# Nitration of arachidonic acid modulates PGHS-1 activity

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Prostaglandin endoperoxide H synthase-1 (PGHS-1) converts arachidonic acid (AA) to prostaglandin G2 (cyclooxygenase activity, COX) and reduces the hydroperoxide at C15 to prostaglandin H2 (peroxidase activity, POX). We have previously demonstrated that AA can be nitrated in the presence of nitrite at acidic pH yielding nitroarachidonic acid ($AANO_2$). $AANO_2$ exhibited antiinflammatory properties including down-regulation of nitric oxide synthase-2 expression during macrophage activation. We hypothesize that during PGHS-1 turnover AA-derived radicals can be sequestered by reactive nitrogen species to form $AANO_2$ which in turn modulates prostaglandin formation, diverting AA from its normal metabolic pathway. Ovine-PGHS-1 incubated in 50 mM phosphate buffer, pH 7.4 at 37ºC with peroxides, phenol and AA showed decreased oxygen consumption when $AANO_2$ was added ($K_i$=141 µM). Similar results were observed when POX was evaluated using $H_2O_2$ as peroxide substrate ($K_i$=135 µM). Enzyme preincubation for five minutes with $AANO_2$ increased its inhibitory effect ($K_i$=7.6 µM for POX). To determine if this inhibition was reversible, PGHS-1 was incubated with $AANO_2$ or the slow reversible inhibitor indomethacin following gel filtration chromatography. In contrast to indomethacin, neither POX nor COX were recovered after gel filtration. Moreover, $AANO_2$-treated PGHS-1 had a reduced capacity to bind heme, suggesting that $AANO_2$ selectively labels PGHS-1 near the heme binding site. Nitroalkenes are potent electrophiles capable of covalently modifying cysteine and histidine residues. Mass spectrometry experiments using a QTRAP 2000 (Applied Biosystems) showed that $AANO_2$ is attached to PGHS-1, suggesting that $AANO_2$ modifies critical histidine residues with the concomitant release of heme, thus inactivating PGHS-1.

PGHS; nitroalkenes; arachidonate