A Spectrophotometric Study of the Interaction of Pentacyanoferrate(II/III) Complexes with Nucleic Bases, Nucleosides and 5′-Mononucleotides

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Abstract

The reactions of the complexes $[Fe^{II}(CN)_5NO]^{2-}$, $[Fe^{II}(CN)_5H_2O]^{3-}$ and $[Fe^{III}(CN)_5H_2O]^{2-}$ with purine and pyrimidine nucleic bases, nucleosides and 5′-nucleotides were studied spectrophotometrically. The influence of light, [ligand], pH and temperature on the complex formation process was studied. In the case of the aqua complexes the observed spectral changes are in line with substitution of the aqua ligand by the entering nucleophile. For acidic, neutral and slightly alkaline ($pH \leqslant 8$) solutions of $[Fe(CN)_5NO]^{2-}$ in the absence of light, no evidence for complex formation was found. However, in more basic solution ($pH > 8$) or in the presence of light (acidic or neutral solutions) the observed reactions are due to subsequent substitution reactions of the thermally and photochemically produced decomposition products of $[Fe^{II}(CN)_5NO]^{2-}$, viz. $[Fe^{II}(CN)_5H_2O]^{3-}$ and $[Fe^{III}(CN)_5H_2O]^{2-}$, respectively.

Introduction

The interaction of metal ions and complexes with nucleosides and their bases, has received considerable attention in recent years [1–5]. Under mild reaction conditions characteristic for living organisms, the nucleic bases are extremely unreactive and complex formation is one of the few reactions they can undergo. Such an interaction differs for different metal ions and depends on the potential binding site on the nucleic base or nucleoside. In this respect, pentacyanoferrate(II/III) ions such as $[Fe(CN)_5NO]^{2-}$, $[Fe(CN)_5H_2O]^{3-}$ and $[Fe(CN)_5H_2O]^{2-}$ are interesting reaction partners [6]. The nitrosyl ligand of $[Fe(CN)_5NO]^{2-}$ is known to be a very active nitrosating agent in neutral and slightly alkaline media [7, 8], where no reaction with free nitrite occurs, and was considered as a possible nitrosating agent for nucleic bases [9]. The reactions of $[Fe(CN)_5NO]^{2-}$ with nucleic bases and nucleosides are especially interesting since nitroprusside is widely used as a hypotensive agent during surgery, in hypertensive emergencies and to improve the heart function after myocardial infarction [10].

In the past, many studies on the reaction of nitroprusside with different ligands were performed without taking care of its photochemical activity. This led to unnecessary complications [10–12]. In order to avoid these complications, the investigated reactions with nucleic bases, nucleosides and 5′-mononucleotides were not only performed with $[Fe(CN)_5NO]^{2-}$ but also with its thermal and photochemical aquation products, viz. $[Fe^{II}(CN)_5H_2O]^{3-}$ and $[Fe^{III}(CN)_5H_2O]^{2-}$, respectively. Both these complexes have one very labile coordination site that can interact with the nucleic base moiety.

Experimental

$Na_2[Fe(CN)_5NO]\cdot 2H_2O$ was used as obtained from Merck (reagent grade). $Na_3[Fe(CN)_5NH_3]\cdot 3H_2O$ was prepared from nitroprusside according to a standard procedure [13] and characterized by UV–Vis, IR and chemical analysis. Solutions of $[Fe(CN)_5H_2O]^{3-}$ (usually less than 2×10^{-4} M) were prepared freshly by dissolving the ammine complex in nitrogen saturated water. $[Fe(CN)_5H_2O]^{2-}$ was prepared in solution from $[Fe(CN)_5NO_2]^{3-}$ as described elsewhere [14]. Solutions of the ligands were prepared using the free bases and the nucleosides as pure compounds and the 5′-mononucleotides as disodium salts, the only exceptions being adenine and adenosine (A) as hemisulfate salts and AMP as sodium salt. The concentration of the pentacyano complexes was usually kept constant and that of the ligand varied.

