ASSESSING THE EFFECT OF LIGAND PROXIMITY ON CHIROPTICAL AND OTHER PROPERTIES IN COBALT(III) MODEL, TYROSINE-LIKE COMPLEXES

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A series of new cobalt(III) octahedral complexes of the general formula $Na[Co(ohb\text{-}aa)_2]$ (ohb-aa = *N*-(*o*-hydroxybenzyl)amino acid anion); amino acid = glycine, (*S*)-α-alanine, α-aminoisobutyric acid, (*S*)-valine, (*S*)-norvaline and (*S*)-leucine) were prepared by the charcoal catalyzed reaction of the appropriate ligand with $[Co(NH_3)_6]^{3+}$ in alkaline aqueous solution. Complexes obtained have, regardless of the amino acid used, the same *facial all-trans* symmetry (CoN_2O_4 chromophore) with the vicinal effects as the entire source of the optical activity. ^{13}C NMR spectra reveal that the leucine derivative has, due to the steric reasons, a different ground state structure. Absorption maxima reflect a positive inductive effect from the amino acid side chain carbon atoms. Complexes of the ligands bearing electrophobic alkyl groups exhibit more negative $E_{1/2}$ in comparison with the glycine derivative, reduction of which occurs at a more positive potential. Reduction potentials do not correlate with cobalt(III) Lewis acidity modulated by ligands.

The fact that numerous proteins such as transferrins, catechol dioxygenases and purple acid phosphatases bind iron[1–3], has necessarily raised questions concenrning the identity of the actual ligands coordinated to iron(III) in these important species. Although the iron coordination centers in some transferrins are now well established[4–6], iron(III) coordination by tyrosine originating from different part of these proteins[7–12], together with the observations that the protein functional groups that are not governed in iron coordination play an important role in determining kinetic and thermodynamic properties of transferrins[13], makes the study of proximal ligand influences particularly important at the present time. Thus, we report the results of a study of the structural diversity of tyrosine-like ligands on the stereochemistry, spectral and redox properties of their octahedral cobalt(III) complexes with the aim of applying the results obtained to the coordination chemistry of transferrins or more generally to other iron tyrosinate proteins. Substitution inert cobalt(III) complexes have been used to avoid the dissociation events which complicate the study of the more labile iron(III) species.

EXPERIMENTAL

Electronic absorption spectra were recorded with either a Perkin–Elmer Lambda Array model 3840 UV-VIS or a Specord M 40 (Zeiss, Jena) spectrophotometer. ^{13}C NMR spectra were obtained in D_2O and recorded on Bruker 400 instruments with tetramethylsilane as the external standard. Circular dichroism spectra were measured on an Aviv 62DS spectrometer. Redox measurements were performed using a Polarograph PA4 (Laboratorni pristroje, Praha) in 0.1 M $NaClO_4$ against a saturated calomel electrode.

All chemicals used were purchased from Aldrich.

Ligand Synthesis

N-((*o*-Hydroxybenzyl)amino acid) ligands abbreviated as ohb-aa (aa is the glycine (Gly), (*S*)-α-alanine (Ala), α-aminoisobutyric acid (Ibu), (*S*)-valine (Val), (*S*)-norvaline (Nva) and (*S*)-leucine (Leu)) were prepared by the reduction of the Schiff bases synthesized in situ from salicylaldehyde and the sodium salts of the appropriate amino acid with $NaBH_4$. Acidification of the reaction mixture to pH 5–6 afforded the desired product, which was washed with water, acetone and characterized by elemental analysis, which gave satisfactory results.

TABLE I
Elemental analysis, electronic absorption spectra (cm^{-1}) and reduction potentials (V) of $Na[Co(ohb\text{-}aa)_2]$ complexes

