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Spectrophotometric Determination of the pK_a Values of Some Aminoacid Complexes of Pentacyanoferrate(II) and Pentacyanoruthenate(II)

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The syntheses, pK_a and spectrospcopic characterization of $[Fe(CN)_5(H_2O)]^{-3}$ and $[Ru(CN)_5(H_2O)]^{-3}$ complexes of alanine(Ala), glycine(Gly), valine(Val), lysine(Lys), arginine(Arg) and cysteine(Cys) complexes were investigated in aqueous solution. The spectra of the complexes containing amino acid ligands were similar to that of $[Fe(CN)_5(NH_3)]^{-3}$. The pK_a values of the complexes were measured by spectrophotometric titration at 22° C and $\mu = 0.100$ M($NaClO_4$).

Introduction

A large number of compounds, both natural and synthetic, contain acid an/or basic groups which govern many of their chemical, physical and biological properties. For such compounds, the properties of species that are present at a particular pH are determined by the pK_a controls many aspects of metabolism, including transport through membranes, which are frequently peculiar to one particular species. Amino acids occupy a special place in the coordination chemistry of transition metal ions[1,3]. In most of these complexes, aminoacid ligands are bound to a metal center in a unidentate fashion by amino nitrogen. In acidic medium, where the amino group is protonated, the formation of oxygen-bound species is observed. Numerous studies have reported the binding of transition metal ions to proteins, from which complexation at the histidyl residue of proteins has often been inferred. The classical example of biological histidine-metal interactions is, of course, the case of hemoglobin, myoglobin and erythocruorin. X-ray structures have in each case revealed bonding of one histidine residue to the porphyrin iron. $[Fe(CN)_5H_2O]^{-3}$ and $[Ru(CN)_5(H_2O)]^{-3}$, low spin

species, represent models for biological systems and have been used for the investigation of specific binding groups in amino acids[4] and peptides. $[Fe(CN)_5H_2O]^{-3}$ and $[Ru(CN)_5(H_2O)]^{-3}$, ions bind only one ligand, thereby forming a well behaved series $[Fe(CN)_5L]^{-3}$ and $[Ru(CN)_5L]^{-3}$ complexes[5,6]. In this paper, we report the results of extensive studies of electronic spectra and the pK_a values of pentacyanoferrate(II) and pentacyanoruthenate(II) complexes of some amino acids.

Experimental

Alanine, glycine, valine, lysine, arginine, cysteine, $Na_2[Fe(CN)_5(NO)]\ .\ 2H_2O$, $NaClO_4$, Br_2, NH_3 were supplied by Merck. $K_4[Ru(CN_6)].2H_2O$ was used as received from Alfa. $Na_3[Fe(CN)_5)(NH_3)].3H_2O$ was prepared from $Na_2[Fe(CN)_5(NO)].2H_2O$ by standard procedure[7]. The compound was recrystallized several times by saturating aqueous solution containing concentrated NH_3 at room temperature and cooling overnight in a freezer maintained at 0° C. The pure solid was collected on a filter, washed several times with ethanol and dried in a vacuum oven at 30° C. The yellow crystalline product was stored in the dark. The sample decomposed in the presence of light and humidity, producing a greenish product. The solution of aquapentacyano ferrate(II) complex was always prepared by dissolving $Na_3[Fe(CN)_5(NH_3)].3H_2$ in argon-saturated water. The complex concentration must be lower than 10^{-4} M. At concentration higher than this value $[Fe(CN)_5(H_2O)]^{-3} \leftrightarrow [Fe(CN)_5(NH_3)]^{-3}$ equilibrium can not be neglected. This equilibrium may be shifted to aqua complex formation by removing NH_3 with a passage of argon gas from the solution, but formation of dimeric species[8] such as $[Fe_2(CN)_{10}]^{-6}$ becomes important under such conditions. The concentration of the $[Fe(CN)_5(H_2O)]^{-3}$ solution can be determined spectrophotemetrically by recording the spectrum of this complex. $[Fe(CN)_5(H_2O)]^{-3}$ has an λ_{max} at 442 nm, but this band will shift to a lower value of about 410 nm in concentrated solutions. $[Ru(CN)_5(H_2O)]^{-3}$ provides a more labile coordination site on the Ru(II) allows for incorporation of the amino acid as in eq. 1

