The pro-radical hydrogen peroxide as a stable hydroxyl radical distributor: lessons from pancreatic beta cells

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Abstract

The toxic potential of H₂O₂ is limited, even if intracellular concentrations of H₂O₂ under conditions of oxidative stress increase to the micromolar concentration range. Its toxicity is mostly restricted to the oxidation of highly reactive thiol groups, some of which are functionally very important. Subsequently, the HO· radical is generated spontaneously from H₂O₂ in the Fenton reaction. The HO· radical is extremely toxic and destroys any biological structure. Due to the high reactivity, its action is limited to a locally restricted site of its generation. On the other hand, H₂O₂ with its stability and long half-life can reach virtually any site and distribute its toxic effect all over the cell. Thereby HO· in spite of its ultra-short half-life (10⁻⁹ s), can execute its extraordinary toxic action at any target of the cell. In this oxidative stress scenario, H₂O₂ is the pro-radical, that spreads the toxic action of the HO· radical. It is the longevity of the H₂O₂ molecule allowing it to distribute its toxic action from the site of origin all over the cell and may even mediate intercellular communication. Thus, H₂O₂ acts as a spreader by transporting it to sites where the extremely short-lived toxic HO· radical can arise in the presence of “free iron”. H₂O₂ and HO· act in concert due to their different complementary chemical properties. They are dependent upon each other while executing the toxic effects in oxidative stress under diabetic metabolic conditions in particular in the highly vulnerable pancreatic beta cell, which in contrast to many other cell types is so badly protected against oxidative stress due to its extremely low H₂O₂ inactivating enzyme capacity.

Keywords Oxidative stress · Hydrogen peroxide · Hydroxyl radical · Pancreatic beta cell

Abbreviations

GPx Glutathione peroxidase
H₂O₂ Hydrogen peroxide
O₂·− Superoxide radical
HO· Hydroxyl radical
Prx Peroxiredoxin
SOD Superoxide dismutase

Introduction

In the process of oxidative phosphorylation, energy is harvested as ATP via a process of reduction of a dioxygen molecule to two water molecules. ROS (reactive oxygen species) are products of incomplete reduction of molecular O₂ (oxygen). H₂O₂ (hydrogen peroxide) as the 2-electron reduction product of O₂ is not fully reduced and more reactive than dioxygen. At variance, the O₂− (superoxide radical anion) and the HO· (hydroxyl radical) are 1-electron reduction products of O₂ and of H₂O₂, respectively [for details, see Lenzen (2017)]:

O₂ + e⁻ + H₂O → H₂O₂; H₂O₂ + e⁻ → HO· + HO· + 2H⁺ → 2H₂O

Although O₂ has the same oxidation state in HO· and H₂O₂, the standard potential of the HO· radicals is exceedingly higher:
The central role of hydrogen peroxide as a pro-radical in the mitochondria and the peroxisomes

H$_2$O$_2$ can traverse lipid membranes (Lushchak 2011), in particular through some members of the aquaporin family, the so-called peroxiporins by facilitated diffusion (Bienert and Chaumont 2014; Laporte et al. 2020; Lenzen 2017), thereby entering the cytosolic compartment irrespective of the subcellular site of its generation, may it be the mitochondria or the peroxisomes. The number of fast reacting high affinity sites, namely the thiol (sulphydryl, SH) groups of the antioxidative enzymes of the glutathione peroxidase (GPx) and peroxiredoxin (Prx) enzyme families is small due to the low expression levels in the mitochondria and the peroxisomes of the pancreatic beta cells (Lenzen 2017). Therefore, only little of the high amounts of H$_2$O$_2$ generated in these organelles during cytokine toxicity in the T1DM scenario (Lenzen 2017) in the mitochondria and glucolipotoxicity in the T2DM scenario (Gehrmann et al. 2010; Lenzen 2017) in the peroxisomes are quickly inactivated at the sites of H$_2$O$_2$ generation. These are optimal prerequisites for longevity and long-distance travel of H$_2$O$_2$. This allows a distribution of H$_2$O$_2$ over the entire organelle of origin and beyond in the cytosolic space across the surrounding lipid membranes through facilitated diffusion via peroxiporins. Under these conditions, H$_2$O$_2$ can travel as long and as far until it meets free iron (II) or copper (I) ions or other suitable electron donors (Lenzen 2017). Instead of an interaction of H$_2$O$_2$ with a protective high affinity thiol, it can hit such a free ion, which acts as a catalyst for the formation of the most highly toxic hydroxyl radical (HO$^\cdot$) (Lenzen 2017). The high toxicity of the HO$^\cdot$ radical has thermodynamic and kinetic reasons. Its very high standard oxidative potential provides the thermodynamic driving force for many oxidations, and its small radius and uncharged state provides a great mobility, thereby allowing high rates of chemical reactions.

