Review

A new cellular function for peroxisomes related to oxygen free radicals?

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Summary. Although in cell biology peroxisomes are still 'young' organelles, it is becoming increasingly clear that they are involved in important cellular functions. Recent results have indicated the presence of the metalloenzyme superoxide dismutase in peroxisomes and the production of superoxide free radicals (O_2^-) in these oxidative organelles. These findings, together with other experimental evidence, point towards the existence of new roles for peroxisomes in cellular active oxygen metabolism, something that has a potential impact in multiple areas of cell biology, particularly in biochemistry and biomedicine.

Key words. Oxygen free radicals; superoxide; superoxide dismutase; peroxisomes; cellular metabolism.

Peroxisomes are subcellular respiratory organelles containing as basic enzymatic constituents catalase, and at least one H_2O_2-producing flavin oxidase[37]. They have the potential to carry out different metabolic pathways, depending upon their origin[15,19,37,38]. These organelles have an essentially oxidative type of metabolism. The biochemical characterization of peroxisomes and their recognition as distinct cellular organelles was accomplished nearly three decades ago by De Duve et al. in animal tissues[3,4]. These organelles were first reported as microbodies, a nonspecific morphological term not implying any biochemical function, but De Duve proposed the term peroxisomes for these organelles because they produced and consumed hydrogen peroxide. Some microscopists still continue to use the term microbody to describe this class of ultrastructurally defined cell organelle bounded by a single membrane. In the following years, after De Duve's characterization of peroxisomes, these organelles were shown to be present in the vast majority of eukaryote cells[20]. Initially, the only function described for peroxisomes was the removal by catalase of toxic hydrogen peroxide produced in the peroxisomal respiratory pathway[4], but in recent years it has been demonstrated that peroxisomes are involved in a range of important cellular functions.

In plant cells, peroxisomes have a well-established role in cellular metabolism, whereas in animal cells their function is less well known. Table 1 shows different functions described so far for peroxisomes in animal cells[9,36]. In plants, peroxisomes have been demonstrated to have a key function in the oxidative photosynthetic carbon cycle of photorespiration, β-oxidation of fatty acids, and the glyoxylate cycle[15,19,37–39] (table 2). Photorespiration is the uptake of O_2 and the formation of CO_2 in the light, resulting from glycolate biosynthesis in chloroplasts and subsequent glycolate metabolism in peroxisomes and mitochondria[38,39]. This complex process occurs by a metabolic pathway that differs from that of dark respiration and appears to be a protective mechanism against light and oxygen toxicity[39]. β-oxidation of fatty acids is thought to take place in most higher plant peroxisomes[15], but is more significant in those organelles from fat-storing tissues of oilseeds. These peroxisomes function to oxidize fatty acids to acetyl CoA through the β-oxidation system; the acetyl CoA is metabolized by the glyoxylate cycle to succinate, and this metabolite is used for gluconeogenesis or synthesis of other biosynthetic intermediates[19,37,38]. In fungi and yeasts, peroxisomes appear to have a certain role in the metabolism of amines, alkanes, alcohols, and oxalate[19]. An important aspect of peroxisomal metabolism is the effect that it can produce upon metabolic pathways in other cell compartments. As indicated by Tolbert et al.[40], peroxisomal metabolism integrates transport systems for peroxisomal substrates, metabolic pathways shared with other compartments (like chloroplasts and mitochondria) and indirect regulation of other metabolic routes by means of the pool size of substrates or products of peroxisomal action. Higher plant peroxisomes have been classified by Huang et al.[19] into four types: unspecialized peroxisomes, glyoxysomes, leaf peroxisomes, and root nodule peroxisomes. The so-called unspecialized peroxisomes occur in a great variety of tissues and contain the basic peroxisomal enzymes, but do not play any known physiological role[19]. The glyoxysomes are specialized peroxisomes, oc-

Table 1. Functions of peroxisomes in animal cells.

Lipid biosynthesis (bile acids, ether-phospholipids, and dolichol)
Lipid β-oxidation
Dicarboxylic acid β-oxidation
Metabolism of glyoxylate
Amino acid metabolism
Metabolism of drugs and chemicals
Metabolic control by peroxisomal oxidases (insulin mechanism)
Catabolism of phytanic acid
Oxidation of polyamines

Table 2. Functions of peroxisomes in plant cells.

