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New perspectives on the role of amine oxidases in physiopathology

Review Article

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Summary. In the paper here presented we summarize some results obtained in our laboratory in the last few years on new structural and functional aspects of some amine oxidases (AOs), which have to be taken into consideration in defining new strategies of controlling the cellular physiopathology.

In particular, the ability of Cu-AO purified from vegetal sources or from bovine serum to bind different cellular targets inducing in them conformational as well as chemical modifications are described and the consequences of this interaction on cellular functions are discussed. This is the case of the protective effect of Cu-AO against the damage induced by free radicals, cell enrichment with Cu-AO, induction of cataract and the leukocyte-endothelia interaction.

The role of Cu and FAD-amine oxidases related as to the protection or damage of cells is also discussed. In this context the involvement of MAOs in the modulation of the mitochondrial functions and in the induction of apoptosis is described and some aspects of the molecular mechanism of AO inhibition by H_2O_2 and metronidazole analyzed.

Keywords: Amine oxidase – Hydrogen peroxide – Polyamine – Aldehyde – Apoptosis – Histamine

Introduction

Amine oxidases (AOs) are a large class of Cu or FAD-containing enzymes (Cu-AO, FAD-AO), distributed among all living organisms, able to oxidize biogenic amines to hydrogen peroxide and aldehydes (McIntire and Hartman, 1992).

A plethora of physiological functions, sometimes in contrast ones with the others, has been ascribed for AOs. Although the exact molecular mechanism of their biological activity is not well defined, a role of these enzymes in various cellular processes throught the action of either their substrates and their reaction products has been postulated (Averill-Bates et al., 1994; Malorni et al., 1998; Maccarrone et al., 2001; Thomas and Thomas, 2001). In fact, evidences have been accumulated on the physiological relevance of polyamines, hydrogen peroxide and aldehydes in the cellular death, proliferation and differentiation. High level of polyamines are typical of actively proliferating cells (Heby, 1989); hydrogen peroxide, depending on its concentration, is able to impair cell growth and proliferation, or to regulate the gene expression and the transduction of cellular signals (Sen and Packer, 1996); cytotoxicity of aldehydes has also been observed, probably due to the inhibition of nucleic acid and protein synthesis (Bachrach et al., 1967; Bachrach, 1970).

In the paper here presented we summarize some results obtained in our laboratory in the last few years on new structural and functional aspects of some AOs, which suggest new biological mechanisms of action of these enzymes.

Protective effect of AOs against heart damage induced by histamine or free radicals

Cardioprotection properties of Cu-AO isolated from animal or vegetal sources have been observed in two different model systems.

In isolated guinea pig hearts, protective effects of Cu-AO purified from pea seedling (P-DAO) against

cardiac anaphylaxis have been observed (Mondovì et al., 2002, in press). The hearts isolated from animals sensitized with crystallized ovoalbumin, were set up in a Langendorff apparatus, challenged with ovoalbumin (OV) and analyzed for the typical changes in cardiac functions associated with a sudden release of histamine in the coronary effluent. The addition to the perfusion fluids of vegetal Cu-AO, free or immobilized on CNBr-activated Sepharose 6 MB, blocked the OV-dependent increase of the rate and the strength of heart contraction and reduced the release of histamine in the coronary effluent. These results indicate that vegetal Cu-AO might be an useful tool to control cardiac anaphylactic reactions, through its efficient histaminase activity.

Cu-AO purified from bovine serum (BSAO) also protected rat hearts, mounted on a modified Langendorff device, from the injury induced by free radicals generated in solution by electrolysis. Native BSAO, added to the perfusion fluid, prevented in a dose-dependent manner the radical-induced alteration of left ventricular pressure, coronary flow, and heart beats (Mateescu et al., 1997). No protection was afforded by heat denaturated enzyme. As either native than heat denaturated BSAO were able to scavenge in vitro directly free radicals generated in solution by electrolysis; the cardioprotective effect of BSAO, observed in ex vivo experiments, might due to a possible binding on specific cellular sites rather than to radical scavenging properties.

