The Influence of Amino Acid Ligands and Vitamin C on the Reduction Potential of Fe(III): Polarographic and Electron Spin Resonance Investigations

Berthold Kiefer, Helmut Sapper, and Wolfgang Lohmann

Institut für Biophysik, Leihgesterner Weg 217, D-6300 Giessen, Bundesrepublik Deutschland

Z. Naturforsch. **39c**, 102–106 (1984); received September 22, 1983

Ferrous Ions, Ascorbic Acid, Redox-Potential, Polarography, ESR-Spectroscopy

The pH dependence of Fe(III)-amino acid complexes has been studied polarographically and by means of ESR spectroscopy.

It could be shown that, at least, two complexes exist: one with a low molecular weight, ESR detectable at acid pH, and one with a high molecular weight at alkaline pH, ESR non detectable. The half wave potential of Fe(III) is lowered by amino acid ligands.

The redox interaction of the Fe(III)-amino acid complexes with vitamin C results in a decrease of the ascorbyl radical concentration. Ascorbic acid also forms a complex with Fe(III) as indicated by a polarographic half wave potential near − 0.55 V *vs.* S.C.E. at pH 7.

Introduction

The biological role of iron is well known, especially in connection with physiological redox processes [1].

Since ascorbic acid is a predominant low molecular weight ligand of iron *in vivo* [2], it should interfere with the Fe(III)/Fe(II) redox system [3]. Such an interaction will be modified by amino acids to which iron ions are bound in many biological instances.

Despite the importance of the iron redox system for biological systems, not much is known about its potentials. Therefore, the influence of a few amino acid ligands on the redox potential and the stoichiometry of the Fe(III)/Fe(II)-system as well as the modification of iron-amino acid complexes by vitamin C has been studied by means of polarographic and electron spin resonance methods.

Materials and Methods

Ascorbic acid (ASC), L-histidine, L-aspartic acid, L-alanine and ferric nitrate ($Fe(NO_3)_3 \cdot 9\,H_2O$) have been purchased from Merck, Darmstadt, and were used without further purification.

A Metrohm Polarecord E261R equipped with a dropping mercury electrode and a saturated calomel reference electrode (S.C.E.) with a potassium nitrate-agarose bridge was used for the polarographic measurements. The polarograms were obtained at room temperature (25 °C) after deoxygenation of the solutions with purified nitrogen and addition of 0.002% gelatine.

The pH values were measured with a Knick pH meter using a glass electrode.

The potentiograms were taken by a Metrohm Potentiograph E436.

The ESR measurements were carried out at 77 K with a Varian E9 100 kHz modulation X-band spectrometer. The modulation amplitude was 0.8 mT and the microwave power 5 mW for all samples investigated.

Results and Discussion

Changes in the molecular characteristics of metal ion complexes in aqueous solutions can be well monitored polarographically by means of the pH dependence of the half wave potentials and diffusion currents [4, 5].

a) Polarographic diffusion current

The half wave potential of the free Fe(III)/Fe(II) redox system is supposedly about +0.77 V (corresponding to +0.53 V *vs.* S.C.E.) [6] and is, thus, not accessible by the dropping mercury electrode. Nevertheless, the concentration of the Fe(III)-aquo complex can be estimated by measuring the total diffusion current at a lower potential (*e.g.* 0 V *vs.* S.C.E.). The pH dependence of such a diffusion current, measured relatively to the value in the strong acid solution, is shown in Fig. 1 a.

Reprint requests to Prof. W. Lohmann.