Oxidative photosynthetic carbon cycle of photorespiration
Fatty acid β-oxidation
Glyoxylate cycle
Metabolism of ureides
Methanol metabolism (fungi, yeasts)
Oxalate synthesis (fungi)
Amine metabolism (yeasts)
Alkane metabolism (yeasts)

curring in the storage tissues of oilseeds, that contain the fatty acid β-oxidation and glyoxylate cycle enzymes to convert the seed reserve lipids into sugars which are used for germination and plant growth[37,38]. The leaf peroxisomes are specialized peroxisomes present in photosynthetic tissues, and carry out the major reactions of photorespiration[19,38,39]. In root nodule peroxisomes from certain tropical legumes the synthesis of allantoin – the major metabolite for nitrogen transport within these plants – is carried out[35].

In cell biology, peroxisomes are still 'young' organelles whose functions have been only partially explored, and it is a general belief among workers in the field that very probably other functions of peroxisomes in cellular metabolism still remain to be discovered[9,19].

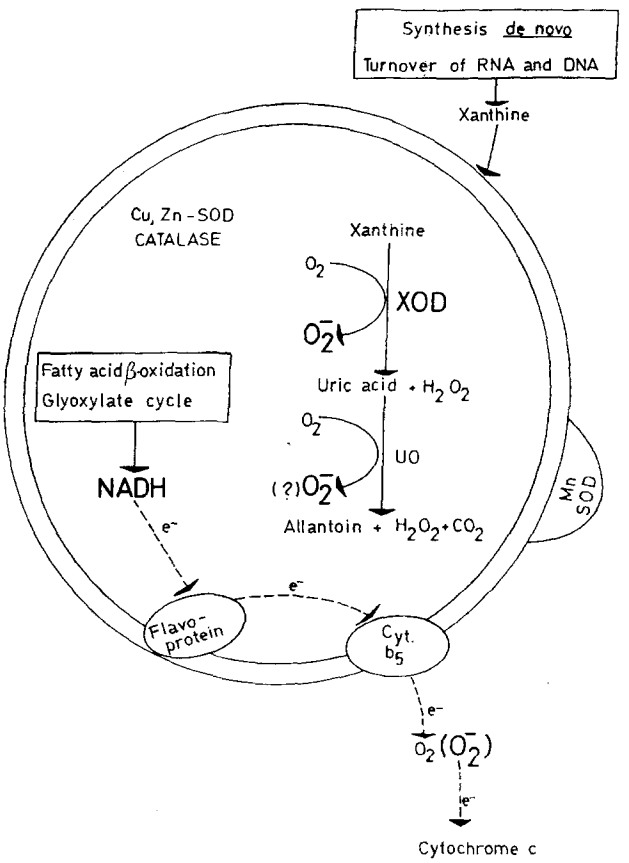
Superoxide dismutase and oxygen free radicals in peroxisomes

Superoxide dismutases (SOD; EC 1.15.1.1) are a family of metalloenzymes that catalyze the disproportionation of superoxide free radicals (O_2^-), produced in different cellular loci, according to the following reaction[13]:

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

The association of superoxide dismutase with peroxisomes passed undetected for a long time, in contrast to the situation for other cell compartments like mitochondria, chloroplasts, and the cytosol, where the presence of superoxide dismutase is well established[13,18]. This situation was probably due to the methodological difficulties involved in the isolation of intact peroxisomes with adequate yields, because of the normally small cellular population of peroxisomes and their considerable lability. Owing to the fragility of peroxisomes, unless very gentle methods of isolation are used and special precautions taken to preserve their integrity, it is very easy to be misled into ascribing a cytosolic localization to superoxide dismutase, when this enzyme might be really present in peroxisomes. In the last few years, the presence of superoxide dismutase has been demonstrated in three kinds of plant peroxisomes: glyoxysomes[31], leaf peroxisomes[6,34], and unspecialized peroxisomes[7]. In general, this cellular location of superoxide dismutase has been established using peroxisomes purified by density-gradient centrifugation techniques, but in the case of leaf peroxisomes, immunocytochemical methods were also used[6]. More recently, the occurrence of superoxide dismutase has also been detected in leaf peroxisomes from other plant species (M. L. Salin, personal communication). Nevertheless, it is known that peroxisomes may show variable enzymatic composition depending not only on the tissue function, but also on substrate induction, stage of development, and genetic and environmental factors[37,38,40]. In this respect, the peroxisomal locus of superoxide dismutase could also change with the tissue metabolism, growth conditions, and during different stages of plant development.