Compound	Calculated/Found			ν_{max} (ε)		$E_{1/2}$
	% C	% H	% N	1st band	2nd band	
$Na[Co(ohb\text{-}Gly)_2] \cdot 0.5\ H_2O$	48.12	4.26	6.23	14.660 (118)	25.660 (2.530)	–0.18
$C_{18}H_{19}N_2CoNaO_{6.5}$ (449.2)	48.20	4.30	6.30	18.149 sh		
$Na[Co(ohb\text{-}Ala)_2] \cdot 2\ H_2O$[a]	47.63	5.20	5.55	14.382 (125)	26.220 (2.530)	–0.34
$C_{20}H_{26}N_2CoNaO_8$ (504.3)	47.33	5.27	5.64	18.160 sh		
$Na[Co(ohb\text{-}Ibu)_2] \cdot H_2O$	51.37	5.49	5.45	14.440 (128)	26.300 (2.543)	–0.33
$C_{22}H_{28}N_2CoNaO_7$ (514.4)	51.29	5.52	5.49	18.169 sh		
$Na[Co(ohb\text{-}Nva)_2] \cdot H_2O$	53.14	5.95	5.16	14.455 (131)	26.380 (2.542)	–
$C_{24}H_{32}N_2CoNaO_7$ (542.4)	52.98	6.00	5.15	18.173 sh		
$Na[Co(ohb\text{-}Val)_2] \cdot 1.5\ H_2O$	52.27	6.03	5.08	14.453 (130)	26.400 (2.550)	–0.32
$C_{24}H_{33}N_2CoNaO_{7.5}$ (551.4)	52.18	6.09	5.00	18.180 sh		
$Na[Co(ohb\text{-}Leu)_2] \cdot 1.5\ H_2O$	53.88	6.44	4.83	14.502 (131)	26.440 (2.549)	–0.38
$C_{26}H_{37}N_2CoNaO_{7.5}$ (579.5)	53.70	6.46	4.79	18.188 sh		

[a] Protonated (sample dissolved in approximately 0.1 M $HClO_4$): 14.454 (120), 26.482 (2.074).

Synthesis of $Na[Co(ohb\text{-}aa)_2]$ Complexes

All cobalt(III) complexes were prepared using $[Co(NH_3)_6]Cl_3$ as the cobalt(III) precursor. A typical preparation of valine complex which describes the general method of synthesis, ohb-Val (4.42 g, 20 mmol) and NaOH (1.60 g, 40 mmol) were dissolved in H_2O (150 ml). To this solution solid $[Co(NH_3)_6]Cl_3$ (2.67 g, 10 mmol) together with charcoal were added. The reaction mixture was stirred and heated on the water bath until NH_3 evolution ceased, cooled to room temperature and filtered. The filtrate was evaporated to dryness on the water bath. The brown residue was dissolved in dry CH_3OH; the solution was filtered and evaporated to dryness using a rotary evaporator. The product was purified by chromatography on an alumina column an ethanol–water 60 : 40 (v/v) mixture as eluant. Evaporation of the main band eluate to dryness in vacuo yielded the complex of the desired composition (Table I). The remaining complexes were prepared in the same manner replacing *N*-(*o*-hydroxybenzyl-Val) with the appropriate ligand. Isomeric purity of the individual complexes was checked by TLC (alumina precoated plates, 2-propanol–water 60 : 40 as the solvent).

RESULTS AND DISCUSSION

Characterization of Complexes

Complexes of the general formula $Na[Co(ohb\text{-}aa)_2]$ were prepared by the displacement of ammonia ligands from $[Co(NH_3)_6]Cl_3$ with the proper ligand in alkaline solution in the presence of charcoal. Although several isomers are possible for each of these complexes, only one isomer was obtained in each case as revealed by the chromatographic results. Their absorption spectra (Fig. 1, Table I), which exhibit the same absorption pattern, show a shoulder on the low-energy side of the intense charge-transfer band. The shoulder is positioned where a second component of the first T_{1g} octahedral complex absorption band is anticipated for a *trans*-CoN_2O_4 complex. (The third expected *d–d* transition is buried under the same charge-transfer band, which occurs at lower

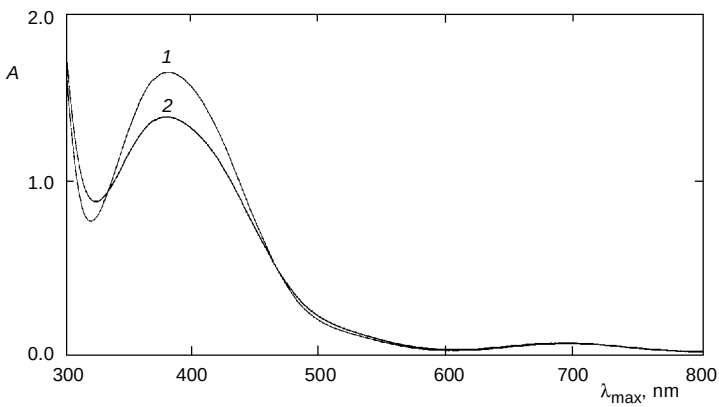
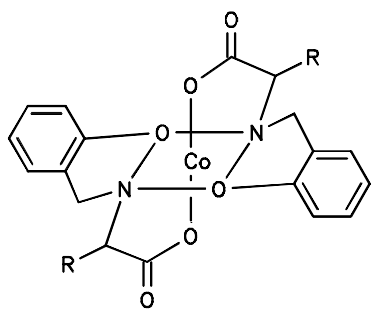


