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Copper induces permeability transition through its interaction with the adenine nucleotide translocase

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Abstract

In this work we examined the effect of low concentrations of Cu^{2+} on the opening of the mitochondrial non-specific pore. The purpose was addressed to further contribute to the knowledge of the mechanisms that regulate the open/closed cycles of the permeability transition pore. Membrane leakage was established by measuring matrix Ca^{2+} efflux and mitochondrial swelling. The experimental results indicate that Cu^{2+} at very low concentrations promoted the release of accumulated Ca^{2+}, as well as mitochondrial swelling, provided 1,10-phenanthroline has been added. Carboxyatractyloside and Cu^{2+} exhibited additive effects on these parameters. After Cu^{2+} titration of membrane thiols, it might be assumed that the blockage of 5.9 nmol of SH/mg protein suffices to open the non-specific pore. Taking into account the reinforcing effect of carboxyatractyloside, the increasing ADP concentrations, and that *N*-ethylmaleimide inhibited the Cu^{2+}-induced Ca^{2+} efflux, it is proposed that the target site for Cu^{2+} is located in the ADP/ATP carrier.

Keywords: Kidney mitochondria; Permeability transition; Calcium; Copper; Carboxyatractyloside

1. Introduction

Kidney injury by heavy metals, like Hg^{2+}, Cd^{2+}, Zn^{2+}, and Cu^{2+}, occurs as a consequence of interactions between these metals and sulfhydryl groups of cell membrane proteins that modulate cation permeability (Kone et al., 1990; Chávez et al., 1991a; Akesson et al., 2005). In mitochondria, such interactions greatly increase their sensitivity to the deleterious effect of massive Ca^{2+} load on membrane leakage (Chávez and Holguín, 1988; Gunter and Pfeiffer, 1990; Belyaeva et al., 2002; Dineley et al., 2005). Besides, heavy metals and a number of thiol-blocking reagents have been introduced as inducers of the non-selective permeability; among them, phenylarsine oxide, *N*-ethylmaleimide, and mersalyl (Halestrap et al., 1997; Kowaltowski and Castilho, 1997; Costantini et al., 1998; Balakirev and Zimmer, 2001; McStay et al., 2002). The so-called membrane permeability transition is characterized by the opening of a non-selective pore with a diameter of approximately 2–3 nm that allows for the efflux of matrix ions and metabolites (Bernardi, 1999; Zoratti et al., 2005). The chemical identity of the non-selective pore has not yet been elucidated; however, a general consensus points to adenine nucleotide translocase (ANT) as the membrane constituent of the pore. The latter is based on the fact that carboxyatractyloside, atractyloside, and agaric acid, inhibitors of ANT, induce permeability transition (Haworth and Hunter, 1980; García et al., 2005) and, conversely, ADP and ATP inhibit pore opening (Haworth and Hunter, 2000). In addition, Brustovetsky and Klingenberg (1996), Tikhonova et al. (1994), and Wieckowski et al. (2000) have shown that the reconstituted ANT can behave as the non-specific pore.

Rat ANT possesses four cysteine residues, i.e., Cys^{56}, Cys^{128}, Cys^{160}, and Cys^{257} (Majima et al., 1993), and as demonstrated by Majima et al. (1995), Cys^{128} is located in the membrane segment and the others in the loops facing the matrix side. Several reports indicate that chemical modification of

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critical cysteine residues of ANT causes permeability transition. To this regard, McStay et al. (2002) showed that phenylarsine oxide induces pore opening by bringing about intramolecular cross-linking between Cys^{160} and Cys^{257} of mitochondrial ANT isolated from liver and heart rat (as pointed out by the same authors, this nomenclature corresponds to Cys^{159} and Cys^{256} of the bovine sequence).

The effect of copper on membrane permeability has been widely studied. Krumschnabel et al. (2005) stated that addition of low copper concentrations to trout hepatocytes induces increased formation of reactive oxygen species that result in mitochondrial permeability transition and cell death. On the other hand, copper, in addition to 1,10-phenanthroline (Op), induces pore opening with (Zazueta et al., 1998) or without (Costantini et al., 1998) dimerization of ANT.

We now inform that copper at a concentration of 6 μM induces a fast matrix Ca^{2+} discharge. However, after addition of Op, nanomolar concentrations of Cu^{2+} were required to promote permeability transition. It should be noted that under these conditions pore opening is dependent on the concentrations of ADP. Furthermore, low concentrations of carboxyatractyloside (CAT), i.e., 0.5 μM, promoted the Cu^{2+}-induced release of matrix Ca^{2+}; this reaction is sensitive to CSA. Based on these results, we suggest that copper induces permeability transition by interacting with critical thiol groups, probably located in ANT.

