ULTRATHIN LIPID-POLYMER MEMBRANE MICROCAPSULES CONTAINING MULTIENZYMES, COFACTORS AND SUBSTRATES FOR MULTISTEP ENZYME REACTIONS

Y. T. YU and T. M. S. CHANG

Artificial Cells and Organs Research Centre, Faculty of Medicine, McGill University, 3655 Drummond Street, Montreal H3G 1Y6, Canada

Received 23 December 1980

1. Introduction

Spherical, ultra-thin lipid–polymer membrane systems of cellular dimensions have been prepared here to retain multienzyme systems, cofactors and selected impermeant substrates. A detailed analysis was carried out to see whether this multienzyme system can act on small external permeant substrates.

Early systems have been prepared but with no lipid in the membrane [1,2]. Although capable of enclosing multiple enzyme systems to carry out multistep enzyme reactions [3,4], the membranes are too permeable to retain cofactors [3,4]. In these cases cofactors had to be covalently linked to macromolecules [5–7] before they can be retained within the microcapsules [8]. Recently, techniques of preparing spherical ultra-thin lipid–polymer membrane systems have become available [2,9]. This report is the first successful attempt to use this new technique for the simultaneous inclusion of multienzyme system, cofactors and selected substrates.

2. Materials and methods

L-Glutamic dehydrogenase (EC 1.4.1.3) from bovine liver, type III (40 U/mg); alcohol dehydrogenase (EC 1.1.1.1) from yeast (330 U/mg) and Sigma grade $NADP^+$ from yeast were obtained from Sigma Chemical Co. Ultra-thin lipid–nylon membrane microcapsules of 100 μm mean diameter were prepared using a modification of the procedure in [2,9]. Each 5 ml microcapsules contains glutamic dehydrogenase (12.5 mg), alcohol dehydrogenase (6.25 mg), ADP (1.18 mg), α-ketoglutarate (56.5 mg), $MgCl_2$ (2.5 mg), KCl (0.93 mg) and either $NADP^+$ (0.52 mg or 21.13 mg) or NADPH (21.13 mg).

Here, the substrate solution consists of 12 mM ammonium acetate and 200 mM alcohol in 0.1 M Tris buffer (pH 9). In testing the 4 types of microcapsules, 2 g supernatant-free microcapsules was added to 4 ml substrate solution. In the stability tests either 2.4 ml washing of microcapsules or 2 g microcapsules suspended in 2.4 ml Tris buffer was added to 4 ml substrate solution.

3. Results and discussion

The present study is to see whether ultra-thin lipid–nylon membrane microcapsules can retain enzymes, $NADP^+$, NADPH and α-ketoglutarate; and whether external ammonia and alcohol can cross the lipid membrane to take part in the multistep reactions shown in fig.1. To answer these questions the following 4 types of microcapsules were studied:

(1) *Control microcapsules:* These microcapsules only contained alcohol dehydrogenase and glutamic dehydrogenase in the amounts described. When 2 g of these control microcapsules were added to 4 ml ammonia–alcohol substrate solution (pH 9), the changes in ammonia levels corresponded to the equilibration of ammonia into the microcapsules along a concentration gradient (fig.2). In other studies carried out at pH 7.4 instead of 9, there was little equilibration. At pH 9 a large proportion of ammonia is in the free form (NH_3) and can equilibrate across the lipid-