0341-0382/84/0100-0102 $ 01.30/0

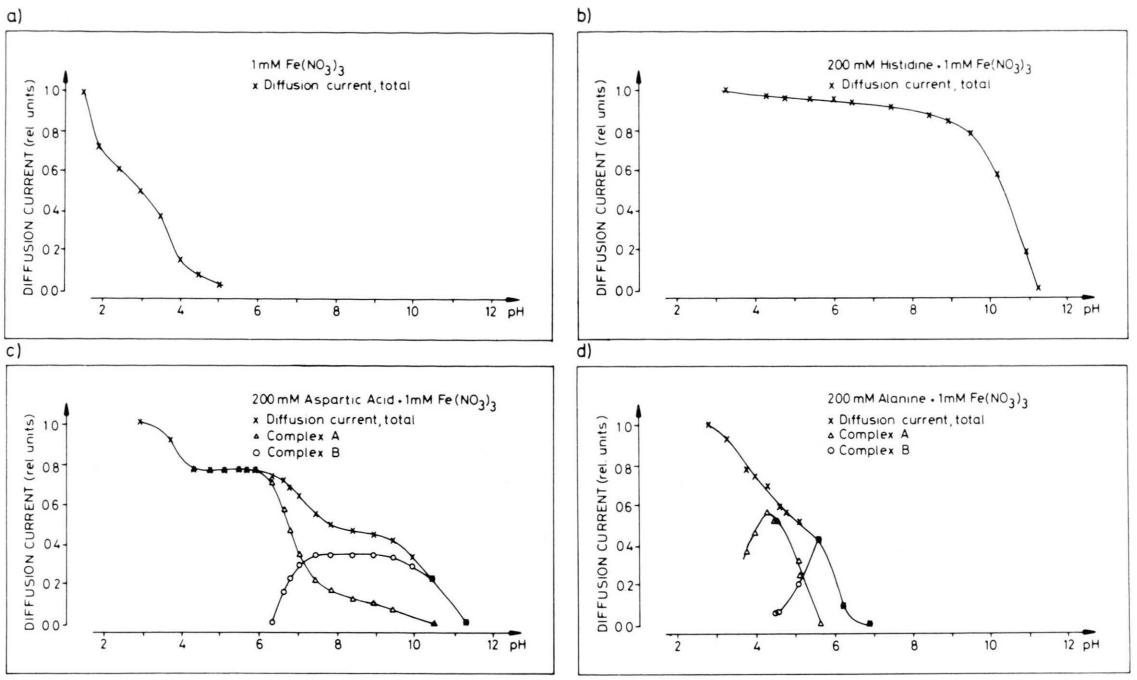

Fig. 1. pH dependence of the total diffusion current (×) of $Fe(NO_3)_3$ (1 mM) (a) complexed with histidine (b), aspartic acid (c), and alanine (d) (200 mM each). Also shown are the diffusion currents contributed by the complexes A (△) and B (○).

It is known that Fe(III) has a strong tendency in forming aquo complexes even at very low pH values. With increasing pH this complexation increases resulting in a lowered diffusion coefficient and, thus, in a diminished diffusion current. In order to maintain Fe(III) in solution at physiological pH, it must be coordinated to suitable ligands. Thus a complexation with histidine, *e.g.*, prevents such a precipitation up to about pH 9 (Fig. 1 b).

In the case of aspartic acid and alanine, the total diffusion current may be explained by a superposition of the currents contributed by at least two complexes, complex A (mainly present at low pH) and complex B (increasing in concentration with higher pH) (s. Fig. 2, 1 c, and 1 d).

The potentials of the polymerization products (complexes B) are always lower than those of the mononuclear complexes (complexes A).

The potential of the Fe(III)-histidine complex cannot be observed by means of this technique. However, the diffusion current already present at electrode potentials as high as possible (higher than 0 V) indicates, that the corresponding half wave potential is higher than this value over the whole pH range studied.

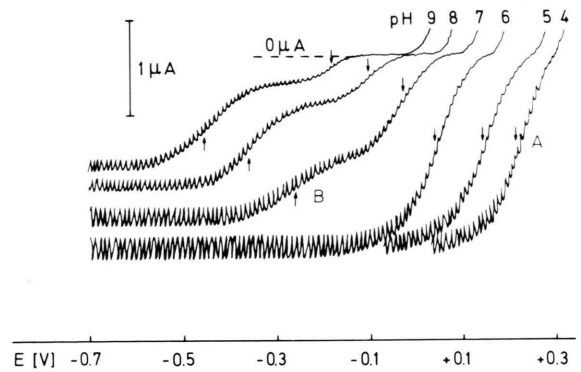

Fig. 2. Polarograms of the Fe(III)-aspartic acid complexes A and B as a function of pH. The potentials are taken *vs.* S.C.E. Concentrations used: 1 mM $Fe(NO_3)_3$, 200 mM aspartic acid, 200 mM KNO_3.

b) ESR-signal at g = 4.3

The electron spin resonance (ESR) signal of high spin Fe(III) depends strongly on the symmetry of the environment [7]. In a rhombic symmetry the

