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Cd^{2+} and Cu^{2+} ions inhibit cytochrome oxidase in the respiratory chain of liver mitochondria

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We have determined earlier that the main target of direct toxic action of two metal ions Cd^{2+} and Cu^{2+} (5 mM) on oxidative phosphorylation is within the respiratory subsystem. With the aim of localizing the effects of these ions in the respiratory chain, modular kinetic analysis on the level of the respiratory subsystem was performed. To this end, the oxidative phosphorylation system was conceptually divided into modules around different intermediates – coenzyme Q (CoQ) (CoQ-reducing and CoQ-oxidizing modules) and cytochrome c (cytochrome c-reducing and cytochrome c-oxidizing modules). Experiments were performed on rat liver mitochondria respiring with succinate (+ rotenone) in state 3. By the changes induced by ions of both metals in the kinetics of the modules we determined that Cd^{2+} and Cu^{2+} did not have any effect on succinate dehydrogenase (SDH) and cytochrome bc_1. The inhibition of the respiratory subsystem in both cases was caused by the action of these ions on cytochrome oxidase (COX).

Key words: liver mitochondria, kinetic analysis, respiratory chain, cadmium ions, copper ions

INTRODUCTION

Virtually all metals can produce toxicity when ingested in sufficient quantities, but there are several which are especially important, because either they are so pervasive or produce toxicity at low concentrations. Transition metals are easily taken up by living organisms and because of a long biological half-life accumulate mainly in the liver and kidney [1]. Cu^{2+} and Cd^{2+} ions accumulate inside mitochondria and interact with important functional groups (in particular, with -SH groups) of a variety of enzymes in the matrix or the inner mitochondrial membrane [1]. Micromolar concentrations of Cd^{2+} and Cu^{2+} uncouple oxidative phosphorylation and inhibit mitochondrial respiration in state 3 [2, 3]. Cd^{2+} directly inhibits SDH, H^+-ATPase and the respiratory chain in isolated liver mitochondria [4]. Despite considerable efforts, separate findings do not resolve the question about the mechanism of Cd^{2+} action on the whole system level. With the aim to distinguish which blocks of oxidative phosphorylation system are affected by Cd^{2+} in rat liver mitochondria, we have applied the modular kinetic analysis (MKA) [5, 6] The results indicated that the main target of direct toxic action of two metal ions, Cd^{2+} and Cu^{2+}, on oxidative phosphorylation lies within the respiratory subsystem.

The aim of this study was to identify and to compare the inhibitory sites of Cd^{2+} and Cu^{2+} within the respiratory chain of rat liver mitochondria. For this purpose, we the divided oxidative phosphorylation system into two modules in two different ways, depending on the choice of connecting intermediate (coenzyme Q (CoQ) or cytochrome c). By changes induced in the kinetics of these modules, we detected what sites within the respiratory subsystem are sensitive to a deleterious action of Cd^{2+} or Cu^{2+}.

MATERIALS AND METHODS

Mitochondria were isolated from the liver of male Wistar rats as described previously [5]. Mitochondria were suspended in a buffer containing 250 mM sucrose, 5 mM Tris-HCl (pH 7.35). Protein was estimated according to Bradford [7].

The experiments were performed at 25 °C using 5 mM succinate (+ 2 μM rotenone) as a substrate. Mitochondrial concentration was 1.0 mg/ml. The rate of mitochondrial respiration was measured using a Clark electrode after preincubation of mitochondria for 3 min in the absence or presence of 5 μM Cd^{2+} or Cu^{2+} in the incubation medium containing 110 mM KCl, 20 mM Tris-HCl, 5 mM KH_2PO_4, 50 mM creatine, excess of creatine kinase, 1 mM $MgCl_2$, pH 7.2.

