

On the Possible Involvement of Ascorbic Acid and Copper Proteins in Leukemia.

III. ESR Investigations on the Interaction between Ascorbic Acid and Some Transition Metal Ions

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The interaction between lyophilized samples of ascorbic acid and Cu^{2+} , Fe^{3+} or Mn^{2+} has been investigated by means of ESR spectroscopy. All of the three transition metal ions form complexes with vitamin C, but only in the case of Cu^{2+} and Fe^{3+} the interaction results in a reduction of the metal ions. Cu^{2+} and ascorbic acid seem to form 2 : 1 complexes with an equilibrium constant of about $K = 1 \times 10^7 \text{ mol}^{-1}$. None of these metal ion complexes exhibits, however, the ESR spectrum obtained with leukemic blood.

Introduction

In a preceding paper we have shown that the addition of ascorbic acid to white ghosts of erythrocytes results in an electron spin resonance (ESR) spectrum similar to the one obtained with leukemic blood [1]. This suggests that the receptor(s) for ascorbic acid must be a constituent of the membrane. Since, however, a similar spectrum was also obtained with plasma, the receptor(s) for ascorbic acid has to be searched for in membrane and plasma as well. It seems to be obvious, that metal ions or metallo-proteins could function as receptors. Atomic absorption studies have shown, that Cu and Fe are present in erythrocytes, their white ghosts, and plasma [1]. Thus, the "leukemic" ESR signal might be caused by an interaction between ascorbic acid and metal ions or the corresponding metallo-proteins. In order to elucidate this mechanism of interaction the effect of some transition metal ions (Cu^{2+} , Mn^{2+} , Fe^{3+}) on ascorbic acid has been investigated by means of electron spin resonance (ESR) spectroscopy.

Materials and Methods

MnCl_2 , CuCl_2 , FeCl_3 , and ascorbic acid were obtained from Merck, Darmstadt. The substances were dissolved in bidistilled water and freeze-dried in or-

der to get identical conditions as used for the investigation of the biological material.

The ESR spectra of these lyophilized samples were obtained as described in a preceding paper [2]. The modulation amplitude varied between 0.1 – 5 G (0.01 – 0.5 mT) and the microwave power was 5 mW for all samples investigated. The ratios $\text{MeCl}_x/\text{ascorbic acid (wt/wt)}$ are marked at the right hand side of each spectrum. For the evaluation of the spin concentration, the metal ion concentration was kept constant. The relative spin concentration was obtained by double integration of the spectra by means of a planimeter.

Results and Discussion

The effect of increasing concentration of ascorbic acid on MnCl_2 is shown in Fig. 1. As can be seen, a lyophilized MnCl_2 sample exhibits only a rather broad singlet centered at about the g -value of the free electron. Vitamin C does not affect the shape of the Mn^{2+} ESR spectrum, if ratios $< 1 : 10$ for $\text{MnCl}_2/\text{ascorbic acid (wt/wt)}$ are used. At higher concentrations of ascorbic acid, the hf-structure of Mn^{2+} appears indicating a change in the relaxation time of the metal ion. The splitting parameter is about 96 G which agrees with the values reported previously [3]. The spin concentration of Mn^{2+} remains constant over the whole concentration range of ascorbic acid used, which means that there is no reduction of Mn^{2+} to Mn^+ .

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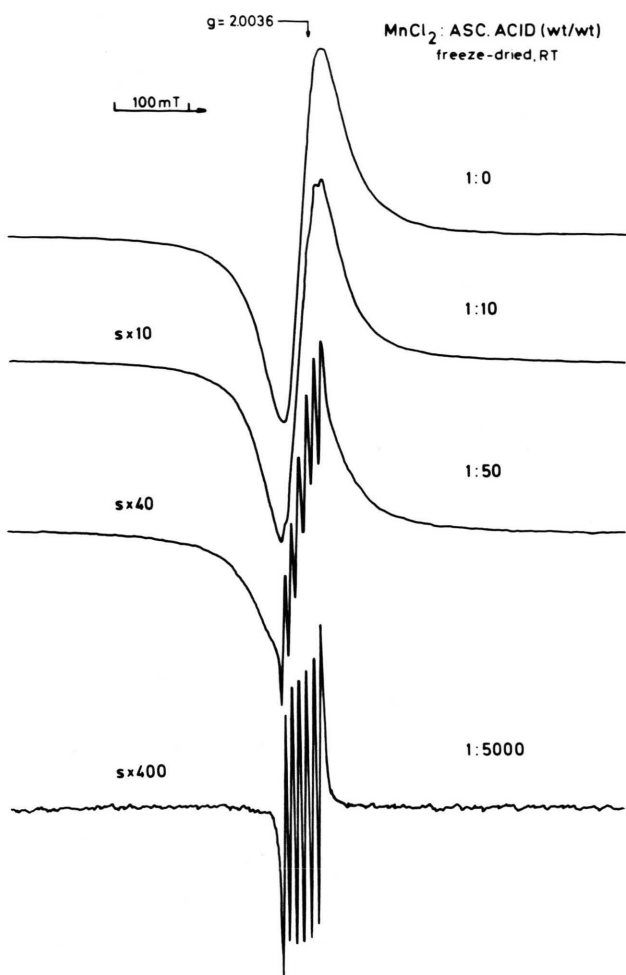


Fig. 1. The effect of different concentrations of ascorbic acid on MnCl_2 . The ratio $\text{MnCl}_2/\text{ascorbic acid}$ (wt/wt) is marked at the right hand side of each spectrum ($s \triangleq$ rel. sensitivities).

