ESR Investigations on the Modification of the Ascorbic Acid-Erythrocyte Interaction by Ferrous Ions

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An ascorbic acid-erythrocyte interaction results in the formation of the semidehydroascorbate (SDA) radical and an increase in spin concentration. Addition of a more than ten-fold concentration of Fe^{2+} (as $FeSO_4$) to such a system compensates the effect produced by ascorbic acid, that is the electron spin resonance spectrum obtained resembles very closely that of erythrocytes only. With the disappearance of the SDA radical a high spin ferric ion signal appears concomitantly at $g = 4.3$.

Introduction

Recently we could demonstrate that the electron spin resonance (ESR) signal at $g = 2.005$ observed in lyophilized native blood and erythrocytes of patients with an acute lymphatic leukemia (ALL) is due to the semidehydroascorbate (SDA) radical [1]. It is formed by an ascorbic acid-copper containing protein interaction resulting in the SDA radical. This radical can cause, among other things, lipid peroxidation. This leads to structural and functional derangements of lipoprotein membranes. In the case of erythrocytes, this peroxidation will result in hemolysis of the red cells. There is, therefore, currently considerable effort being made to convert this SDA radical to the reduced form of ascorbic acid with a concomitant reduction in spin concentration.

Since in tumor tissue an increase in the copper level with a concomitant decrease in the iron level has been observed [2], we assumed that an application of iron ions might produce the effect desired.

The interaction between vitamin C and iron ions is well established [3]. Thus, ascorbate deficiency causes major abnormalities in human iron metabolism. The mechanism by which iron concentrations are affected by vitamin C remains, however, still uncertain. In order to elucidate its molecular mechanism of interaction the effect of Fe^{2+} on the SDA radical produced by the ascorbic acid-erythrocyte interaction has been investigated by means of ESR spectroscopy.

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Materials and Methods

Erythrocytes were obtained from fresh 1:10 ACD-blood (acid-citrate-dextrose anticoagulant solution) of healthy volunteers and prepared according to a method described previously [4]. $FeSO_4$ (Merck, Darmstadt, Germany) was added in different concentrations (up to 28 mM) to erythrocytes treated prior with 0.2 mM of ascorbic acid (Merck, Darmstadt, Germany). Thereafter, the samples were lyophilized and their ESR spectra measured at room temperature. The ESR spectra were obtained with a Varian E-9, 100 kHz modulation X-band spectrometer. The modulation amplitude was $\leqq 0.2$ mT and the microwave power 5 mW for all samples investigated. The spectra of 50 mg samples each were recorded at different sensitivities marked at the left-hand side of each spectrum. The relative spin concentration was obtained by double integration of the spectra by means of a planimeter.

Results and Discussion

The effect of ascorbic acid on the ESR spectrum of healthy erythrocytes is shown in Fig. 1, second curve from top. As has been pointed out, this spectrum resembles that obtained with erythrocytes from patients with ALL. The peak located at $g = 2.005$ is due to the SDA radical. Addition of different concentrations of $FeSO_4$ to healthy erythrocytes treated with 0.2 mM of vitamin C results in a reduction of spin concentration with a concomitant disappearance of the SDA radical (s. Fig. 1, two lower curves). The ESR spectrum obtained after the addition of 8.5 mM of $FeSO_4$ resembles very closely that obtained with erythrocytes only (compare with upper curve, Fig. 1).

The effect of $FeSO_4$ on the spin concentration is seen in Fig. 2. According to these results, about 2 mM of $FeSO_4$ (that is about ten-fold concentration of ascorbic acid) are sufficient to compensate the vitamin C effect.

