation and warrants a comment. The electronic spectrum of each compound was recorded in three different solvents, *viz.* H_2O, MeOH and DMSO. (DMF was observed to have the same effect on the compounds as DMSO.) For each compound, the energy of the LMCT increased in the order H_2O < MeOH < DMSO. Table 4 exemplifies the variation of the energies of the charge transfer bands with the nature of the solvent. The solvent dependence of the electronic spectra of these compounds provided evidence for the existence of the higher-energy ($^1A_{1g} \rightarrow {}^1T_{2g}$) d–d band by shifting the LMCT band hypsochromically thereby exposing the previously obscured ligand-field band in question, albeit as a shoulder.

The existence of the two d–d bands is consistent with the pseudo-octahedral geometry around the low-spin cobalt(III) ion; thus the solvents are not expected to interact directly with the metal ion at the centre of the complex ions, but rather they are expected to interact with the donor atoms of the ligands. The related ligand *N*,*N*′-ethylenebis[(*o*-hydroxyphenyl)-glycinate], EHPG, exhibited similar behaviour for *meso*-Fe^{III}EHPG and *rac*-Fe^{III}EHPG in H_2O and DMF.[9] This phenomenon was explained, after exhaustive research, using the solvent donor–acceptor approach. The strong donor DMF solvent (or DMSO in the present study) was proposed to interact with the H atom of the ethylenediamine nitrogen atom causing lengthening of the N–H bond and shortening of the Fe–N bond. As a result, the positive charge at the metal centre would decrease, shifting the LMCT transition to higher energy.[10] In contrast, interaction of a strong acceptor solvent (such as H_2O or MeOH) with the carboxyl oxygen atoms would have the effect of increasing the net positive charge at the metal

Table 4 Illustration of solvent dependence of charge transfer bands

Compound	DMSO (λ_{max}/nm)	MeOH (λ_{max}/nm)	H_2O (λ_{max}/nm)
$[C_5H_{10}NH_2][Co(5\text{-}Cl\text{-}hbg)_2]$ **2**	371	383	397
$[C_5H_{10}NH_2][Co(5,3\text{-}Cl,Me\text{-}hbg)_2]$ **4**	375	396	406
$[C_5H_{10}NH_2][Co(3,5\text{-}Me_2\text{-}hbg)_2]$ **5**	388	414	422

centre (by withdrawing electron density), thus facilitating the transference of charge from the phenolate oxygen to the metal d_{σ^*} orbitals and shifting the LMCT transition to lower energy. The X-ray crystal structure of *rac*-Fe^{III}EHPG has shown water molecules hydrogen-bonded to the ligand carboxyl oxygens.[11] We have also shown for the related $[MnL_2]^-$ system [H_2L = *N*-(2-hydroxynaphthalen-1-yl)sarcosine] using single-crystal X-ray crystallography that MeOH does indeed interact with the co-ordinated carboxyl oxygen,[12] providing compelling evidence for the donor–acceptor explanation[9] for the solvent dependence.

However, the solvation of the complex anion by the dipolar solvent is probably another contributing factor.[13] In the ground state, the solvent is organised such that its dipole is oriented to interact with the ground state dipole of the complex anion. Owing to the CT nature of the electronic transition, the distribution of charge in the complex anion changes from the ground state to the excited state. The dipole of the excited CT state usually differs considerably from the ground state dipole. Hence the solvation sphere suitable for the ground state will be less favourable for the excited state, which in turn affects the energy of the electronic transition. The energy of the transition depends on the polarity of the solvent.

It is also noteworthy that the nature of the substituent group positioned *para* to the phenolate oxygen has a significant influence on the energy of the LMCT band as can be seen in Table 4. Unsurprisingly, the lowest-energy LMCT transition for a fixed solvent occurs in *trans*,*trans*,*trans*-$[Co(3,5\text{-}Me_2\text{-}hbg)_2]^-$ due to the presence of the electron-donating methyl groups which increases the basicity of the phenolate oxygen and thus facilitates the phenolate-to-cobalt(III) charge transfer. By taking into account the solvent dependence of, and the effect of the nature of the substituent groups on, the energy of the LMCT transition, we have synthesised the complex anion *trans*,*trans*,*trans*-$[Co(5,3\text{-}Cl,Me\text{-}hbg)_2]^-$ which gives the closest spectroscopic match with [Co$^{III}_2$BTf] and the corresponding complexes of the other members of the transferrins (Table 5).

