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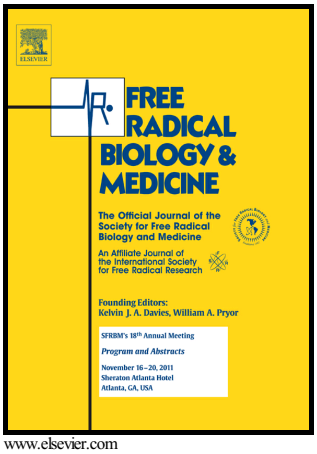

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The high affinity of small-molecule antioxidants for hemoglobin

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Abstract

Hemoglobin has previously been shown to display ascorbate peroxidase and urate peroxidase activity, with measurable Michaelis-Menten parameters that reveal a particularly low Km for ascorbate as well as for urate – lower than the respective in vivo concentrations of these antioxidants in blood. Also, direct detection of a hemoglobin-ascorbate interaction was possible by monitoring the 1H-NMR spectrum of ascorbate in the presence of hemoglobin. The relative difference in structures between ascorbate and urate may raise the question as to exactly what the defining structural features would be, for a substrate that binds to hemoglobin with high affinity. Reported here are Michaelis-Menten parameters for hemoglobin acting as peroxidase against a number of other substrates of varying structures – gallate, caffeate, rutin, 3-hydroxyflavone, 3,6-dihydroxyflavone, quercetin, epicatechin, luteolin – all with high affinities (some higher than those of physiologically-relevant redox partners of Hb – ascorbate and urate). Moreover, this high affinity appears general to animal hemoglobins. ^{1}H-NMR and ^{13}C-NMR spectra reveal a general pattern wherein small hydrophilic antioxidants appear to all have their signals affected, presumably due to binding to hemoglobin. Fluorescence and calorimetry measurements confirm these conclusions. Docking calculations confirm the existence of binding sites on hemoglobin and on myoglobin for ascorbate as well as for other antioxidants. Support is found for involvement of Tyr42 in binding of three out of the four substrates investigated in the case of hemoglobin (including ascorbate and urate, as blood-contained relevant substrates), but also for Tyr145 (with urate and caffeate) and Tyr35 (with gallate).

Graphical abstract