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Composition of the redox environment of the endoplasmic reticulum and sources of hydrogen peroxide

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ABSTRACT

The endoplasmic reticulum (ER) is a metabolically active organelle, which has a central role in proteostasis by translating, modifying, folding, and occasionally degrading secretory and membrane proteins. The lumen of the ER represents a separate compartment of the eukaryotic cell, with a characteristic proteome and metabolome. Although the redox metabolome and proteome of the compartment have not been holistically explored, it is evident that proper redox conditions are necessary for the functioning of many luminal pathways. These redox conditions are defined by local oxidoreductases and the membrane transport of electron donors and acceptors. The main electron carriers of the compartment are identical with those of the other organelles: glutathione, pyridine and flavin nucleotides, ascorbate, and others. However, their composition, concentration, and redox state in the ER lumen can be different from those observed in other compartments. The terminal oxidases of oxidative protein folding generate and maintain an "oxidative environment" by oxidizing protein thiols and producing hydrogen peroxide. ER-specific mechanisms reutilize hydrogen peroxide as an electron acceptor of oxidative folding. These mechanisms, together with membrane and kinetic barriers, guarantee that redox systems in the reduced or oxidized state can be present simultaneously in the lumen. The present knowledge on the in vivo conditions of ER redox is rather limited; development of new genetically encoded targetable sensors for the measurement of the luminal state of redox systems other than thiol/disulfide will contribute to a better understanding of ER redox homeostasis.

Introduction

The redox environment of a subcellular compartment is determined by the concentration and redox state of the major redox-active compounds (i.e., electron carriers composed of electron donors and acceptors). The redox conditions in the ER lumen became a focus of interest upon the recognition of oxidative folding and its major oxidoreductases [for recent reviews see 1–3]. It was soon recognized that the thiol/disulfide redox system is present in an oxidized state compared to the cytosol [4]. After the application of genetically encoded ER-targetable fluorescent sensors, the in vivo real-time investigation of the thiol/disulfide redox system became feasible. However, the knowledge of the other luminal redox systems [5,6] is regrettably scanty. Although indirect proofs show the presence of pyridine and flavin nucleotides, ascorbate, and other redox-active compounds, their source, luminal concentration, and redox state have not been defined. Thus, our present viewpoint on ER redox has an oxidative folding-dependent focus. Indeed, the redox conditions in the ER seem to be organized according to the requirements of oxidative protein folding:

- The main low-molecular-weight electron carriers are not synthesized in this compartment; they are taken up by—molecularly mostly undefined—transporters, creating a point for the regulation of luminal redox.
- The thiol/disulfide redox system is separated from the cytosolic one by a membrane barrier and from the pyridine nucleotide

Abbreviations: AA, ascorbic acid; DHA, dehydroascorbic acid; ER, endoplasmic reticulum; ERAD, ER-associated degradation; Gpx, glutathione peroxidase; GR, glutathione reductase; H6PD, hexose-6-phosphate dehydrogenase; Nox, NADPH oxidase; PDI, protein disulfide isomerase; Prx, peroxiredoxin; ROS, reactive oxygen species; VKOR, vitamin K epoxide reductase; 11βHSD1, 11β-hydroxysteroid dehydrogenase type 1.

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system by the absence of adequate reductases. Thus, the luminal thiol/disulfide redox system can keep its oxidized state, crucial for oxidative folding.

- Despite the intense oxidative activity, the ER lumen is not equipped with the common antioxidant enzymes; instead, special luminal enzymes utilize protein thiols as electron donors and reactive oxygen species (ROS) as electron acceptors. These conditions also promote oxidative folding.

Disturbance of the luminal redox homeostasis in the ER is an almost universal consequence of various human pathologies affecting the organelle [7,8]. Redox imbalance by itself and by altering key ER functions is perceived as ER stress [9], which activates various adaptive signaling pathways. Among these cellular responses, the unfolded protein response (UPR) is the best known, in accordance with the prominent role of the ER in the synthesis and folding of secretory proteins [10–12].

However, oxidative protein folding is not the only ER function that employs and affects luminal redox systems. The aim of the present review is to delineate the major redox systems of the ER and their connections and disconnections from a broader aspect including some points of view beyond oxidative folding. The main luminal hydrogen peroxide-producing reactions and their impact on local redox conditions as well as some experimental tools allowing their investigation are also covered.

Glutathione and its disulfide, the main redox buffer in the ER lumen

The major redox buffer of the ER lumen, similar to other subcellular compartments, is composed of the glutathione/glutathione disulfide (GSH/GSSG) couple. However, the luminal GSH–GSSG pool has been found to be different from the cytosolic one regarding both the total concentration and the ratio of its two components. A pioneering study revealed a GSH:GSSG ratio of 1:1–3:1 in the ER and in the whole secretory pathway in hybridoma cells by using a small thiol-containing glycopeptide that can undergo thiol/disulfide exchange with glutathione [4]. The calculated redox potential values were between −170 and −185 mV in the ER lumen in contrast to approximately −230 mV in the cytosol. Similar GSH:GSSG ratios (3:1–6:1) were observed by using ER-derived microsomal vesicles [13,14].