Conclusion

Definitive evidence for the existence of the compounds of the type $[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(hbg)_2]$ has been obtained from single-crystal X-ray crystallography. The spectroscopic features of the *trans*,*trans*,*trans*-$[Co(hbg)_2]^-$ series of complex ions resemble those of the metalloproteins [Co$^{III}_2$BTf], [Co$^{III}_2$HLf], [Co$^{III}_2$HTf] and [Co$^{III}_2$OTf]. The interactions of the phenolates or tyrosinates with the cobalt(III) ion give rise to a LMCT band which is the dominant feature in the visible region of each of the electronic spectra of these compounds. As expected for low-spin Co(III) octahedral complexes, there is an occurrence of two ligand-field transitions assignable as $^1A_{1g} \longrightarrow {}^1T_{1g}$ and $^1A_{1g} \longrightarrow {}^1T_{2g}$. However, in the electronic spectra recorded in protic, strong-acceptor solvents, such as H_2O or MeOH, the higher-energy ligand-field band is obscured by the intense LMCT band. The extent of the solvent dependence of the LMCT is considerable with $\Delta\lambda_{max}$ ranging from 8 to 26 nm and accompanied by discernible colour changes. Acceptor solvents are presumed to interact with the carboxyl oxygens and increase the net positive charge at the metal centre thereby promoting the transference of charge from the phenolate oxygen to the cobalt(III) ion. On the other hand, donor solvents interact with the amine H atom causing a decrease in the net positive charge at the metal centre and hence shifting the LMCT band to higher energies. However, solvation effects are also thought to contribute to the observed solvent dependent shifts of the CT bands. Furthermore, substituent groups in the *para* position on the phenolic ring have been shown to have a considerable influence on the energy of the LMCT transition with electron-donating groups enabling the phenolate-to-cobalt(III) charge transfer transitions to occur at lower energies.

Table 5 Comparison of electronic spectra of $[C_5H_{10}NH_2][Co(hbg)_2]$ **1**–**5** with those of the Co(III) ternary complexes of the transferrin family of proteins

Compound	λ_{max}/nm (ε/M^{-1} cm^{-1})[a]
$[C_5H_{10}NH_2][Co(5\text{-}Br\text{-}hbg)_2]$ **1**	398 (5320)
$[C_5H_{10}NH_2][Co(5\text{-}Cl\text{-}hbg)_2]$ **2**	397 (5210)
$[C_5H_{10}NH_2][Co(3,5\text{-}Cl_2\text{-}hbg)_2]$ **3**	398 (5250)
$[C_5H_{10}NH_2][Co(5,3\text{-}Cl,Me\text{-}hbg)_2]$ **4**	406 (4400)
$[C_5H_{10}NH_2][Co(3,5\text{-}Me_2\text{-}hbg)_2]$ **5**	422 (5490)
[Co$^{III}_2$BTf][b]	405 (4400)[f]
[Co$^{III}_2$HLf][c]	405 (5170)[f]
[Co$^{III}_2$OTf][d]	405 (*ca.* 4300)[f]
[Co$^{III}_2$HTf][e]	405 (*ca.* 3950)[f]

[a] Electronic spectra recorded in aqueous medium. [b] BTf = bovine serum transferrin (present work). [c] HTf = human lactoferrin (ref. 2*c*). [d] OTf = ovotransferrin (ref. 2*d*). [e] HTf = human serum transferrin (refs. 2*b* and 2*d*). [f] Molar absorptivity per metal-binding site.

Experimental

Materials and physical measurements

Glycine, paraformaldehyde and the various derivatives of phenol and salicylaldehyde were purchased from Tokyo Kasei Kogyo; bovine serum transferrin (BTf) (in the apo form), *N*-(2-hydroxyethyl)piperazine-*N*′-(ethanesulfonic acid) (HEPES) as its sodium salt, sodium hydrogen carbonate and trisodium nitrilotriacetate (NTA) from Sigma; and $Co(ClO_4)_2 \cdot 6H_2O$ from Aldrich.

Infrared (IR) spectra were recorded on a Jasco IR-700 spectrophotometer in the range 4000–400 cm^{-1} with the samples compressed as KBr pellets. UV-visible spectra were measured in the range 250–900 nm using either a Shimadzu UV-3100 spectrophotometer or a Cary 1E UV-Vis spectrophotometer. Elemental analyses were carried out on a Perkin-Elmer 2400 Series II CHNS/O analyser and conductivity measurements were performed on a Horiba DS-14 conductivity meter.

