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THE SYRINGE NITROXIDE FREE RADICAL – PART II

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Some plastic syringes introduce a nitroxide free radical into the fluids they contain. The EPR spectrum shows a triplet with $a^N = 17.0 \pm 0.1$ G in plasma. We report here that this nitroxide/hydroxylamine couple serves as an antioxidant system, whose redox potential appears to be between that of vitamin C and vitamin E. This adventitious antioxidant can alter the course of free radical processes.

KEY WORDS: Free radical, Nitroxide radical, Antioxidant, Electron paramagnetic resonance, EPR.

INTRODUCTION

We have previously found that some disposable plastic syringes can contaminate solutions with a nitroxide free radical ($a^N = 16.9$ G in water, $a^N = 13.9$ G in chloroform)[1]. In that work we cautioned researchers that the presence of this species might alter free radical reactions because the nitroxide radical and its associated hydroxylamine may serve as additional antioxidants. We report here that this is indeed the case.

MATERIALS AND METHODS

Heparinized blood was collected with a sterile 30 cc syringe (Becton Dickinson & Co, Rutherford, NJ). Plasma was separated and kept on ice until use. This plasma was subjected to continuous oxidative stress by adding hypoxanthine (0.5 mM) and then xanthine oxidase (10 mU/ml, Sigma Chemical Co., Grade IV). The rate of superoxide production with this level of hypoxanthine and xanthine oxidase was determined to be ≈ 70 nM/s in phosphate buffer using cyctochrome *c* (Sigma Chemical Co., Type III) and UV-visible spectroscopy.[2,3]

Using an infusion pump, the plasma being stressed was continuously recirculated through an EPR aqueous flat cell, thereby allowing continuous aeration. Repeated EPR scans for ascorbate and/or nitroxide radical were obtained using a Bruker ESP 300 EPR spectrometer and a TM_{110} cavity. Ascorbate and nitroxide free radical concentrations were determined using the method of Buettner;[4] 3-carboxyproxyl was the standard. Because the nominal microwave power used (40 mW) produced saturation effects in both the ascorbate and nitroxide radicals, appropriate corrections were made.[5]

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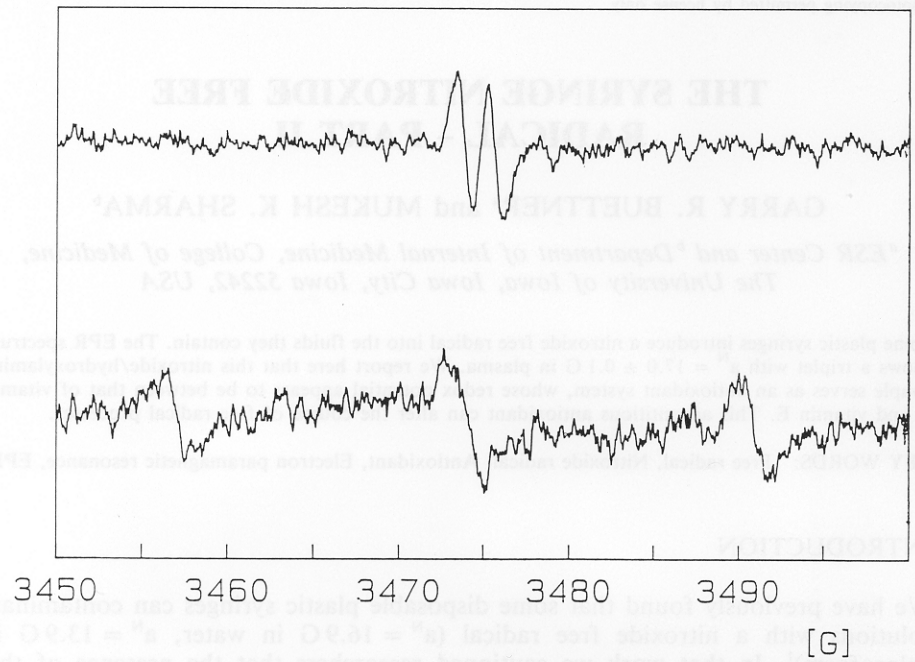

FIGURE 1 EPR spectra: *Top:* Ascorbate free radical (a^H = 1.8 G) observed initially when plasma was subjected to oxidative stress. *Bottom:* The "syringe nitroxide" free radical (a^N = 17.0 ± 0.1 G) observed after depletion of ascorbate and the disappearance of $Asc\cdot^-$ following prolonged oxidative stress. EPR instrument settings: modulation amplitude, 0.9 G; time constant, 0.67 s; receiver gain, 2×10^6; power, 40 mW; scan, 40 gauss/167 s.