The pH of the reaction mixture was adjusted with the aid of $HClO_4$ and NaOH, and most investigations were performed in the range $5.5 \leqslant pH \leqslant 6.5$. This pH range is significantly far away from the

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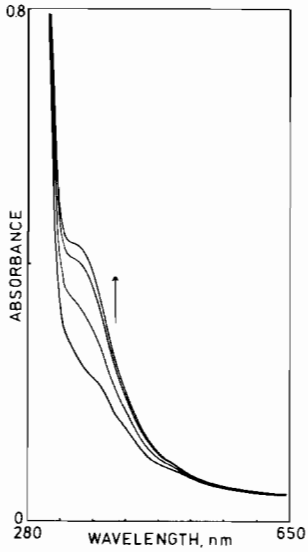
deprotonation constants of the aqua complexes, viz. pK_a = 8.3 and > 13 for $[Fe(CN)_5H_2O]^{2-}$ and $[Fe(CN)_5H_2O]^{3-}$ respectively [15, 16], as well as the protonation of the coordination sites of the nucleosides, viz. pK_a (N3) = 4.3 for cytidine, pK_a (N1) = 3.6 for adenosine and pK_a (N7) = 1.2 for inosine [2]. In some experiments the ionic strength of the medium was adjusted with the aid of $NaClO_4$.

UV–Vis spectra were recorded on a Perkin-Elmer Lambda 5 spectrophotometer equipped with a thermostated cell changer. pH measurements were performed on a Metrohm E 250 pH meter of which the reference electrode was filled with 3 M NaCl in order to prevent the precipitation of $KClO_4$ when KCl is used as electrolyte.

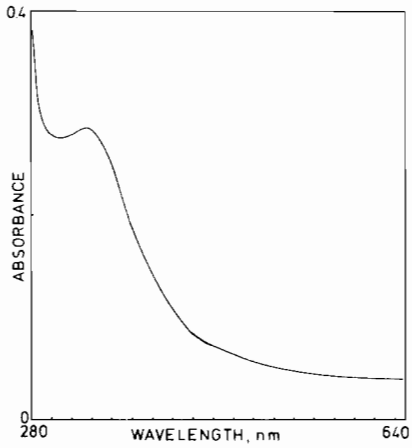
Results and Discussion

In a series of preliminary experiments the reactions of $[Fe(CN)_5NO]^{2-}$ with nucleic bases and their nucleosides were studied spectrophotometrically as a function of ligand concentration (1:1, 1:5, 1:10 and 1:50), pH (3, 5.5–6.5, 8 and 10–11) and temperature (25 and 40 °C). The reaction solutions were protected from light to prevent possible photoreactions of $[Fe(CN)_5NO]^{2-}$. For the reactions at pH ⩽ 8 and 25 or 40 °C, no significant spectral changes could be detected after several hours. This was not only observed for pyrimidine type of bases [9], but also for purine bases. Significant spectral changes and evidence for the occurrence of substitution reactions were observed at pH > 8 (i.e. 10–11) or when the solutions at lower pH were not protected from light or even irradiated. Under these conditions $[Fe(CN)_5NO]^{2-}$ decomposes thermally and photochemically to $[Fe^{II}(CN)_5NO_2]^{4-}$ (and its aquation product $[Fe^{II}(CN)_5H_2O]^{3-}$ [17] and $[Fe^{III}(CN)_5H_2O]^{2-}$ [18, 19], respectively. The observed spectral changes for alkaline solutions of $[Fe(CN)_5NO]^{2-}$ are similar in character for nucleic bases and nucleosides, and are most significant for adenine and adenosine (shown in Fig. 1(a)). Very similar spectral changes were observed during the reaction of $[Fe(CN)_5H_2O]^{3-}$ with adenine or adenosine at pH 5.5 (see Fig. 1(b)), indicating that this is the actual species reacting in alkaline solution, i.e. produced during base hydrolysis of nitroprusside. The product spectrum shows an absorption band at 330 nm with $\epsilon \geqslant 3 \times 10^3$ M^{-1} cm^{-1}, which is assigned to MLCT as found for similar complexes summarized in Table 1 [20–22].