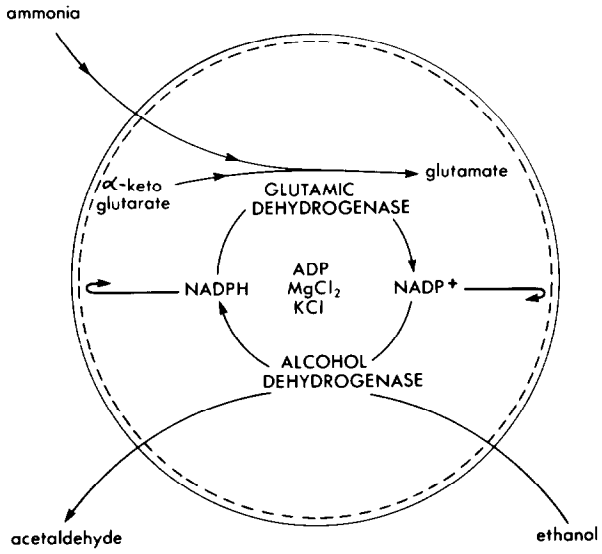

Fig.1. Schematic representation of ultra-thin lipid–nylon membrane microcapsules. Dotted line (. . . .) nylon component of membrane. Solid line (——) lipid component of membrane. Glutamate dehydrogenase, alcohol dehydrogenase, NADPH, $NADP^+$, α-ketoglutarate, ADP are all retained within the microcapsules. External ammonia entering the microcapsules is converted to glutamate.

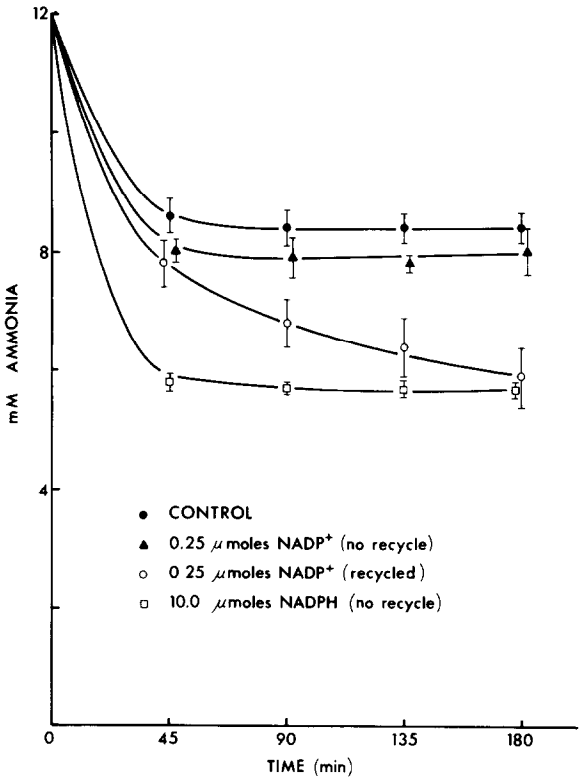

Fig.2. Rate of conversion of ammonia by 4 different types of ultra-thin lipid–nylon membrane microcapsules.

coated microcapsules, however, at pH 7.4 most of the ammonia would be in the impermeant ionized form (NH_4^+).

(2) *High NADPH microcapsules with no recycling:*

Each 2 g of these microcapsules contained 10 μmol NADPH. These microcapsules also contained glutamic dehydrogenase, α-ketoglutarate, KCl, $MgCl_2$ and ADP in the amounts described earlier. When 2 g of these 'high NADPH microcapsules with no recycling' was added to 4 ml ammonia–alcohol substrate solution (pH 9), the ammonia levels fell rapidly. Ammonia (10 μmol) was converted into glutamate by the microcapsules which contained a total of 10 μmol NADPH (fig.2).

(3) *Low $NADP^+$ microcapsules with no recycling:*

Each 2 g of these microcapsules contained 0.25 μmol $NADP^+$. These microcapsules also contained glutamic dehydrogenase, α-ketoglutarate, $MgCl_2$, KCl and ADP in the amounts described earlier. When the 'low $NADP^+$ microcapsules with no recycling' were added to 4 ml substrate solution, the change in ammonia levels was not significantly different from the control microcapsules. This showed that, without recycling, the 0.25 μmol $NADP^+$ in the microcapsules did not result in any significant conversion of ammonia to glutamate (fig.2).

