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ORIGINAL ARTICLE

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Effect of neurotoxic metal ions on the proteolytic activities of the 20S proteasome from bovine brain

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Abstract The effect of oxidative stress induced by neurotoxic metal ions on the properties of the brain 20S proteasome or multicatalytic proteinase complex (MPC) has been studied. Exposure of the 20S proteasome to increasing amounts of Fe(III), Fe(II), Cu(II) or Zn(II) affects its main hydrolytic activities: trypsin-like (T-L), chymotrypsin-like (ChT-L), peptidylglutamyl-peptide hydrolase (PGPH), branched-chain amino acid preferring (BrAAP) and caseinolytic activities, although in different ways. T-L activity showed gradual activation by both iron ions but inhibition by Cu(II) and Zn(II). ChT-L and PGPH activities were inhibited whereas BrAAP activity was widely activated by all the tested metal salts except for zinc ions. Moreover, the exposure to ferrous salt increased the degradation rate of casein. The functional effects appear to be linked to oxidation-induced modifications, as demonstrated by an increase of carbonyl groups following the exposure to metal ions. In addition, modifications induced by ferrous salt on the catalytic subunits were also supported by western blot analyses performed using anti-X, anti-Y and anti-Z antibodies. The results obtained clearly indicate that metal-catalyzed oxidation strongly affects the functions of the brain 20S proteasome, even though the catalytic subunits seem to be differently influenced by oxidative phenomena.

Keywords 20S proteasome · Metal ions · Metal-catalyzed oxidation · Neurodegenerative disorders

Abbreviations *AD* Alzheimer's disease · *AGE* advanced glycation end-products · *BrAAP* branched-chain amino acid preferring · *ChT-L* chymotrypsin-like · *CNS* central nervous system · *MCA* 7-methylcoumarylamide · *MCO* metal-catalyzed oxidation · *MPC* multicatalytic proteinase complex · *2NA* 2-naphthylamide · *NFT* neurofibrillary tangles · *pAB* *p*-aminobenzoate · *PGPH* peptidylglutamyl-peptide hydrolyzing · *pNA* *p*-nitroaniline · *PVDF* poly(vinylidene difluoride) · *ROS* reactive oxygen species · *SNAAP* small neutral amino acid preferring · *SOD* superoxide dismutase · *T-L* trypsin-like · *Z* benzyloxycarbonyl

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by irreversible cognitive and physical deterioration [1]. It is a multifactorial pathology and several biochemical mechanisms seem to be involved in its etiology. Among them, recently, an important role has been attributed to the oxygen-derived free radicals also known as ROS (reactive oxygen species), which induce oxidative stress in the brain cells [2]. Experimental evidence supports this hypothesis, since in the AD-affected brain there have been observed increased levels of metals (as Fe, Cu and Hg) [3, 4] able to stimulate free radical generation, increased lipid peroxidation and decreased polyunsaturated fatty acids, increased protein and DNA oxidation, diminished energy metabolism and decreased cytochrome *c* oxidase, advanced glycation end-products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase-1 and superoxide dismutase-1 (SOD-1) in neurofibrillary tangles (NFT), and AGE, hemeoxygenase-1 and SOD-1 in senile plaques [2]. In addition, the amyloid β-peptides contribute to the oxidative environment typical of the AD brain because they are able to generate free radicals as a consequence of their aggregation in the senile plaques [4] which are, together with NFT (see below), the major morphological characteristics of the AD brain. The latter arise from intraneuronal accumulation of paired helical filaments mainly constituted by an

abnormal tau protein, with some more mature inclusions being found to contain ubiquitin. This finding suggests a role for the ubiquitin/proteasome system in the intraneuronal events leading to the neuritic plaques and NFT formation. The proteasome is a barrel-shaped proteinase particle responsible for the degradation of intracellular proteins [5, 6, 7, 8]. Proteasomes consist of a 20S catalytic core (MW = 700 kDa) which associates with regulatory particles, e.g. PA28 (or 11S) and the 19S complex (or PA700). The 20S core or multicatalytic proteinase complex (MPC) is composed of four stacked heptameric rings, each of them containing seven different subunits. The α-subunits are assembled in the two outer rings while the two inner rings are composed of seven different β-subunits that harbour the catalytically active subunits [9, 10]. The mammalian MPC shows at least five distinct peptidase activities, which, on the basis of the residue in the P1 position, are classified as: chymotrypsin-like activity (ChT-L), trypsin-like activity (T-L), peptidylglutamyl-peptide hydrolyzing (PGPH), branched chain amino acid preferring (BrAAP) and small neutral amino acid preferring (SNAAP), cleaving bonds on the carboxyl side of hydrophobic, basic, acidic, branched chain and small neutral amino acids, respectively [7]. Work with irreversible inhibitors has indicated that the β-subunits X, Y and Z of the mammalian proteasome are associated with expression of the ChT-L, PGPH and T-L activities, respectively [11]. On the other hand, it is still unclear if the BrAAP and SNAAP activities have to be ascribed to one or more cooperating β-subunits.