Syntheses of ligands and complexes

The ligands H_2hbg were synthesised either by condensation of the appropriate aldehyde with glycine followed by reduction of the resultant Schiff-base with sodium borohydride or by the Mannich reaction of the appropriate phenol with paraformaldehyde and glycine as described by Wilson.[14]

The cobalt(III) complexes were synthesised by reaction of the appropriate hbg ligand with piperidine and cobalt(II) perchlorate hexahydrate in stoichiometric amounts. (**Caution**: Perchlorate salts are notorious for being explosive. Extra care must be exercised when handling them.) A detailed description of the procedure for the preparation of the complexes is given below using $[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(3,5\text{-}Cl_2\text{-}hbg)_2]$ **3** as an example.

$[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(3,5\text{-}Cl_2\text{-}hbg)_2]$ 3. To a suspension of $H_2(3,5\text{-}Cl_2\text{-}hbg)$ (0.10 g, 0.40 mmol) in a mixture of MeOH (3.5 cm^3) and EtOH (3.5 cm^3) was added piperidine (0.068 g, 0.80 mmol) with stirring to give a lemon-coloured solution. Then $Co(ClO_4)_2 \cdot 6H_2O$ (0.073 g, 0.20 mmol) was added, whereupon an orange colour developed. On further stirring and swirling at room temperature, the colour of the solution intensified. After about 15 min the resultant dark orange solution was filtered and left standing at room temperature. Red-orange blocks of crystals were deposited after two days. These were dried over P_4O_{10} *in vacuo*. Yield: 83 mg (65%); Λ_M (MeOH): 86 S cm^2 mol^{-1}. Calc. for $C_{23}H_{26}N_3Cl_4O_6Co$ (M = 641.23): C, 43.08; H, 4.09; N, 6.55. Found: C, 43.00; H, 4.09; N, 6.51%.

$[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(5\text{-}Br\text{-}hbg)_2]$ 1. Minute brownish yellow crystals were obtained overnight. Yield: 67 mg (51%); Λ_M (MeOH): 90 S cm^2 mol^{-1}. Calc. for $C_{23}H_{28}N_3Br_2O_6Co$ (M = 661.23): C, 41.78; H, 4.27; N, 6.35. Found: C, 41.41; H, 4.27; N, 6.30%.

$[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(5\text{-}Cl\text{-}hbg)_2]$ 2. Red-brown clusters of crystals were obtained overnight. Yield: 63 mg (55%); Λ_M (MeOH): 91 S cm^2 mol^{-1}. Calc. for $C_{23}H_{28}N_3Cl_2O_6Co$ (M = 572.33): C, 48.27; H, 4.93; N, 7.34. Found: C, 48.17; H, 4.92; N, 7.22%.

$[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(5,3\text{-}Cl,Me\text{-}hbg)_2]$ 4. Minute dark orange crystals were obtained overnight. Yield: 82 mg (68%); Λ_M (MeOH): 89 S cm^2 mol^{-1} Calc. for $C_{25}H_{32}N_3Cl_2O_6Co$ (M = 600.38): C, 50.01; H, 5.37; N, 7.00. Found: C, 49.97; H, 5.35; N, 6.98%.

$[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(3,5\text{-}Me\text{-}hbg)_2]$ 5. Red-orange blocks of crystals were obtained after two days. Yield: 68 mg (61%); Λ_M (MeOH): 90 S cm^2 mol^{-1}. Calc. for $C_{27}H_{38}N_3O_6Co$ (M = 559.55): C, 57.96; H, 6.84; N, 7.51. Found: C, 57.89; H, 6.81; N, 7.48%.

Single-crystal X-ray diffraction analysis

Crystals of $[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(3,5\text{-}Cl_2\text{-}hbg)_2]$ and $[C_5H_{10}NH_2]$-*trans*,*trans*,*trans*-$[Co(3,5\text{-}Me_2\text{-}hbg)_2]$ suitable for X-ray crystallography were obtained from MeCN and MeOH solutions, respectively. Reflection data were recorded on an Enraf-Nonius CAD-4 diffractometer equipped with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). Intensity data were collected by the ω–2θ technique. The structures were solved by direct methods and refined by full-matrix least squares on F^2. Hydrogen atoms were fixed at calculated positions whereas non-hydrogen atoms were treated anisotropically. Calculations were performed using the programme package MoLEN crystal structure analysis.[15]

CCDC reference numbers 180730 and 180731.

See http://www.rsc.org/suppdata/dt/b2/b202161g/ for crystallographic data in CIF or other electronic format.

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