FIG. 1
Absorption spectra of the deprotonated (*1*) and protonated (*2*) $Na[Co(ohb\text{-}Ala)_2]$

energy than normal for cobalt(III) complexes because of the low-energy π*-levels of the aromatic systems.) It should be noted that the absorption spectra pattern is not affected (except for values of both molar absorption coeficients cf. Fig. 1), contrary to cobalt(III) amino alcohol complexes[14,15], by the protonation. The splitting of the T_{1g} band into two separate peaks suggests that all of the isomers prepared have a *trans*(*N*) geometry, either with *facial* or *meridional* topology for the chelate rings. (Splitting into separate peaks would not be expected for a *cis*(*N*) geometry). To distinquish between the two *trans*(*N*) topologies, ^{13}C NMR spectroscopy has been used, and the results are presented in Table II, from which it follows that the isomers (except for leucine – vide infra) give NMR spectra corresponding to structures with the respective carbon atoms in both ligands in chemically equivalent environments (aminoisobutyric acid has two nonequivalent methyl groups). This equivalency is satisfied only for the symmetrical "*all-trans*" *trans*(*N*)-*facial* geometry*(*I*) with several planes of symmetry.



I

Coordination of the secondary N-atoms of unsymmetrical ohb-aa ligands leads to the nitrogen atoms fixed stereochemistry and when both N-atoms adopt an (*R*) absolute configuration, a minimum of intramolecular nonbonding interactions (based on Dreiding models) occur between the amino acid side chains and the H-atoms of the hydroxybenzyl moiety. Interactions gradually increase in the series of *N*-atom configurations: (*R*,*R*) < (*R*,*S*) < (*S*,*S*).

Although these interactions are generally reduced for the (*R*,*R*) configurations, increasing the steric volume of the substituents on the chiral atoms leads to steric strain, which is most striking in the case of the (*S*)-leucine ligand, where the *facial* isomer would have large nonbonding interactions between the isobutyl groups and the H-atoms of CH_2N. To reduce this potential steric crowding, the two facially coordinated

* This geometry has been recently estimated by X-ray analysis of $[Co(ohb\text{-}Leu)_2]^-$ anion (ref.[16]).

o-hydroxybenzyl-(*S*)-leucine ligands are forced to deviate from their equivalent positions. As a result, the two ligands are in nonequivalent environments and the leucine complex shows double the number of signals observed for the less hindered systems. Since the optical activity of the complexes described arises primarily from the *N*- and *C*-vicinal effects (vide infra), the optical activity of the leucine complex is very similar to that of the other systems.

With respect to the geometry of these complexes, the results clearly demonstrate that *o*-hydroxybenzylamino acidato ligands coordinate with geometric specificity giving rise to *facial* isomers. Furthermore, the fact that only *facial* "*all-trans*" isomers were obtained, even for the glycine complex that is free of any steric interactions, suggests that the coordination mode of the two flexible tyrosine-like tridentate ligands in these complexes, except for leucine, is primarily dictated by the mutual orientation of six-membered chelate rings rather than by the steric requirements of amino acid nonpolar side chains. This tendency, i.e. the in-plane coordination of angle expanding six-membered chelate rings is common feature of metal complexes with fused chelate rings[17,18].

Circular dichroism (CD) spectra (Fig. 2, Table III) exhibit two high intensity peaks with negative signs in the T_{1g} absorption band region. The fact that both peaks have the same sign indicates that the CD spectra of this region are dominated by the vicinal effects (generally two negative Cotton effects are observed in the T_{1g} region for the (*S*)-amino acids[19]) because configurational chirality effects would lead to two CD bands of opposite sign for the *trans*-CoN_2O_4 chromophore. However, it must be emphasized that the CD band intensity significantly increases when an amino acid is a part of a linear rigid tridentate ligand that coordinates to a metal forming five- and six-mem-

