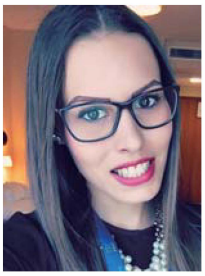
Dojindo Products in Mechanistic Studies of Reactive Sulfur Species



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The common phrase 'Reactive Oxygen Species' is used to describe a number of reactive molecules and free radicals derived from molecular oxygen, mostly generated as by-products during mitochondrial electron transport. They have long been known to lead to oxidative stress causing cellular damage, however recent evidence have shown that these species may play a key role in redox regulation of cellular signaling[1]. They are also primary components of the antimicrobial stratagem of phagocytic cells in their host defense mechanisms. These important mediatory and protective functions of reactive oxygen species serve as foundations for recent developments in redox based medicine[2].

The new kid on the block in redox biology is the small signaling molecule hydrogen sulfide (H_2S). Although sulfide related research remained for a long time limited to toxicology, H_2S is now considered as an essential signaling molecule with crucial roles in health[3] and disease[4]. These important functions have recently been underpinned by novel posttranslational modifications that were shown to be induced by hydrogen sulfide or related sulfur species (now commonly called Reactive Sulfur Species, RSS)[5-10]. Extensive research has already identified a number of pathways for the underlying molecular actions of RSS, which are summarized below:

1. The most widely suggested pathway with respect to sulfide-mediated biological functions is persulfide formation on protein cysteine residues[11]. This posttranslational modification has been proposed to have a protecting function on Cys residues against oxidative damage[9], as well as modulating distinct enzyme activities[8]. A recent study from the Akaike laboratory in collaboration with us reported a pathway of translation-coupled protein persulfide biosynthesis, introducing a new direction on the pivotal roles of these modifications in cellular homeostasis[12].

2. Due to its strong nucleophilic character and reducing capacity, sulfide readily coordinates to metal centers of enzymes, and can potentially reduce these metal centers, representing another pathway of regulating enzymatic functions.[8] For example favorable interactions were reported with cytochrome C oxidase (which is also coupled to sulfide toxicity at high endogenous concentrations of H_2S), hemoglobins or myoglobins[13, 14] as well as heme peroxidases like myeloperoxidase (MPO) [15, 16], lactoperoxidase[17], catalase[18, 19] or superoxide dismutase[20].

3. Another exciting new direction in sulfide biology focuses on the chemical interactions of sulfide with NO and the cross-talk of NO mediated and H_2S mediated signaling[21]. Accumulating evidence suggest that biochemical and pharmacological interactions between these two signaling molecules occur in numerous different ways, where they reciprocally regulate the expression and function of distinct proteins. Recent research from the Feelisch laboratory in collaboration with us led to the discovery and identification of novel hybrid S/N molecules, which were proposed to be key bioactive reaction products of these small signaling molecules[22].

In order to gain deeper insights into the molecular mechanisms of RSS biology, rigorous mechanistic studies require reliable chemicals, which are commercially available and can be used relatively easily. Dojindo Molecular Technologies offers a number of different reagents, which are extensively used by us and gaining increasing interest in numerous research laboratories all over the world. Here we introduce a few of them that are routinely used in our laboratory:

SSP4 (Sulfane Sulfur Probe 4) is a novel fluorescent probe, which can selectively detect sulfane sulfurs. The thiosalicyl analogue itself is not fluorescent, but fluorescein is released during the chemical interaction with sulfane sulfurs, emitting a strong green fluorescent signal. Thus, high sensitivity of fluorescence detection and convenient imaging of sulfane sulfur species can be performed with this product.

Figure 1 Reaction of SSP4 with sulfane sulfurs
(figure was adopted from Dojindo homepage)

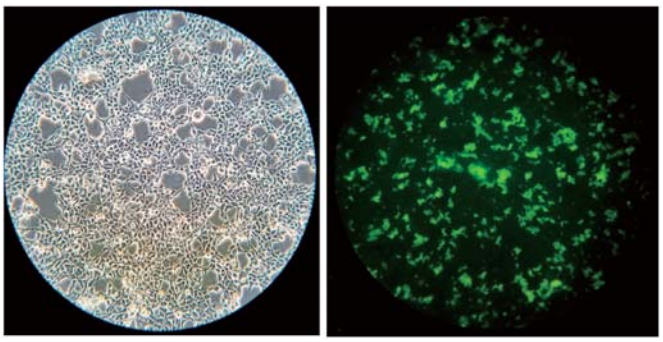

Figure 2 HEK293 cells stained with 50 μM SSP4, after treatment with sulfide

SSP4 has for example been used in our research laboratory for the detection of cellular persulfide production in HEK293 cells (Figure 2), as well as to follow sulfane sulfur generation during the MPO catalyzed oxidation of sulfide by hydrogen-peroxide[15), 23)].

