CARTAS AL EDITOR

Lytic Effect of Cysteine and Glutathione on Inner Mitochondrial Membranes

In previous communications (4, 5) we have already reported the effect of ascorbate on inner mitochondrial membranes obtained from rat liver. Ascorbate produced lysis of the inner mitochondrial membrane and three subfractions were obtained by differential centrifugation which were morphologically and chemically different. This lysis was accompanied by phospholipid changes.

Since ascorbate is a mitochondrial swelling and lysis inducer (1, 2), the question arises whether other swelling inducers such as cysteine and reduced glutathione (1, 2) would also affect the inner mitochondrial membrane structure.



FIG. 1. Effect of different concentrations of cysteine and reduced glutathione on inner mitochondrial membranes.

Inner mitochondrial membranes from male Wistar rat livers were prepared by the method of PARSONS *et al.* (3) slightly modified by us (5). Incubations were carried out at 30° C in a medium containing 0.020 M Tris-HCl buffer, pH 7.4, and 0.25 M sucrose. Additions were made of cysteine or reduced glutathione (GSH) to give final concentrations of 0.08 mM, 0.8 mM, or 4 mM. The structural disaggregation was followed by optical density changes of the membrane suspension in a Zeiss PMQII Spectrophotometer at 520 m μ (5).

As can be seen in Fig. 1, both glutathione and cysteine produced an appreciable decrease in optical density. This decrease was more pronounced with cysteine than with GSH for all the concentrations tested. In the case of cysteine the lowest optical density reading was obtained the 0.08 mM concentrations. However, in the case of GSH the 4 mM concentration gave the lowest value.

It is interesting to note that the optical density starts to decrease only after a lag of 45 to 50 minutes. These results contrast with those observed with ascorbate (5) which acted after a lag of approximately 5 minutes. The final optical density reached with inner membranes incubated in ascorbate was much lower than that obtained in cysteine or glutathione.

Preliminary observations indicate that these two swelling agents, cysteine and reduced glutathione, alter the phosphatidyl ethanolamine and phosphatidyl choline content. Furthermore, preliminary electron microscopic studies show parallel disaggregations of the inner membrane structure and present studies will define the precise morphological and chemical nature of the fragments.

References

- 1. HUNTER, F. E., JR., LEVY, J. F., FINK, J., SCHUTZ, B., GUERRA, F., and HURWITZ, A.: J. Biol. Chem., 234, 2176, 1959.
- HUNTER, F. E., JR., SCOTT, A., HOFFSTEN, P. E., GUERRA, F., WEINSTEIN, J., SCHNEI-DER, A., SCHUTZ, B., FINK, J., FORD, L., and SMITH, E.: J. Biol. Chem., 239, 604, 1964.
- 3. PARSONS, D. F., WILLIAMS, G. R., and CHANCE, B.: Ann. N.Y. Acad. Sci., 137, 643, 1965.
- SANTIAGO, E., GANSER, A., MACARULLA, J. M., and GUERRA, F.: R. csp. Fisiol., 24, 37, 1968.
- SANTIAGO, E., VÁZQUEZ, J., GUERRA, F., and MACARULLA, J. M.: *R. esp. Fisiol.*, 24, 31, 1968.
 - E. Santiago
 - F. GUERRA *
 - J. Eugui

J. M. MACARULLA

Centro de Investigaciones Biológicas «Félix Huarte» Department of Biochemistry University of Navarra Pamplona (Spain)

(Received for publication on December, 2, 1967)

* Present address: Centro de Estudos de Bioquimica do Instituto de Alta Cultura, Faculdade de Farmacia, Porto (Portugal).

48