$$Ru(CN)_5H_2O^{-3} + AA \rightarrow Ru(CN)_5AA^{-3} + H_2O \quad (1)$$

The reaction of $[Ru(CN)_6]^{-4}$ with Br_2 results primarily in the oxidation state of the Ru(II) center[9]. The reaction of Br_2 with $[Ru(CN)_6]^{-4}$ is rapid and produces the yellow color of $Ru(CN)_5H_2O^{3-}$ that has λ_{max}:312 nm eq.2.

$$Ru(CN)_6^{-4} + Br_2 \rightarrow Ra(CN)_5H_2O^{-3} + BrCN + Br^- \quad (2)$$

At higher concentrations $> 10^{-4}$ M) the $[Ru(CN)_5(H_2O)]^{-3}$ ion is observed to undergo a slow dimerization reaction, presumably to yield a cyanide-bridged $[Ru_2(CN)_{10}]^{-6}$ ion[10] and λ_{max} shifts to lower values $\sim$ 290 nm, as observed with the pentacyanoferrate(II) system.

Preparation of Aminoacid Complexes of $[Ru(CN)_5(H_2O)]^{-3}$

The aquapentacyanoruthenate(II) ion, $[Ru(CN)_5(H_2O)]^{-3}$, was prepared by mixing equimolar concentrations of 0.0468 M (0.1 mmol) $[Ru(CN)_6]^{-4}$ and Br_2 in 800 ml of argon-saturated water in the presence of ten-fold excess of KBr (0.1mmol Br_2+ 1mmol KBr). The reaction of Br_2 with $[Ru(CN)_6]^{-4}$ is rapid and produces a pale yellow color of $[Ru(CN)_5(H_2O)]^{-3}, \lambda_{max}$312 nm. To this solution 200 ml 0.1 mmol aminoacid solution was added while the mixture was stirred on a magnetic stirrer. Half an hour was allowed for the reaction with the aminoacid to be complete. As $[Ru(CN)_5\ (AA)]^{-3}$ was produced, the yellow color of the solution intensified. Completion of the reaction was checked spectrophotometrically. Al of the solution were prepared at an ionic strength of 0.100 M with $NaClO_4$.

Preparation of the Aminoacid complexes of $[Fe(CN)_5(H_2O)]^{-3}$

The solution of the $[Fe(CN)_5(H_2O)]^{3-}$ were always freshly prepared. 0.0320 g (0.1 mmol) $Na_3[Fe(CN)_5(NH_3)].3H_2O$ was dissolved in 800 ml of argon-saturated water. NH_3 was eliminated by argon bubbling for an hour. With slow stirring, 200 ml of 0.1 mmol aminoacid was added to the aquapentacyanoferrate(II) solution. Half an hour allowed for the reaction with aminoacid to be complete.

Electronic Spectra

UV-VIS spectra of the complexes were recorded on a Bausch-Lomb spectronic 2000 spectrophotometer, modified with a cell block thermostated by means of an external water bath. An Orion 520 A pH meter was used for pH measurements.

Determination of pK_a

The pK_a's of the complexes were determined by spectrophotometric titration. Ten solutions containing identical quantities (1×10^{-4} M) of the complex were prepared. Various amount of $HClO_4$ was added to each solution and left for only a brief period for equilibration to 20° C. The UV-VIS spectrum was taken for the solutions at high acid concentration to insure that the complex was sufficiently stable under these conditions for obtaining reliable absorbance data. At a fixed λ and pH, absorbance of each of the solutions was measured. The pK_a values of the complexes were determined from the absorbance vs. $[H^+]$ data by graphical and numerical methods. Each of the pK_a values is the mean of four measurements.