As a result of this interaction the SDA radical will be reduced to ascorbic acid while Fe^{2+} will be oxidized to Fe^{3+}. With the disappearance of the SDA signal a high spin ferric ion signal appears at

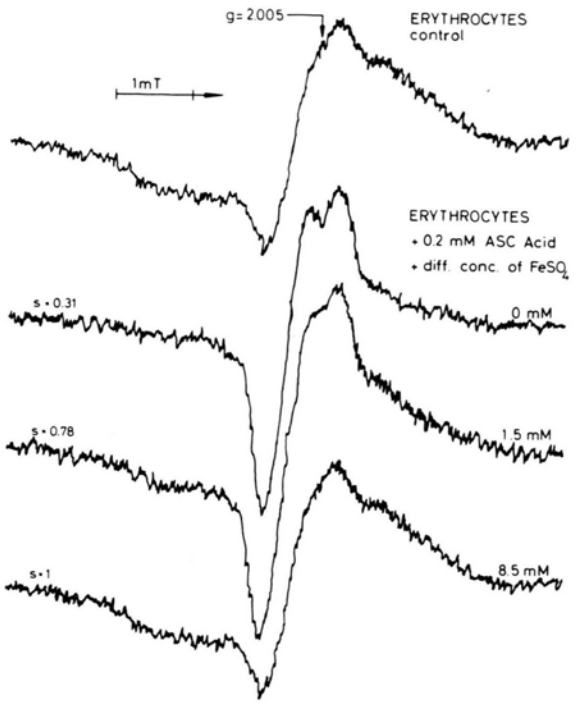

Fig. 1. The effect of different concentrations of $FeSO_4$ on the ESR spectrum of healthy erythrocytes treated with 0.2 mM of ascorbic acid. $s \triangleq$ sensitivity factor.

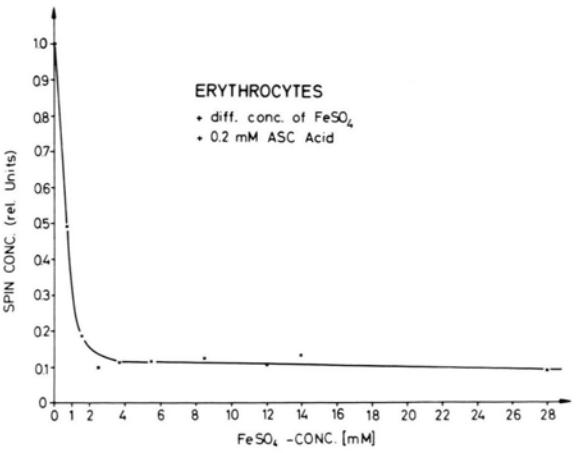

Fig. 2. The effect of different concentrations of $FeSO_4$ on the spin concentration of healthy erythrocytes treated with 0.2 mM of ascorbic acid. SD $\leqq$ 5%.

$g = 4.3$ [5]. As expected, Fe^{3+} can effect the SDA radical only if it has been reduced to Fe^{2+} first. This can happen by the SH compounds present in erythrocytes. Fe^{3+}, which is complexed, *e.g.* an Fe^{3+}-Na-gluconate complex or catalase, and can hardly be reduced, is without any effect even up to concentrations of about 100 mM. The most suitable complex seems to be Fe^{3+}-histidine which is almost as effective as Fe^{2+}. As has been shown polarographically, such complexes have a high solubility also in the alkaline pH region, a positive half wave potential, and do not form polymerisation products up to pH 9.5. The toxic effect exerted by iron alone is greatly diminished. Addition of small concentrations of histidine (10 mM) to an Fe^{3+}-ascorbic acid solution (1 mM:6 mM) results in a decrease of the Fe^{3+} concentration [6].

There is also the possibility that Fe^{3+}, whose half wave potential $E_{1/2}$ for its reduction is +0.77 V [7], can oxidize both ascorbic acid and SDA, whose $E_{1/2}$ are +0.33 V and −0.2 V resp. [8].

There might be also the possibility, that the added Fe^{2+} will be oxidized first by the oxygen dissolved in the blood. Fe^{3+}, produced in this way, like the addition of the Fe^{3+}-histidine complex will oxidize ascorbic acid to SDA which will decay to dehydro-ascorbic acid and ascorbic acid by a disproportionating reaction. At present, it cannot be distinguished between these two mechanisms.

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