In this paper we investigate the role of metal-catalyzed oxidation on the biological activity of the brain 20S proteasome, looking at the effects of exposure to increasing amounts of metals which have been found in altered levels in AD brains [3, 4]. The enzyme was treated with iron and copper, which are able to generate ROS [2], and zinc, which seems to favor β-amyloid aggregation [12, 13]. The results obtained indicate that the tested metals strongly affect some of the proteasome proteolytic activities. These effects are mediated by oxidative phenomena, reinforcing the hypothesis of a key role for oxidative stress in the etiology of AD.

Materials and methods

Materials

Bovine brain was obtained from the local slaughterhouse. It was rapidly frozen in liquid nitrogen and maintained at –70 °C. The anti-subunit X, Y and Z antibodies were purchased from Affiniti Research Products (Mamhead, Exeter, UK). The Oxidized Protein Detection Kit (OxyBlot) was purchased from Appligene-Oncor (Strasbourg, France). Substrates for assaying the MPC proteolytic activities (Z-GGL-pNA, Z-LLE-2NA, Z-LLR-2NA, Z-GPALG-pAB, Z-GPAGG-pAB; Z = benzyloxycarbonyl, pNA = *p*-nitroaniline, 2NA = 2-naphthylamide, pAB = *p*-aminobenzoate) were the kind gift of Prof. M. Orlowski. Other reagents were obtained from Sigma.

Isolation and purification

Isolation and purification of MPC were carried out following an experimental protocol very similar to that previously utilized for MPC isolation from other bovine organs [14, 15] and essentially based on fractionation from 40 to 60% in ammonium sulfate, an ion exchange chromatography and two gel-filtration columns which favor the removal of lower molecular weight contaminants. A higher degree of purification was obtained by adding a hydrophobic interaction chromatography step, which seems to improve the separation of MPC from the copurifying chaperonine Hsp90.

Determination of proteolytic activities

The ChT-L, T-L, PGPH, BrAAP and SNAAP activities of the brain 20S proteasome were determined as reported previously [14, 16, 17], using Z-GGL-pNA, Z-LLR-2NA, Z-LLE-2NA, Z-GPALG-pAB and Z-GPAGG-pAB, respectively, as substrates. Aminopeptidase N (EC 3.4.11.2), used for the coupled assay utilized for detecting the BrAAP and SNAAP activities [17], was purified from pig kidney, as reported elsewhere [18, 19].

Exposure to metal ions

$CuSO_4 \cdot 5H_2O$ (Merck), $Fe(NO_3)_3 \cdot 9H_2O$ (Aldrich), $ZnCl_2$ and $FeSO_4 \cdot 7H_2O$ (J. T. Baker) were dissolved in water or in Tris-HCl (50 mM, pH 8.0) and were incubated with the enzyme at 37 °C for different incubation times.

Preliminary assays were performed in order to establish the salt concentration ranges in which the proteasome maintains its activities even if slight variations of pH occur. The ferric salt was used up to 1 mM because it produced a significant pH variation over that concentration. Moreover, influences of the different anions (Cl^-, SO_4^{2-} and NO_3^-) were checked, measuring the effects of three salts of the same metal [$Zn(NO_3)_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$ and $ZnCl_2$] on the ChT-L activity. No differences were observed (data not shown).

Specific substrates were incubated with the enzyme (1.43×10^{-11} M) in the presence of increasing amounts of salts at 37 °C, for different incubation times, in 50 mM Tris-HCl (pH 8.0) in a final volume of 250 µL. The activities were stopped and measured colorimetrically as described above. When SOD and catalase were used, they were added to the reaction mixture at concentrations of 1 mg mL^{-1} and 1.4 mg mL^{-1}, respectively. Control assays were performed in the presence and absence of 0.5 mM metal salts.

Determination of casein degradation by the 20S proteasome

The brain MPC was preincubated at 37 °C with $CuSO_4 \cdot 5H_2O$ (1.0 and 1.5 mM) and $FeSO_4 \cdot 7H_2O$ (1.0 and 2.0 mM) in 50 mM Tris-HCl, pH 8.0. Then 22.5 µg of treated proteasome, dialyzed against Tris buffer in order to remove free metal ions, were incubated with 100 µg of β-casein and 0.05 M Tris-HCl, pH 8.0, in a final volume of 100 µL. The mixture was incubated at 37 °C, and 20 µL aliquots were withdrawn at different times (from 0 to 90 min) and subjected to HPLC on a Hamilton PRP-3 column (4.1×150 mm). Elution was carried out with a linear gradient established between 10 and 50% acetonitrile, containing 0.1% of trifluoroacetic acid, at a flow rate of 1 mL min^{-1}. The rate of casein degradation was determined by measuring the peak height of the casein ($\lambda = 210$ nm) in relation to controls in which the enzyme was not treated with metals [20].