Relation between the subcellular organelles in the generation of hydrogen peroxide and the hydroxyl radical in the pancreatic beta cell

The volume of the beta cell covered by the mitochondria and the peroxisomes is very low, 4% and less than 1% of the cytosolic ground substance, respectively (Dean 1973; Lenzen and Panten 1983). The cytosolic ground substance comprises more than 50% of the beta cell volume (Dean 1973). The consequence of the very low beta cell mitochondrial volume and the even smaller peroxisomal volume is that any crossing of H$_2$O$_2$ into the surrounding cytoplasmic space
results in a decrease of the H$_2$O$_2$ concentration in these organelles through the dilution in a larger cellular space. This can result in a lower H$_2$O$_2$ toxicity in the organelle of origin. Vice versa, it has been shown that HO· radicals do not diffuse via a proton tunneling. Of course, in the case of the HO· radicals a hydrogen atom abstraction should occur instead of a proton abstraction (in fact, only bonds are moving). Very recently, it has been shown that HO· radicals do not diffuse via a hydrogen atom abstraction (Vassilev et al. 2005). Rather the diffusion rate of the HO· radical (like that of H$^+$ and HO$^-$ ions) is typical for its physical size. An exceptionally high diffusion rate would also contribute to a higher reaction rate with whatever targets. Thus, the high reactivity of the HO· radical, which also extends to the noble metals (Au, Pt, Pd, Ag) (Nowicka et al. 2010a, b, 2011), has to be solely attributed to its radical nature.

Thus, H$_2$O$_2$ plays the role of a pro-radical in the weakly protected beta cell (Lenzen 2008). That means that virtually any protein, carbohydrate, lipid or nucleic acid is a potential target for the toxic action of the highly toxic HO· radical with its extremely high reactivity (Lenzen 2017; Lushchak 2014). This explains the universal unspecific toxicity of the HO· radical towards any chemical structure at the site of its generation against which no protection whatsoever is possible. A protection is possible only by elimination of H$_2$O$_2$, which, however, is limited in the pancreatic beta cell due to the low level of expression of H$_2$O$_2$-eliminating enzymes (Lenzen 2017).

The role of the superoxide radical anion

When the primary reactive oxygen species generated is not H$_2$O$_2$ but the O$_2$·$^-$ radical anion, O$_2$·$^-$ is efficiently transformed into H$_2$O$_2$ through SOD isoenzymes (Lortz et al. 2005). This is in particular true for the highly expressed MnSOD in the beta cell mitochondria (Lortz et al. 2005) so that also when O$_2$·$^-$ is initially generated, it is instantaneously available as H$_2$O$_2$ owing to the action of SOD (Mehmeti et al. 2011). Thus SOD acts as a pro-oxidant enzyme (Lortz et al. 2005; Lushchak et al. 2005), whenever the conversion of H$_2$O$_2$ into the HO· radical is fostered in a cell type like the beta cell with its low abundance of H$_2$O$_2$-eliminating enzymes (Lenzen 2017). The hydrophilic O$_2$·$^-$ as a precursor of the hydrophilic H$_2$O$_2$ is a pro-oxidant, which as an anion is restricted in its mobility to the compartment of generation. In contrast to the negatively charged O$_2$·$^-$, the mobility of the electroneutral H$_2$O$_2$ molecule is virtually not restricted and thus reaches more sites than O$_2$·$^-$ for mediating toxicity.

The special situation in the pancreatic beta cell ER

The ER in secretory cells such as the pancreatic beta cells is a subcellular compartment in which a very oxidized state prevails. The strongly oxidative milieu in the ER is the result of high amounts of H$_2$O$_2$ generated as well as of the low GSH/GSSG ratio (Appenzeller-Herzog 2011). This is achieved in an oxidation step mediated by PDI oxidoreductases (protein disulfide isomerases) [for details, see Lenzen (2017)]. The ER acts as a H$_2$O$_2$ store, keeping a greater amount of the generated H$_2$O$_2$ in the ER due to a limited efflux of H$_2$O$_2$ into the cytosolic space. Insulin biosynthesis is a process which takes place constantly in the pancreatic beta cells. Its rate is high in particular at increased blood glucose concentrations in the postprandial state. Further accelerated is the increased rate of insulin biosynthesis in the prediabetic phase during the development of the T2DM state due to the prevailing insulin resistance under these conditions. This goes along with an increased rate of insulin misfolding, to which other islet cell hormones in other islet cell types are not prone, thus further accelerating ER stress (Lenzen 2017). In the prediabetic state, the insulin resistance induced hyperinsulinemia can be compensated by increased rates of insulin biosynthesis until the compensatory mechanisms ultimately collapse along with a gradually decreasing insulin synthesizing capacity. This results in continuously decreasing plasma insulin levels in the circulation due to progressive development of beta cell dysfunction along with an open state of ER stress.

