On the Origin of the Non-Haemic Iron Transferrin ESR Signal: ESR Investigations on Histidine-Iron-Citric Acid Systems

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Z. Naturforsch. **38 c**, 926–928 (1983); received May 2, 1983

ESR, High Spin Ferric Ion Complex, Citric Acid

The nature of the high spin ferric iron complex located at $g = 4.3$ has been investigated by means of electron spin resonance spectroscopy and polarography. It could be shown that two complexes each exist in the acid and alkaline pH region, and that the iron is bound to two histidines, three citric acids, and probably to one bicarbonate. These results agree well with previous findings according to which the ligand field of iron should be composed mainly of oxygen and nitrogen atoms. Another low-field signal located at $g = 3.6$ appears in the pH range from 2 to 7 only and exhibits its maximum where the $g = 4.3$ signal has its minimum. Its exact nature is still unknown but it seems to represent some intermediate state of the ternary Fe^{3+}-histidine-citric acid complex. When citric acid is used, the spin concentration seems to be always larger than in the case of ascorbic acid. Since the effect obtained with ascorbic acid and citric acid seems to be similar, it may be concluded that the biological function of both of the acids might be somehow related to each other.

Introduction

The asymmetric ESR signal at $g = 4.3$, observed in many biological systems [1], is indicative of high spin ferric iron atoms in a rhombic ligand field. Since its zero-field splitting parameter D was determined to be less that 1 cm^{-1}, its ligand field should be composed mainly of oxygen and nitrogen atoms. Previous suggestions proposed that five of the six groups to which Fe^{3+} is bound are derived from the protein-3 tyrosines and two nitrogen ligands, probably from the imidazole part of histidine [2]. More recently, we could show, however, that in this complex which is responsible for the signal observed at $g = 4.3$, Fe^{3+} is bound mainly to histidine and ascorbic acid [3].

Whether or not ascorbic acid is a unique ligand for Fe^{3+} in this complex remained an unresolved question. Other compounds which participate in electron transfer reactions in biological systems, esp. in the respiratory chain, might be able to replace ascorbic acid as a ligand. Thus, the enzyme succinate-dehydrogenase, which oxidizes succinate to fumarate, requires iron whose oxidation state might be changed from Fe^{2+} to Fe^{3+} during this process. Furthermore, it is well-known that for the reversible transformation of citrate to isocitrate by aconitase the enzyme requires an Fe^{2+} which forms a stable complex with citric acid [4]. This mechanism is known as the "ferrous wheel" and it is assumed that iron is present in the ferrous state. There are certain indications that ferric irons might be involved. Thus, it seems to be of interest to find out if the complex iron-citric acid-histidine will also exhibit the $g = 4.3$ signal.

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0341-0382/83/1100-0926 $ 01.30/0

Materials and Methods

The ESR spectra were obtained with the Varian E-9, 100-kHz modulation X-band spectrometer. The modulation amplitude was 1.25 mT and the microwave power 5 mW for all samples investigated. The relative spin concentrations were obtained by taking the peak-to-peak heights of the signals at $g = 4.3$.

Citric acid, ascorbic acid, and $FeCl_3$ were purchased from Merck Co., Darmstadt, and were dissolved in bidistilled water in concentrations given in the Figures. Shortly after preparation they were frozen at 77 K and their ESR spectra measured at that temperature. The pH value was adjusted by adding either HCl or NaOH.

Results and Discussion

As we have shown recently [3], histidine and $FeCl_3$ ($Fe(NO_3)_3$ can be used instead of $FeCl_3$ without modifying the results) exhibit a $g = 4.3$ signal at a very acid pH value only (pH < 4).

The disappearance of the signal with increasing pH is due to a precipitation caused by the polymerization of the Fe^{3+} species via hydroxy-bridges

[5]. In this way, Fe^{3+} is coupled antiferromagnetically resulting in a reduction in spin concentration [5, 6].

Addition of citrate to a histidine-iron solution results in a stable complex at pH values up to about 9 depending on the concentration ratios used in the ternary system (s. Fig. 1). As can be seen the spin concentration increases with pH. A similar pH dependence can be observed for the citric acid-Fe^{3+} complex, its spin concentration is, however, much smaller than obtained with the histidine-citrate-Fe complex. The spin concentration for the corresponding histidine-ascorbate-Fe^{3+} complex is also given for comparison.