Polyacrylamide gel electrophoresis and western blotting

Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) [21] was run on a 12% acrylamide separating gel. Immunoblot experiments using anti-subunit X, Y and Z antibodies were performed, electroblotting 20 µg of the enzyme, previously run on a SDS gel, onto poly(vinylidene difluoride) (PVDF) membranes

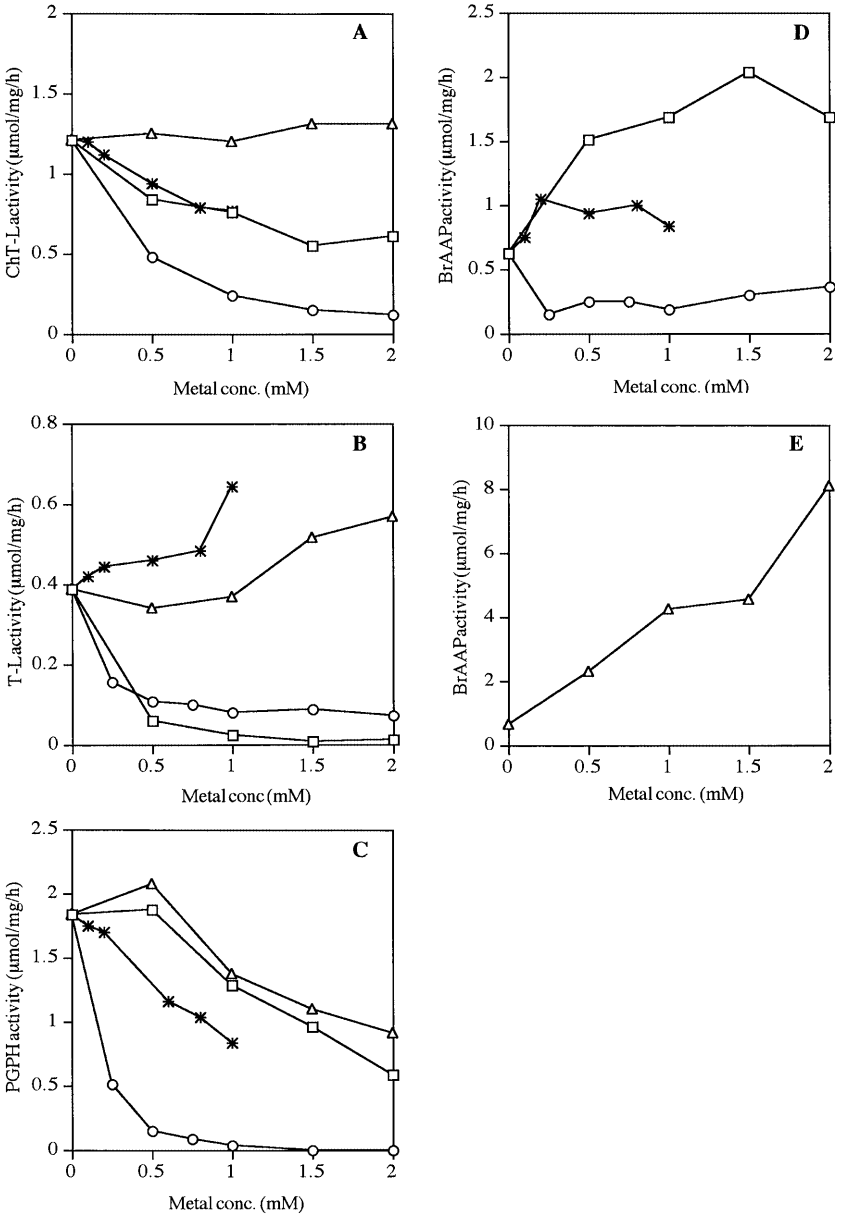

Fig. 1A–E. Effect of increasing concentrations of metal salts on the MPC peptidase activities. 10 µg of 20S proteasome were incubated in 50 mM Tris-HCl, pH 8.0, with synthetic substrates (**A**, Z-GGL-pNA for ChT-L activity; **B**, Z-VGR-pNA for T-L activity; **C**, Z-LLE-2NA for PGPH activity; **D**, **E**, Z-GPALA-pAB for BrAAP activity) in the presence of increasing concentrations of $CuSO_4 \cdot 5H_2O$ (*squares*), $ZnCl_2$ (*circles*), $Fe(NO_3)_3 \cdot 9H_2O$ (*asterisks*) or $FeSO_4 \cdot 7H_2O$ (*triangles*) at 37 °C for 30 min. The activities were measured colorimetrically as previously described [16]. Each data point is the mean value ± 4% standard error and comes from four separate determinations. Error bars are not shown because they correspond to the size of the symbols

(Millipore, Bedford, Mass., USA) according to Towbin et al. [22] and Burnette [23].