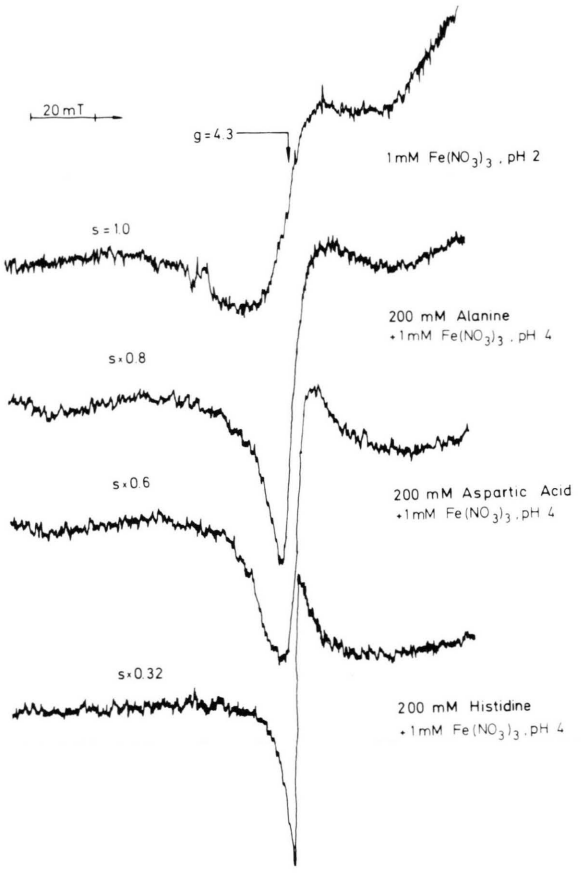

Fig. 3. ESR-spectra of $Fe(NO_3)_3$ (1 mM) (pH 2, top spectrum) with alanine, aspartic acid, and histidine as ligands (pH 4, 200 mM each), taken at 77 K. s: relative sensitivities.

signal is very sensitive to any deviation of the perfect rhombic symmetry (s. Fig. 3) [8, 9]. The line width of the ESR signal depends on the kind of the ligand; it is nearly constant over the pH range used.

The signal of the Fe(III)-histidine complex is very small (only 6 mT line width) and of a symmetrical line shape, indicating a nearly perfect rhombic symmetry.

The pH dependence of the spin concentration is shown in Fig. 4. A comparison with the polarographic diffusion currents (s. Fig. 1), shows that the ESR signal is detectable only in those pH regions where the complexes A can be recorded polarographically. Complexes B of both aspartic acid and alanine are undetectable by the ESR method. These complexes might be polynuclear with an antiferromagnetic coupling between the Fe(III) ions by −O− or −OH− bridges [7, 10].

The decrease of the spin concentration of the Fe(III)-histidine system above pH 3.5 might be caused by a change in complexation state from rhombic to another symmetry without changing molecular size or redox potential.

c) Interaction with vitamin C

The vitamin C redox system exists of two one electron transitions. Their reduction potentials are +0.32 V and −0.20 V at pH 7 for the semidehydroascorbic acid/ascorbic acid (SDA/ASC) and dehydroascorbic acid/semidehydroascorbic acid (DHA/SDA) couple [11].

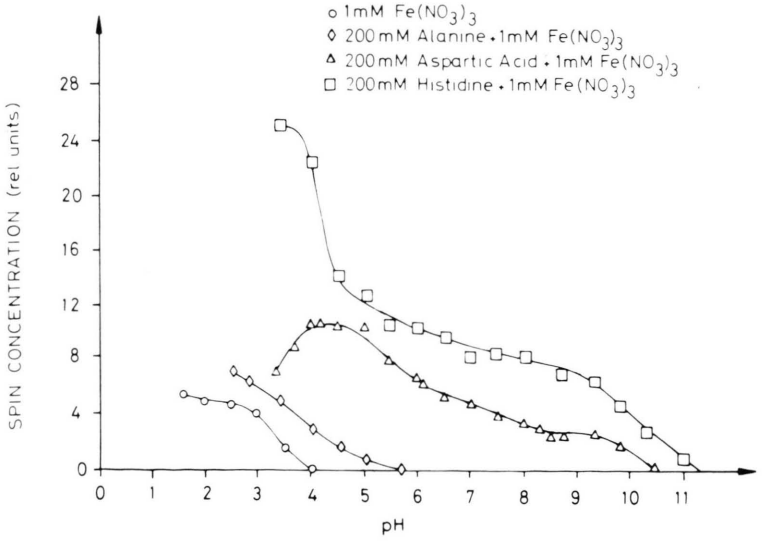

Fig. 4. pH dependence of the relative spin concentration (measured as peak height because shape and width of the signals remain constant) of the ESR signal at $g = 4.3$ of $Fe(NO_3)_3$ (1 mM) (○) with alanine (◇), aspartic acid (△), and histidine (□) as ligands (200 mM each).