TABLE II
Resonance frequency assignments (δ, ppm) in the ^{13}C NMR spectra of Na[Co(ohb-aa)$_2$] complexes

aa	α-C	β-C	γ-C	δ-C	CH_2N	CO_2^-	C_{ipso}–CH_2	C_{ipso}–O
Gly	55.4	–	–	–	53.4	186.0	126.2	164.0
α-Ala	61.7	16.6	–	–	51.5	186.6	128.6	163.0
Ibu	66.4	23.0	–	–	47.9	190.4	128.5	162.9
		27.0	–					
Val	71.1	30.6	19.3	–	52.9	185.6	128.6	162.8
Nva	66.0	30.4	18.6	13.9	51.5	185.7	128.3	162.8
				14.3				
Leu	63.7	51.4	25.6	22.1 22.8	40.4	186.8	125.6	163.0
	64.8	52.3	26.2	23.5 23.7	42.6	188.9	128.6	163.7

bered chelate rings[18]. This suggests that the strain imposed by a rigid six-membered fused chelate ring exerts strong influence on the vicinal effect, the intensity of which is related to the distortions of the octahedron angles. In this connection, it should be noted that the carbon-atom vicinal effect thoroughly dominates the CD spectra of heteroleptic amino acid linear-triamine cobalt(III) chiral complexes in which the deviation of donor atoms from the Cartersian coordinates is the result of six-membered fused chelate ring formation[20]. Thus, it can be concluded that CD spectra for this type of cobalt(III) complex show a close relationship between the chelate ring size and the vicinal effect intensity. However, it must be emphasized that the vicinal effect reflects other, not well understood contributions from intra- and interligand interactions, the importance of which varies as expected. When the degree of these interactions which diminish in the series Leu > Val > Ala (based on Dreiding models), are taken into account, a possible reason for the gradual decrease in the CD peaks intensities (Ala > Val > Leu) becomes apparent. Besides this, the CD peaks intensities of the $[Co(ohb\text{-}aa)_2]^-$ complexes are also affected by the protonation of phenolic oxygen atoms (Fig. 2, Table III). Deprotonated forms display, in comparison with their conjugate acids, rather enhanced CD magnitudes. The observed CD peak intensity dependence on pH probably arise from conformational changes.

The CD spectra of the $Na[Co(ohb\text{-}aa)_2]$ complexes also display a two high intensity positive peaks congruent with the intense charge-transfer bands at about 300 and 400 nm (Table III). These peaks are also observed in the CD spectrum of iron(III) transferrin[21,22].

Amino Acid Side Chain Effects

The absorption maxima of the homologous complexes under investigation are sensitive to the number of carbon atoms in the amino acid side chain (Table I). The absorption

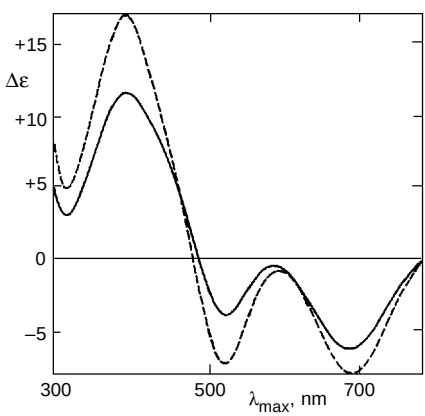


FIG. 2
Circular dichroism spectra of the deprotonated (– – –) and protonated (——) *trans*(*N*)-*facial* ("*all-trans*") $Na[Co(ohb\text{-}Ala)_2]$ isomer

maxima of both the T_{1g} and CT transitions shift to higher energy as the ring substituents become more electron donating, suggesting that the ligand-field strength at the cobalt(III) is inductively affected by the nature of the amino acid located in the tyrosine-like ligand moiety (Fig. 3). Since the first absorption band energies of homologous complexes of the same central atom reflect the ligand-field strength, these results demonstrate that the ligand donor ability increases in the series Ala < Ibu < Val = Nva < Leu (the absorption of the glycine complex, which occurs at higher energies is in line with the greater ligand-field strength obtained with the unsubstituted chelate rings because of the absence of steric strain). As far as the energy of the CT band is concerned (Fig. 3), it follows that the increasing electron density (associated with increasing ligand basicity) on the cobalt atom makes the charge-transfer t_{2g} to π^*-transition more favorable. It is interesting to speculate whether the charge-transfer spectra and other properties of the natural transferrins may also be affected inductively by the amino acids in the vicinity of the metal binding. According to our opinion, which is supported by the dependence of natural transferrin CT band energy on the Lewis basicity of synergistic

TABLE III
Circular dichroism data for the *trans*(*N*)-*facial* ("*all-trans*") Na[Co(ohb-aa)$_2$] complexes

Complex	λ_{max}, nm ($\Delta\varepsilon$)
Na[Co(ohb-Ala)$_2$][a]	295 (+10.1), 391 (+17.1), 524 (–7.1), 690 (–8.0)
Na[Co(ohb-Ala)$_2$][b]	394 (+11.7), 526 (–3.8), 685 (–6.2)
Na[Co(ohb-Val)$_2$]	299 (+8.9), 395 (+11.0), 520 (–6.8), 685 (–7.1)
Na[Co(ohb-Leu)$_2$]	299 (+8.7), 395 (+7.6), 524 (–6.9), 680 (–6.1)

[a] Deprotonated. [b] Protonated (sample dissolved in approximately 0.1 mM $HClO_4$).