During the reaction of alkaline solutions of $[Fe(CN)_5NO]^{2-}$ with cytosine (cytidine) or guanine (guanosine), an absorbance increase in the range 300–550 nm with a good resolved maximum at 420 nm, is followed by a decrease in this band and an increase in the range 300–370 nm. The final



(a)



(b)

Fig. 1. Repetitive scan spectra observed for the system (a) $[Fe(CN)_5NO]^{2-}$ (1×10^{-3} M) and adenine (5×10^{-2} M) pH = 10, T = 40 °C, Δt = 15 min; (b) $[Fe^{II}(CN)_5H_2O]^{3-}$ (1×10^{-1} M) and adenine (4×10^{-2} M) pH = 5.5, T = 25 °C.

TABLE 1. Maxima of the absorption bands for some pentacyanoferrate(II) complexes

L	λ_{max} (nm)	log ϵ_{max}	Reference
4-Aminopyridine	320	3.64	20
Adenine	330	⩾3.47	this work
Pyridine	362	3.57	21
Pyrimidine	410	3.49	22
Isonicotinamide	435	3.66	21
Pyrazine	452	3.70	21
Quinoxoline	545	3.78	22
N-Methylpyrazinium	655	4.08	21

spectra are once again similar to those obtained on starting with $[Fe(CN)_5H_2O]^{3-}$. These results further underline the importance of base hydrolysis of nitroprusside to produce $[Fe^{II}(CN)_5H_2O]^{3-}$, which is followed by substitution by L to produce $[Fe^{II}(CN)_5L]^{3-}$.

The reactions at pH < 8 are more interesting from a medical point of view. The substitution reactivity observed only in the presence of light must be due to the photochemical formation of $[Fe^{III}(CN)_5H_2O]^{2-}$ [18, 19, 23], followed by a rapid substitution to produce $[Fe^{III}(CN)_5L]^{2-}$.

$$[Fe^{II}(CN)_5NO]^{2-} \xrightarrow[H_2O]{h\nu} [Fe^{III}(CN)_5H_2O]^{2-} + NO \quad (1)$$

$$[Fe^{III}(CN)_5H_2O]^{2-} + L \longrightarrow [Fe^{III}(CN)_5L]^{2-} + H_2O \quad (2)$$

The spectral changes observed previously [9] for the reaction of adenine or guanine with $[Fe(CN)_5NO]^{2-}$ at pH 8 and 40 °C in the presence of light, may also in fact be due to the suggested photochemical formation of the aqua species and the subsequent substitution step. In order to check this possibility, we have studied the reactions of $[Fe^{III}(CN)_5H_2O]^{2-}$ with nucleic bases, nucleosides and 5′-mononucleotides in more detail. Both purine and pyrimidine nucleic bases react with this complex, and the reactions were studied at pH 5.5–6.5. The spectral changes observed for the reactions with nucleosides and nucleotides are similar in character to those observed for the nucleic bases, although in most cases smaller than in the case of the nucleobases, probably due to steric hindrance and the charge on the ligand. Nevertheless, all the observed changes are consistent with the formation of $[Fe^{III}(CN)_5L]^{2-}$ as shown in eqn. (2).