Result and Discussion

Amino acids, having general formula $NH_2CH(R)$-COOH, form two classes of ligands for coordination with pentacyanoferrate(II) and pentacyanoruthenate(II). In the first class, R is not expected to bind to $[Fe(CN)_5(H_2O)]^{-3}$ and $[Ru(CN)_5(H_2O)]^{-3}$. R may be neutral, H− in glycine, CH_3− in alanine, $(CH_3)_2CH$− in valine; cationic $+NH_2C(NH_2)NH(CH_2)_3$− in arginine. In the other class, R is expected to bind strongly to $Fe(CN)_5H_2O^{-3}$ and $Ru(CN)_5H_2O^{-3}$. This group includes $H_2N(CH_2)_4$− in lysine and $HSCH_2$− in cysteine.

Electronic Spectra

The lowest energy band in thespectrum of each of the $[Fe(CN)_5(L)]^{-3}$ and $[Ru(CN)_5(L)]^{-3}$ complexes is assigned to the metal-ligand charge transfer transition, MLCT. λ_{max} values of the amino acid complexes of $[Fe(CN)_5(L)]^{-3}$ and $[Ru(CN)_5(L)]^{-3}$ are the same as those of NH_3 complexes. Cysteine is bound to $[Fe(CN)_5(H_2O)]^{-3}$ are the same as those of NH_3 complexes. Cysteine is bound to $[Fe(CN)_5(H_2O)]^{-3}$ and $[Ru(CN)_5(H_2O)]^{-3}$ by its sulfur atom. The presence of a sulfur-iron and sulfur-ruthenium bond is responsible for the dark orange color. The spectra of these complexes features S-Fe and S-Ru charge transfer transitions. These lower energy bads are not observed in the spectra of the amino acid caboxylate complexes of $[Fe(CN)_5(L)]^{-3}$ and $[Ru(CN)_5(L)]^{-3}$ [11,12] (Table 1 and Table 2).

pK_a Values

When strong acid, $HClO_4$, is added to a solution of $[Fe(CN)_5(L)]^{-3}$ and $[Ru(CN)_5(L)]^{-3}$ changes in the electronic spectrum occur as shown in Figure 1. This series of spectra may be compared to the less complica-

ted absorbance changes that occur upon the acidification of $[Fe(CN)_5(AA)]^{-3}$ and $[Ru(CN)_5(AA)]^{-3}$ solutions. When these solutions are titrated with $HClO_4$, the coordinated CN^- is protonated. pK_a values of the aminiacids studied ranged from 1.7 for cysteine up to 2.34 for alanine and glycine. Measured pK_a of the amino acid complexes are higher than free amino acids. A complex stability constant is greater than the corresponding stability of the acid K_a because a complex is more stable than acid when the complex is formed. Since measured pK_a's are higher, the protonation may not be related to the amino acid protonation

Table 1. Electronic spectra of $[Fe(CN)_5(L)]^{-3}$ and $[Ru(CN)_5(L)]^{-3}$

Complex	λ_{max}	$\epsilon_{max} M^{-1}$ cm^{-1}
Alanine	398 (304)	462 (992)
Glysine	396 (302)	485 (945)
Valine	391 (308)	605 (770)
Lysine	394 (305)	415 (810)
Arginine	395 (298)	573 (782)
Cysteine	438 (467)	510 (640)

Data for $Ru(CN)_5L^{-3}$ are given in parenthesis

Table 2. Spectral and pK_a data for amino acid complexes of $[Fe(CN)_5(H_2O)]^{-3}$

	MLCT abs, nm (10^{-3} cm^{-1}			
Complex	Unprotonated	Protonated	ΔE cm^{-1}	pK_a
Ala	398(25.13)	378(26.45)	-1.32	2.53±0.02
Gly	396(25.25)	381(26.25)	-1.00	2.65±0.03
Val	391(25.57)	371(26.95)	-1.35	2.58±0.02
Lys	394(25.38)	376(26.59)	-1.21	2.50±0.01
Arg	395(25.32)	373(26.81)	-1.49	2.63±0.04
Cys	438(22.83)	391(25.57)	-2.74	3.47±0.03