Immunoblot detection of carbonyl groups was performed with the OxyBlot oxidized protein detection kit, according to the manufacturer. Briefly, 12 µg of metal-exposed enzyme were incubated for 15 min at room temperature with 2,4-dinitrophenylhydrazine (DNPH) to form the dinitrophenylhydrazone carbonyl derivate and separated on 12% SDS/PAGE [24, 25, 26]. The modified proteins, blotted on a PVDF membrane, were revealed by anti-DNP antibodies. The immunoblot detection was carried out with an ECL (Enhanced ChemiLuminescence) western blotting analysis system (Amersham-Pharmacia-Biotech) using peroxidase conjugated anti-rabbit or anti-mouse secondary antibodies.

Results and discussion

Over the past few years a large number of publications have reported that alterations in cerebral metal ion concentration and oxidative metabolism play a fundamental role in many neurological diseases, like AD, Parkinson's disease, Huntington's disease and prion diseases [3]. It seems that neurodegeneration results from increased oxidative damage, which could be related to a modification in neuronal metal ion homeostasis. Furthermore, increasing evidence suggests that oxidative stress may cause changes in the central nervous system (CNS) proteasome functionality, even though these effects are CNS region-specific and they occur in many, but not all, neurodegenerations; in fact, it has been observed that in AD the proteasome does not undergo oxidative damage [27, 28]. The 20S proteasome is considered the major proteolytic enzyme involved in the removal of oxidized proteins and its activities are affected by exposure to oxidants such as hydrogen peroxide, peroxynitrite or hypochlorite [29, 30, 31]. In

Table 1. Effect of 0.5 mM metal salt concentrations on the 20S proteasome peptidase activities[a]

Component	Activity (μmol mg^{-1} h^{-1})				
	Metal salt concentration (mM)				
	0	0.5			
		Cu(II)	Zn(II)	Fe(II)	Fe(III)
ChT-L	1.207 ± 0.048	0.839 ± 0.034	0.471 ± 0.019	1.243 ± 0.050	0.938 ± 0.038
T-L	0.386 ± 0.015	0.057 ± 0.002	0.105 ± 0.004	0.337 ± 0.013	0.459 ± 0.018
PGPH	1.835 ± 0.073	1.872 ± 0.075	0.149 ± 0.006	2.068 ± 0.083	1.151 ± 0.046
BrAAP	0.620 ± 0.025	1.506 ± 0.060	0.246 ± 0.010	2.273 ± 0.091	0.930 ± 0.037

[a]20S proteasome (10 μg) was incubated in 50 mM Tris-HCl, pH 8.0, with synthetic substrates in the presence of 0.5 mM of $CuSO_4 \cdot 5H_2O$, $ZnCl_2$, $Fe(NO_3)_3 \cdot 9H_2O$ or $FeSO_4 \cdot 7H_2O$ at 37 °C for 30 min. Control experiments were performed in the absence of metal salts. Data represent the activities expressed in μmol mg^{-1} h^{-1} in the presence and absence of 0.5 mM metal salts. The activities were measured colorimetrically as previously described [16]. Data are mean values ± standard error for four separate determinations

this study we have examined the effect, on the 20S proteasome purified from bovine brain, of the oxidative stress induced by metals found at enhanced levels in various neurodegenerative disorders. For example, Atwood et al. [3] reported that in cerebral amyloid deposits in AD the amounts of Zn, Cu and Fe are highly enriched (Cu around 0.4 mM, Zn and Fe around 1.0 mM).

The effect of metal ions on the ChT-L, T-L, PGPH and BrAAP activities of the 20S proteasome is shown in Fig. 1 and Table 1. The ChT-L activity was inhibited by all the cations except for $FeSO_4 \cdot 7H_2O$, which, on the contrary, induced a slight activation (Fig. 1A). The T-L component was completely inactivated by Zn and Cu salts, whereas the presence of both iron salts produced a gradual activation (Fig. 1B). The PGPH activity was inhibited by all the assayed metals, each with a specific inhibition constant (Fig. 1C). The BrAAP activity increased significantly with the exposure to all the metal salts [especially Fe(II), see Fig. 1E] with the exclusion of $ZnCl_2$, which did not affect this activity (see Fig. 1D).

The effect of 0.5 mM metal salts on the proteasome activities were also monitored over time; in Fig. 2 the effects of 0.5 mM $CuSO_4 \cdot 5H_2O$ on the ChT-L and BrAAP activities are shown. The effect of inhibition or activation induced by Cu(II) was invariant over 90 min, demostrating that the proteasome was not able to recover its initial activities (Fig. 2A, B).

