The functional and molecular consequences of oxidation in the skeletal muscle myofilament

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THESIS ABSTRACT

It is becoming increasingly evident that redox state leading to post-translational modifications of structural proteins, enzymes and ion channels can cause activation or inhibition of cellular function (Andrade et al., 1998a, Jackson, 2008, Kelly et al., 1996). While low levels of nitric oxide (NO) synthesised by endothelial and neuronal nitric oxide synthase have been shown to provide a beneficial effect to tissues, the elevated release of NO accompanying inflammation has a detrimental effect, resulting in dysfunction (Khanna et al., 2005). We investigated the functional consequence and molecular substrate of NO and another potentially harmful reactive oxygen species, H_2O_2, on the skeletal muscle myofilament.

In a rat model we used functional myography of demembranated single fast- and slow-twitch skeletal muscle fibers to examined the consequence of the addition of the free radical NO and reactive oxygen species H_2O_2 on the Ca^{2+} sensitivity of the myofilament. The reversibility of oxidative modifications following NO or H_2O_2 treatment was examined using the general anti-oxidant dithiothreitol. Isoelectric focusing combined with SDS-PAGE separation of proteins investigated the post-translational modification of free-radical exposed myofilament proteins. Molecular substitution of endogenous troponin C (TnC) with WT cardiac/slow TnC or C84S TnC, incapable of being oxidized at Cys84, investigated the molecular and functional consequence of oxidation of TnC at Cys84.

Exposure of fast-twitch muscle fibers to NO resulted in a decrease in Ca^{2+} sensitivity, while H_2O_2 had the opposite effect, increasing Ca^{2+} sensitivity. In contrast, slow-twitch fibers were insensitive to both NO and H_2O_2. Following myofilament exposure to NO (~2 μM) proteomic analysis revealed that many proteins underwent post-translational modification, including myosin light chain (LC_{20}) and TnC. Molecular substitution of endogenous fast-twitch TnC with WT-cardiac/slow TnC demonstrated a similar sensitivity to NO as WT skeletal muscle. In contrast TnC, non-oxidizable at Cys84, rendered fast-twitch skeletal muscle insensitive to NO.

Many myofilament proteins, including myosin light chains were identified as being post-translationally modified by NO exposure, however, molecular substitution experiments clearly identify TnC, specifically residue Cys84 as the functional substrate responsible for fast-twitch skeletal muscle sensitivity to NO. Although slow-twitch muscle contains the same isoform of TnC, it was insensitive to NO. This suggests that slow-twitch muscle may have a greater capacity for anti-oxidant defense than fast-twitch muscle. The contrasting increase in Ca^{2+} sensitivity following H_2O_2 to the decline caused by NO demonstrates that not all oxidative molecules act alike, possibly targeting differing substrates and causing differing post-translational modifications.

COMMON ABBREVIATIONS

BH4	tetrahydrabiopterin
$[Ca^{2+}]_{cyt}$	cytoplasmic calcium concentration
CICR	calcium-induced-calcium-release
DHPR	dihydropyridine receptor
DTT	dithiothreitol
GSNO	S-nitroso-glutathione
H_2O_2	hydrogen peroxide
IEF	isoelectric focusing
IRI	ischemia-reperfusion injury
MHC	myosin heavy chain
MI	myocardial infarct
NOS	nitric oxide synthase
$O_2^{\cdot -}$	super oxide
OH^-	hydroxyl radical
ONOO	peroxynitrite
pCa	$-\log [Ca^{2+}]$
RNS	reactive nitrogen species
ROS	reactive oxygen species
RyR	ryanodine receptors
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SNAP	sodium nitroprusside
SOD	super oxide dismutase
SR	sarcoplasmic reticulum
TnC	troponin C
TnI	troponin I
TnT	troponin T