Insulin comprises the major portion of the proteins synthesized in the pancreatic beta cell. For each proinsulin molecule folded in the ER three molecules of H$_2$O$_2$ are generated (Lenzen 2017) giving rise to a concentration of H$_2$O$_2$ higher than in many other cell types. This high level of H$_2$O$_2$ is also due to the fact that the ER membrane is rather
impermeable for H$_2$O$_2$ (Konno et al. 2015). This is advantageous, since a significant proportion of H$_2$O$_2$ generated in the ER during proinsulin folding is consumed again for PDI re-oxidation (Hudson et al. 2015; Lenzen 2017). At the same time, high H$_2$O$_2$ levels in the ER are a risk factor with potential for beta cell dysfunction, as documented by the fact that an increase of the low expression level of the antioxidative enzyme Prdx4 in rodent beta cells through overexpression goes along with enhanced glucose-induced insulin secretion due to increased proinsulin mRNA transcription and insulin content (Mehmeti et al. 2012, 2014). An increased expression of the peroxiredoxin Prdx4 (Mehmeti et al. 2014, 2012) and the glutathione peroxidases GPx7 and GPx8 (Mehmeti et al. 2017) resident in the ER of many cell types can also improve the antioxidative capacity in the ER of rodent beta cells, though GPx7 and GPx8 are not expressed constitutively in their ER (Mehmeti et al. 2017). At variance from Prdx4, however, expression of these glutathione peroxidases in the ER has no positive effect on proinsulin folding (Lenzen 2017; Mehmeti et al. 2017).

Such a reinforcement of the antioxidative and functional capacity of the ER is thus an option to improve the resistance of the pancreatic beta cell against the challenges of diabetic metabolic stress. Exactly, this is the option which has been exercised by the ER in the human beta cell. This is likely the best possible choice of the human beta cell to protect the ER thereby allowing the human beta cell to remain viable over the long lifespan of the human being. Nevertheless, a Westernized lifestyle that is often associated with insufficient exercise and overweight remains a constant challenge also for a well-protected ER. Even the best protected proinsulin-folding machinery in the human ER can be overwhelmed through a lifestyle, which overcharges the functional capacity of the beta cell.

Though the rough ER compartment is relatively large, comprising one seventh of the beta cell volume (Dean 1973), the H$_2$O$_2$ generation during protein oxidative folding and its consumption during PDI re-oxidation takes place around the proinsulin molecule. The ER as a whole is occupied by the proinsulin-folding apparatus and rather oxidized through limited H$_2$O$_2$ transition into the surrounding cytosol. Due to this, there is no need for a long-distance travel of H$_2$O$_2$ within the ER.

**ER stress under type 1 and type 2 diabetic conditions: a comparison**

When glucose tolerance starts to deteriorate towards the end of the prediabetic phase along with the start of a shrinking of the beta cell mass in the pancreas the pressure on the remaining beta cells through metabolic stress increases steadily. This poses an increased functional demand on the ER in each remaining beta cell, both in the quickly decreasing beta cell mass under T1DM conditions as well as in the gradually decreasing beta cell mass with its continuously increasing dysfunction under T2DM conditions (Lenzen 2017). In particular in the type 2 scenario, this ER stress can induce a process lasting many years or even decades, comprising both an initial phase of compensatory hyperinsulinemia due to insulin resistance and a subsequent phase of a slowly developing hypoinsulinemia due to deterioration of the insulin biosynthetic capacity in the beta cell ER and the gradual decease of the beta cell volume (Lenzen 2017).

This is a scenario which over stresses the remaining beta cells, which are still capable of providing insulin in the diabetic metabolic state and thus gives rise also to more misfolded proinsulin (Lenzen 2017). Therefore, the induction of a beta cell rest through provision of insulin by exogenous administration or by a blockade of insulin release by administration of a K$_{ATP}$ channel opener such as diazoxide (Lenzen 2017) is a feasible measure. The desired result of this resting state of the insulin biosynthesis apparatus can be ideally a cessation of proinsulin folding and thus a reduction of H$_2$O$_2$ generation, thereby reducing the ER stress.

**Conclusions**

The pancreatic beta cell is characterized by a number of unique features (Lenzen 2021). One of these is the extreme vulnerability to ROS toxicity due to its weak antioxidative defense equipment compared to many other better protected cell types (Lenzen 2008, 2017; Lenzen et al. 1996; Tiedge et al. 1997). This is particularly true in states of diabetic metabolic stress. A crucial element of the beta cell demise in the developing diabetic state is the extreme toxicity of the HO radical. All biological structures in the beta cell are potential targets for this toxic action. In contrast, H$_2$O$_2$ causes only limited cellular dysfunction. However, due to its longevity and capability to reach all sites in the cell, H$_2$O$_2$ acts as a spreader for the toxicity of the HO radical reaching virtually all sites of the cell, where H$_2$O$_2$ in the presence of “free iron” can generate the HO radical (Fig. 1). This allows HO to execute its extremely toxic action leading to pancreatic beta cell dysfunction and ultimately to beta cell death in the vulnerable beta cell with its low enzymatic capacity for H$_2$O$_2$ inactivation as firmly documented (Grankvist et al. 1981; Lenzen 2008, 2017; Lenzen et al. 1996; Tiedge et al. 1997).
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Declarations

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