Superoxide dismutases are found in virtually all organisms and have an important role in the protection of aerobic cells against O_2^--derived oxygen toxicity[13]. The production of superoxide free radicals has been clearly demonstrated in certain cellular and subcellular systems including neutrophils, monocytes and macrophages, chloroplasts, mitochondria, microsomes and nuclei[8,18,25]. Very recently, the generation of superoxide radicals in two classes of plant peroxisomes (glyoxysomes and leaf peroxisomes) was reported for the first time[5,33]. The production of O_2^- radicals in soluble fractions from glyoxysomes was found to be induced by either xanthine or hypoxanthine, whereas in membrane fractions the generation of superoxide radicals was stimulated by NADH[33]. The system responsible for O_2^- production in glyoxysomal supernatants was identified as xanthine oxidase (XOD; EC 1.1.3.22), which was found to be present mainly in the soluble fraction of gly-



Hypothetical scheme to explain the production of O_2^- radicals in glyoxysomes, partly based on the model of the glyoxysomal membrane electron transport components proposed by Fang et al.[10].

In the soluble fraction (matrix) of glyoxysomes, xanthine oxidase (XOD) is responsible for the generation of superoxide radicals although the O_2^--producing capacity of urate oxidase (UO) cannot be ruled out since the production of O_2^- was detected in vitro in the enzymatic reaction of urate oxidase by spin trapping ESR[33]. The glyoxysomal electron transport system consists of a flavoprotein NADH reductase which transfers electrons to cytochrome b_5[10]. The fact that when NADH was used as metabolic inducer in glyoxysomal pellets the cytochrome c reduction was inhibited by SOD, suggests that cytochrome b_5 could perhaps use O_2 as an electron acceptor with the production of O_2^- as an intermediate which would eventually bring about the final reduction of the exogenous acceptor cytochrome c.

oxysomes, while in membranes the generation of O_2^- could be a consequence of the NADH-dependent electron transport system of glyoxysomal membranes[10] (fig. 1). In these peroxisomes, two different superoxide dismutases were localized: Cu, Zn-SOD in the organelle matrix and Mn-SOD in the membrane[32]. This different location of SODs appears to be logical from a physiological point of view, considering the O_2^--producing sites found in these peroxisomes and shown in figure 1.

In leaf peroxisomes, where only a Mn-containing SOD is present[34], NADH induced the production of O_2^- radicals in membranes, but in the soluble fraction no generation of superoxide was observed on incubation with xanthine, although xanthine oxidase was found to be located predominantly in the matrix of leaf peroxisomes[5]. The failure of xanthine to induce O_2^- generation was probably due to the inability to fully suppress the endogenous Mn-SOD activity by inhibitors which were inactive against xanthine oxidase. The generation of superoxide radicals in glyoxysomes[33] and leaf peroxisomes[5] suggests that O_2^- production could be a common metabolic property of plant peroxisomes.

Activated oxygen species produced in peroxisomes: a role in cell injury?

In the last few years, superoxide free radicals and other reactive oxygen intermediates derived therefrom have been implicated in the toxic mechanism of a wide range of diseases and tissue injuries including inflammatory diseases, cancer, atherosclerosis, emphysema, many types of liver injury, photosensitization, diabetes, cataract, retrolental fibroplasia, aging, cardiovascular disorders, radiation damage, and the reoxygenation injury following reperfusion of ischemic tissues[11, 17, 18, 22, 26, 30].

Although the generation of superoxide radicals in purified animal peroxisomes has not yet been investigated, the presence of xanthine oxidase in peroxisomes from rat liver, and beef liver and kidney, has been recently demonstrated using cytochemical and biochemical techniques[1]. The production of O_2^- radicals in plant peroxisomes by endogenous xanthine oxidase[33] suggests a role for animal peroxisomes in the post-ischemic injury following reperfusion, which has not been taken into account until now. One of the more commonly accepted mechanisms for post-ischemic damage involves the interconversion of soluble xanthine dehydrogenase into the O_2^--generating xanthine oxidase as a result of ischemic metabolic conditions[12, 21]. In this context, oxygen free radicals produced in animal peroxisomes by xanthine oxidase could also be involved in the post-ischemic tissue injury. This peroxisome-derived toxic mechanism could be parallel to that proposed by Turrens et al., consisting of an enhanced production of O_2^- radicals by mitochondria during reoxygenation of ischemic tissues[41].