The ability of BSAO to bind cellular effectors is further substained by other studies performed by our group. In a neuroblastoma cell line (N1E-115) a voltage-clamp study revealed that native BSAO, but not heat denaturated enzyme, in a time dependent manner, significantly amplified the outwardly rectifier K^+ channel current. The effect of BSAO was not mediated via the antioxidant or free radical scavenging properties since conditions permitting the generation of oxygen derived free radicals were not present in our experiments (Wu et al., 1996).

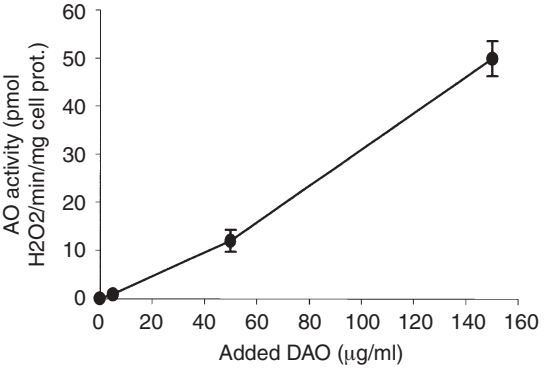

Fig. 1. Enrichment of K562 cells in AO activity. K562 cells (1×10^6 cells/ml) were exposed for 6 hrs in PBS + glucose to different concentrations of P-DAO. Means +/− SD of one assay in triplicate

Cell enrichment with Cu-AO

We also observed that enrichment of cells with Cu-AO may occur as a consequence of the binding properties of the animal or vegetal enzymes to the cellular membrane. In fact, an electron microscopic study performed on cultured hepatocytes exposed to BSAO-gold complexes has revealed that the BSAO binds cells in small clusters of gold granules, not bound in a specialized region of the plasma membrane; it is then internalized by endocytosis (Dini et al., 1991). In this model system, however, no enhancement of Cu-AO activity was observed, probably due to the high endogenous Cu-AO activity of the hepatocytes. To prove that the internalized enzyme can maintain its catalytic activity, we performed a study on a cultured cellular model which did not have appreciable Cu-AO activity, and used P-DAO which more efficiently than BSAO oxidizes biogenic amines (Marcocci, et al., 2001a). Homogenates of K562 human leukemia cells treated with P-DAO were able to efficiently oxidize putrescine (the best substrate for this AO) indicating that the exposure of cells to exogenous vegetal Cu-AO resulted in cellular enrichment with catalitically active enzyme. The enrichment resulted higher in RPMI 1640 without fetal calf serum (FCS) than in PBS or RPMI 1640 with 10% serum. It was affected by the exposure time and was dependent on the concentration of added enzyme (Fig. 1). The data we obtained indicate that purified BSAO or P-DAO may enter the cells. The consequence of Cu-AO enrichment on cellular functions is currently under investigation in our laboratory.

Modification of proteins by AOs

The interaction of Cu-AO with target molecules may also induce in them conformational as well as chemical modifications. In fact, BSAO-mediated oxidation of amino groups of some peptides as well as of proteins has been observed. This is the case of poly-lysines at various molecular weight as well as of lysozyme and

ribonuclease A (Wang et al., 1996). In agreement with these results the oxidation by BSAO of aminoexyl groups of some chromatographic supports has also be demonstrated, which may explain the irreversible retention of the enzyme during preparative affinity chromatography on aminoexyl (AH)-Sepharose (Befani et al., 1998).

The Cu-AO-mediated modification of amino groups in cellular components may be physiopathologically relevant. A direct correlation has been observed between the level of cellular Cu-AO activity and the opacity grade of lens and, the post-translational modification of lens proteins by AOs has been claimed to be responsible for the induction of cataractogenesis (Befani et al., 1999).

In this context it should be pointed out that recently a surface expressed endothelial glycoprotein (VAP-1) has been identified as a Cu-AO. In fact, the enzyme is sensitive to the inhibition of semicarbazide, active against substrates such as benzylamine and methylamine and has a surprising high identity with BSAO (85%) (Smith et al., 1998). It has been postulated that the AO catalytic activity of VAP-1 against surface bound amines, such as N-termini of proteins, NH_2-containing aminoacid side chains, amino sugars etc., may be crucial in the process of leukocyte adhesion and extravasion (Salmi et al., 2001).

