Ascorbate Oxidase and Its Possible Involvement in Cancer

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The electron spin resonance (ESR) spectrum of erythrocytes of a healthy male volunteer exhibited 2 min after intravenous administration of 1 g of ascorbic acid a considerable increase in spin concentration and a new signal at about $g = 2.005$ which we previously had found to correlate to the semidehydroascorbate (SDA) radical and which is not identical with the O_2^- radical. Moreover, the vitamin C concentration in erythrocytes and plasma was considerably higher than in comparable samples of other volunteers treated identically. In the latter cases, the ESR spectrum of the erythrocytes was not modified at all. These findings suggest that there must be a substance which reacts with ascorbic acid specifically. It can be assumed that the enzyme ascorbate oxidase plays this decisive role in the ascorbic acid metabolism. For this reason, different amounts of ascorbate oxidase have been added to healthy erythrocytes treated *in vitro* with ascorbic acid and to tissue samples of lung cancer. As expected, the vitamin C effect as expressed by the appearance of the SDA signal and the increase in spin concentration could be reversed. It is suggested, therefore, that in special types of cancer, such as acute lymphatic leukemia and lung cancer, the concentration of ascorbate oxidase or of an enzyme acting like it is, primarily, diminished, while in other types it might be enlarged.

Introduction

Recently we succeeded in identifying the electron spin resonance (ESR) signal at about $g = 2.005$ which is present in lyophilized native blood and its constituents of patients with acute lymphatic leukemia [1–4]. We could demonstrate that it is due to the semidehydroascorbate (SDA) radical. In *in vivo* experiments, in which 1 g of ascorbic acid was administered intravenously to healthy volunteers, the ESR spectrum of native blood is identical to that obtained in the case of acute lymphatic leukemia [5]. While it prevails in untreated leukemic patients, it returns to normal within several hours to one day in these healthy volunteers. From these results and previous findings [2, 3] one might conclude that a substance which reacts with ascorbic acid specifically exists in healthy individuals regulating the interaction between vitamin C and blood constituents, but which is present in patients with acute lymphatic leukemia only in a diminished quantity.

There were some indications that ascorbate oxidase or an enzyme acting similarly might be the substance in question. Therefore, the effect of ascorbate oxidase on erythrocytes treated with ascorbic acid and on cancerous lung tissue has been investigated by means of ESR spectroscopy, in order to elucidate its molecular mechanism of interaction. Normal erythrocytes treated with ascorbic acid were used as a model since their ESR signal is identical to that obtained in the cases of acute lymphatic leukemia [2].

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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 [2]. Ascorbate oxidase from Cucurbita species (L-Ascorbate: oxygen oxidoreductase, EC 1.10.3.3; Boehringer, Mannheim, Germany) was added in different concentrations (up to 20000 U) to 0.2 ml of erythrocytes treated prior with either 0.5 or 1.0 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. A DPPH (diphenylpicrylhydrazil) standard (g = 2.0036) was used as a reference for marking resonance positions. 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.

In one case, 5 ml of a solution containing 1 g of ascorbic acid (Merck, Darmstadt, Germany) have been administered intravenously to a 26 year old healthy male volunteer. Shortly before and 2, 5, 15, 60 min, 4, 24, and 48 h after the administration, 0.5 ml of native blood and 10 ml of 1:10 ACD-blood were collected. The latter samples were used for separation of erythrocytes and plasma. All were subsequently lyophilized for the ESR studies. Two ml of EDTA-blood were obtained simultaneously for the hematologic examinations.

The concentration of ascorbic acid in erythrocytes and plasma was determined after derivatization with 2,4-dinitrophenylhydrazine according to the method described by Omaye *et al.* [6].

In order to test the effect of ascorbate oxidase on cancerous lung tissue, 5 g samples were soaked with 0.2 ml of an aqueous solution containing 10000 U of the enzyme immediately following surgical removal. Obviously, such an experiment will only provide qualitative data; it might be, however, indicative for the action of this enzyme.

Results

Recently we have shown that 1 g of ascorbic acid, administered intravenously to young healthy volunteers, didn't affect the ESR spectrum of erythrocytes while that of native blood was modified in shape and spin concentration as well [5]. Furthermore, ascorbic acid concentration was found to be unchanged in erythrocytes, while it showed an initial increase in plasma. This result could be confirmed in additional experiments with the exception of one case. Here, also the ESR spectrum of the erythrocytes exhibited an increase in spin concentration and the SDA signal located at about $g = 2.005$ two minutes after ascorbic acid administration (s. Fig. 1). Concomitantly, only immediately after injection a drastic increase in ascorbic acid concentration could be observed in erythrocytes and plasma (about 3.5 times of the increase observed in other volunteers after injection of 1 g of ascorbic acid). However, five and more minutes after administration of ascorbic acid all of the values were comparable to those obtained with the other volunteers [5]. From this, it might be concluded that in this individual, after the injection of ascorbic acid, there might be a shortlasting break-down (for a few minutes only) of the system regulating the ascorbic acid metabolism which results in an additional release of vitamin C from an unidentified pool. Immediately thereafter the complete system seems to function normally indicated by the same time response in spin- and vitamin C-concentration as observed in the other volunteers [5]. These findings suggest that there might be an enzyme which regulates the vitamin C metabolism and maintains its concentration at an optimum value. There are