RESULTS

Only a low level of ascorbate free radical ($Asc\cdot^-$, Figure 1) was present in the plasma before the initiation of the oxidative stress (Figure 2 at time 0). Upon introduction of hypoxanthine/xanthine oxidase the intensity of the $Asc\cdot^-$ EPR signal rapidly increased followed by its decay and eventual disappearance (Figure 2). This observation is consistent with a role for ascorbate ($AscH^-$) as an antioxidant.[6,7] The hypoxanthine/xanthine oxidase system produces superoxide and hydrogen peroxide that initiate free radical processes in the plasma. Ascorbate "repairs" these radicals by donating an electron, thereby producing the resonance-stabilized ascorbate free radical, which we then observe by EPR:

$$R\cdot + AscH^- \longrightarrow Asc\cdot^- + RH$$

With a continuous source of initiating free radicals, as supplied by the hypoxanthine/xanthine oxidase system, the ascorbate radical concentration in the plasma will decrease with time, because ascorbate is being consumed by the on-going free radical oxidation reactions.

When ascorbate was nearly depleted, a three line (1:1:1) nitroxide free radical (a^N = 17.0 ± 0.1 G) appeared (Figure 1). Because the blood had been collected using a syringe from a product line that had previously been shown to impart nitroxide

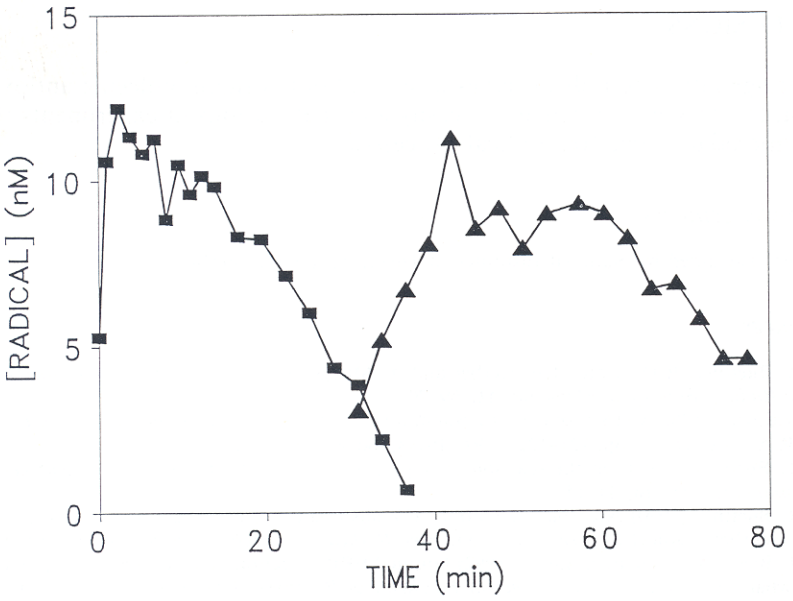

FIGURE 2 Time course for the change in the ascorbate free radical (■) and in the nitroxide free radical (▲) concentrations in the plasma being oxidatively stressed with hypoxanthine/xanthine oxidase.

radicals to the solutions they contained, and because plasma that was obtained without the use of this type of syringe did not show the presence of this nitroxide radical, we attribute the source of this free radical to the syringe.

Nitroxides are easily reduced by ascorbate to their corresponding EPR-silent hydroxylamines,[8]

$$R_2NO\cdot + AscH^- \longrightarrow R_2NOH + Asc\cdot^-$$

Therefore, we reasoned that prior to the introduction of the oxidative stress in the plasma, all of the syringe nitroxide radical had been reduced to its hydroxylamine, and therefore was not initially detectable by EPR. However, after the depletion of ascorbate by the ongoing oxidative stress the hydroxylamine became the next line of antioxidant defense.

$$R\cdot + R_2NOH \longrightarrow RH + R_2NO\cdot$$

This observation is consistent with the thermodynamics of nitroxides:

$$E^{1/2}(R_2NO\cdot, H^+/R_2NOH) \approx +100 \text{ to } -400\,mV^{7,9}$$

Thus, as long as ascorbate is present the nitroxide/hydroxylamine couple will be predominately in the reduced form. Upon depletion of ascorbate, R_2NOH will readily serve as an antioxidant. In fact, its potential of only ≈ 100 to -400 mV will result in it serving as the next line of defense among the various small molecule antioxidants present, such as vitamin E.[7] Indeed, when this nitroxide/hydroxylamine couple is not present in plasma, the chromanoxyl radical of vitamin E is observed after the depletion of ascorbate and the associated disappearance of the ascorbate free radical.[10]

CONCLUSIONS

The syringe nitroxide radical serves as an additional small molecule antioxidant in plasma, the presence of which can easily alter conclusions in experiments that deal with antioxidants and free radical processes.

Acknowledgements

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