Since Fe(III) ions in their complexes with histidine are reduced completely by ASC, it might be concluded that the reduction potential of the Fe(III)/Fe(II)-histidine system is much higher than that of SDA/ASC, which agrees well with the polarographic experiments. ASC is oxidized to SDA, which, then, is converted to DHA by either oxidation or disproportionation.

The potential of the Fe(III)/Fe(II)-aspartic acid system, however, is comparable to that of the SDA/ASC system. Therefore, it oxidizes ASC only partially. This partial oxidation allows the evaluation of the SDA concentration. It will result in 0.5 μM by using the equation

$$\lg[\mathrm{SDA}] = \frac{E_0^{\mathrm{Fe}} - E_0^{\mathrm{ASC}}}{0.059} + \lg\frac{[\mathrm{Fe(III)}][\mathrm{ASC}]}{[\mathrm{Fe(II)}]} \quad (\mathrm{pH}\ 7, 25\,^\circ\mathrm{C})$$

with [ASC] = 1.73 mM, [Fe(II)] = 0.54 mM and [Fe(III)] = 0.04 mM as obtained for complex A from the polarograms, $E_{1/2}^{\mathrm{Fe}} = E_0^{\mathrm{Fe}} = +0.18$ V (pH 7) as measured polarographically for the fully reversible Fe(III)/Fe(II)-aspartic acid system (reduction potential of complex A), and $E_0^{\mathrm{ASC}} = +0.32$ V (pH 7).

By using the disproportionation reaction

$$2\,\mathrm{SDA} \rightleftharpoons \mathrm{ASC} + \mathrm{DHA}$$

with the equilibrium constant $K = 1.5 \times 10^{-9}$ (pH 7, 25 °C) [11], the SDA concentration should be 30 nM only if one assumes [DHA] = 1/2 [Fe(II)].

A comparison of these two results suggests, that the potential of the Fe(III)/Fe(II)-aspartic acid system, as determined polarographically, is too high. Assuming [SDA] = 30 nM is correct, then, $E_0^{\mathrm{Fe}} = +0.11$ V (pH 7) would be the appropriate reduction potential of the Fe(III)-aspartic acid complex A in the presence of ascorbate.

Oxidation of SDA to DHA by Fe(III)-complexes will also decrease the SDA concentration. Due to the potential difference of the redox systems, this oxidation should be more pronounced than it has been observed. It is very likely that the SDA radicals, contrary to the ascorbate ions, are not affected by the Fe(III)-complexes [12], probably due to sterical reasons resulting from the special bicyclic structure of the SDA molecules [13].

It is well known that Fe(III) ions catalyze the autoxidation of ASC presumably by forming an intermediate complex [14]. The potential of such an Fe(III)-ASC complex can be detected by a cathodic polarographic wave near −0.55 V *vs.* S.C.E. (Fig. 5a) in the presence of oxygen at neutral or slightly alkaline pH. The complex also exhibits a broad absorption band near 550 nm ($\varepsilon_{550} = 1400\ \mathrm{M^{-1}\,cm^{-1}}$ at pH 7.4) which can be used for quantitative derivation of Fe(III) and ASC in aqueous solution [15].

The complexation described seems to inhibit any further oxidation probably due to the incorporation of OH^- or to the formation of other complexes, as indicated by an acidification of the solution (Fig. 5b) and by the time dependence of the Fe(III) catalyzed oxidation of ASC (anodic wave).

Conclusions

The bio-availability of iron in living cells requires the solubilisation of the Fe ions by complexation. This complexation, however, influences also the redox potential of the Fe(III)/Fe(II) redox system.