The effect of ascorbic acid on FeCl_3 is shown in Fig. 2. The lyophilized FeCl_3 sample exhibits a singlet superimposed on a rather broad singlet. With increasing concentration of ascorbic acid several intermediate complexes must be formed as can be concluded from the ESR spectra. They could not be identified yet. At the highest concentration used (1: 200 = $\text{FeCl}_3/\text{ascorbic acid}$, wt/wt), a Mn^{2+} spectrum can be clearly seen. The splitting parameter is about 96 G. Mn^{2+} is obviously an impurity in the FeCl_3 sample. The spin concentration of the FeCl_3 sample decreases considerably with increasing con-

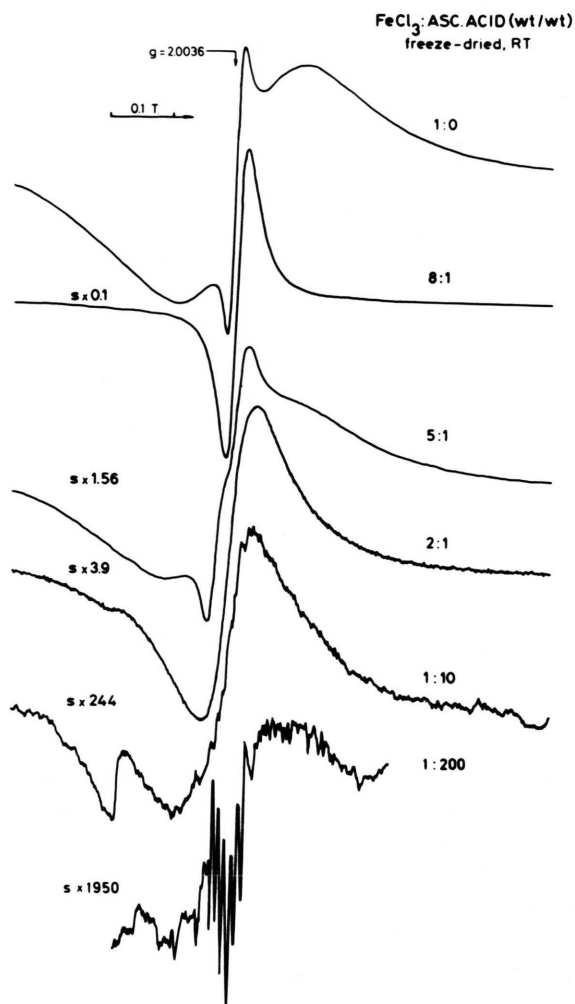


Fig. 2. The effect of different concentrations of ascorbic acid on FeCl_3 . The ratio $\text{FeCl}_3/\text{ascorbic acid}$ (wt/wt) is marked at the right hand side of each spectrum ($s \triangleq$ rel. sensitivities).

centration of ascorbic acid, as can be seen by the change in sensitivity. Since all Fe containing samples were, however, rather sticky after lyophilization, their weight could not be determined and, therefore, a quantitative evaluation was not possible.

In the case of Cu^{2+} , a singlet (s. Fig. 3, upper spectrum) seems to be superimposed on the Cu^{2+} ESR spectrum. The Cu^{2+} hf-structure is indicated only when ascorbic acid is added to CuCl_2 (s. e. g. 1.9: 1 spectrum). Already small amounts of ascorbic acid change the shape as well as the spin concentration of the Cu^{2+} ESR spectrum considerably. At a molar

Fig. 3. The effect of different concentrations of ascorbic acid on CuCl_2 . The ratio CuCl_2 /ascorbic acid (wt/wt) is marked at the right hand side of each spectrum.

