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Environmentally Persistent Free Radicals (EPFRs) – 2. Are Free Hydroxyl Radicals Generated in Aqueous Solutions?

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Dependence of DMPO-OH adduct generation on biological reducing equivalents. The generation of hydroxyl radical in biological systems is usually enhanced by the presence of H-donors, e.g. NADPH and ascorbates etc. [1, 2]. The effectiveness of NADPH and ascorbic acid on generation of DMPO-OH adducts in sample solutions is presented in **Figure S1**. Addition of 200 µM NADPH resulted in a ~ 20% increase in DMPO-OH adduct generation (**Figure S1A**), while addition of 100 µM of ascorbic acid increased the DMPO-OH concentration ~ 100 % (**Figure S1B**). Above these concentrations, the DMPO-OH adduct concentration decreased, and, in the case of ascorbic acid, a characteristic doublet line of ascorbyl radicals appeared (cf. **Figure S2A**). The observed α_H = 1.81 G and g = 2.0057 match very well with literature EPR data of ascorbyl radicals generated by oxidation of ascorbic acid by hydroxyl radicals[3]. DMPO-

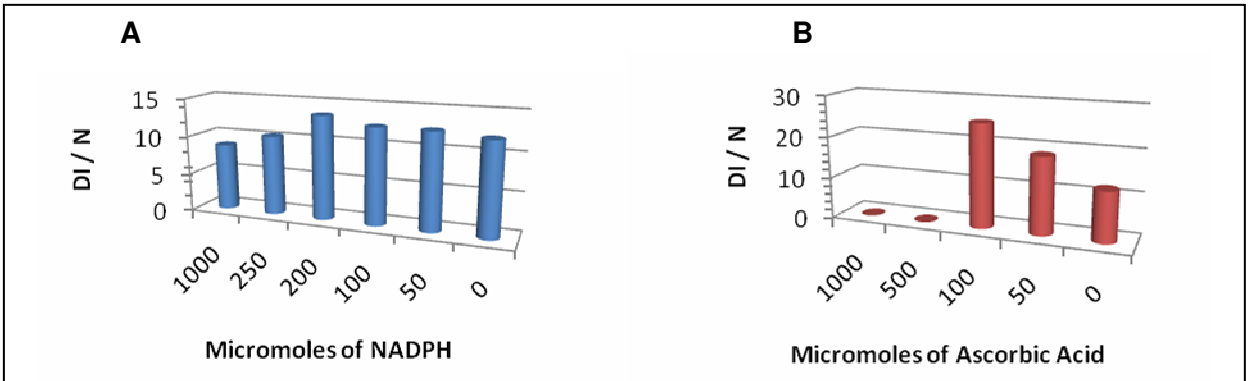

Figure S1. The dependence of EPR signal intensity of DMPO-OH adducts on concentration of NADPH (**A**) and ascorbic acid (**B**) in water solution of EPFRs (50ug/ml) + DMPO (150 mM) + PBS for incubation time of 50 minutes.

OH adducts may also convert ascorbic acid into ascorbyl radical [4].

It is interesting that ascorbyl radicals are suddenly generated in a > 500µM solution of ascorbic acid. Ascorbyl radical concentrations were measured in EPFR and non-EPFR solutions containing 1 mM Ascorbic acid (cf. **Figure S2A**), and ascorbyl radicals were immediately observed in both samples. The ascorbyl signal decayed completely during first 120 minutes of incubation in both EPFR (black line) and non-EPFRs(red line) solutions (**Figure S2 A**). DMPO-OH adducts were generated after 120 minutes of incubation time (cf. **Figure S2B**). However, the intensity of DMPO-OH adduct signal is again higher in EPFRs (black line) than non-EPFR (red line) solutions. The presence of ascorbyl radicals in the non-EPFRs solution has been explained as formation of silica – ascorbate complexes, which are oxidized by active surface centers of silica or iron impurities [5]. This explanation is very close to our theory of EPFR formation, and Cu(II) in our system probably oxidizes the ascorbate to ascorbyl radical.

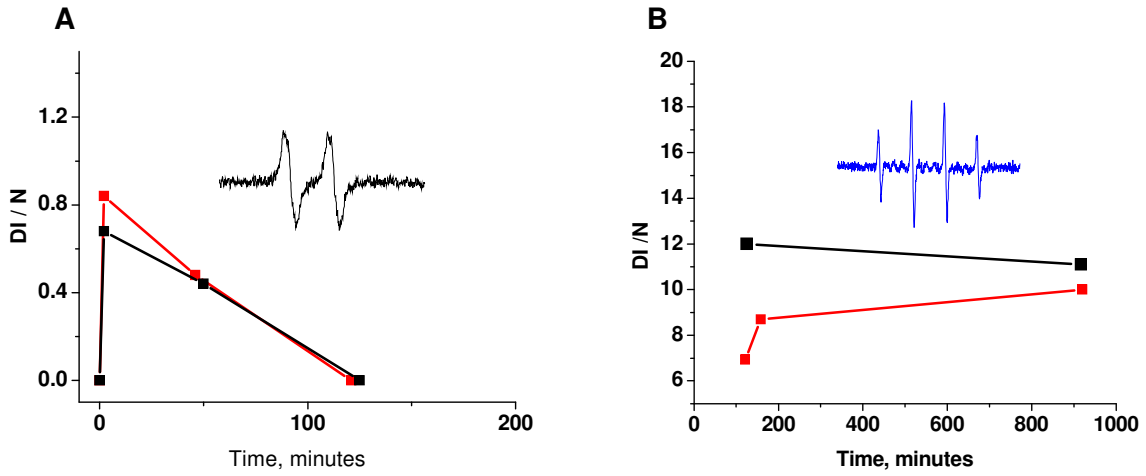

Figure S2. **A.** EPR signal intensity from ascorbyl radicals for EPFR (black line) and non-EPFR controls (red line) solutions in the presence of 1mM ascorbic acid. The inset is the ascorbyl radical EPR spectrum (α_H = 1.81 G, g = 2.0057). **B**. EPR signal intensity of DMPO-OH adducts (inset spectrum) for EPFR (black line) and non-EPFR controls (red line) solutions in the presence of 1mM ascorbic acid after ~ 120 minutes incubation.

References

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