The metal concentrations indicated in Fig. 1 are referred to the added salts and not to the free metal ion concentration available in solution, which, in principle, should be lower than the total added salt concentration, owing to unspecific buffering capacity of proteins and other metal chelating systems [32]. Therefore, the observed change in the proteasome catalytic activities should be induced by a free metal ion concentration lower than the actual added salt.

In order to evaluate if the observed effects came from metal-catalyzed oxidation or metal binding to the enzymatic molecule, we assayed the influence of metal ions on the proteolytic activities in the presence of reactive oxygen scavengers, such as SOD and catalase.

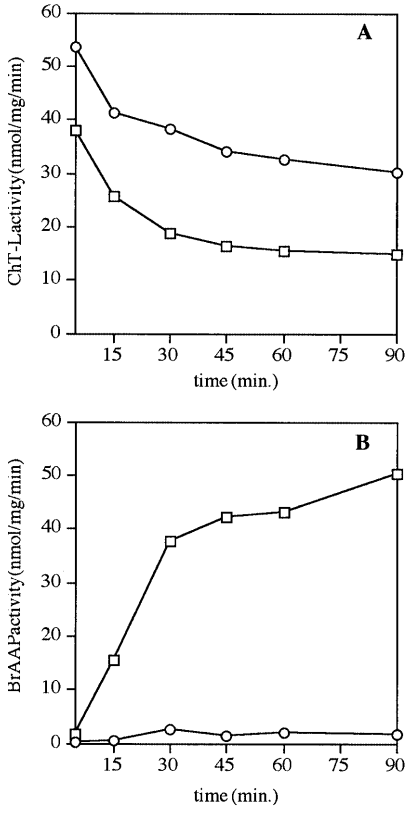

Fig. 2. Effect of 0.5 mM $CuSO_4 \cdot 5H_2O$ on the ChT-L (**A**) and BrAAP (**B**) activities over time. The 20S proteasome was incubated, from 0 to 90 min, with the appropriate substrates in the presence (*squares*) and absence (*circles*) of 0.5 mM $CuSO_4 \cdot 5H_2O$ at 37 °C. Data are affected by ±4% standard error and derive from three separate measurements. For other details, see Materials and methods

Our results showed that, in the presence of SOD and catalase, the activities of the 20S proteasome with all assayed substrates were not affected by the presence of 0.5 mM metal salts. In Table 2 the data relating to ChT-L and BrAAP activities in the presence of 0.5 mM Cu(II) are reported. The same results were obtained using Zn(II), Fe(II) and Fe(III) salts (data not shown).

Table 2. Effect of reactive oxygen scavengers on the ChT-L and BrAAP activities in the presence of 0.5 mM Cu(II)[a]

Component	[$CuSO_4 \cdot 5H_2O$] (mM)	Activity remaining (%)	
			SOD-catalase
ChT-L	–	100	100
ChT-L	0.5	82.8	100.4
BrAAP	–	100	100
BrAAP	0.5	339	81.9

[a]The ChT-L and BrAAP activities were measured in the presence and absence of SOD (1.0 mg mL^{-1}) and catalase (1.4 mg mL^{-1}) and 0.5 mM Cu(II) in a final volume of 250 μL. The mixture was incubated at 37 °C for 30 min. Data are reported as % activity remaining. For other details, see Materials and methods

Figure 3 shows the effect of increasing concentrations of Cu(II) (Fig. 3A) and Fe(II) (Fig. 3B) on the caseinolytic activity of the brain 20S proteasome. Surprisingly, incubation with Cu(II) did not influence the degradation of casein, whereas Fe(II) increased the rate of proteolysis, especially at longer incubation times (60 and 90 min). Probably Cu(II) produced oxidative modifications which were not sufficient to affect the caseinolysis; however, it was able to cause a three-fold increase of the BrAAP activity, measured with a synthetic pentapeptide.

In order to estimate the degree of oxidation of the complex induced by the metal salts, the amount of carbonyl groups was detected by immunoblot analyses using the Oxyblot kit (see Materials and methods). The results obtained, shown in Fig. 4, clearly indicate that increasing amounts of $CuSO_4 \cdot 5H_2O$ and $FeSO_4 \cdot 7H_2O$ induce a gradual increase of carbonyl groups, more evident for the latter salt, within 30 min incubation at 37 °C (see Fig. 4A and B, respectively); $Fe(NO_3)_3 \cdot 9H_2O$ leads to a relevant effect only after 1 h of incubation (see Fig. 4C), whereas $ZnCl_2$ causes a slight oxidation of the proteasome subunits (see Fig. 4D).

