The Effect of Alcohol Oxidase on the Ascorbic Acid-Erythrocyte Interaction: ESR Investigations

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Addition of alcohol oxidase to erythrocytes treated with ascorbic acid reverses the vitamin C effect, as expressed by the appearance of the semidehydroascorbate signal, and the increase in spin concentration. The original erythrocyte electron spin resonance (ESR) signal is, however, never restored completely, as it is in the case if ascorbate oxidase is used. Additional interaction between the enzyme alcohol oxidase and haemoglobin iron must occur, since both of the iron ESR signals at $g = 4.3$ and $g = 6$ disappear and the erythrocyte ESR spectrum at $g \approx 2$ is changed characteristically. The spin concentration of the latter spectrum increases again above a certain concentration of alcohol oxidase.

Introduction

Recently we could show that 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 (ALL) [1–4], is due to the semidehydroascorbate (SDA) radical. From this we concluded that the ascorbic acid metabolism is disturbed, at least in some types of diseases. This might be caused by changes in the concentration of an enzyme which reacts specifically with ascorbic acid. Ascorbate oxidase, when added *e.g.* to erythrocytes of patients with ALL, produced a disappearance of the SDA signal with a concomitant reduction in spin concentration [5].

In additional experiments the molecular mechanism of interaction could be determined [6]. Its effect seems to be due to an oxidation of ascorbic acid to its dehydro form in the presence of oxygen. Whether or not this mechanism is unique, is an unresolved question. Other oxidases, *e.g.* alcohol oxidase, will generate labile radicals in the presence of their substrates. The presence of ascorbic acid will, however, reduce subsequent chemical events due to these radical intermediates. For this reason, the effect of alcohol oxidase on the SDA radical has been investigated by means of ESR spectroscopy. Healthy erythrocytes treated with ascorbic acid were used as a model since their ESR spectrum exhibits the SDA radical, which is identical to that obtained in the cases of ALL [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]. Alcohol oxidase from yeast (alcohol oxidase: oxigen oxido-reductase, E.C.1.1.3.13; Phillipps Chemical Company, Bartlesville, USA) was added in different concentrations (up to 280 IU) to 0.2 ml of 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.

Results and Discussion

Part of the ESR spectrum of healthy erythrocytes is shown in Fig. 1, upper curve. The different signals could not be assigned yet. Obviously, part of it must belong to a high spin ferric ion which exhibits signals between $g = 2$ to 6 depending on the ligands [7]. The two signals located at $g = 4.3$ and $g = 6$ are not shown in this figure. When ascorbic acid is added to these erythrocytes, the new signal at $g = 2.005$ is produced which we could assign to the ascorbyl radical. In addition, the spin concentration is increased.

Addition of alcohol oxidase to an erythrocyte suspension treated with 0.2 mM of ascorbic acid results in a decrease in spin concentration and disappearance of the ascorbyl radical as well. The original erythrocyte control ESR spectrum can never

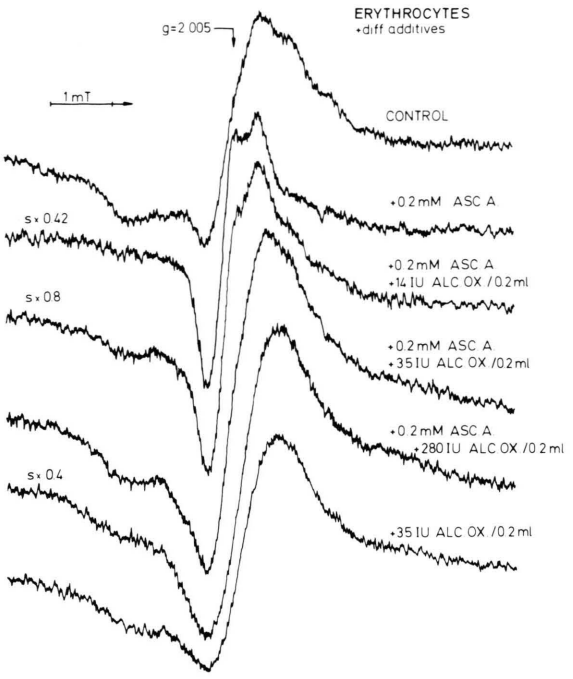

Fig. 1. The effect of alcohol oxidase on the ESR spectrum of healthy erythrocytes treated with or without 0.2 mM of ascorbic acid. $s \triangleq$ sensitivity factor.

be obtained again since additional changes at the erythrocytes, *e.g.* at those which are responsible for the ESR spectrum, occur. At concentrations of alcohol oxidase larger than 35 IU, the signal which appears as a shoulder in the control spectrum is much more pronounced and the spin concentration is increased again.

The effect produced by the enzyme is enlarged if the erythrocytes are not treated with ascorbic acid first (s. bottom curve, Fig. 1). This effect cannot be explained yet. Apparently, the enzyme can penetrate the membrane and will interact, then, with haemoglobin. This assumption is supported by the fact that already at small enzyme concentrations (> 10 IU/0.2 ml) both of the iron signals at $g = 4.3$ and $g = 6$ disappear. This never happened when ascorbate oxidase was added to erythrocytes treated with or without ascorbic acid. Thus, iron seems to be involved in the action mechanism of alcohol oxidase.

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