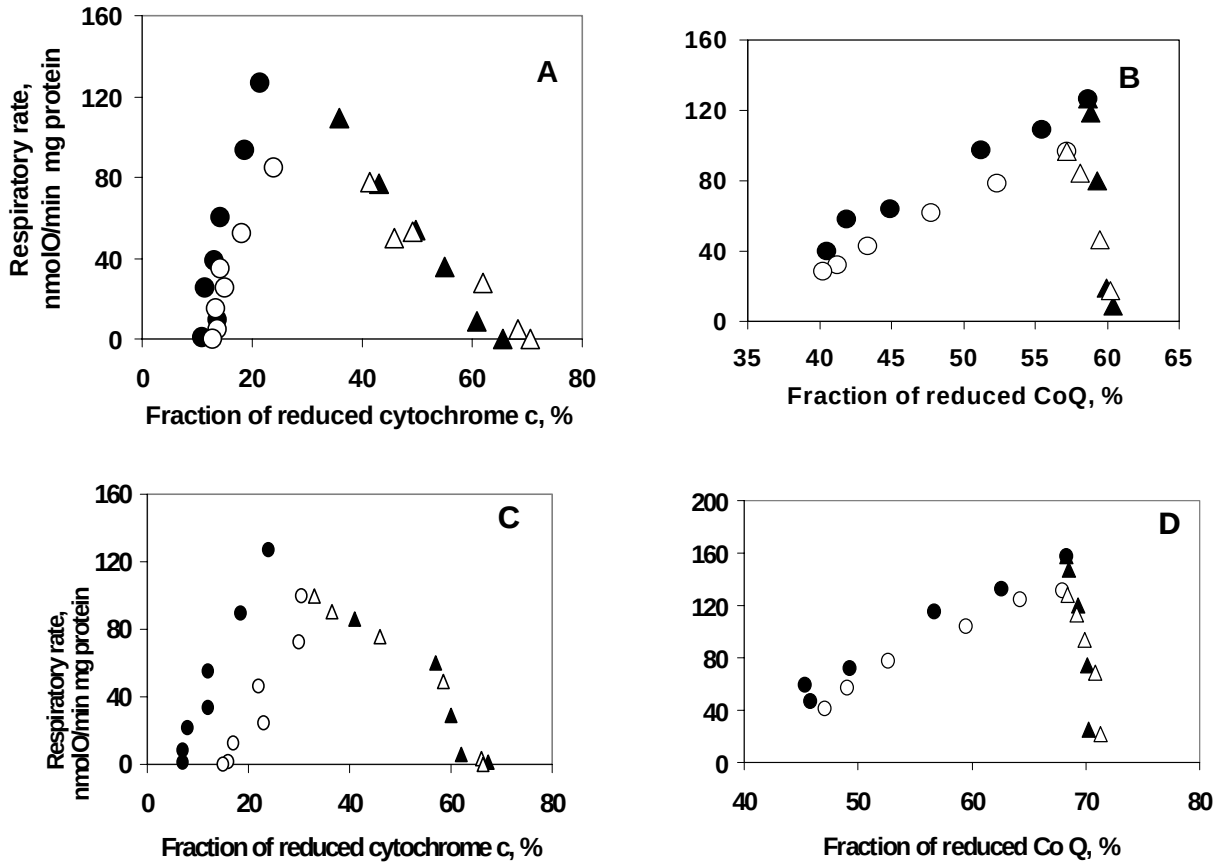

Figure. **Effects of Cd^{2+} and Cu^{2+} on the kinetics of cytochrome c and CoQ reducing and oxidizing modules in the respiratory chain.** A – Cd^{2+} effect on cytochrome c kinetics. B – Cd^{2+} effect on CoQ kinetics. C – Cu^{2+} effect on cytochrome kinetics. D – Cu^{2+} effect on CoQ kinetics. ●,○ – oxidizing module kinetics (obtained by titration with malonate); ▲,△ – reducing module kinetics (obtained by titration with myxothiazol and KCN); ●,▲ – in the absence of Cu^{2+} or Cu^{2+}; ○,△ – 5 μM $CdCl_2$ (in A and B) or $CuCl_2$ (in C and D) present. The results of typical experiment are presented, the same results were obtained in two other experiments

The fraction of reduced CoQ was measured concomitantly with mitochondrial respiration in a vessel fitted with both a Clark type oxygen electrode and a Q-selective electrode. The output from the polarograph and the CoQ electrode was connected to a Macintosh computer via a MacLab/4e interface (AD Instruments, UK). At desired steady states, 1 ml samples were taken from the vessel and extracted with petroleum ether, and the CoQ amount was calibrated by HPLC.

Cytochrome c reduction was followed simultaneously with oxygen uptake. The oxygen electrode was plugged into a multipurpose thermostated vessel mounted in a laboratory-built automated dual wavelength spectrophotometer [8]. Spectral scans were between 520 and 590 nm, with 578 nm as reference. To determine the fraction of reduced cytochrome c, spectra were recorded in the absence of substrate (oxidized), after addition of substrate (steady state) and after addition of sodium dithionite (reduced). Data were gathered and rates calculated using the CHART and the PowerChrom programs supplied with MacLab [9].

RESULTS AND DISCUSSION

Using the MKA approach, a complex process is simplified by division into a small number of modules connected by the common intermediates [10]. The components of the system that are influenced by an effector are detected by the effector-induced shift in kinetic dependencies of each module on the concentration of the intermediate.

In order to localize the inhibitory sites of Cd^{2+} and Cu^{2+} within the respiratory chain, we performed a modular kinetic analysis by dividing the oxidative phosphorylation system into two modules around different intermediates, CoQ (CoQ reducing and CoQ oxidizing modules) and cytochrome c (cytochrome c reducing and cytochrome c oxidizing modules). We analyzed changes in the kinetics of the obtained modules induced by Cd^{2+} and Cu^{2+}. For this, the steady-state rate of oxygen consumption and steady-state reduction level of an intermediate (CoQ or cytochrome c, respectively) were measured simultaneously. The kinetics of the CoQ reduction was determined by modulating the activity of the CoQ-oxidizing module by myxothiazol titration and

the kinetics of CoQ oxidation by malonate titration of the CoQ reducing module. The kinetic dependencies of cytochrome c-reducing and -oxidizing modules on cytochrome c reduction state were determined by measuring the number of steady states in which the rate of cytochrome c oxidation and reduction was varied by titration with KCN and malonate, respectively.

