On a Possible Mechanism of Action of Interferon

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The effect of interferon on the ESR spectra of erythrocytes treated with ascorbic acid has been investigated. This model system has been chosen since it represents identically the spectra obtained in cases with acute lymphatic leukemia. The data obtained show that small interferon concentrations increase, while larger concentrations decrease the effect produced by ascorbic acid resulting, finally, in the original erythrocyte ESR spectrum. Atomic absorption studies reveal the presence of copper which might be part of the active principle.

Introduction

Increasing evidence suggests that interferon not only confers protection against virus but also inhibits cellular growth. Not much is known, however, about its molecular mechanism of action. Therefore, we have studied its effect on the electron spin resonance (ESR) signal located at about $g = 2.005$ in biological samples which is indicative for *e.g.* acute lymphatic leukemia [1] and lung tumor [2]. We could demonstrate recently that it is due to the semidehydroascorbate (SDA) radical and that the change in spin concentration is caused by an increased concentration of ascorbic acid [3–5]. From these findings it was concluded that in certain types of cancer the metabolism of ascorbic acid is, at least, disturbed which seems to be due to the diminished quantity of ascorbate oxidase, an enzyme which reacts specifically with vitamin C. Addition of ascorbate oxidase to healthy erythrocytes treated with ascorbic acid or to tissue samples with lung tumor reestablished in both cases such an ESR spectrum as has been obtained with healthy samples [6]. 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 [3]. In this report, the effect of interferon on such a model system has been investigated.

Materials and Methods

Human fibroblast (diploid) interferons of two specific activities were used: one with a specific activity of 1×10^5 U/mg protein, marked IF, and one, a highly purified sample, with a specific activity of about 1×10^6 U/mg protein, marked IF_{pur}. The interferon was added in different amounts to 0.2 ml of erythrocytes treated with 0.5 mM of ascorbic acid.

Erythrocytes were obtained from fresh 1 : 10 ACD-blood (acid-citrate-dextrose anticoagulant solution) of healthy volunteers and were prepared according to a method described previously [3]. The samples were lyophilized and, thereafter, their ESR spectra determined.

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 0.2 mT and the microwave power 5 mW for all samples investigated. The spectra were recorded at different sensitivities marked at the left-hand side of each spectrum. All measurements were done at room temperature. The relative spin concentration was obtained by double integration of the spectra by means of a planimeter.

The Cu concentration was determined by a Zeiss atomic absorption spectrometer model FMD 3 using a graphite furnace HGA 72 accessory.

Results and Discussion

As can be seen in Fig. 1, addition of interferon to healthy erythrocytes treated with ascorbic acid results in a gradual change in spin concentration and of the SDA signal depending on the concentration used. An interesting effect can be noticed: at small concentrations of IF or IF_{pur}, the spin concentration

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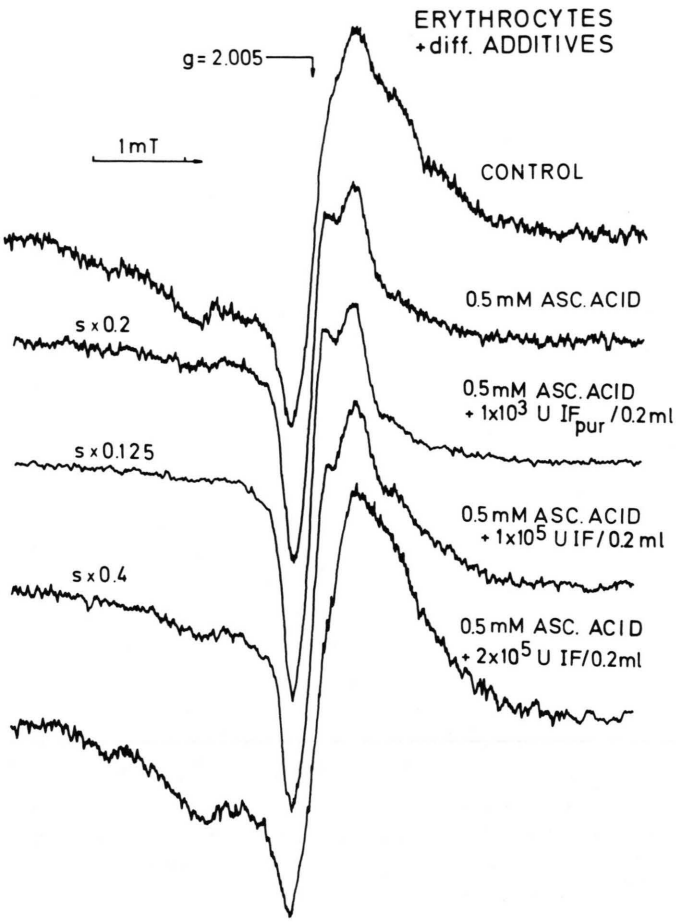

Fig. 1. Effect of interferon on the ESR spectra of erythrocytes treated with 0.5 mM of ascorbic acid.

increases and the SDA peak is more pronounced (s. 3rd spectrum from top). At larger concentrations ($> 4 \times 10^4$ U IF/0.2 ml; $> 5 \times 10^3$ U IF_{pur}/0.2 ml) a gradual decrease in spin concentration and disappearance of the SDA signal, proportional to the concentration used, can be observed. Finally, the ESR spectrum of healthy erythrocytes is restored (compare upper and lower spectrum). It should be pointed out that IF added to erythrocytes only has almost no effect at all on the erythrocyte spectrum.

Since similar results – except the initial increase mentioned above – were obtained with ascorbate oxidase [6], it is temptatious to assume that interferon acts either like ascorbate oxidase or its active principle is ascorbate oxidase. Therefore, IF should also contain copper.

Atomic absorption studies revealed a concentration of copper of about 18 ppm in IF and about 174 ppm in IF_{pur}. The ratio of both, 9.7, agrees very well with the ratio of the specific activities of the two interferons used. Thus, copper might, indeed, be a part of the active principle of interferon just like in the case of ascorbate oxidase.

A preliminary determination of the enzymatic activity of IF according to the method described for ascorbate oxidase by Boehringer, Mannheim, Germany, failed to give any positive result.

The initial increase in spin concentration and the stronger pronounciation of the SDA peak of the erythrocyte ESR spectrum obtained at small IF concentrations cannot be explained yet. Further experiments have to be conducted in order to elucidate this phenomenon. For therapeutic purposes, this observation, however, seems to be important for the selection of the appropriate concentration of IF in order to obtain an optimal success. The results obtained suggest that interferon might act very similarly to ascorbate oxidase. Its effect, as deter-

mined by ESR spectroscopy, has to be investigated in some more detail on tumor samples both *in vitro* and *in vivo*.

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