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Forum Editorial

Biological Redox Switches

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Abstract

Research over the last decade has substantially advanced our understanding of cellular redox chemistry and introduced new terms to redox signaling and biological redox switches. It emerges that redox switching affects a plethora of biological processes and seems to be necessary to counterbalance oxidative stress and guarantee cellular survival in oxidative conditions. Despite intensive studies, the mechanisms of redox switching and its consequences are poorly understood, which points towards an extremely complex nature of these phenomena. Indeed, in contrast to classical signalling cascades, cellular redox signaling seems to affect the whole cellular redox environment and a large number of different redox switches. A majority of biological redox switches rely on the oxidation of thiol group(s) of cysteine residue(s); however, thiolates also bind transition metal ions like Zn(II), Cu(I), and iron, and these metal-thiolate motifs also function as redox switches. It follows that a deeper understanding of redox signalling and redox switches could be achieved by a multidisciplinary approach combining advances in the redox chemistry of sulphur, the chemistry of reactive oxygen and nitrogen species, as well as the bioinorganic chemistry of metal complexes. Many of these aspects are reviewed in the current forum issue on biological redox switches with the aim to promote the understanding of cellular redox phenomena at system biology level. *Antioxid. Redox Signal*. 11, 981–983.

Introduction

Since the dawn of biochemistry, it has been acknowledged that redox processes play a crucial role in the Chemistry of Life and especially in the production of biological energy. Indeed, oxidation of organic molecules and ultimate transfer of gained electrons to molecular oxygen can release huge amounts of free energy that is exploited by aerobic organisms for production of biological energy. Other more primitive life forms also utilize redox processes to fuel production of biological energy. Quite early it was also acknowledged that overproduction of by-products of aerobic oxidation, especially reactive oxygen species (ROS), might cause toxic effects on cells, which are counterbalanced by numerous biological antioxidant defense systems. Another biological purpose of redox chemistry was linked with biosynthesis of different metabolites catalyzed by different oxidoreductases, and thus redox chemistry has been considered vitally important but potentially dangerous. Recent developments in the field of cellular redox chemistry have enriched this dualistic, largely black and white, scheme with many new redox phenomena, and thus added other intermediary colors to the palette. The current forum issue on biological redox switches gives overviews of these recent developments in cellular redox chemistry and also envisages trends for future development.

We underline three types of new cellular redox phenomena that were discovered in recent decades and have revolutionized our understanding of cellular redox chemistry. First, immense evidence from biochemical and physiological studies has iconed the paradigm of redox signaling, where mild oxidant substances (such as NO and hydrogen peroxide) act as signaling molecules and affect a variety of crucial cellular functions, including gene transcription, translation, metabolism, proliferation, and ultimately apoptosis. Second, it has been recognized that the intracellular redox environment, created mainly by glutathione (GSH) and its oxidized counterpart GSSG, is not just reducing, which is opposite to the oxidizing extracellular milieu, but it differs in various subcellular compartments and, more importantly, it is also

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subject to substantial changes during redox signaling and in oxidative stress conditions. The most reliable information from intracellular redox environments has been mediated by *in situ* redox-active GFP-based sensors that have been targeted to different cellular compartments and tested in various cellular conditions. Although there are still problems with accuracy of these measurements, discussed later, it is important to know that redox signaling affects the whole cellular redox environment, and not only a few specific signaling proteins as it occurs in classical signaling cascades. This view is supported also by a third breakthrough discovery demonstrating that intracellular Cys-containing proteins are not always fully reduced but hundreds of them might become reversibly oxidized during redox signaling and by other oxidative stimuli (3, 4). Taking these phenomena together, we see that during redox signaling and oxidative stress, numerous cellular proteins and also low-molecular Cys-containing ligands such as GSH function like biological redox switches. Transient oxidation of these switches cause modification of active site residues or introduce changes in conformation of proteins by affecting their biological activity.

