0273–2289/84/9000–0301$02.00

Excitable Artificial Cells of Proteinoid

ALEKSANDER T. PRZYBYLSKI AND SIDNEY W. FOX*

NFCR Laboratory of the Institute for Molecular and Cellular Evolution, University of Miami, Coral Gables, FL 33134, USA

Received November, 1983; Accepted December, 1983

ABSTRACT

The proteinoid cells are assembled of thermal polymers of amino acids. Typically, an appropriate mixture of amino acids containing aspartic or glutamic acid is heated at 190°C for 6 h, stirred with water for 2 h, dialyzed during 2 d, and lyophilized. Spheroidal cells are made from such polymer by dissolving it in the water by boiling, and then cooling. Many of them can be made by sonication at room temperature.

These artificial cells, ranging from microns to tens of microns in diameter (depending on composition and preparation), have double membranes and various internal compositions. The spherules can microencapsulate dyes, oxidant–reductant compounds or acceptor–donor substances, and can be packed together.

Such spherules display electrical polarization and electrical discharges and respond to intra- and extracellular ionic and electric influence upon membrane and action potential. These properties arise from the double membrane structure, asymmetric membrane permeability, and channeling phenomena.

Such features as exponential dependence of the steady-state conductance and capacitance as well as negative resistance of the membrane seem to be responsible for the flip-flop alternations of the membrane polarization, rhythmic electric oscillations, and all-or-none action potentials.

The presence of such chromophores as pteridine and flavin in polymers constituting these cells is responsible for their photosensitivity.

Index Entries: Artificial cell, of proteinoids; proteinoid cells; excitable cells, of proteinoid; encapsulation; proteins, thermal encapsulation of; thermal polymers, of amino acids; polymers of amino acids, thermal.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

The proteinoids (thermal polymers of amino acids), elaborated in order to simulate the terrestrial origins of cells, i.e., the emergence of the protocell (*1*) are also relevant to microencapsulation. They make in aqueous solution phase-separated systems (*2*). The various degrees of hydrophobicity and hydrophilicity of the amino acids in the polymers determine also compartmentalization properties of the vesicles (*3*). Various types of microspheres have been prepared using variations in pH and temperature. This property and the chemical composition of the proteinoids make them useful materials for microencapsulation purposes. Such advantages as almost endless variability of composition of the polymer, high degree of purity, self-ordered homogeneity, and the occurrence of emergent properties (selective permeability, catalytic activity, photoactivity, osmobility, ultrastructure, and electrical features) have been emphasized already (*4*). The proteinoid morphological units displaying double membrane structure (*5*) are endowed with electrical properties and with selective permeability. This may be considered promising both in the process of microencapsulation and in the development of artificial neurons.

The present paper indicates some electrical properties of the proteinoid artificial cells from both of these points of view.

MATERIALS AND METHODS

The 2:2:1 proteinoid was prepared by heating in an oil bath at 190°C for 6 h a mixture of 50 g of each individual amino acid treated with 50 g of an equimolar mixture of 18 amino acids under a nitrogen blanket. The product was stirred mechanically with water for 2 h and filtered. The dissolved fraction was dialyzed during 2 d in a cold room with two changes of water per day. The nondiffusate was lyophilized (*6*).

Vesicles were prepared by solution of 60 mg of the proteinoid and 60 mg of lecithin in 3 mL of water with glycerol in the volumetric ratio 1:1. K_2HPO_4 was added to this mixture to adjust the pH to 5.8. This was then boiled in a water bath followed by heating twice for 1–2 s over an open flame. The tube was then allowed to cool to room temperature.

Electrical properties of the vesicles were monitored by means of a standard intracellular technique using glass microelectrodes filled with 3*M* KCl, using platinum wire as a reference electrode. The membrane potential of vesicles was measured in the mother liquor, after compensation of the microelectrode tip potential, by means of high input impedance WPI 701 amplifier, displayed on the oscilloscope and recorded on a chart recorder. Details were described previously (*7*).

RESULTS

The structure of the proteinoid vesicle and its membranous properties with controlled permeability, depending on polymer composition and physical parameters of the aqueous solution, permits the microencapsulation of various substances.

Figure 1 presents an encapsulated α-chlorophyll. The chromophore is evenly located in the body of the vesicle except its membrane. The encapsulated chlorophyll remains in the vesicle for weeks. The electrical potential across the membrane of the vesicle is correlated with retention of the encapsulated substance. The high membrane potential is an indication of the quality of the membrane and also of the encapsulating capacity of the vesicle. An absence of membrane potential is correlated with discontinuity of the membrane and with loss of encapsulating capacity of the vesicle.