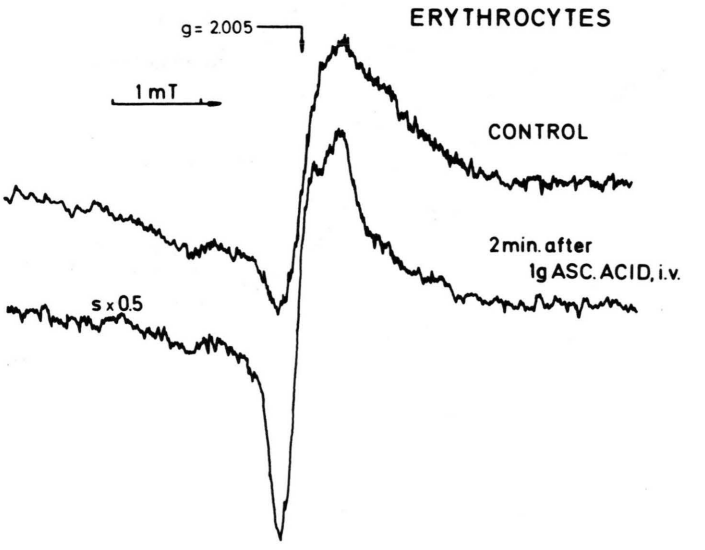
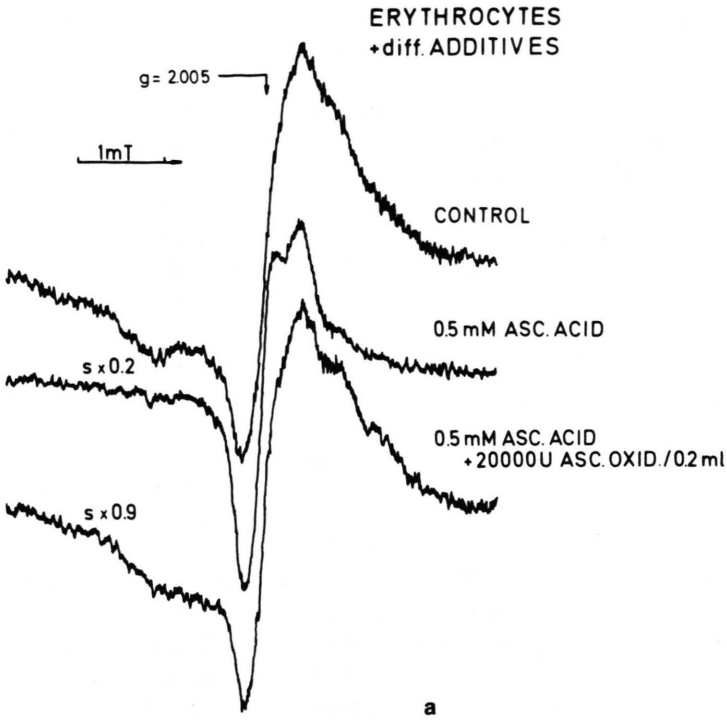


Fig. 1. ESR spectra of erythrocytes of controls and 2 min after intravenous injection of 1 g of ascorbic acid. $s \triangleq$ rel. sensitivity.



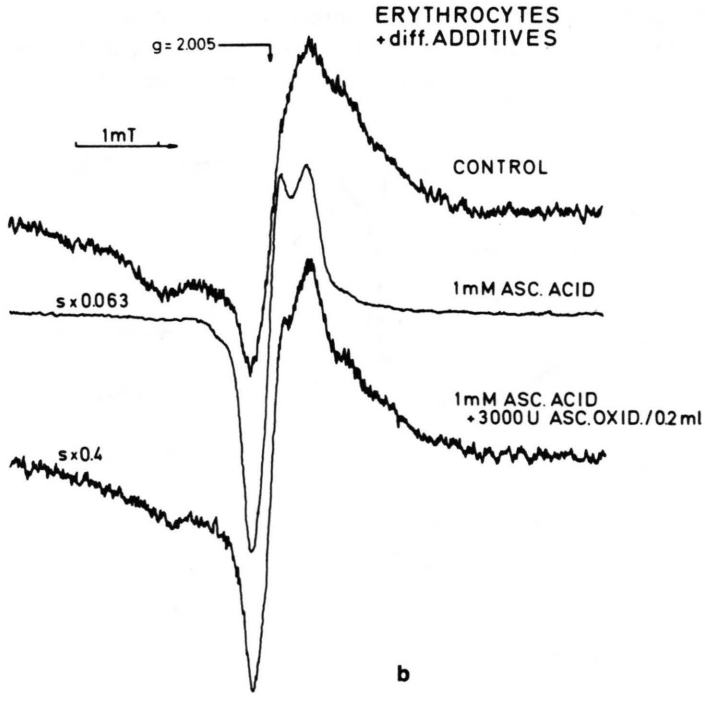


Fig. 2. Effect of ascorbate oxidase on the ESR spectra of erythrocytes treated with either 0.5 mM (Fig. 2a) or 1.0 mM of ascorbic acid (Fig. 2b). $s \triangleq$ rel. sensitivities.

certain indications that this enzyme, using ascorbic acid as a substrate, is ascorbate oxidase or an enzyme acting similarly.