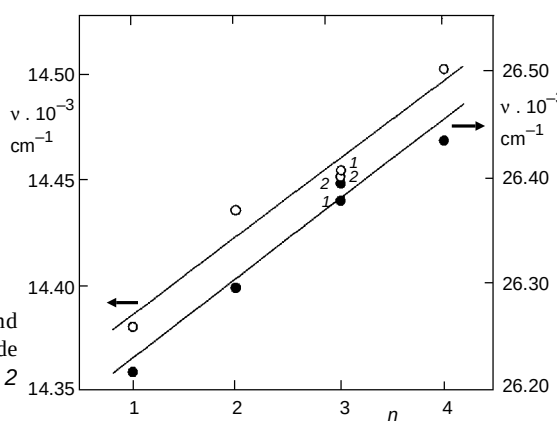


FIG. 3
Plots of the 1st and 2nd absorption band maxima vs number of the amino acid side chain carbon atoms (*n*). *1* (*S*)-norvalin, *2* (*S*)-valin

anion[23], at least the spectral properties reflect the character of proximal ligand uncoordinated groups.

Additional insight into the electronic structure of the model complexes is provided by electrochemical information. Results of polarographic reduction show (Table I) that the half-wave potential of complexes bearing nonpolar alkyl groups exhibit more negative values in comparison with the glycine derivative suggesting that these groups render the metal center difficult to reduce. The less negative potential for the glycine derivative may be thus related to relative ease with which the electron can penetrate the complex ion relative to other amino acid derivatives with their electrophobic alkyl groups. Because the reduction potentials do not correlate either with the amino acids p*K* or with the Lewis acidity of the cobalt(III) center modulated by the ligands, it is reasonable to assume that the reduction is controlled primarily by the complex solvation which is aided by the lack of alkyl groups on the complex. As can be further seen in Table I, the reduction potentials are, with the exception both of $[Co(ohb\text{-}Gly)_2]^-$ and $[Co(ohb\text{-}Leu)_2]^-$ ions, virtually identical, especially considering the nonreversible (or pseudoreversible) nature of the cobalt(III)/(II) redox couple that requires bond length changes during the process. However, a closer look on the reduction potentials reveals that the leucine derivative $E_{1/2}$ is more negative than for the rest of the nonpolar series. We ascribe this shift (which exceeds the estimated standard deviation of ± 0.01 V) to the different ground state structure of the $[Co(ohb\text{-}Leu)_2]^-$ ion, as is evident from both its NMR spectrum and the results of single-crystal X-ray analysis[16]. If this complex ion had and identical structure with the others, its extrapolated $E_{1/2}$ value should be approximately –0.31 V.

CONCLUSIONS

Though the models used here are simplified in comparison with transferrins, the fact that the iron coordination sites are very similar in individual type of transferrins[6,24] in spite of they differ in their function[25–29] and the kinetic of iron release[13] suggests that the proximal uncoordinated groups as well as distal groups can play an important roles (not yet studied) in the chemistry of transferrins at the molecular level. Present results show that an increasing number of C-atoms is manifested in a spectral blue shift, which may explain the well known color differences exhibited by iron-tyrosine proteins. In addition to this, the presence of alkyl groups which increases the hydrophobic character of complexes, shifts the reduction to more negative value, suggesting that the redox potential of a metal can be altered by proximal ligands. One of the intriguing features to emerge from this study is that the reduction potential is influenced by the polarity (solvation) of the metal coordination site exerted by the proximal ligand substituent rather than by the metal Lewis acidity. It should be pointed out in this connection that the reduction potentials for the C- and N-terminal sites of human serum transferrin are different[30]. This, together with the recent finding that the iron removal from lactoferrin

causes significant conformational change in the N-terminal lobe, but not in the C-terminal one[4,31,32] makes the study of proximal ligand effects particularly important. Further work concerning this problem is in a progress.

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