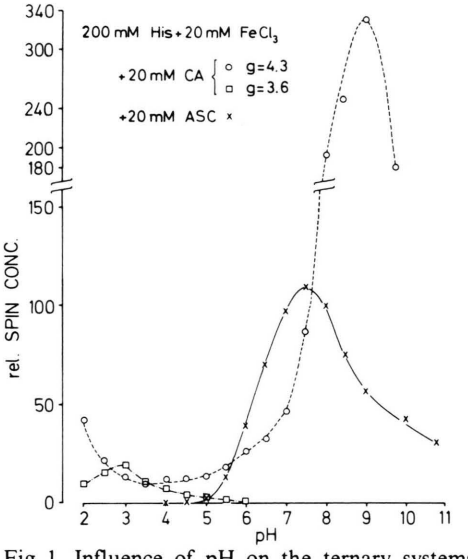

Fig. 1. Influence of pH on the ternary systems histidine (His)-Fe^{3+}-citric acid (CA) and histidine-Fe^{3+}-ascorbic acid (ASC) as determined by the Fe^{3+} ESR signals at $g = 4.3$ and $g = 3.6$ measured at 77 K.

As in the case of ascorbic acid, another low-field signal located at $g = 9.5$ with a low intensity appears always concomitantly with the $g = 4.3$ signal. This signal is due to a transition between the two sublevels of the low-lying Kramers doublet in one principal direction.

In a certain pH interval (pH 2 to 6), an additional signal at $g = 3.6$ accompanied by a weak signal at $g = 4.7$ can be observed (its change with pH is shown also in Fig. 1). These two signals seem to belong to Fe^{3+} complexes with a different complex symmetry and/or with a different magnetic state of Fe^{3+}.

It should be pointed out that at low pH values (2 to 4.5) the ESR spectrum is rather complex exhibiting several unresolved signals with g factors between 3 and 10. At higher pH (> 5) the signal at $g = 4.3$ remains only. Furthermore, the intensity dependence at the $g = 4.3$ signal with pH is similar to that observed in the Fe^{3+}-citric acid system. Histidine seems to influence the absolute spin concentration only.

To obtain some more detailed information about the ternary complex in regard to symmetry and composition, first the iron-citrate complex was investigated polarographically. The modification of these results by histidine or other amino acids will be published elsewhere [7]. The reduction of complexed Fe^{3+} to Fe^{2+} on the dropping mercury electrode occurs in two steps between -0.019 V and -0.19 V followed by the complete reduction to Fe^0 at -1.35 V (*vs.* S.C.E.). The half wave potential of

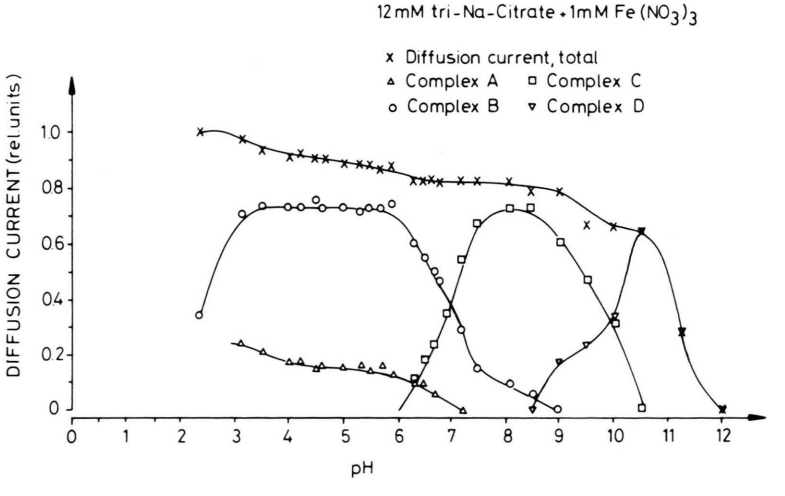

Fig. 2. The variation of the total diffusion current as well as the height of the different waves, resembling the concentration of different Fe^{3+}-citrate complexes, as a function of pH.