2. Materials and methods

Mitochondria from rat kidney cortex were prepared after homogenization of the tissue in 0.25 M sucrose-1 mM EDTA, adjusted to pH 7.3. After following the centrifugation pattern, the last pellet was suspended in EDTA-free sucrose medium. Mitochondrial protein was measured according to the method of Lowry et al. (1951). Calcium movements were followed by changes in absorbance at 675–685 nm, using the external metallochromic indicator Arsenazo III (Scarpa et al., 1978). Mitochondrial swelling was analyzed at 540 nm. Cross-linking of membrane proteins was explored by electrophoresed 100 μg proteins in SDS/PAGE (12%), in non-reducing slab gels. Membrane thiol groups were measured by using the Ellman's reagent DTNB (Ellman, 1958). ADP exchange reaction was measured by incubating 1 mg of mitochondrial protein in 1 ml of medium containing 125 mM KCl, 10 mM HEPES, 3 mM phosphate, 1 μg oligomycin, 5 μg rotenone, and 60 μM [3H]-ADP (sp. act. 1796 cpm/nmol), adjusted to pH 7.3. After 1 min of incubation, an aliquot of 0.2 ml was withdrawn and filtered through a Millipore filter of 0.45 μm pore diameter. The radioactivity contained in mitochondria and retained in the filter was measured. Malondialdehyde (TBARS) from membrane mitochondria was measured according to the method of Ohkawa et al. (1979). Reduced glutathione was measured in mitochondrial extracts by high-performance liquid chromatography following the method described by Lakritz et al. (1997). Basic incubation media, adjusted to pH 7.3 with Tris, contained 125 mM KCl, 10 mM succinate, 10 mM HEPES, 3 mM phosphate, 100 μM ADP, 50 μM $CaCl_2$, 5 μg rotenone, and 2 μg oligomycin.

3. Results

In a previous work, we showed that 5 μM Cu^{2+} is unable to induce matrix Ca^{2+} efflux from mitochondria incubated in 250 mM sucrose (García et al., 2000). However, as we also demonstrated, mitochondria are more susceptible to permeability transition when incubated in potassium medium (Chávez et al., 1991b). Thus, the effect of Cu^{2+} on mitochondria incubated in KCl was explored. Fig. 1A shows that, in these conditions, 4 μM Cu^{2+} initiated the release of accumulated Ca^{2+}. Nevertheless, a fast rate of Ca^{2+} efflux was observed at Cu^{2+} concentrations higher than 6 μM. On the other hand, several reports indicate that high concentrations of Cu^{2+}, i.e., 5 μM plus Op open the non-specific pore (Costantini et al., 1998; Zazueta et al., 1998; Sheline and Choi, 2004). Fig. 1B shows that, in KCl medium, very low concentrations of Cu^{2+}, i.e., 0.4 μM, in addition to Op, suffice to initiate matrix Ca^{2+}

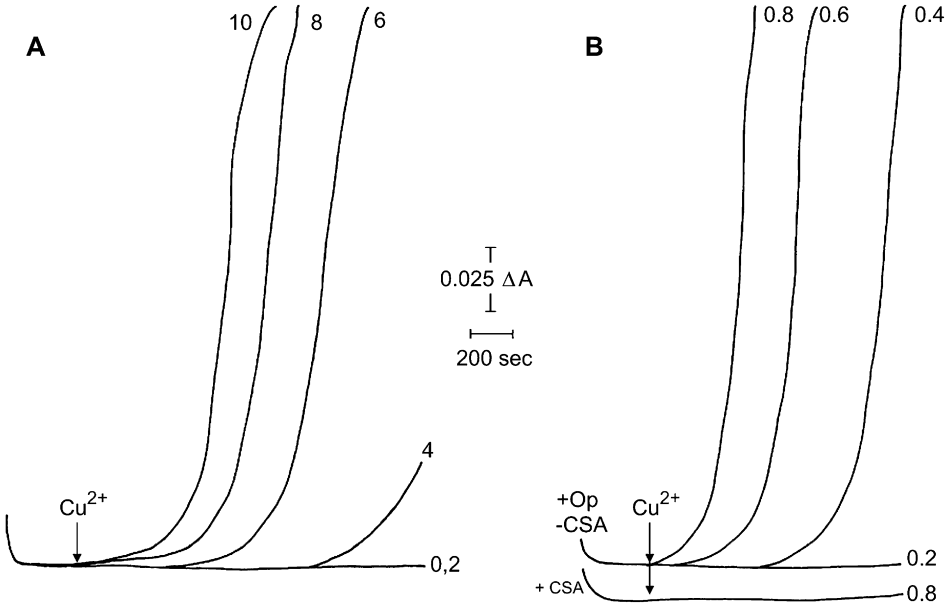

Fig. 1. The effect of increasing concentrations of copper on mitochondrial Ca^{2+} content. Mitochondrial protein (2 mg) was incubated in 3 ml of the basic medium, described in Section 2. The numbers at the side of the figures indicate the added copper concentrations (in μM). Where indicated, 100 μM ortho-phenanthroline (Op) or 0.5 μM cyclosporin A (CSA) was added. Temperature 25 °C.