Measuring redox-active species in cells and tissues. Focus on “A case of mistaken identity: are reactive oxygen species actually reactive sulfide species?”

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The generation of reactive oxygen species (ROS) is inevitable for aerobic organisms and generally occurs at a controlled rate, with endogenous mechanisms tightly regulating ROS production and metabolism. Alterations in the balance between oxidant production and metabolism can lead to enhanced oxidative stress, which promotes oxidative damage to a wide range of biomolecules. The accumulation of oxidized products is associated with aging and the development of pathologies, such as atherosclerosis (11, 14), diabetes mellitus (9), cardiovascular disease (1), and neurodegenerative disorders (12). This perceived importance of ROS generation in disease progression is supported by a myriad of available methods for measurement of various ROS species (13). However, the assays themselves are not without limitations, including a limited ability to identify the specific ROS responsible for a given footprint of biological damage (4).

Maintenance of intracellular redox status requires alteration of both oxidant production and thiol-reducing capacity to permit specific regulatory modifications of protein thiols. Recently, ROS-mediated oxidative modification to organic sulfur has been linked to the regulation of protein structure, function, and cellular trafficking (3, 7, 8). Therefore, understanding factors that regulate protein thiold redox status has moved center stage with both reversible and irreversible formation of reactive sulfide species (RSS) potentially playing a crucial controlling role in signaling mechanisms involved in cellular homeostasis. For example, redox regulation of key thiol residues in Kelch-like ECH-associated protein 1 (KEAP-1) is associated with transcriptional activation of nuclear factor 2 (NRF-2) (5). Therefore, KEAP-1 is considered to be a major sensor of RSS with transcriptional activation of NRF-2 which promotes thioredoxin system activity and cellular redox status.

In their paper in the current issue of the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology, DeLeon et al. (2) suggest that oxidative stress and redox signaling is strongly linked to another intracellular stressor, RSS. Given the similarities between ROS and RSS, the authors hypothesized that current methods employed for measuring ROS also indiscriminately detect RSS. Here, the authors compared the relative sensitivity of five methods commonly employed to measure cellular ROS [redox-sensitive green fluorescent protein (roGFP), 2,7-dihydro-dichlorofluorescein, MitoSox Red, Amplex Red, and amperometric electrodes, the latter detecting nitric oxide as well as ROS] and examined whether RSS were also detected using these methods.

In summary, all methods demonstrably detected RSS and were generally equal or more sensitive in detecting RSS compared with the relevant added ROS. Most notably, roGFP, debatably considered one of the “gold standard” methods for assessing intracellular ROS production, was ~200-fold more sensitive to the mixed polysulfide H2Sn (n = 1–8) than to added hydrogen peroxide (H2O2). Therefore, RSS may be a confounder in assigning a role for intracellular ROS in cellular signaling processes.

Tellingly, the authors’ data showed amperometric detection of polysulfide H2Sn using electrodes “specific” for H2O2 and nitric oxide. Indeed, H2Sn was detected at markedly greater sensitivity than the prescribed agents. In contrast, electrodes designed to specifically detect H2S(g) and molecular oxygen did not exhibit appreciable cross-sensitivity to H2O2 or the NO-releasing agent S-nitroso-N-acetylpenicillamine. The authors conclude that these findings suggest that RSS may be more important to intracellular signaling than previously appreciated. If so, then it follows that under conditions in which both RSS and ROS are produced, the contribution of RSS to these commonly measured readouts is substantially unrecognized or, at least, substantially underestimated.

The study by DeLeon et al. (2) addresses an important issue in relation to the specificity of probes for the measurement of ROS. Often, these probes are used to imply ROS involvement, and specificity for ROS is generally assumed without parallel experiments that assign identity unambiguously (4, 13). Overall, the outcomes from the present study indicate that this assumption can lead to misleading interpretations and that RSS should also be considered when measuring cellular ROS. Indeed, the outcomes provided in this study indicate that it is imperative to consider whether RSS are present and contribute to the physiological and pathophysiological processes previously assigned mainly to ROS. Understanding the fundamental regulation of RSS and their relationship with ROS and whether these species may both play a role in promoting pathological processes, may guide the development of the next generation of therapies to reduce morbidity and mortality associated with oxidative stress.

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