DEPLETION OF Mg\(^{2+}\) AND PERMEABILITY INCREASE OF THE MITOCHONDRIAL INNER MEMBRANE BY PRIMYCIN

L. Mészáros, L. Hoffmann*, M. Paróczai, T. König and I. Horváth

Second Institute of Biochemistry, Semmelweis University Medical School Budapest, Hungary
*Department of Petrology and Geochemistry, Eötvös L. University, Budapest, Hungary

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Recently we have shown\(^1\) that primycin below 2 ~ 3 nmoles/mg protein concentration (“low dose”) renders their inner membrane permeable to K\(^+\), Na\(^+\) and Tris\(^+\) but not to Tea\(^+\). This effect was seen only under energized conditions. It has been concluded that this effect of the antibiotic is due to its ionophore-like action. This conclusion has been supported by the experiments of BLASKÓ et al.\(^2\) made on erythrocytes and artificial lipid membranes. On the other hand, we have also reported\(^1\) that primycin at a concentration higher than 2 ~ 3 nmoles/mg protein (“high dose”) can also interact with non-respiring (i.e. deenergized) mitochondria increasing their inner membrane permeability to protons and chloride, too. Since the ATPase activity induced by a “high dose” of primycin was significantly higher in the presence of added Mg\(^{2+}\) than in its absence, the depletion of Mg\(^{2+}\) from the mitochondria by the antibiotic has been proposed to be responsible for the non-selective permeability increase. In this note we present direct evidence in favour of this proposal.

As it can be seen in Fig. 1 primycin in a “high dose” (6.6 nmoles/mg protein) induces a rapid—and in the presence of EDTA practically complete—depletion of Mg\(^{2+}\) from mitochondria, while adding it in “low dose” (1 n mole/mg protein) the Mg\(^{2+}\) loss is significantly less and slower.

Our previous proposal that primycin in a “high dose” depletes Mg\(^{2+}\) from mitochondria is now experimentally verified. Thus the non-selective permeability changes can be well explained by the depletion of Mg\(^{2+}\) from mitochondria as suggested by others\(^3\)~\(^7\).

To see whether primycin in a “high dose” also induces a Mg\(^{2+}\) influx, swelling experiments were made in isoosmotic Mg(NO\(_3\))\(_2\). Figs. 2 a, b, c show that in the presence of “low dose” of the antibiotic neither energized nor deenergized mitochondria swell in magnesium nitrate, though they do swell in isoosmotic KNO\(_3\). On the contrary, primycin in a “high dose” induces a high amplitude swelling of non-respiring mitochondria in magnesium nitrate (Fig. 2a). Thus it can be concluded that primycin only in a “high dose” renders the membrane permeable to Mg\(^{2+}\).

The question arises whether primycin in “high dose” acts as a bivalent cationophore.

As it is shown in Fig. 1 primycin in “low” and “high dose” equally induces a Ca\(^{2+}\) efflux from mitochondria. The rate of this efflux is increased in the presence of EDTA presumably preventing the mitochondrial reuptake of Ca\(^{2+}\).

Non-respiring mitochondria swell in isoos-
motic calcium acetate in the presence of protonophore, and this swelling can be prevented by ruthenium red which inhibits the natural Ca-translocator of the inner membrane (Figs. 2d and e). The synthetic Ca-ionophore, as found by CARONI et al.8), induces a swelling of mitochondria also in the presence of ruthenium red, by facilitating the Ca²⁺ influx through the inner membrane. On the contrary, primycin does not induce a swelling of ruthenium red treated mitochondria indicating that it does not facilitate the transport of Ca²⁺ (Figs. 2e and f).

These experimental findings make very unlikely that primycin in “high dose” acts as a bivalent cationophore. Thus, the mechanism by which the antibiotic increases the Mg²⁺ permeability of the inner membrane of mitochondria requires further experiments. In this respect it should be mentioned, that olefinic, an antibiotic of quite different structure, increases the Mg²⁺ permeability of the mitochondrial inner membrane in a similar manner⁹).

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References