Therefore there is no tight correlation between the oxidative strength measured as the amount of carbonyl groups and the influence of the utilized salts on the activities. On one side, Fe(II) and Cu(II) induce the most evident oxidative modifications but they do not show a unique effect on the activities; on the other side, Zn(II), which does not seem to have a relevant oxidative strength, inhibits all the assayed activities.

Western blot analyses were performed using anti-X, anti-Y and anti-Z antibodies in order to detect changes, following metal-catalyzed oxidation (MCO), in the antigen-antibody recognition process, probably due to the epitopes masking or unmasking. It was observed that only 2 mM $FeSO_4 \cdot 7H_2O$ produced a different antibody binding to the subunits compared to the untreated MPC (Fig. 5).

In conclusion, our results show that the assayed metal salts did not have the same effects on the 20S proteasome activities and a correlation between bi- and trivalent salts, or between their relative oxidative strengths, did not occur. The incubation of the 20S proteasome

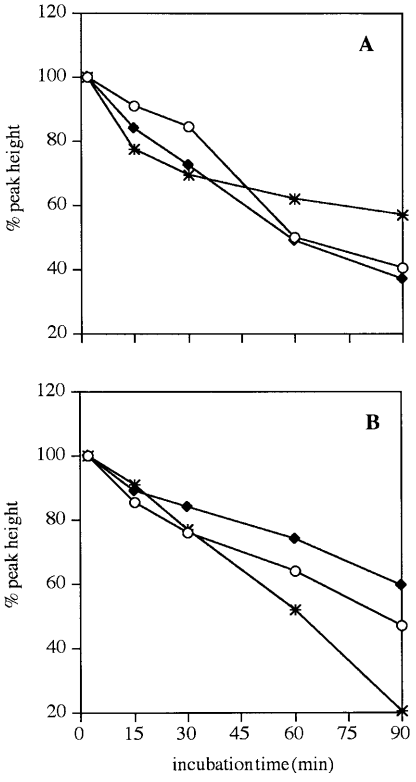

Fig. 3. Effect of $CuSO_4 \cdot 5H_2O$ [**A**: control (*open circles*), 1 mM (*solid diamonds*) and 1.5 mM (*asterisks*)] and $FeSO_4 \cdot 7H_2O$ [**B**: control (*open circles*), 1 mM (*solid diamonds*) and 2 mM (*asterisks*)] on casein degradation by the 20S proteasome. Each data point is the mean value ± 4% standard error and comes from three separate determinations

with $ZnCl_2$ drastically inhibited all the assayed activities and did not produce a significant increase of carbonyl groups, indicating that oxidation is not the base of the zinc inhibitory effect, which might result from the displacement of Mg^{2+} from its binding sites inside the catalytic chamber [33]; further experiments will be necessary to demonstrate this hypothesis.

As for the other assayed metal ions, even though they increase the carbonyl group content in the 20S proteasome, they do not produce the same effect on its catalytic activities, suggesting that the tested metals induce various oxidative conditions which influence, in a different way, the degradative functions of the MPC. Interestingly, incubation with ferrous ions induces the most significant oxidation of the enzymatic molecule and a decrease of the subunit-antibody recognition. Besides that, it causes great activation of the BrAAP component, the one apparently involved in protein substrate degradation. This finding, together with an oxidation-induced structural modification of the molecule, which renders it more accessible to macromolecular substrates, could be responsible for the increase of the rate of caseinolysis, suggesting a possible role of the metal ions on the gating of the 20S proteasome.

As a matter of fact, the results presented here indicate that metal-catalyzed oxidation represents the major