AOs as modulators of mitochondrial functions

Taking into consideration that mitochondria are important points in the pathways of cellular death we have analyzed the role of mitochondrial AO (MAO) on mitochondrial functions. In particular, we analyzed the consequences of the catalytic activity of MAOs on the induction of membrane permeability transition (MPT) in rat isolated hepatic mitochondria (Marcocci et al., 2001b). The data we obtained indicate that octopamine as well as benzylamine, substrates of MAO A and MAO B respectively, induced at concentrations of 50–100 μM the matrix swelling and the collapse of membrane potential. However, the lack of correlation between the onset of membrane permeability transition and the production of H_2O_2 with higher concentration of substrates (1–2 mM), suggests that the disruption of mitochondrial membrane function might occur also through MAO-independent mechanisms.

A role of MAO inhibitors on the maintenance of mitochondrial homeostasis and the induction of the cellular apoptosis has also been demonstrated. Treatment of melanoma cell line (M14) with clorgyline, a specific inhibitor of MAO A, for 48 hrs decreased in a dose dependent manner the induction of apoptosis as well as the mitochondrial membrane depolarization upon serum deprivation. In the same experimental conditions deprenyl, a MAO B inhibitor, was ineffective probably due to the low MAO B content in the mitochondria of M14 cells; pargyline, at concentrations higher than clorgyline, in accordance with its low affinity for MAO A, partially protected the cells (Malorni et al., 1998). Althought the correlation between inhibition of MAO-A and B activity and the protective effect of MAO inhibitors indicate that the catalytic activity of MAO is important in the process, an effect of the MAO-A inhibitors by themselves can not be excluded.

Molecular mechanism of AO inhibition by H_2O_2 and metronidazole

The modulation of cellular Cu-AO activity may be crucial for the regulation of cellular growth. In particular, mucosal Cu-AO activity has been claimed to be important as proliferation terminating factor. In fact, administration of the Cu-AO inhibitor aminoguanidine has been shown to increase in rats the rate of tumor growth (Kusche et al., 1988). As a consequence it may be important to identify factors that may affect the cellular Cu-AO activity. In this context, we have analyzed the effect of metronidazole on various AOs. Metronidazole, an antibacterial and antiprotozoal drug, has a relatively low toxicity when used topically, but can induce acute toxicity, mutagenesis, carcinogenesis and co-carcinogenesis when used for the treatment of gastroenteritis due to *Entamoeba histolytica* (Davis et al., 1992). Furthermore, the treatment of patients with metronidazole for prolonged periods has also been shown to cause some neurotoxic effects such as dizziness, vertigo, headache and, very rarely, convultions, incoordination and ataxia (Tracy and Webster, 1996). We observed that the drug was a non-competitive inhibitor of Cu-AOs from man, rabbit, rat intestine as well as of swine kidney (Befani et al., 1995). It also was a non-competitive inhibitor of purified bovine brain MAO (Befani et al., 2001). The inhibitory effect of metronidazole on Cu-AO and MAO may explain the clinical adversal effects of this drug and suggests that the use of metranidazole in therapy

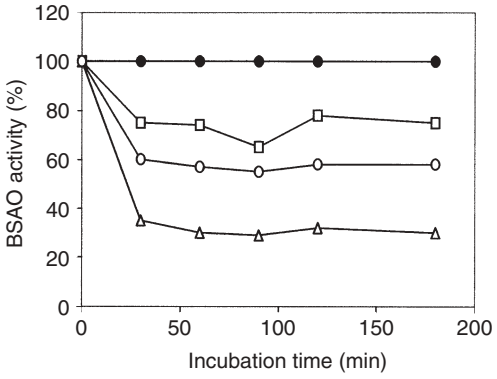

Fig. 2. Effect of catalase and exogen H_2O_2 on inactivation of BSAO by spermine. 0.6 μM BSAO in 0.1 M potassium phosphate buffer, pH 7.2, was incubated at 37°C with spermine without stirring or shaking. The same experiment was performed in the presence of 100 U/ml catalase or 0.5 mM H_2O_2. Aliquots of the solutions, diluited to approximately 0.06 μM BSAO were tested for activity with 5.0 mM benzylamine. (●) 0.5 mM spermine + catalase or 0.5 mM H_2O_2 (□); 0.5 mM spermine; (○) 0.5 mM spermine + 0.5 mM H_2O_2; (△) 1.0 mM spermine. Means of three experiments