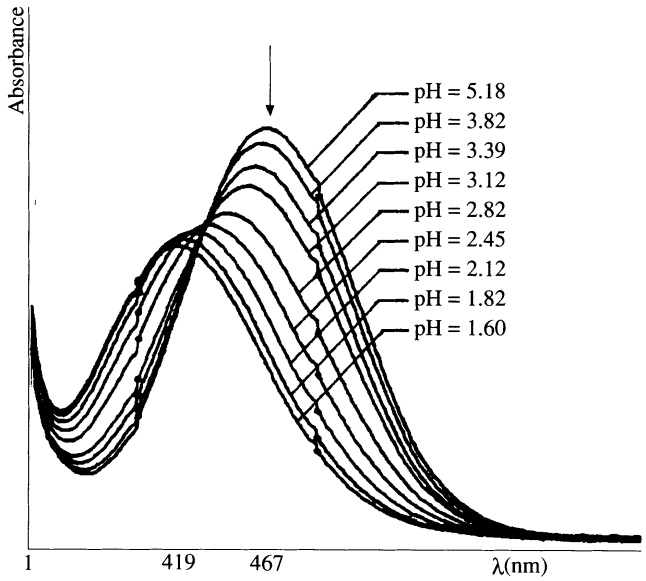

Figure 1. Spectrophotometric titration of $Ru(CN)_5Cys^{-3}$ with perchloric acid at 22° C, μ = 0.100 M $NaClO_4$. The pH values are indicated in the figure. The arrow indicates the direction of absorbance changes as the acid is added.

$$Ru(CN)_5(AA)^{-3} + H_3O^+ \iff Ru(CN)_4(HCN)(AA)^{-3} + H_2O$$
$$Fe(CN)_5(AA)^{-3} + H_3O^+ \iff Fe(CN)_4(HCN)(AA)^{-3} + H_2O$$

The MLCT bands shift to higher energy without an isobestic point. The higher energy of this transition upon protonation is consistent with the MLCT nature of the absorption[13]. $[Fe(CN)_5(AA)]^{-3}$ and $[Ru(CN)_5(AA)]^{-3}$ complexes offer two potential sites for protonation; amino acid and cyanide. There are four species during the course of the titration $[Fe(CN)_5(AA)]^{-3}$, $[Fe(CN)_4(HCN)(AA)]^{-2}$, $[Fe(CN)_5(HAA)^{-2}$ and $[Fe(CN)_4HCNHAA]^-$. Protonation of amino acid is not achieved even at low pH values. Spectral and pK_a data for the $[Fe(CN)_5AA]^{-3}$ and $[Ru(CN)_5AA]^{-3}$ complexes are given in Table 2 and Table 3. Determination of the pK_a's of amino acid complexes of $[Fe(CN)_5]^{-3}$ and $[Ru(CN)_5]^{-3}$ was done by graphical method (Figure 2) and plot of $[M]/\Delta A$ vs $1[H^+]$ as the ratio of slope to intercept (Figure 3) according to eq.3[14,15].

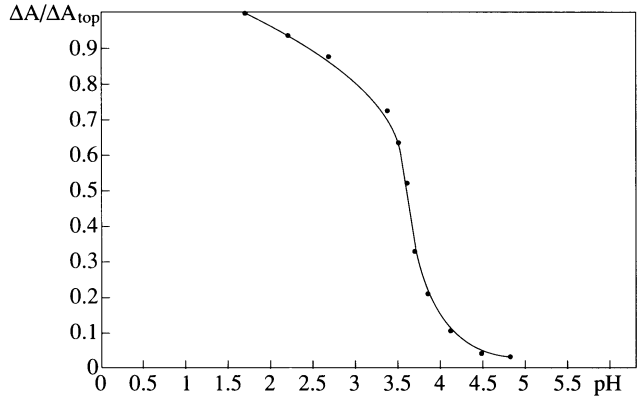

Figure 2. Determination of pK_a of cysteine complexes of $Ru(CN)_5^{3-}$ by graphical method.