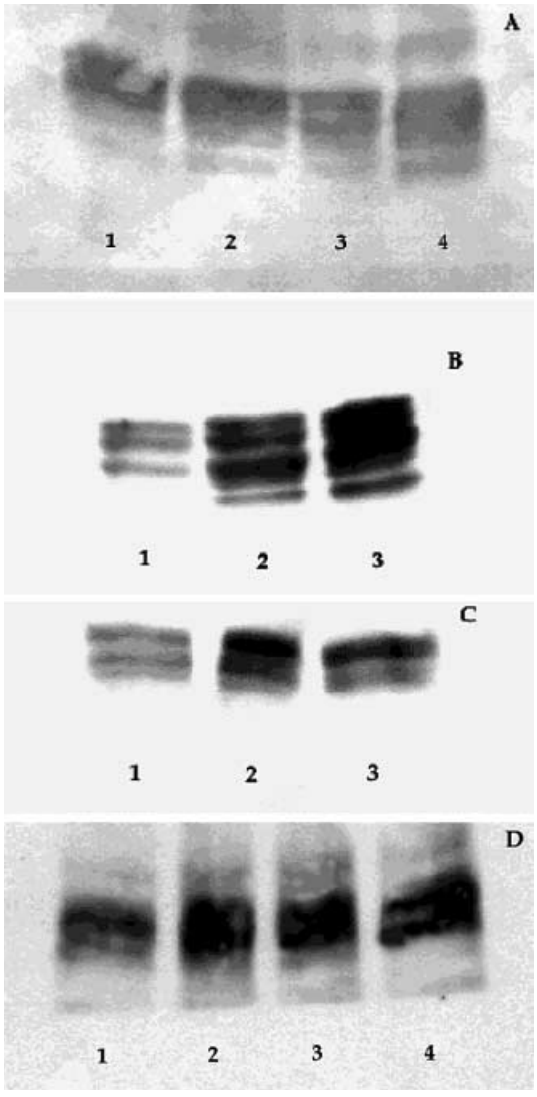

Fig. 4. Purified MPC from bovine brain was incubated at 37 °C with: **A**, $CuSO_4 \cdot 5H_2O$ [0 mM (*lane 1*); 0.5 mM (*lane 2*); 1.5 mM (*lane 3*); 3 mM (*lane 4*)] for 30 min; **B**, $FeSO_4 \cdot 7H_2O$ [0 mM (*lane 1*); 1 mM (*lane 2*); 2 mM (*lane 3*)] for 30 min; **C**, $Fe(NO_3)_3 \cdot 9H_2O$ [0 mM (*lane 1*); 0.4 mM (*lane 2*); 1 mM (*lane 3*)] for 1 h; **D**, $ZnCl_2$ [0 mM (*lane 1*); 0.5 mM (*lane 2*); 1 mM (*lane 3*); 1.5 mM (*lane 4*)] for 1 h. The reactions were stopped by adding the same volume of 20% sodium dodecyl sulfate. Samples were treated according to the OxyBlot procedure. Western blotting onto PVDF membrane and incubation with HRP-conjugated secondary antibody were performed according to the standard procedures

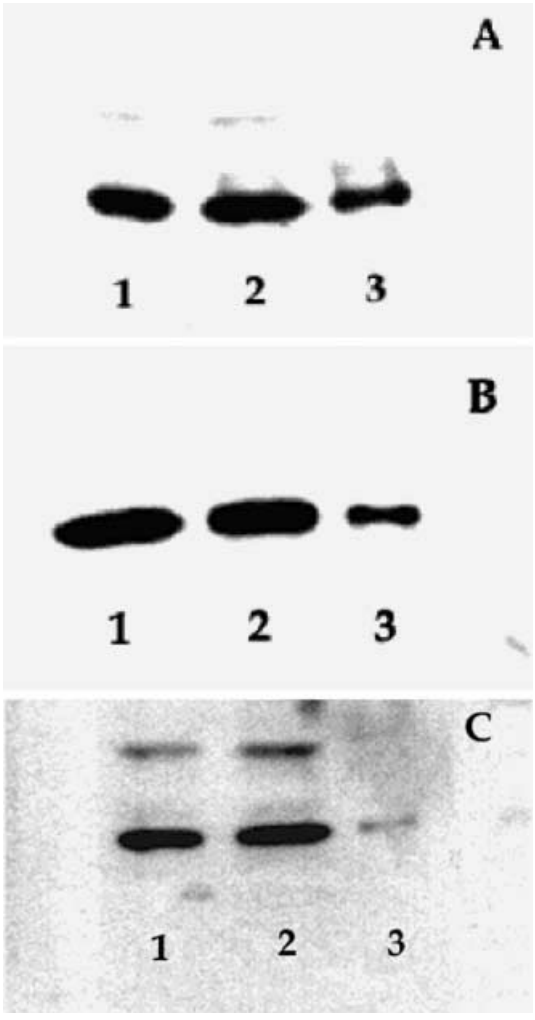

Fig. 5. Effect of the incubation of 20S proteasome from bovine brain with $FeSO_4 \cdot 7H_2O$ [0 mM (*lane 1*); 1 mM (*lane 2*); 2 mM (*lane 3*)] at 37 °C for 1 h; the immunoblot membranes were revealed with anti-X (**A**), anti-Y (**B**) and anti-Z (**C**) antibodies

contribution to the observed changes in the catalytic activities of the brain 20S proteasome. These results could support Davies' [31] hypothesis that oxidized proteins are mainly degraded in an ATP- and ubiquitin-independent manner by the 20S proteasome. Furthermore, one of the components of the brain 20S proteasome, most likely involved in the removal of protein substrates, the BrAAP activity, is activated by all the oxidant metal ions. These results do not appear in line with those obtained by other authors on similar systems. Strack et al. [30] in fact reported that exposure of the erythrocyte 20S proteasome to $FeSO_4$-EDTA-ascorbate induces a stimulation of the Suc-LLVY-MCA (MCA = 7-methylcoumarylamide) hydrolyzing activity as well as of the T-L, the PGPH and the caseinolytic activities, while Reinheckel et al. [29] showed a partial inhibition of the Suc-LLVY-MCA and oxidized ferritin hydrolysis by the 20S isolated from erythrocytes and treated with oxidants such as H_2O_2, peroxynitrite and hypochlorite. These apparent contradictory results could derive either from the source of the proteasome or by the different systems utilized for inducing oxidation or from the substrates used for measuring the same proteolytic activity.