(4) *Low $NADP^+$ microcapsules with recycling:* Each 2 g of these microcapsules contained 0.25 μmol $NADP^+$. These microcapsules also contained alcohol dehydrogenase, glutamic dehydrogenase, α-ketoglutarate, $MgCl_2$, KCl and ADP in the amounts described earlier. When 2 g of these 'low $NADP^+$ microcapsules with recycling' was added to the substrate solution, the change in ammonia levels when compared to the control, indicated that 10 μmol ammonia was converted into glutamate. Thus, the 0.25 μmol $NADP^+$ retained in 2 g microcapsules could be recycled 40 times to convert 10 μmol ammonia (fig.2).

3.1. *Stability tests*

The ability of the microcapsules to retain enzymes, $NADP^+$ and α-ketoglutarate was studied. Briefly, each

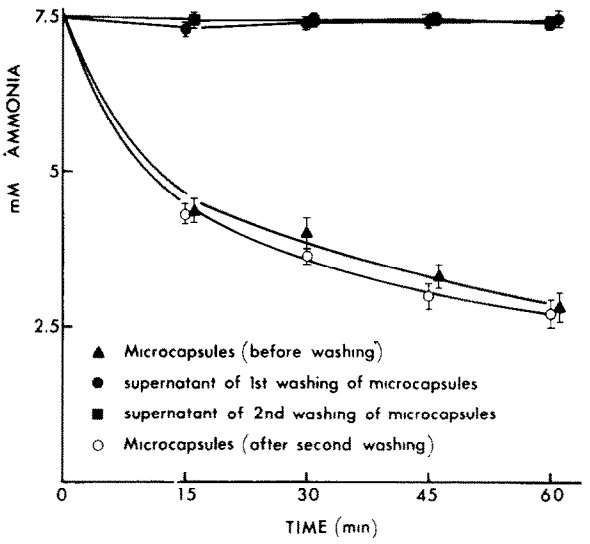

Fig.3. Leakage test: No enzymes or cofactors detectable in the washing solution. Microcapsules retained same activity after 2 washings.

2 g microcapsules was prepared to contain 10 μmol $NADP^+$, and also glutamic dehydrogenase, alcohol dehydrogenase, α-ketoglutarate, $MgCl_2$, KCl and ADP in the amounts described earlier. The freshly prepared microcapsules converted ammonia into glutamate at the rate shown in fig.3. In other studies freshly prepared microcapsules were placed in 2 vol. buffer solution and shaken for 30 min. The supernatant when analyzed, showed no leakage of enzymes, $NADP^+$ or α-ketoglutarate. When the same procedure was repeated with fresh buffer solution for another 30 min, the supernatant from this second washing also showed no leakage of enzymes, $NADP^+$ or α-ketoglutarate.

After these two washings these microcapsules still retained the same rate of conversion of ammonia when compared to the original microcapsules (fig.3). These results therefore show that the cofactor $NADP^+$ and α-ketoglutarate, did not leak out from the microcapsules despite extensive washings.

The above results show that recycling cofactors and substrates required for multienzyme reactions can be retained within ultra-thin lipid–nylon microcapsules to act on permeant external substrates.

Acknowledgement

The support of the Medical Research Council of Canada (MRC-SP-4) to TMSC is gratefully acknowledged.

References

[1] Chang, T. M. S. (1964) Science 146, 524–525.
[2] Chang, T. M. S. (1972) Artificial Cells, monograph, Charles C. Thomas, Springfield IL.
[3] Campbell, J. and Chang, T. M. S. (1975) Biochim. Biophys. Acta 397, 101–109.
[4] Chang, T. M. S. and Malouf, C. (1978) Trans. Am. Soc. Artif. Intern. Organs 24, 18–20.
[5] Wykes, J. R., Dunnill, P. and Lilly, M. D. (1972) Biochim. Biophys. Acta 286, 260–268.
[6] Larsson, P. O. and Mosbach, K. (1974) FEBS Lett. 46, 119–122.
[7] Fuller, C. W. and Bright, H. J. (1977) J. Biol. Chem. 252, 6631–6639.
[8] Grunwald, J. and Chang, T. M. S. (1979) J. Appl. Biochem. 1, 104–114.
[9] Rosenthal, A. M. and Chang, T. M. S. (1980) J. Membr. Sci. 6, 329–338.