In order to verify this hypothesis, different amounts of ascorbate oxidase have been added to 0.2 ml of healthy erythrocytes treated *in vitro* with either 0.5 mM or 1.0 mM of ascorbic acid (Figs. 2a and b). With increasing concentration of the enzyme the spin concentration as well as the SDA peak decrease, as seen in the middle spectra of both Figures. While an intermediate state can be seen in Fig. 2b, lower curve, a return to the original state can be observed after the addition of 20000 U of ascorbate oxidase to 0.2 ml of erythrocytes treated with 0.5 mM of ascorbic acid (Fig. 2a, compare upper and lower curve). Addition of this concentration of the enzyme to erythrocytes only has almost no effect at all on the spectrum.

In a preliminary study the effect of ascorbate oxidase on cancerous lung tissue has also been investigated. Immediately following surgical removal, 5 g of such a tissue have been soaked in 0.2 ml of an aqueous solution containing 10000 U of the enzyme. This qualitative study shows (Fig. 3) that this concentration of ascorbate oxidase diminishes the SDA peak considerably. As a matter of fact, this concentration is already too high comparing this spectrum with that of healthy tissue (see lower spectrum).

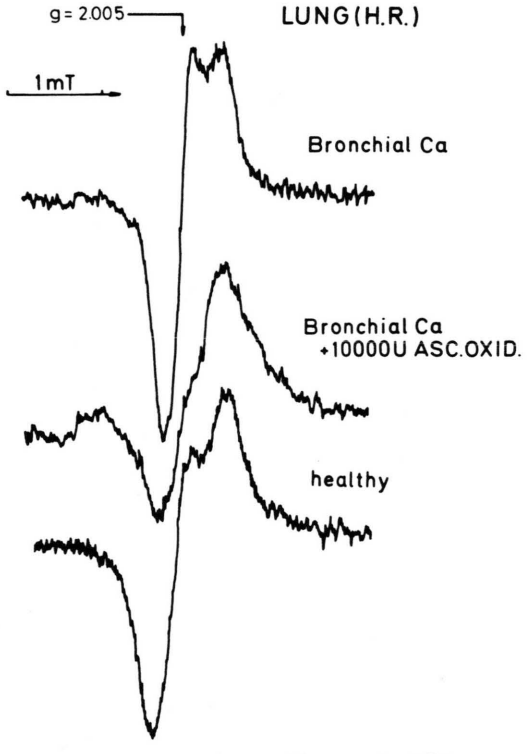


Fig. 3. Effect of ascorbate oxidase on the ESR spectrum of a lung sample affected with cancer. For comparison: lower spectrum represents the ESR response of a healthy lung sample.

Discussion

From the results obtained the following conclusions might be drawn: In a few types of cancer, *e.g.* acute lymphatic leukemia and lung tumor, the ESR spectra exhibit the semidehydroascorbate radical and a characteristic change in spin concentration. This effect can be also obtained if ascorbic acid is added to healthy tissue. Ascorbate oxidase reverses this effect and reestablishes ESR spectra as obtained with healthy tissue depending, of course, on the concentration used. Therefore, it seems very likely, that in these two types of cancer – and in all the ones, in which an increased ascorbic acid concentration can be measured – the concentration of ascorbate oxidase or of an enzyme which acts like it is, primarily, diminished. This results in a rather uncontrolled rise of the concentration of ascorbic acid and, thus, leads to an increased interaction with its receptors (probably Cu^{2+}-proteins). Such an interaction will result in the formation of the semidehydroascorbate radical which, in turn, will cause lipid peroxidation [7–9].

If ascorbate oxidase is a regulatory enzyme of the type discussed above, then it should also be responsible for the compensation of the effect produced in healthy volunteers after i.v. administration of ascorbic acid [5]. This suggests very strongly its existence in living systems. It has been detected in and extracted from plants; its existence in humans is still unknown. Within the blood system this enzyme ought to be located in the erythrocytes, since no SDA signal could be observed in such a sample of volunteers who had been injected intravenously with 1 g of vitamin C [5]. In the one case described above, these erythrocytes seem to have a reduced concentration of ascorbate oxidase or of a comparable enzyme.

If this mechanism is correct, then, there should be cases in which the opposite effect occurs. There should be some types of malignancies in which primarily the concentration of ascorbate oxidase or of a comparable enzyme is increased and, thus, the ascorbic acid concentration decreased [10]. In a few cases of acute myeloic leukemia studied thus far, the SDA signal could never be observed and the ascorbic acid concentration was too low compared with the controls.

Finally, it should be pointed out that the signal located at $g = 2.005$ is not identical with the O_2^- radical.

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