Peroxisome proliferation and oxidative stress

Some xenobiotics, including hypolipidemic drugs and certain phthalate-ester plasticizers, when fed to rodents and certain primates, induce the proliferation of the peroxisomal population as well as the activity of some enzymes of these organelles, particularly the H_2O_2-producing acyl-CoA oxidase[29]. Hypolipidemic drugs (clofibrate, nafenopin, Wy-14643, BR-931, tibric acid, etc.) are a class of compounds used for the treatment of high levels of blood lipoproteins (hyperlipidemia)[27]. Some of these peroxisome proliferators, like clofibrate (ethyl-α-p-chlorophenoxyisobutyrate) are proven carcinogenic agents in animals, probably acting by a mechanism involving a single biological receptor or a set of receptors[28]. The determination of enzymatic activities in peroxisomes purified from plants treated with clofibrate showed, apart from the proliferation of the peroxisomal population, a significant drop in catalase and Mn-SOD which was simultaneous with a rise in H_2O_2-producing acyl-CoA oxidase and O_2^--producing XOD[23]. Under the same conditions, an increase in the NADH-dependent O_2^- production by peroxisomal membranes was observed[23]. This implies an overproduction of oxygen free radicals in peroxisomes as a result of clofibrate treatment, and strongly suggests the participation of activated oxygen species (O_2^-, H_2O_2, and possibly ·OH) in the molecular mechanism by which this hypolipidemic drug exerts its toxic effect in plants and perhaps also in animals.

Recent electron microscope studies on the senescence of plant tissues have shown an increase in the number of peroxisomes coinciding with the appearance of symptoms of cell degeneration[7]. This implies the existence of a relationship between peroxisome proliferation and plant senescence. On the other hand, the possibility has been suggested that sustained oxidative stress resulting from the continued proliferation of peroxisomes might serve as an initiator and promoter in carcinogenesis, with the participation of highly reactive oxygen free radicals[16, 28]. In fact, the involvement of oxygen free radicals in the general mechanism of carcinogenesis has been postulated and there is expanding evidence linking oxy radicals with tumor promotion in several organs[17, 22, 30].

Metal toxicity

As far as metal toxicity is concerned, the effect of high nutrient levels of copper on the activity of a number of metalloenzymes present in peroxisomes was studied in leaves of two pea varieties with different sensitivities to copper. Results obtained showed that the peroxisomal Mn-SOD activity was considerably higher in Cu-tolerant than in Cu-sensitive plants, and the activity of catalase was also increased in peroxisomes of Cu-tolerant plants[24]. This implies the involvement of reactive oxygen intermediates in the mechanism of copper toxicity, and a function for peroxisomal Mn-SOD in the molecular mechanisms of tolerance to copper.

Conclusion

All these apparently isolated pieces of experimental evidence point towards the existence of hitherto unknown

functions for peroxisomes in cellular metabolism related to oxygen free radicals. Moreover, under certain conditions of depressed catalase activity or acatalasemia, superoxide free radicals (O_2^-) generated in peroxisomes could react with H_2O_2, which is quite abundant in these organelles, by the metal-catalyzed Haber-Weiss reaction [13, 18], and give rise to the extremely reactive hydroxyl radicals (·OH). This strongly oxidizing species, if not controlled, could seriously damage biological membranes and react with practically all the compounds present in biological systems [8, 18]. It must be said that apart from the bactericidal activity of leukocytes, where O_2^- plays an important role [18], activated oxygen species are usually considered as undesirable products of oxygen metabolism produced by the leakage of electrons from oxidative reactions towards oxygen [2, 13, 14]. Nevertheless, oxygen free radicals could also have some useful function(s) in the metabolism of peroxisomes. In this respect, the peroxisomal superoxide dismutase could modulate the O_2^--dependent site-specific formation of ·OH radicals so that they could be effectively used for reactions of these oxidative organelles which require strong oxidizing agents. Further studies are necessary in order to determine those oxidative reactions of the peroxisomal metabolism where these highly reactive oxygen radicals could be used beneficially.

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