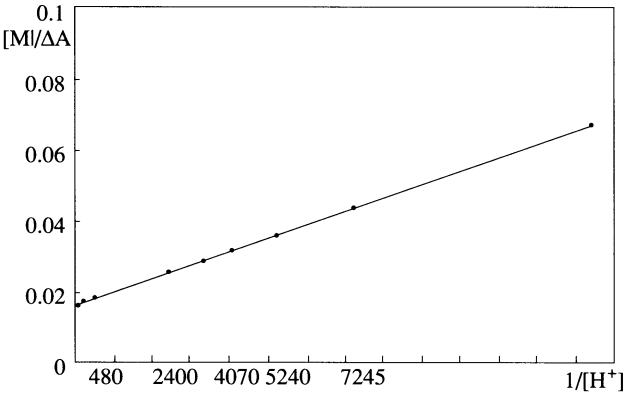

Figure 3. Determination of pK_a of cysteine complexes of $Ru(CN)_5^{3-}$ by linaer least-squares analysis.

Table 3. Spectral and pK_a data for amino acid complexes of $[Ru(CN)_5(H_2O)]^{-3}$

	MLCT abs, nm (10^{-3} cm^{-1}			
Complex	Unprotonated	Protonated	ΔE cm^{-1}	pK_a
Ala	304(32.89)	288(34.72)	-1.83	2.63±0.01
Gly	302(33.11)	292(34.25)	-1.14	2.92±0.03
Val	308(32.47)	289(34.60)	-2.13	2.69±0.03
Lys	305(32.79)	287(34.84)	-2.05	2.87±0.02
Arg	298(33.56)	282(35.46)	-1.90	2.78±0.03
Cys	467(21.41)	419(23.87)	-2.46	3.65±0.02

ΔE is energy difference between MLCT absorption of base and acid forms of the complexes.
ΔE is negative if the energy of MLCT transition shifts to higher energy upon protonation.

$$[M]/\Delta A = K_a/\Delta\epsilon[H^+] + 1/\Delta\epsilon \quad (3)$$

[M] is the total concentration of the protonated and unprotonated forms, ΔA is the difference between the initial absorbance and the absorbance after the addition of acid and $\Delta\epsilon$ is the difference between molar absorptivities for the unprotonated and protonated forms. The slopes and intercepts are determined by least-square analysis. The results of this method are in good agreement with those obtained by graphical method.

The pK_a reported in this paper should be considered approximations. Because of the existence of many species involved in solution, accurate individual determinations of the pK_a's were precluded. There are also some experimental difficulties that hinder an accurate determination. Complexes are not so stable the at very high acid concentration (pH < 1) needed for the protonation of amino acid in the complex. Despite these difficulties, the pK_a's of amino acid complexes of $[Fe(CN)_5]^{-3}$ and $[Ru(CN)_5]^{-3}$ evaluated in this paper must be taken as composite pK_a's, and the equilibria may include several CN^- protonated species. The pK_a's of amino acid complexes of $[Fe(CN)_5(H_2O)]^{-3}$ and $[Ru(CN)_5(H_2O)]^{-3}$ were interpreted to indicate a substantial ground-state interaction between a low-lying π^* orbital of the CN^- ligand and the metal t_{2g} orbital[16]. Within the group Fe(II) and Ru(II), the highest pK_a values for the amino acid complexes were expected for the Ru(II) complexes. The 4d orbitals were higher in energy than the 3d orbitals and therefore closer in energy to those of the cyanide antibonding orbital This is consistent with the expected order of the back bonding capability of Ru(II) $>$ Fe(II).

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