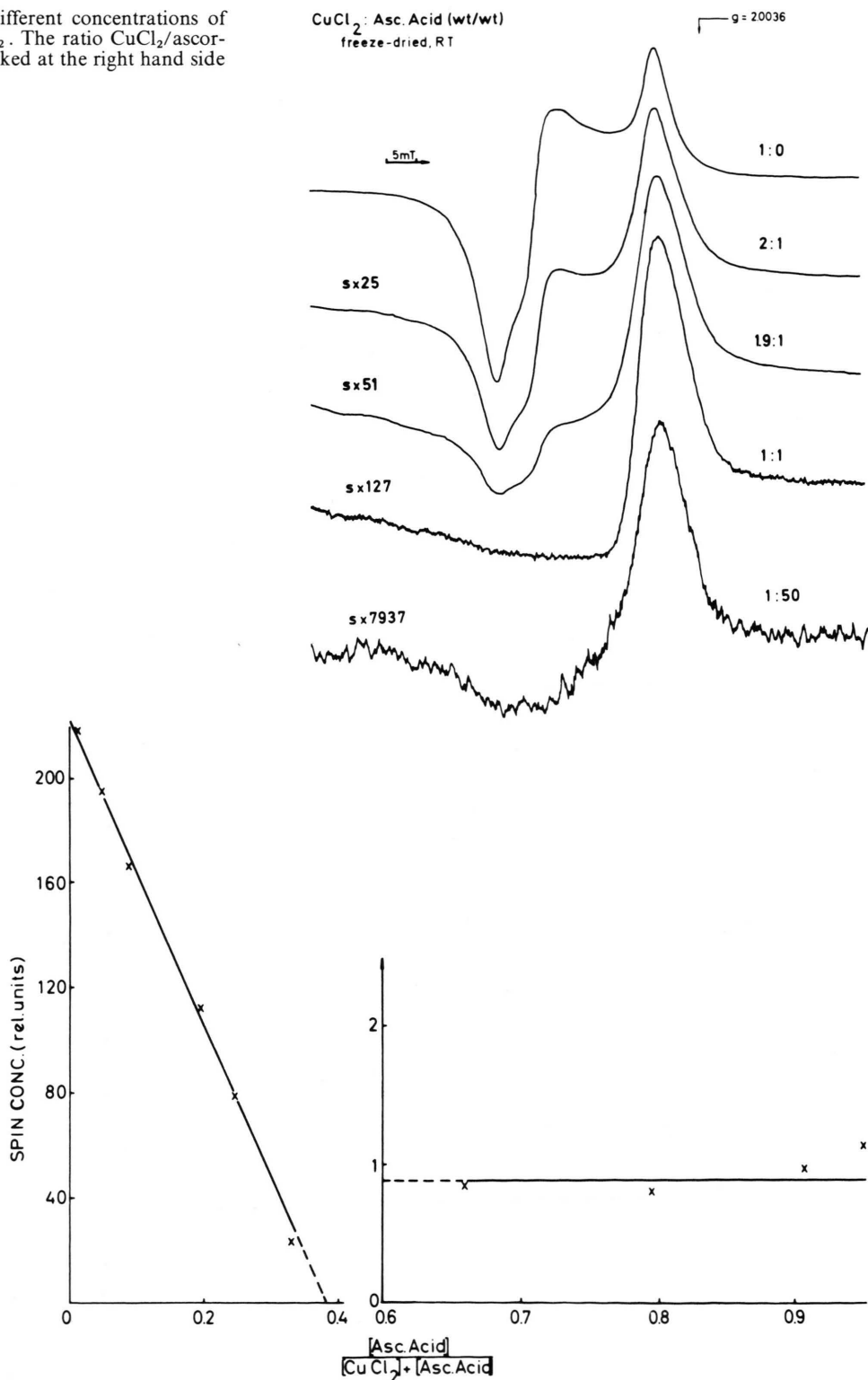


Fig. 4. The effect of different concentrations of ascorbic acid (expressed as molar fraction) on the Cu^{2+} spin concentration.

fraction of about 0.35, the spin concentration is about zero indicating that 2 Cu^{2+} ions might be bound to one ascorbic acid molecule (s. Fig. 4). From the plot obtained it might be concluded that Cu^{2+} is reduced to Cu^+ . The right hand graph showing the change in spin concentration at higher concentrations of ascorbic acid indicates that a minute spin concentration remains. This one seems to be independent of the vitamin C concentration. The equilibrium constant based on this remaining Cu^{2+} content of 0.45% is about $K = 1 \times 10^7 \text{ mol}^{-1}$. It is assumed that this content is not due to Cu^{2+} which has been reoxidized by dissolved oxygen. At molar fractions between 0.4 and 0.6 the samples are sticky and could, therefore, not be evaluated in regard to spin concentration.

Since none of the spectra obtained for the interaction between Fe^{3+} , Mn^{2+} , or Cu^{2+} with ascorbic acid

resembles the one obtained with leukemic blood or with blood fractions of healthy persons treated with ascorbic acid, one can conclude that none of these transition metal ions acts, per se, as receptor for vitamin C. Because of the strong interaction between ascorbic acid and Cu^{2+} it seems that this metal ion might be involved in this interaction, perhaps in the form of a copper-containing protein. For this reason, the mechanism of interaction between ascorbic acid and some copper-proteins has been investigated. Details of these experiments will be described elsewhere [4].

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