SDH Activity in Heavy Metal-treated Malpighian Tubules of *Triatoma infestans* Klug (Hemiptera, Reduviidae)

F. S. Kubrusly and M. L. S. Mello¹

Department of Cell Biology, Institute of Biology, UNICAMP, 13081
Campinas (SP), Brazil

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The localization of mitochondria in the Malpighian tubules of *Triatoma infestans* Klug, which varies preferentially as a function of the organ region and of its physiology, has been established ultrastructurally (Mello and Dolder 1977). Cytochemical detection of succinic dehydrogenase (SDH) activity using a rather simple technique at the light microscopy level (Wegmann and Tordet-Caridroit 1960) has confirmed the preferential distribution of the mitochondria in the apical region of the distal cells and basal region of the proximal cells of the Malpighian tubules of this insect species (Azeredo-Oliveira and Mello 1985).

Heavy metals are known to affect some cell enzyme activities in the Malpighian tubules of *T. infestans*, including those of Mg^{2+} -dependent ATPases and acid phosphatases (Kubrusly 1984). It is suspected that mitochondrial SDH activity may also be disturbed by these agents. Furthermore, if the cytoskeleton elements of the Malpighian tubule cells were affected by heavy metals (Mello and Dolder 1977), the distribution of mitochondria along the cells may also be affected (Smith *et al.* 1975, Birky Jr. 1983).

Considering that a cytochemical investigation of SDH activity may clarify both questions, this analysis was carried out on the Malpighian tubules of *T. infestans* subjected to treatment with Cu^{2+} and Hg^{2+} ions.

Material and methods

Unfixed Malpighian tubules removed from 5th instar nymphs of *Triatoma infestans* Klug (Hemiptera, Reduviidae) were used 2h after the injection of 0.05 ml of 10^{-5} M (2.5 ppm) and 2×10^{-5} M $CuSO_4$ and 1.56×10^{-4} M (40 ppm) CH_3HgCl_2 solutions. Controls were injected with an equal volume of Ringer's solution or distilled water. Two specimens were used for each experimental condition.

The concentrations of $CuSO_4$ used were those known to be toxic in other cell systems (Sullivan and Cheng 1975) and even to destabilize microtubules (Mello and Dolder 1987) and increase Mg^{2+} -dependent ATPase activity (Kubrusly 1984) in the Malpighian tubule cells of *T. infestans*. The CH_3HgCl_2 concentration was that which induces an increase in acid phosphatase activity in *T. infestans* cells (Kubrusly 1984).

In a parallel assay, none of 60 specimens of *T. infestans* tested were found to have died 2 h after the injection of $CuSO_4$ or CH_3HgCl_2 .

The material was subjected to the method of Nachlas and coworkers for the detection of SDH activity, modified by Wegmann and Tordet-Caridroit (1960). Tubules incubated in the absence of substrate were used as a control of the reaction.

¹ Reprint requests to Dr. M. L. S. Mello

Results

SDH activity detected by the visualization of microcrystalline deposits of bluish-violet formazan was observed in the cytoplasm of the Malpighian tubule cells of both control and experimental insect specimens.

Figs. 1-4. SDH activity in the Malpighian tubule cells of *T. infestans*. The arrows indicate regions of intense reaction in the proximal (1) and distal (2-4) regions of the tubules. Insects were treated with 10^{-5} M (1, 4) and 2×10^{-5} M (2, 3) CuSO_4 solutions. Bar = 50 μm .

Controls exhibited higher SDH activity in the apical region of the distal cells and in the basal region of the proximal cells, in agreement with previous studies reporting preferential mitochondria localization as a function of the Malpighian tubule regions in *T. infestans* (Mello and Dolder 1977, Azeredo-Oliveira and Mello 1985).

The same pattern of intensity and distribution of the reaction due to the SDH activity was detected in part of the distal and proximal cells of the tubules dissected from the insects treated with the metal ions (Figs. 1 and 2). However, other cells, especially those in the distal region of the tubules, showed a positive reaction only in their middle parts, exhibiting dense and irregularly-shaped formazan deposits (Figs. 3 and 4). In contrast, part of the cells of the distal region and most cells of the proximal region exhibited a very faint reaction not predominantly concentrated in a specific cellular zone (Fig. 4).

No striking differences between the effects promoted by Cu^{2+} and Hg^{2+} ions could be detected.

The above-mentioned characteristics of the reaction were absent when the substrate was omitted from the incubation medium.

Discussion

The results showed that the SDH activity of the Malpighian tubule cells of *T. infestans* is not homogeneously affected by metal ion treatment. There are distal and proximal cells in the organ which continue to present the same intensity of enzyme activity in the same places as in control cells. However, a decrease in SDH activity was also verified, especially in the proximal cells, which under ordinary conditions are known to exhibit mitochondria situated in the basal cellular region (Mello and Dolder 1977).

As regards the cells which were unaffected by the metal ions, they may possibly have resistance mechanisms for coping with mercury and copper ions under the experimental conditions to which they were subjected. Even in terms of insect survival, resistance to copper and mercury toxicity varies among individuals in a given population (Kubrusly 1984). Special mechanisms of resistance have also been reported to have evolved in certain free-living bacterial cells (Silver and Misra 1984).

In the cells with decreased SDH activity a blockade of the citric acid cycle with accumulation of acetyl CoA may have occurred, and the efficiency of the electron-transport chain may have been reduced (Rawn 1989).

The displacement of the enzyme reaction products from the apical cellular zone to the middle of the cells in part of the distal region of the tubules is suggested to indicate a change in the spatial distribution of the mitochondria. This phenomenon may well mean that the microtubules and other cytoskeleton elements, which are assumed to hold mitochondria in fixed positions (Smith *et al.* 1975, Birky Jr. 1983), were affected by the Cu^{2+} and Hg^{2+} ions. Cu^{2+} ions have been reported to disorganize microtubules in several cell systems, including the Malpighian tubule cells of *T. infestans* (Mello and Dolder 1987). However, no mention has been made of a similar action promoted by Hg^{2+} ions.

Heavy metal pollutants, such as copper sulphate and methylmercury chloride are thus to be considered as potential candidates for a direct effect on mitochondrial performance and for an indirect effect on other cell functions in special cellular domains, which require a specific mitochondrial spatial distribution.

Summary

A variable effect of Cu^{2+} and Hg^{2+} ions on the intensity and localization of the SDH

activity was cytochemically detected in the Malpighian tubule cells of the blood-sucking insect, *Triatoma infestans*. Unaffected and decreased enzyme activities as well as a change in the spatial distribution of mitochondria were detected on the basis of the cytochemical localization of the enzyme. It is assumed that these metal ions could affect not only the mitochondrial performance but also other cell functions which require a specific mitochondrial distribution.

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