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The effect of copper on membrane enzymes

Copper has a variety of effects on cells, modifying the facilitated diffusion of glycerol across erythrocytes[1,2], Schwann cell[3] and squid axon membranes[3] and altering the Cl^- permeability of frog skin[4] and molluscan neurones[5]. $CuCl_2$ is the copper salt frequently used in permeability studies and so was used in the present investigation, although it is little dissociated in aqueous solution. However, $CuCl_2$ is still able to attack lysine, histidine and cysteine residues[6]. Cu(II) also induces mitochondrial swelling[7] and inhibits the oxygen consumption of brain homogenates[8], and subarachnoid injections in pigeons cause the rapid onset of convulsions and death[9]. This convulsive action is believed to be due to a direct effect on the plasma membrane of brain cells. These workers conclude that it is primarily the microsomal Mg^{2+}-ATPase and not the (Na^+-K^+-Mg^{2+})-ATPase which is inhibited by copper[10] although EPSTEIN AND MCILWAIN[11] found both enzymes to be sensitive to 150 μM Cu(II).

We have suggested elsewhere[12,13] that both these enzyme systems could be implicated in the passage of ions across the cell membrane and have therefore studied their differential sensitivity to $CuCl_2$ in greater detail, using a microsomal preparation from rat brain, and rat erythrocyte ghosts. We have not used a Tris buffer system when the preparation is exposed to Cu(II), since these two agents interact strongly in dilute solution[14].

Microsomal preparations were made from rat brain as previously described[15] except that EDTA was not used at any stage, and the microsomal pellet was gently homogenised in 10 mM Mg^{2+} and recentrifuged to reduce Ca^{2+} concentration to a level which did not inhibit ATPase activity. The final microsomal pellet was suspended in neutralised, deionised water, and the resulting specific activity and the (Na^+-K^+-Mg^{2+})-ATPase: Mg^{2+}-ATPase activity ratio both proved to be the same as that found previously[15]. 1-ml aliquots of the preparation were mixed with 1 ml of $CuCl_2$ of appropriate concentration and left for 10 min. 0.5-ml samples were then taken and assayed for ATPase activity over 10 min at 37°. The reaction medium was made up in 50 mM histidine–HCl buffer.

Ghosts were prepared from rat erythrocytes by haemolysis of washed cells in 3 mM $MgCl_2$ *plus* 0.5 mM EDTA, followed by three washings with 10 mM $MgCl_2$ and one wash with deionised water. The ghosts were suspended in deionised water, and 0.5-ml samples were mixed with 0.5 ml $CuCl_2$ of appropriate concentration and left for 10 min at 37°. The reaction was started by addition of 1 ml of ions *plus* ATP in 1 mM imidazole–HCl buffer and was stopped after 30 min.

The effect of $CuCl_2$ on the brain microsomal preparation is shown in Fig. 1. The concentration of copper given is that in the preincubation medium, the concentration in the ATPase reaction being 0.25 of this value. EPSTEIN AND MCILWAIN[11] have shown that a critical factor is the quantity of copper per unit weight of tissue, rather than the concentration of the ion. However, under our conditions, it is clear that both the Mg^{2+}-ATPase and the $(Na^+-K^+-Mg^{2+})$-ATPase are inhibited by Cu(II), and further that the $(Na^+-K^+-Mg^{2+})$-ATPase is the more sensitive. Thus, inhibition of the $(Na^+-K^+-Mg^{2+})$-ATPase begins at 5 μM (13 μatoms/g protein) compared with the very small inhibition of the Mg^{2+}-ATPase seen at 10 μM. A plot of percentage inhibition against log $CuCl_2$ concentration produced a sigmoidal curve with both enzymes, the critical concentrations being in the range 10–100 μM. However, the graphs are very different; above 100 μM the $(Na^+-K^+-Mg^{2+})$-ATPase is almost completely inhibited, whereas the activity of the Mg^{2+}-ATPase is reduced by less than 50% in the range 0.1–1 mM. These results are in contrast with those of earlier workers[10], who found the Mg^{2+}-ATPase to be the more sensitive. The addition of 50 mM histidine–HCl (pH 7.2) to the preincubation medium completely protects both enzymes against Cu(II) inactivation at concentrations 0.001–100 μM.