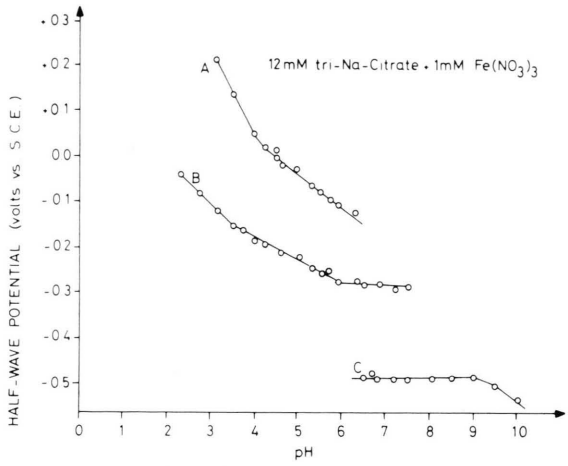

Fig. 3. The pH dependence of the half wave potentials of the different Fe^{3+}-citrate complexes.

the Fe^{3+} to Fe^{2+} wave is characteristic for the different complex species. The variation of the total diffusion current as well as the height of the different waves, resembling the concentration of different Fe^{3+}-citrate complexes, with pH is shown in Fig. 2. As can be seen, there are several iron-citrate complexes existing, however, at different pH values. The complex "A" seems to be identical with the one representing the $g = 3.6$ signal. No other correlation between the pH response and the $g = 4.3$ signal is possible.

The pH dependence of the half wave potentials (s. Fig. 3) gives some more additional information. For its analysis it is assumed that the examined Fe^{3+} complex with p ligands, which are tied in a deprotonated state, will be reduced to an Fe^{2+} complex with p−1 ligands whereby the cleaved ligand will pick up protons according to its pK values. Between pH 3.2 and 4.1, the slope of complex "A" is 180 mV/pH indicating the participation of 3 protons at the electrode reaction. In the case of the iron-citrate complex "B", the corresponding values are: 100 mV/pH for pH 2.3 to 3.5 indicating the possible replacement of two protons of the carboxyl groups by Fe^{3+}; 50 mV/pH for pH 3.5 to 5.9, followed by a pH independent segment.

$E_{1/2}$ of complex "C" is mainly pH independent. The shift towards a more negative potential at rather high pH values results from an increasing irreversibility of the electrode reaction. A similar effect applies to the iron-citrate complex "D" whose $E_{1/2}$ could not be determined. The results obtained show the existence of two complexes in each the acid and alkaline pH region. This agrees well with results reported previously [8–11]. Using redox potential measurements, Timberlake [9] could show, that one complex, in the acid region, must be a dimer ($Fe_2Cit_2^{2-}$). In these complexes, the three protons of the carboxyl groups and the proton of the hydroxyl group of the citrate are replaced by Fe^{3+}. According to the strong pH dependence of $E_{1/2}$, complex "A" described above must be identical with this dimer. In the case of complex "B", the present results suggest the binding of 3 citrate molecules by one Fe^{3+} which seems to be contradictory to a 1:1 complex proposed previously [8].

In the alkaline region, the existence of an $Fe(Cit)_2^{5-}$ complex has been proposed which is in competition with the formation of a polymer [10, 11]. In this case, the existence of an iron-hydroxid nucleus is assumed at which surface the citrate ions are bound. Complexes "C" and "D" reported above seem to be identical with these two complexes. The existence of the $g = 4.3$ signal in the iron-citrate complexes investigated suggests that Fe^{3+} is present in a high spin state with a rhombic environment. This state is caused by a weak ligand field, that is a small crystal field splitting parameter.

Acknowledgements

The excellent technical assistance of Miss P. Bräunlich and Miss P. Ferber is appreciated. This work was supported in part by grants from the Bundesministerium für Forschung und Technologie and from the Fonds der Chemischen Industrie.

[1] N. J. F. Dodd, Metal Ions in Biological Systems, Vol. **10,** p. 95–128 (H. Sigel, ed.), Marcel Dekker, New York 1980.
[2] E. Frieden, J. Chem. Educat. **52,** 754–761 (1975).
[3] W. Lohmann, D. Holz, B. Kiefer, and D. Schmidt, Z. Naturforsch. **38c,** 90–93 (1983).
[4] A. L. Lehninger, Biochemistry, Worth publ., 1970, p. 272–273.
[5] S. A. Fairhurst and L. H. Sutcliffe, Prog. Biophys. Mol. Biol. **34,** 1–79 (1978).
[6] B. Bleaney and K. W. H. Stevens, Rep. Prog. Phys. **16,** 108–159 (1953).
[7] to be published.
[8] R. C. Warner and I. Weber, J. Am. Chem. Soc. **75,** 5086–5094 (1953).
[9] C. F. Timberlake, J. Chem. Soc. **1964,** 5078–5085.
[10] T. G. Spiro, L. Pape, and P. Saltman, J. Am. Chem. Soc. **89,** 5555–5559 (1967).
[11] T. G. Spiro, G. Bates, and P. Saltman, J. Am. Chem. Soc. **89,** 5559–5562 (1967).