The kinetic dependencies of fluxes through each module were determined in the absence of metal ions and in the presence of 5 μM $CdCl_2$ or 5 μM $CuCl_2$. The results showed that Cd^{2+} decreased the rate of oxygen consumption by 22% (from 133 ± 10 to 104 ± 10 nmol O/min per mg, n = 7) and Cu^{2+} by 21% (from 139 ± 7 to 110 ± 6 nmol O/min per mg, n = 7).

In Figure B, D the kinetic dependences of the CoQ-reducing module are presented. It was obvious that the kinetic dependences of this module coincided in the absence and in the presence of Cd^{2+} or Cu^{2+} in the incubation medium. These results indicated that neither Cd^{2+} nor Cu^{2+} had any effect on the kinetics of the CoQ-reducing module consisting of a dicarboxylate carrier and succinate dehydrogenase (SDH). The kinetic curve for CoQ oxidation was shifted by both metal ions towards the lower rates at the same level of cytochrome c reduction (Fig. B, D). This indicated that Cd^{2+} and Cu^{2+} ions inhibited activity of complexes involved in CoQ oxidizing module. From these experiments the conclusion has been derived that inhibition of the respiratory chain by Cd^{2+} and Cu^{2+} is explainable by the effect on cytochrome bc_1 and COX or on both of them.

To clear up this case, we repeated the strategy described above by dividing the respiratory chain into two modules around cytochrome c (Fig. A, C,). Then the part of the system upstream the cytochrome c (including cytochrome bc_1) and COX behave as different modules within the same respiratory chain. We evaluated the effect of Cd^{2+} and Cu^{2+} on the relationship of the redox state of cytochrome c and the respiratory rate. Our results indicated that ions of both metals had no effect on cytochrome c-reducing module (dicarboxylate carrier, SDH and cytochrome bc_1) and inhibited the process catalysed by cytochrome oxidase (Fig A, C). Since the experiments were performed in state 3, the module oxidizing cytochrome c was comprised by COX operating together with the two subsystems consuming the membrane potential – the phosphorylation module and the proton leak module. Thus, we concluded that Cd^{2+} and Cu^{2+} had no effect on SDH and cytochrome bc_1, but inhibited the process catalyzed by COX. Since 5 μM Cd^{2+} and 5 μM Cu^{2+} have no effect on the phosphorylation subsystem and membrane permeability in liver mitochondria oxidizing succinate in state 3 [5, 6], this indicated that inhibition of the cytochrome c oxidizing module by both metal ions was caused by COX inhibition. Thus, Cd^{2+} and Cu^{2+} ions decrease activity of the respiratory subsystem in state 3 by acting on the same site: both metal ions inhibit COX but do not of afect SDH and bc1 complex. It remains to elucidate further by the synergism of interaction whether the mechanism of toxic Cu^{2+} and Cd^{2+} action on COX is identical or different.

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CD^{2+} IR CU^{2+} JONAI INHIBUOJA CITOCHROMOKSIDAZÆ KEPENØ MITOCHONDRIJØ KVËPAVIMO GRANDINËJE

Santrauka

Anksèiau nustatëme, kad pagrindinë Cd^{2+} ir Cu^{2+} jonø (5 μM) poveikio vieta þiurkës kepenø oksidacinio fosforilinimo sistemoje yra kvëpavimo posistemë. Taikydami moduliø kinetinæ analizæ (MKA) kvëpavimo posistemei, nustatëme ðiø metalø jonø poveikio vietas. Tam tikslui oksidacinio fosforilinimo sistemà suskirstëme á modulius, jungiamus skirtingø tarpiniø metabolitø – kofermento Q (KoQ) (KoQ redukuojantá ir KoQ oksiduojantá modulius) ir citochromo c (citochromà c redukuojantá ir citochromà c oksiduojantá modulius). Eksperimentus atlikome su sukcinatà (+ rotenonà) oksiduojanèiomis mitochondrijomis, esant 3-iai metabolinei bûsenai. Išvada – Cd^{2+} (5 μM) ir Cu^{2+} (5 μM) jonai nepaveikë sukcinato dehidrogenazës ir citochromo bc_1. Maþëjantá kvëpavimo posistemës aktyvumà, veikiant ðiø metalø jonams, lemia Cd^{2+} ir Cu^{2+} jonø inhibuojantis poveikis citochromo oksidazei.