In this issue, we give an overview of three types of biological redox switches: thiol-based switches (1), zinc fingers (6), and iron-based redox switches (7). Directly or indirectly, all these motifs utilize redox chemistry of thiol groups of amino acid cysteine, which can exist in an uncomplexed state or participate as thiolate anion in binding of metal ions like Zn(II) in zinc finger motifs or iron in iron–sulfur clusters or mononuclear iron sites. The thiol group is present also in the structure of cellular redox buffer glutathione (GSH), which creates a cellular redox environment. Thus it follows that new cellular redox phenomena such as cellular redox signalling and regulation of redox environment rely largely on redox chemistry of sulfur, in contrast to classical organic carbon redox chemistry. As full understanding of these phenomena requires a solid background in redox chemistry of sulfur, we might ask is it available or not? The honest answer would be rather not, as chapters dedicated to sulfur chemistry are rare in biochemistry and organic chemistry textbooks. The review by Jensen *et al*. (5) attempts to partially fill this cap. General knowledge was that SH groups can be oxidized to disulfide bonds, which can be reduced to SH groups by the influence of reducing agents. Until recently it had also been suggested that protein thiols and thiols of GSH compose rather separate thiol pools, which do exchange disulfide bonds and form only intermediary mixed disulfides. Recent research by using correct chemistry for trapping of reaction intermediates and adequate mass spectrometric identification techniques demonstrates, however, that hundreds of cellular proteins become glutathionylated by oxidative stimuli (3). It is important to mention that nitrosative stimuli lead to similar large scale nitrosylation of protein thiols (4) and also of GSH thiol by forming of S-nitrosoglutathione (GSNO). It can be concluded that protein thiols and GSH thiols do compose a rather unified cellular pool of thiols, which participates "*in corpore*" in redox signaling and antioxidative defense. To our surprise, it appears that the protein thiol pool is as large or even larger than the thiol pool in GSH, which is usually present in high millimolar concentrations (3). Taking into account Cys content in cellular proteins, which is $\sim$2%, and a cellular average protein concentration of 10 mg/ml, we can calculate that cellular concentration of protein thiols is indeed in the millimolar range. The majority of these SH groups are potential partners for oxidants, whereas apparently many protein SH groups are more reactive as those in GSH, which is reflected in their lower pK_a values as compared to pK_a of GSH thiol group equal to 9.4 (1). At the moment it is not known whether large-scale protein glutathionylation is catalyzed by enzymes or occurs through spontaneous chemical reactions, and whether these reactions are regulated by thermodynamic or kinetic control mechanisms; however, as these questions are formulated (5), we might get answers in the near future.

It is certainly known that major changes in thiol-based biological redox switches are reversible and that protein intra- and intermolecular as well as mixed disulfides with GSH are eliminated by enzymatic systems, composed of a variety of thioredoxins and glutaredoxins. These enzymes are present in most living organisms, often in many isoforms and in diverse cellular compartments, being of vital importance for cellular functioning. A closer look at the catalytic mechanisms of their functioning (2) shows that by catalyzing disulfide exchange reactions with high specificity to protein-bound GSH but not to proteins, these enzymes can speed up reaching of thermodynamic equilibrium at proteome level, with profound functional consequences (2). However, as these enzymes also contain functional pairs of Cys residues, with redox potential slightly below physiological, it follows that besides other numerous mechanisms of their regulation (2) these enzymes also fulfill the criteria of biological redox switches and might also be affected by redox signaling and oxidative stress (2). It is also not excluded that glutaredoxins work in both directions of biological redox switching by catalyzing glutathionylation of proteins in slightly oxidizing conditions. Such a scenario is feasible as thioredoxins and glutaredoxins get their reducing power from reduced GSH, which is also largely depleted in oxidative conditions, at the same time the level of GSSG is increased and direction of enzyme action might be reversed. It also follows that thioredoxins and glutaredoxins alone can not reverse the oxidative consequences of redox signaling and oxidative stress, which requires restoration of the pool of reduced GSH. The latter task could be performed by glutathione- and glutaredoxin reductases, which get their reducing equivalents from the classical redox cofactor NADPH, which is a mediator molecule between the metabolic carbon and signaling sulfur redox chemistry. Rules of cross-talk between these two types of redox chemistry are largely unknown; however, they might be vitally important.