Water-soluble tetrazolium salts (WSTs) are now widely used to study cellular redox events in a quick and convenient way. They can, for example, aid activity measurements of mitochondrial dehydrogenases. Dojindo offers several newly developed phenylazotype tetrazolium salts, which are easily reduced by NADH or other cellular reducing agents to give colorful formazan dyes. An important step in the wider utilization of tetrazolium salts was the increase of their solubility in water by incorporating hydroxide groups and positive or negative charges to the phenyl ring. Dojindo's WSTs have additional sulfonate groups introduced directly or indirectly to the phenyl ring to reach even higher water-solubility. Due to the achieved high water solubility, concentrated (up to 100 mM) solutions can be prepared and used for NADH, NADPH or superoxide detection by simply measuring the absorbance of the dye solutions at the indicated absorption maxima. Besides its high sensitivity, another advantage of this method is that it can be performed in a microplate, making it a simple and time-saving protocol. We routinely use Dojindo's WST compounds for example to measure the enzymatic activity of SOD[15)], as well as the superoxide producing activity of NOX2 upon stimulation of human neutrofils[23)].

Sodium-sulfide (Na_2S) and different polysulfide salts (Na_2S_2, Na_2S_3, Na_2S_4) allow to conveniently study sulfide or polysulfide induced processes. Sodium polysulfides are sulfane sulfur species, which have simple structures, and exist as different chain length hydropolysulfides in an aqueous solution, depending on the concentration conditions[8)]. Preparation of inorganic polysulfide solutions has for a long time been based on their in situ generation by reacting hypochlorite[24)] or glycine monochloramine[25)] reagents with excess of sulfide. Dojindo's sodium polysulfide salts, which dissolve in ultrapure water, provide a new and more convenient way to produce polysulfide solutions. These commercially available polysulfide salts were tested in our laboratory during the preparation of persulfidating solutions, which were used to develop the ProPerDP method[5), 26)]. This highly selective semiquantitative protocol allows the detection of protein per- and polysulfide species on isolated proteins, in intact cells as well as in blood plasma or tissue samples.

PEG-PCMal is a reagent to visualize the redox states of proteins by quantitative analysis of their free thiol groups using gel-electrophoresis based techniques. Modification of thiol residues is one of the most important redox post-translational modifications occurring on proteins inside cells. It has recently been revealed that such modifications control numerous cellular functions such as transcription, expression or cell death. Hence, determining protein redox states is a useful mechanistic tool in redox biology.

PEG-Maleimide is a conventional reagent, which is used to visualize the redox states of proteins by a gel shift assay (Figure 3). It has a maleimide group, which binds covalently to protein thiols. This interaction results in a mobility shift upon electrophoretic separation (Figure 4). Hence, the number of free thiol groups on a protein can be assessed after PEG-Maleimide labeling followed by SDS-PAGE. A technological challenge is that PEG labeled protein chains transfer with lower efficiency and often present diminished antibody recognition during western blot analyses. Dojindo's PEG-PCMal has a UV photocleavable moiety in the molecule, which allows the cleavage of the PEG chains off the protein in the gel when exposed to UV irradiation after electrophoresis. Therefore, alkylated proteins that were treated with UV irradiation can be transferred from the gel to PVDF membrane and detected by antibodies with much higher efficacy and sensitivity.