Taking into account what is known on the CNS proteasomes, Keller et al. [27, 28] reported CNS region-specific inhibition of ChT-L and PGPH activities under oxidative conditions, typical of neurodegenerative disorders and aging; although our proteasomal system was not isolated from specific regions of the brain, we also observed inhibition of the ChT-L and PGPH activities, under MCO. Moreover, the BrAAP and T-L compo-

nents were measured, with the former being significantly activated by the strongest oxidant metal ions used. Considering the supposed role of BrAAP activity on the removal of protein substrates, our findings could give insights into the involvement of the proteasome in neuropathologies.

References

1. Gasparrini L, Racchi M, Binetti G, Trabucchi M, Solerte SB, Alkon D, Etcheberrigaray R, Gibson G, Blass J, Paoletti R, Govoni S (1998) FASEB J 12:17–34
2. Markesbery WR (1997) Free Radical Biol Med 23:134–147
3. Atwood CS, Huang X, Moir RD, Tanzi RE, Bush AI (1999) Met Ions Biol Syst 36:309–364
4. Huang X, Atwood CS, Hartshorn MA, Multhaup G, Goldstein LE, Scarpa RC, Cuajungco MP, Gray DN, Lim J, Moir RD, Tanzi RE, Bush AI (1999) Biochemistry 38:7609–7616
5. Baumeister W, Walz J, Zühl F, Seemüller E (1998) Cell 92:367–380
6. DeMartino GN, Slaughter CA (1999) J Biol Chem 274:22123–22126
7. Orlowski M, Wilk S (2000) Arch Biochem Biophys 383:1–16
8. Grune T, Reinheckel T, Davies JA (1997) FASEB J 11:526–534
9. Löwe J, Stock D, Jap B, Zwickl P, Baumeister W, Huber R (1995) Science 268:533–539
10. Groll M, Ditzel L, Löwe J, Stock D, Bochtler M, Bartunik HD, Huber R (1997) Nature 386:463–471
11. Dick TP, Nussbaum AK, Deeg M, Heinemeyer WMG, Schirle M, Keilholtz W, Stevanovic S, Wolf DH, Rammensee HG, Schild H (1998) J Biol Chem 273:25637–25646
12. Esler WP, Stimson ER, Jennings JM, Ghilardi JR, Mantyh PW, Maggio JE (1996) J Neurochem 66:723–732
13. Miura T, Suzuki K, Kohata N, Takeuchi H (2000) Biochemistry 39:7024–7031
14. Orlowski M, Michaud C (1989) Biochemistry 28:9270–9278
15. Eleuteri AM, Angeletti M, Lupidi G, Tacconi R, Bini L, Fioretti E (2000) Protein Express Purif 18:160–168
16. Wilk S, Orlowski M (1983) J Neurochem 40:842–849
17. Orlowski M, Cardozo C, Michaud C (1993) Biochemistry 32:1563–1572
18. Pfleiderer G (1970) Methods Enzymol 19:514–521
19. Almenoff J, Orlowski M (1983) Biochemistry 22:590–599
20. Eleuteri AM, Kohanski RA, Cardozo C, Orlowski M (1997) J Biol Chem 272:11824–11831
21. Laemmli UK (1970) Nature 277:680–685
22. Towbin H, Staehelin T, Gordon J (1979) Proc Natl Acad Sci USA 76:4350–4354
23. Burnette WN (1991) Anal Biochem 112:195–203
24. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER (1990) Methods Enzymol 186:464–478
25. Levine RL, Williams JA, Stadtman ER, Shacter E (1994) Methods Enzymol 233:346–357
26. Shacter E, Williams JA, Lim M, Levine RL (1994) Free Radical Biol Med 17:429
27. Keller JN, Hanni KB, Markesbery WR (2000) J Neurochem 75:436–439
28. Ding Q, Keller JN (2001) Free Radical Biol Med 5:574–584
29. Reinheckel T, Sitte N, Ullrich O, Kuckelkorn U, Davies KJA, Grune T (1998) Biochem J 335:637–642
30. Strack PR, Waxman L, Fagan JM (1996) Biochemistry 35:7142–7149
31. Davies KJA (2001) Biochemie 83(3–4):1–8
32. Baker OJ (1988) Methods Enzymol 158:33–55
33. Hung HC, Chang GG (2001) Protein Sci 10:34–45