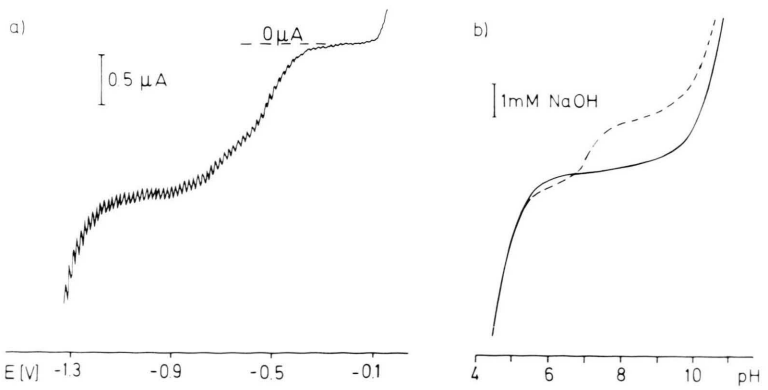

Fig. 5. a) Polarogram of $Fe(NO_3)_3$ (1 mM in 200 mM KNO_3, pH 7) taken in the presence of ascorbic acid (20 mM). The potentials are given *vs.* S.C.E.; b) Potentiograms of 20 mM ascorbic acid without (——) and with (- - -) 1 mM $Fe(NO_3)_3$.

As shown by means of the polarographic technique, there are at least two types of complexes formed between Fe(III) and amino acids: one of a relatively low molecular weight and one highly polymerized. The polymer complexes precipitate at higher pH values due to their high molecular weight and remain undetectable by ESR probably due to an antiferromagnetic coupling of their Fe-centers. The low molecular weight complexes predominate at acid pH, are detectable by ESR, and exhibit a more or less rhombic symmetry.

The reduction potentials of the polymer complexes are lower than those of the low molecular weight complexes. All of these potentials, however, are lower than that of free Fe(III) ions. These ions catalyze the one electron oxidation of ascorbic acid to the ascorbyl radical. This oxidation, however, seems to be restricted by a complexation between Fe(III) and ASC and depends, thus, on the concentration ratio between these two complexes.

The interaction between Fe(III)-amino acid complexes and ascorbate results in a reduced SDA level. This might be caused by a diminished effective redox potential of the Fe(III)-complex as shown *e.g.* with aspartic acid as a ligand.

The redox potential in living cells as maintained by the vitamin C redox system might be directly or indirectly influenced by Fe(III) ions complexed with amino acids.

Acknowledgements

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

[1] H. Sigel, Metal Ions in Biological Systems, **Vol. 7**. Marcel Dekker, New York 1978.
[2] P. M. May and D. R. Williams, in: Metal Ions in Biological Systems, **Vol. 7** (H. Sigel, ed.), Marcel Dekker, New York 1978.
[3] W. Lohmann and R. Lange, Z. Naturforsch. **34c**, 546–549 (1979).
[4] D. R. Crow, Polarography of Metal Complexes, Academic Press, London 1969.
[5] S. Inoue, J. Sci. Hiroshima Univ., Ser. A, **39**, 249–257 (1975).
[6] F. Seel, Grundlagen der analytischen Chemie, Verlag Chemie, Weinheim 1976.
[7] A. Fairhurst and L. H. Sutcliffe, Prog. Biophys. Mol. Biol. **34**, 1–77 (1978).
[8] H. H. Wickman, M. P. Klein, and D. A. Shiley, J. Chem. Phys. **42**, 2113–2117 (1965).
[9] W. E. Blumberg, in: Magnetic Resonance in Biological Systems (A. Ehrenberg, B. G. Malmstrom, and T. Vanngard, eds.). Pergamon Press, New York 1967.
[10] W. F. Tucker, R. O. Asplund, and S. L. Holt, Arch. Biochem. Biophys. **166**, 433–438 (1975).
[11] H. Sapper, S. O. Kang, H. H. Paul, and W. Lohmann, Z. Naturforsch. **37c**, 942–946 (1982).
[12] W. Weis, Z. Zubrzycki, and H. Staudinger, Hoppe-Seylers's Z. Physiol. Chem. **349**, 279–282 (1968).
[13] H. Sapper, A. Pleyer-Weber, and W. Lohmann, Z. Naturforsch. **37c**, 129–131 (1982).
[14] M. M. Taqui Khan and A. E. Martell, J. Am. Chem. Soc. **89**, 4176–4185 (1967).
[15] P. Van den Winkel and J. Mertens, Bull. Soc. Chim. Belg. **90**, 381–390 (1981).