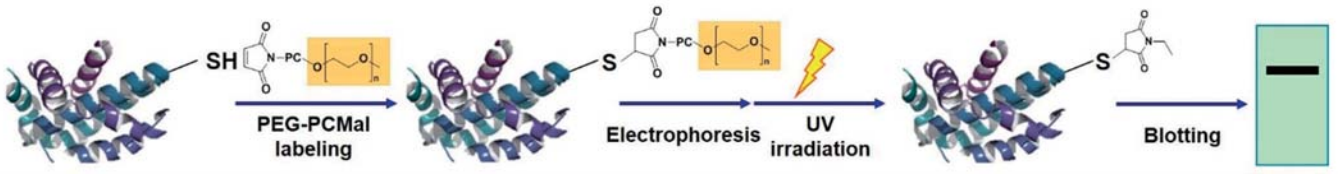

Figure 3 Schematic protocol for the determination of protein redox states by PEG-PCMal labeling, gel shift assay and western blotting (figure was adopted from Dojindo homepage)

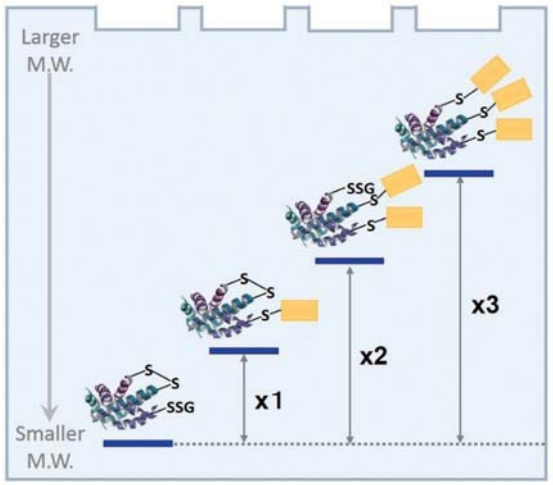

Figure 4 Mobility shift assay mechanism by PEG-PCMal depending on the redox state of the protein (figure was adopted from Dojindo homepage)

PEG-PCMal can also be utilized in a recently developed methodology to detect protein polysulfides, called polyethylene glycol-conjugated maleimide-labeling gel shift assay (PMSA, Figure 5), which is based on the unique redox property of polysulfides[12]. This technology is similar to the method mentioned above, but allows to quantify the protein polysulfide levels according to differences in band mobility upon SDS-PAGE. Initially, all sulfhydryl groups are blocked by iodoacetamide. In the second reaction, polysulfidated Cys residues are labeled by the conventional reagent, biotin-PEG-maleimide which can cleave alkylated polysulfide chains[12]. Thus, the degree of protein polysulfidation is determined as a change in band mobility upon PEG-maleimide labeling. Low sensitivity drawbacks are also resolved by using Dojindo's PEG-PCMal (see Figure 3 and 4). Recently we have extensively validated this methodology and found that it is indeed appropriate for the detection of protein per/polysulfidation levels.

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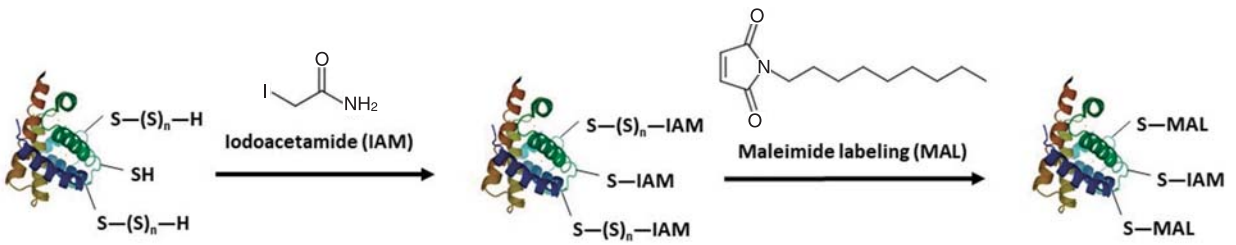

Figure 5 The principle of polyethylene glycol-conjugated maleimide-labeling gel shift assay (PMSA)

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2015- Cancer Center Accreditation Auditor for OECI
2013- Director of International Relations at the National Institute of Oncology, Hungary
2011- Head of Department - Department of Molecular Immunology and Toxicology at the National Institute of Oncology, Hungary

Recent major awards

2017 Doctor of Science of the Hungarian Academy of Sciences
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2015- Honorary Associate Professor at Debrecen University, Hungary
2015- Honorary Senior Research Fellow at the University of Otago, Christchurch, Department of Pathology, Free Radical Research Group, New Zealand
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