Applications of genetically encoded targetable redox sensors have recently resulted in slightly different values. A GSH/GSSG redox potential of -208 ± 4 mV was measured using a glutathione-specific redox-sensitive variant of green fluorescent protein in the ER lumen of intact HeLa cells [15]. The authors concluded that the GSH:GSSG ratio was equal to or above 11:1, or the total luminal concentration of glutathione exceeded the overall cellular concentration. The results were also confirmed in a recent study by the same group, using an ER-targeted single-cysteine glutaredoxin, which rapidly attains equilibrium with GSH/GSSG through autocatalytic glutathionylation. The calculated GSH:GSSG ratio in the ER lumen of HeLa cells was below 7:1 and the luminal concentration of total glutathione was about twofold higher than the overall cellular concentration [16]. The cytosolic GSH:GSSG ratio is about 3 orders of magnitude higher than the ER luminal one in cultured human cells and reflects a redox potential between −280 and −320 mV [17]. Therefore, it can be concluded that the composition of the ER luminal glutathione redox buffer is robustly shifted toward the oxidized/oxidizing direction compared to the cytosolic conditions, and glutathione (mainly GSSG) accumulates in the ER lumen.

Because the enzymes of glutathione synthesis are absent from the ER, the luminal glutathione pool must be derived from the cytosol. The preferential uptake of GSSG was suggested to be responsible for the peculiar intraluminal milieu and to provide the oxidizing power for disulfide bond formation in secretory proteins [4]. However, considering a gradient across the ER membrane, GSSG import would imply an active pumping activity. Systematic analysis of microsomal glutathione traffic revealed that GSH rather than GSSG is preferentially transported across the ER membrane by facilitated diffusion [18]. Although such a transport is theoretically bidirectional, the physiological GSH concentrations favor the uptake of GSH into the ER lumen. In addition to these contradictory findings, the theory of GSSG import was further weakened by the discovery of endoplasmic reticulum oxidoreductin (Ero) enzymes, which catalyze the final step of the electron transfer chain, which drives protein disulfide bond formation in the ER lumen [19,20].

GSH entering the ER lumen can be utilized in various local reactions, among which protein processing involves the most powerful oxidizing machinery. GSH is connected to the electron transfer chain of oxidative protein folding at multiple levels. Thiol/disulfide exchange can occur with substrate proteins or various oxidoreductases including the initiator oxidases of the process. Thus, it was supposed that glutathione and protein thiols compete for the oxidizing power, which drives disulfide bond formation [21]. GSH acts as an electron donor for PDI; an in vitro study showed a rapid GSH-dependent reduction of the isolated α domain in either the human or the yeast enzyme protein [22]. Spontaneous, uncontrolled thiol/disulfide exchange between GSH and protein disulfides would remarkably worsen the efficiency of oxidative folding. Nevertheless, the GSH-dependent protein disulfide reductions are hampered by both membrane and kinetic barriers and are restricted to carefully balanced enzymatic activities. Thus, oxidoreductases involved in the oxidative protein folding depend on the redox state of their partner enzymes rather than on the actual state of the luminal redox buffer [23].

Members of the PDI family can reduce and isomerize inappropriate protein disulfides by using GSH as the source of electrons [24,25]. Similarly, GSH is also utilized in the reductive unfolding of irremediably misfolded proteins directed to ER-associated protein degradation (ERAD). Because even a partial folding prevents these proteins from being retrotranslocated into the cytosol, the place of proteasomal degradation, all disulfides in the ERAD substrates must be first reduced [26]. Oxidoreductases catalyzing these reactions (e.g., ERdJ5) utilize GSH as an electron donor [27]. It should be noted that ERdJ5 disulfide reductase is also involved in the isomerization of nonnative disulfides as was revealed in a study on the folding process of the low-density lipoprotein receptor [28].

Ero1, the terminal oxidase of oxidative folding, can also catalyze GSH oxidation. Indeed, the rate of GSH oxidation was shown to correlate with Ero1p activity in yeast: regeneration of GSSG after dithiothreitol treatment was delayed by Ero1p deficiency and accelerated by Ero1p overexpression [21]. However, in vitro experiments demonstrated that GSH is a poor substrate for Ero1 [29], suggesting that Ero1 activity promotes GSH oxidation indirectly through PDI or hydrogen peroxide.

One of the major GSH-utilizing pathways of the cell is the elimination of hydrogen peroxide by glutathione peroxidases (GPx's). This enzyme family is represented by GPx7 and 8 in the ER lumen, which have been proved to be effective PDI peroxidases (i.e., they can use reduced PDI instead of GSH as electron donor) [30–32]. However, at least in the case of Gpx7, GSH can be an alternative substrate in the reaction, with a relatively low rate [33].

Owing to the poor permeability of the ER membrane to GSSG [18], the intense local GSH oxidation in the aforementioned reactions results in the luminal accumulation of GSSG, which can still leave the compartment via the secretory pathway. GSSG can also be engaged in thiol/disulfide exchange reactions with substrate proteins of oxidative folding, although these reactions are kinetically unfavorable. In vitro experiments showed that supplementation with an appropriate glutathione redox buffer facilitated the folding of proteins, even in the absence of oxidoreductases [34]. However, the addition of