It emerges that cellular redox switching during redox signaling and oxidative stress seems to occur largely by virtue of fluctuations in cellular redox potential, which is undoubtedly the most fundamental and important parameter of cellular redox environment. It is understood that total cellular redox potential is a very complex parameter determined by multiple redox couples, including GSH/GSSG, hundreds of different protein thiol/disulfide couples, as well as the NADPH/$NADP^+$ couple, which all are not necessarily in thermodynamic equilibrium. There is currently a consensus that cellular redox potential is calculated taking into account the GSH/GSSG, redox couple, which is the most prominent low-molecular component of the cellular redox system. By using analytical data or *in situ* redox-active GFP-based sensors, which are in equilibrium with the GSH/GSSG couple, cellular redox potential in normal conditions has been found to lie in the range from -230 to -260 mV, whereas substantial shifts

up to −170 mV could be observed during oxidative signaling/stress and also during biological processes such as apoptosis. Despite progress, there are still many aspects that have to be taken into account in further refinement of our understanding about the cellular redox potential. First, taking into account the recent finding that GSH comprises approximately half of the cellular thiol pool (3), it is evident that the protein thiol pool should also be considered in the estimation of total cellular redox potential and capacity. Apparently it is an extremely difficult task as concentrations of individual proteins and their redox potential values are largely unknown, as well as it is not known which of them are in fast equilibria with GSH/GSSG couple. However, even if these parameters are known, it is might be still difficult to get a reliable estimation of the cellular redox potential. Redox potentials of GSH as well as different proteins are currently determined in *in vitro* conditions for protein components alone; however, cellular conditions transition metal ions such as Zn(II), Fe(II) and Cu(I) are present. There are many examples showing that transition metal ions like Zn(II) can substantially modulate thiol–disulfide redox equilibria. Zn(II) ions and other soft metal ions have high affinity towards thiolates, whereas two spatially close thiolates in biological redox switches can create a zinc-binding site of substantial affinity. Zn(II) complexes, if formed, protect SH groups from oxidation and therefore shift standard redox potentials of proteins to more negative values. These principles might apply not only for zinc- (6), iron- (7), or copper-binding proteins (8) but also to a variety of other redox switches and also to GSH. Such a modulation of cellular redox potentials might have many different physiological consequences such as protection from oxidation, regulation of protein activities (6), or translocation (8). The extent of modulatory effect of metal ions on redox properties of various proteins remains to be established, and such studies could be speeded up by application of new and reliable methods as presented in the current issue (8). There is also one important methodologic aspect connected with the latter work, namely, metal ions might modulate also redox equilibria of *in situ* GFP-based redox sensor proteins hat also contain thiol-based redox switches. It remains to be determined whether redox equilibria of currently used *in situ* redox sensors are metal sensitive in cellular conditions. However, it is not excluded that currently accepted cellular redox potential values have to be revised and updated.

In addition to the modulatory role of metal ions on biological redox switches, release of metal ions from variety of thiol-containing metalloproteins (metal-containing redox switches) during redox signaling and oxidative stress is an inseparable part of cellular redox signaling. It is known that redox signaling and oxidative stress lead to release of substantial amounts of zinc ions from metallothioneins and zinc finger proteins (6), and biological consequences of such metal release are under intensive investigations. Similar release of metal ions might occur also from iron and copper proteins, with potentially devastating consequences as these free redox-active metal ions can, in the presence of oxygen metabolites, initiate production of highly reactive ROS and cause oxidative stress.

Future Perspectives

It appears that we are beginning to understand the whole complexity of cellular redox chemistry and signaling, which relies largely on sulfur chemistry, chemistry of ROS and NOS, as well as on bioinorganic chemistry of metal complexes. Rapid developments in these new emerging fields, combined with achievements obtained by new powerful proteomic techniques, allow us to predict that critical mass of new important aspects of cellular redox chemistry will be discovered in the near future. Hopefully, such integrated data will allow application of a system biology approach to cellular redox chemistry and lead to discovery of new fundamental biological redox pathways for cellular functioning and survival of Life in the harsh oxidizing environmental conditions on current Earth.

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