should be carefully controlled especially when the drug is administred for a long time.

A modulation of Cu-AO activity may also be achieved through an autoregolatory process mediated by H_2O_2. Inhibition by H_2O_2 of Cu-AO from pig kidney was already described (Mondovì et al., 1967) and more recently we have characterized the molecular mechanism of this inhibition on BSAO (Pietrangeli et al., 2000). We observed that the enzyme, reduced by excess amines under limited turnover conditions, is over 80% inactivated by H_2O_2 upon oxygen exhaustion. In the inactivated protein the cofactor TPQ resulted irreversibly reduced and with decreased ability to bind reagents of the carbonyl group, such as phenylhydrazine or semicarbazide. Figure 2 shows the time course of BSAO inactivation by an eccess of spermine. The presence of catalase fully protected the enzyme, thus confirming that H_2O_2 produced during the catalytic reaction was responsible for the inactivation. Exogenously added H_2O_2 was able to inhibit the enzyme only when, in the presence of the substrate, the cofactor was in the reduced status.

Among the possible factors responsible for the low catalytic activity reported for VAP-1 (Smith et al., 1998) the inhibition mediated by H_2O_2 in condition of low oxygen concentration may be hypothized. Also the H_2O_2 produced by the catalytic activity of mucosal Cu-AO may determine its inhibition and concurs as a risk factor for several pathologies of intestine such as inflammation, ulcerative colitis and tumors.

Discussion

The results above reported suggest some new biological functions of AOs, in addition to the their classical one as oxidases of biogenic amines and sources of H_2O_2 and aldehydes. In particular, we observed the ability of Cu-AO purified from vegetal sources or from bovine serum to bind different cellular target and thus modifing cellular functions. This is the case of the activity of BSAO on specific sites of rat hearts challanged with free radicals generated in solution by electrolysis, the effect on the outwardly rectifier K^+ channel current, and more specifically the modifications of amino residues of peptides and proteins such as poly-lysine, lysozime and ribonuclease A and eventually lens proteins and surface bound amines of leukocytes. Important consequences of these interaction may be the protection of rat hearts from damages induced by oxygen radicals, the induction of cataract and the regulation of the process of leukocytes adhesion and extravation. The binding of Cu-AO to cell components is also responsible of the enrichment of cells with AO activity whose consequences on the cellular function is currently under investigation in our laboratory.

As concern the more classical role of AO as sources of H_2O_2 our data indicate the importance of MAO in the homeostasis of mitochondria and consequently in the pathways of cell death for apoptosis. In fact, the catalytic activity of MAO on different substrates has been shown to induce MPT in isolated rat liver mitochondria and to be involved in the induction of apoptosis in M14 cells; however, in both the systems a role of MAO substrates and inhibitors by themself can not be excluded.

In our studies we also define a regulatory activity of H_2O_2 derived from AO catalyzed reaction on the Cu-AO activity itself, and described the inhibitory effect of metronitrazole on Cu and FAD-AO which can explain the neurological and carcinogenic side effects of this antiprotozoal and antibacterial drug.

Furthermore our studies also indicate the ability of Cu-AO purified from vegetal sources to protect from cardiac anaphylaxis through the reduction of the concentration of released histamine and suggest a possible therapeutical use of this enzyme.

Conclusion

All the data above reported support the crucial role of AOs in the cellular metabolism and delineate new mechanisms of action of these enzymes, that have to be taken into consideration in defining new strategies of controlling the cellular physiopathology.

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