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Brief Communication

THE CATALYTIC ACTIVITY OF IRON IN SYNOVIAL FLUID AS MONITORED BY THE ASCORBATE FREE RADICAL

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Abstract—Human synovial fluid, from a patient with synovitis disease, was examined by electron spin resonance spectroscopy for evidence of free radicals. The ascorbate free radical was observed and its intensity was affected by iron chelating agents, demonstrating that the iron in the synovial fluid is indeed available for oxidative catalysis.

Keywords—Ascorbate, Iron, Synovial fluid, Free radicals, Electron spin resonance

INTRODUCTION

Synovial fluid from patients with synovitis disease has been found to have elevated levels of iron compared to controls.[1–6] This iron is thought to be a key element in the catalytic production of hydroxyl radical from superoxide and hydrogen peroxide, which can cause membrane damage, hyaluronic acid depolymerization, and other oxidations associated with this inflammatory disease.

The levels and redox state of ascorbate are also consistent with increased oxidative stress in synovitis disease. For example, total ascorbate in the sera of rheumatoid arthritis patients is approximately 39% of that found in sera of controls.[7] In control patients approximately 80% of the ascorbate is in the fully reduced form. However, in rheumatoid arthritis patients, approximately 80% of the total ascorbate in sera and synovial fluid is in the oxidized form, dehydroascorbic,[7,8] consistent with increased oxidative stress in synovitis disease.

It is well known that transition metals, such as iron and copper, catalyze the oxidation of ascorbate. We have recently demonstrated that the true autoxidation of ascorbate, that is, in the absence of catalytic metals, is a very slow process ($k < 6 \times 10^{-7}$ s^{-1} in air-saturated phosphate buffer pH 7).[9] The rate of ascorbate oxidation is a function of the concentration and nature of the catalytic metals present.[9,10] The concentration of ascorbate free radical ($A^{\cdot-}$), an intermediate in the oxidation of ascorbate, is a function of the rate of oxidation, that is, the catalytic metal concentration.[‖] Thus, the ascorbate radical concentration can be used as a probe for the presence of catalytic metals in solution. We report here that the iron in synovitis synovial fluid is available for oxidative catalysis as monitored by the ascorbate radical concentration.

MATERIALS AND METHODS

Desferal® was from CIBA Pharmaceutical Co. (Summit, NJ). All other reagents were from Sigma Chemical Co. (St. Louis, MO) and were used as received. Synovial fluid was aspirated from a knee joint of a patient with pigmented villonodular synovitis. Samples were immediately put on ice and then centrifuged to separate blood components after which they were frozen until analysis. Samples containing hemoglobin from red cell rupture were not used. ESR spectra were collected using a Bruker ER200-D spectrometer equipped with a TM cavity and an aqueous flat cell at room temperature.

RESULTS AND DISCUSSION

The electron spin resonance spectra of the air-saturated synovial fluid showed the characteristic doub-

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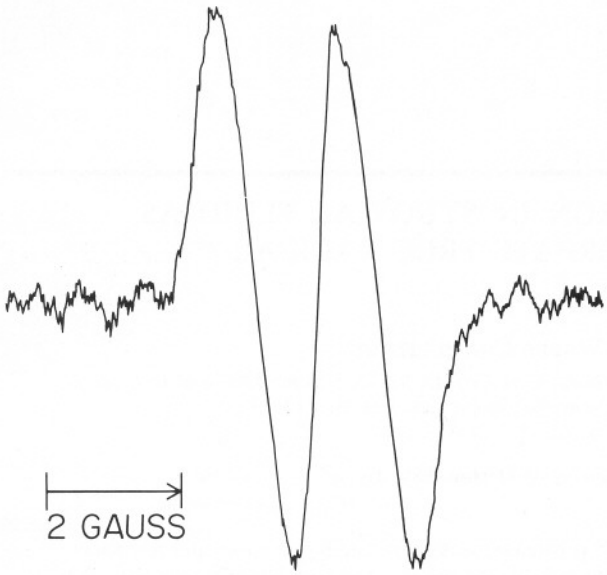

Fig. 1. Electron spin resonance spectrum of $A^{\cdot}$ (a = 1.76 G) observed in human synovitis synovial fluid. Instrument settings were: modulation amplitude, 1.0 G; time constant, 2.5 s; scan 2.4 G/min; receiver gain, 2×10^6; power, 10 mW. This modulation amplitude does not allow for the resolution of the additional hyperfine splittings of $A^{\cdot}$.

let of the ascorbate free radical, $A^{\cdot}$ (Fig. 1). When the metal chelating agents DETAPAC (500 μM; diethylenetriaminepentaacetic acid) or Desferal® (500 μM) were added to the synovial fluid, the concentration of $A^{\cdot}$ was decreased by about 50%, Table 1. This is consistent with catalytic copper and/or iron being present in the fluid because both DETAPAC and Desferal® drastically reduce the catalytic activity of these metals in the air oxidation of ascorbate.[10] However, addition of EDTA (500 μM) increased the signal intensity by 10%.

The addition of 10 μM Fe(III) to the synovial fluid increased the $A^{\cdot}$ concentration by 100%; whereas, addition of 10 μM Fe(III)-EDTA increased the $A^{\cdot}$ concentration approximately 300%. In general, EDTA stimulates the catalytic activity of iron and drastically reduces that of copper in ascorbate air oxidation.[10] Thus, we conclude that the metal-catalysis of ascorbate oxidation in these samples is due primarily to iron, and that the catalytic efficiency of this iron is similar to that of Fe(III)-EDTA.

We believe that this is the first direct demonstration that the iron in synovitis synovial fluid is available for oxidative catalysis. This catalytic iron can easily be responsible for the high proportion of oxidized ascorbate observed in synovitis synovial fluid. Because of the parallels between the iron-driven Haber–Weiss reaction and the involvement of iron in ascorbate oxidation,[10] this iron may also be available for catalysis of oxygen radical-induced oxidations. In addition we believe this approach offers a new method to study catalytic metals in biochemical and biological fluids.

Table 1. $A^{\cdot}$ ESR signal intensity in synovitis synovial fluid (SSF)

Sample	$A^{\cdot}$ Signal Height (Arbitrary Units)
SSF	100
SSF + 500 μM DETAPAC	53
SSF + 500 μM Desferal	52
SSF + 500 μM EDTA	110
SSF + 10 μM Fe(III)	235
SSF + 10 μM Fe(III)-EDTA	306

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