Electronic spectra of $[Fe^{III}(CN)_5L]^{2-}$ are characterized by two bands at 333–360 and 400–435 nm

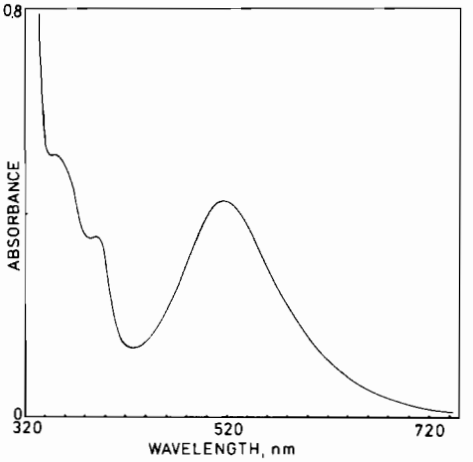


Fig. 2. UV–Vis spectrum of the $[Fe^{III}(CN)_5(cytidine)]^{2-}$ complex, pH = 6.3.

that are relatively independent of L [23, 24]. These bands are assigned to the CN → Fe and LF transitions. In addition, these complexes can exhibit LMCT bands when L = imidazole, pyrazole and pyridine substituted with functional groups that have a lone pair in conjugation with the aromatic ring [24]. In these systems the lone pair can interact (LMCT) with the half empty d_π orbital of the low spin d^5 configuration.

From all the pyrimidine nucleic bases (nucleosides and 5′-nucleotides) the best resolved spectral changes were observed for cytosine (cytidine, CMP). On mixing $[Fe^{III}(CN)_5H_2O]^{2-}$ with these ligands, a pink–violet product is formed which is accompanied by an absorption increase in the ranges 300–400 and 420–620 nm. The good solubility of cytidine made it possible to shift the equilibrium (2) over to the product side such that the final spectrum in Fig. 2 can be assigned to $[Fe^{III}(CN)_5(cytidine)]^{2-}$. From a comparison with the spectral data for related complexes in Table 2, the very strong absorption

TABLE 2. UV–Vis spectra of some pentacyanoferrate(III) complexes

L	λ_{max} (nm) (ϵ_{max} (M^{-1} cm^{-1}))	Reference
Imidazole	475(376), 403(1180), 356(981)	24
2-Methylimidazole	505(350), 404(1530), 358(1050)	24
Histamine	487, 400, 354	24
Histidine	484, 401, 352	24
Hypoxanthine	500(br), 429	24
	≈500(v.br), ≈410–420, 340	this work
Pyrazole	450, 349	24
Benzimidazole	505(1650), 382(700), 352(950)	24
5,6-Dimethylbenzimidazole	527, 385, ≈357(sh)	24
4-Aminopyridine	560(2750), 320(2910)	20, 24
Cytosine	510	this work
Cytidine	515(≥2000), 393, 350	this work
CMP	518	this work
Adenine	550, ≈405, ≈350	this work
Adenosine	560	this work

band at 515 nm ($\epsilon > 2000\ M^{-1}\ cm^{-1}$) can be assigned to LMCT, which can be interpreted as $(\pi_1, \pi_2)_L \rightarrow \pi_d$ [24]. A single LMCT band is also characteristic for the 4-NH_2py complex [20, 24], which seems to be the best suitable comparison for cytidine (cytosine, CMP). The authors [20] postulated that LMCT is facilitated by conjugation of the NH_2 lone pair with the pyridine π^* orbitals, which are of the appropriate symmetry to interact with the half-filled Fe(III) acceptor orbital (d_{xz} or d_{yz}). The 4-NH_2py ligand is coordinated through the pyridine N to the Fe(III) center, which on the basis of the very similar observed spectra, suggests that cytidine (cytosine, CMP) are bound through N3 of the ring. Further support comes from the fact that pyrimidine is also coordinated through the ring nitrogen to $[Fe^{III}(CN)_5]$.