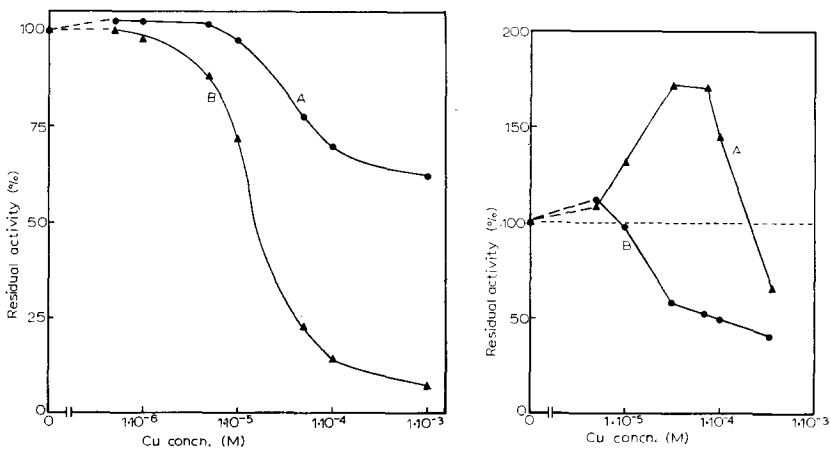

Fig. 1. Effect of preincubation with $CuCl_2$ on ATPases of rat brain microsomal preparation. Conditions: 50 mM histidine–HCl (pH 7.25), 3 mM ATP (Tris salt), 37°. A. 4 mM Mg^{2+}. B. Curve obtained as the difference between activity in presence of 100 mM Na^+, 20 mM K^+, 4 mM Mg^{2+} and 4 mM Mg^{2+} alone. Specific activities in absence of Cu(II): A, 340 μmoles/mg protein per min; B, 376 μmoles/mg protein per min.

Fig. 2. Effect of $CuCl_2$ on ATPases of rat erythrocyte ghosts. Conditions: 1 mM imidazole–HCl (pH 7.25), 2 mM ATP (Tris salt), 37°. A. 4 mM Mg^{2+}. B. Curve obtained as the difference between activity in presence of 100 mM Na^+, 20 mM K^+, 4 mM Mg^{2+} and 4 mM Mg^{2+} alone.

As is evident from Figs. 1 and 2, Cu(II) has differing effects on the ATPases of erythrocyte ghost and brain microsomal preparations. However, both preparations are similar in that they are unaffected by copper at concentrations below 5 μM, although the concentration of Cu(II) relative to protein was lower in the erythrocyte ghost preparations owing to the contamination by haemoglobin. The Cu(II) concentrations given are those during preincubation; in the reaction medium concentrations were half these values. The Mg^{2+}-ATPase of ghosts was markedly activated by

10–100 μM $CuCl_2$, but higher concentrations were inhibitory. The (Na^+-K^+-Mg^{2+})-ATPase, on the other hand, was inhibited over the same concentration range. A consistent but small activation of the (Na^+-K^+-Mg^{2+})-ATPase was seen at concentrations of about 5 μM $CuCl_2$.

The (Na^+-K^+-Mg^{2+})-ATPase is almost certainly implicated in the active transport of cations across the plasma membrane[16]; we have suggested[12,13,15] that the Mg^{2+}-ATPase enzyme complex has a role in the control of passive permeability in excitable cells and erythrocytes. Both these enzymes may be actomyosin-like proteins which are capable of configurational changes associated with ATPase activity[17]. A Mg^{2+}-ATPase with such properties may also be concerned in the control of the shape of erythrocytes[18–21].

The results obtained in this study can be compared with the effects of Cu(II) on intact cells. In the majority of these experiments the concentration of copper in the bathing medium only is quoted, but it is clear that the facilitated diffusion of glycerol across the mammalian erythrocyte membrane is sensitive to very low concentrations of this ion; inhibition can be detected at 0.1 μM (ref. 1) although 10 μM is a commonly used concentration[2]. Blockade of axonal function was produced by 0.1 (lobster axon) and 0.2 mM $CuCl_2$ (frog sciatic nerve)[22], and reduction of Cl^- permeability of frog skin[4] was reported at 10 μM and of snail neurones at 0.1 mM (ref. 5). Cu(II) was shown to affect specifically the passive permeability to Cl^- in the latter experiments. Critical concentrations for a modification of the shape of red cells are 0.5–1 mM (ref. 23). Effective Cu(II) concentrations, therefore, are comparable with those modifying ATPase activity in the present experiments.

The activation–inhibitory effect of Cu(II) on the Mg^{2+}-ATPase of erythrocyte ghosts corresponds closely with its action on the ATPase activity of myosin A, where, at pH 7.1, concentrations of 6–100 μM activate, and higher concentrations inhibit[24]. The results obtained in this study are, therefore, consistent with the suggestion that the Mg^{2+}-ATPase is capable of configurational changes and serves in the control of ionic permeability and in the maintenance of cell shape in erythrocytes. We have also obtained parallel results[25] between the action of photodynamic damage of cells and its effect on membrane ATPases and on actomyosin. It is noteworthy that Cu(II) induced swelling in mitochondria[7], where changes in shape are also believed to be associated with an actomyosin-like Mg^{2+}-ATPase. Swelling was again dependent on the quantity of copper per unit weight of tissue, but the greatest swelling rate was observed at concentrations from 10 to 70 μM, with a significantly lower rate at 2–10 μM (ref. 7).

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