The pH dependence of eqn. (2) was studied for L = cytosine and cytidine in the range 2.2 to 8. At pH 2.2 only the decomposition of $[Fe^{III}(CN)_5H_2O]^{2-}$ was observed. In the range 3.5 to 4.5 a small increase in the absorption band characteristic for the formation of $[Fe^{III}(CN)_5L]^{2-}$ was observed. The largest shift towards the product side was observed at pH 5.5–6.5. At higher pH a subsequent decomposition started to interfere. These observations once again support the coordination of L through N3 since the corresponding pK_a values are 4.3 and 4.6 for cytidine and cytosine, respectively [2]. The other pyrimidine nucleic bases (uracil and thymine) and their nucleosides and 5'-nucleotides are very poor ligands and the observed spectral changes are too small to allow any meaningful interpretation.

For purine nucleic bases (nucleosides and 5'-nucleotides) different coordination sites seem to be possible depending on the nature of the base. A comparison of the spectra of $[Fe^{III}(CN)_5L]^{n-}$ for L = adenine and hypoxanthine with those available for similar complexes (see Table 2), suggests that adenine is coordinated via N(1) and hypoxanthine via N(7). Due to the very poor solubility of guanine (guanosine and GMP) more dilute solutions had to be employed. Consequently, the spectral changes are too small for detailed analysis.

The UV–Vis spectrum of the yellow hypoxanthine complex is characterized by two absorption bands at *c.* 500 and 410–420 nm, which is in good agreement with that observed before [24]. The analogy of these bands with those of the imidazole complexes leads to the LMCT band assignment [24]. The spectra of the corresponding violet adenine (adenosine, AMP) complexes are similar to those of the corresponding cytidine complexes and do not resemble the spectra of the hypoxanthine complexes at all (see Fig. 3).

The suggestion that adenine coordinates to pentacyanoferrate(III) via N(1) was further supported by

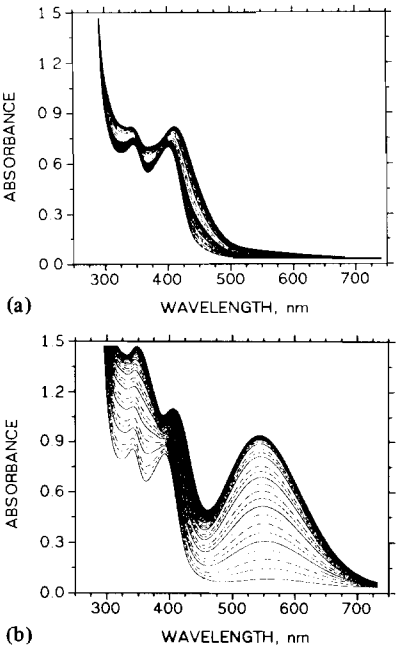


Fig. 3. Repetitive scan spectra illustrating the formation of (a) $[Fe^{III}(CN)_5(hypoxanthine)]^{2-}$ (Δt = 10 and 60 min, as indicated by break in spectral series); (b) $[Fe^{III}(CN)_5(adenine)]^{2-}$ (Δt = 30 min) from $[Fe(CN)_5H_2O]^{2-}$ (1×10^{-3} M) and hypoxanthine (5×10^{-3} M) or adenine (1×10^{-2} M); pH = 5.3, T = 40 °C.

a study of the pH dependence of the complex formation step. At pH ≈ 2, almost no complex formation was observed. With increasing pH more product is formed and a maximum is reached at pH 5.3–6.3. At higher pH decomposition occurs. Considering the fact that pK_a (N7) = −1.6, it follows that the observed results can be interpreted in terms of coordination through N3 since pK_a (N3) = 3.6.

In conclusion, the results of this study clearly show that $[Fe^{II}(CN)_5H_2O]^{3-}$ and $[Fe^{III}(CN)_5H_2O]^{2-}$, which are both decomposition products of $[Fe^{II}(CN)_5NO]^{2-}$, can account for the observed substitution reactions with the investigated nucleic bases, nucleosides and 5'-nucleotides. A kinetic study of the involved substitution processes is presently underway. This information is of interest for biological systems where $[Fe^{II}(CN)_5NO]^{2-}$ is widely employed as mentioned in the 'Introduction'.

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