Electrical phenomena of the proteinoid-only and the proteinoid–lecithin vesicles consist of membrane polarization, spike-like potentials, and periodic oscillations. When recorded intracellularly, the amplitude of electrical discharge exceeds the zero-line of electrical polarization of the membrane (Fig. 2). The membrane of the vesicle displays self-resealing ability, as seen under the microscope and monitored on the oscilloscope (Fig. 3). The frequency distribution of the membrane amplitude of measured vesicles is presented in the histogram of Fig. 4. The

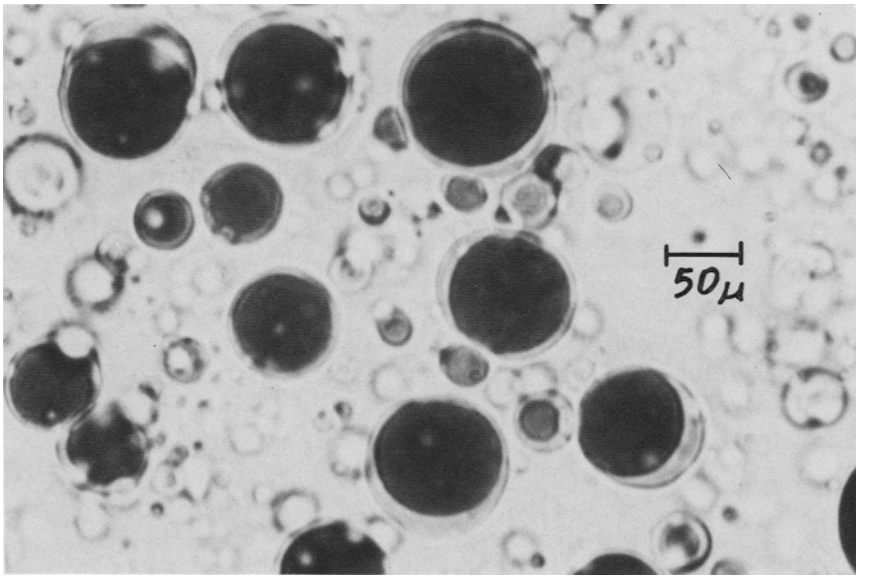

Fig. 1. Encapsulated α-chlorophyll in the poly(Asp, Glu) proteinoid–lecithin vesicle.

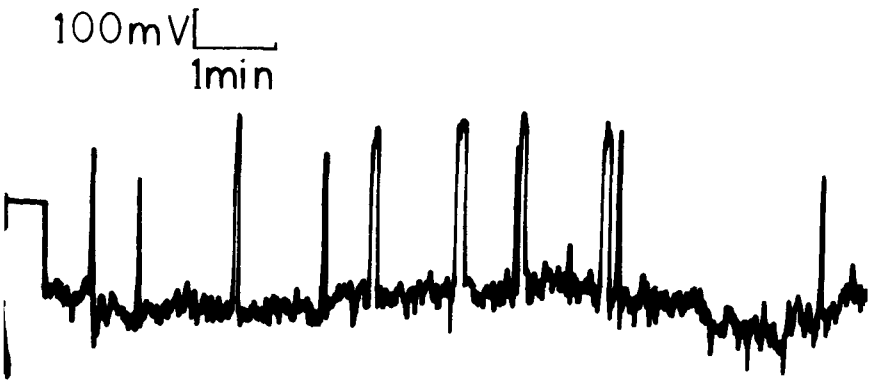

Fig. 2. Electrical discharges of the poly(Asp, Glu) proteinoid–lecithin vesicle with encapsulated chlorophyll exceeding the zero-line of the membrane polarization.

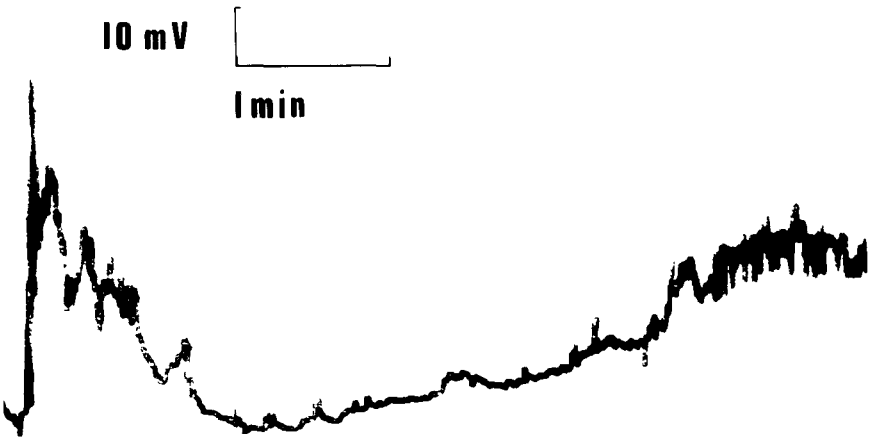

Fig. 3. Membrane potential recovery after microelectrode impalement of the poly(Asp, Glu) proteinoid–lecithin vesicle.

excitability of proteinoid–lecithin vesicles is shown by the electrical current (Fig. 5) and light induced potentials (Fig. 6).

DISCUSSION

The data obtained extend our previously reported observation on electrical properties of the proteinoid vesicles (*4*). Further studies on proteinoid–lecithin (*7*) and on proteinoid-only vesicles (*8*) indicate that proteinoid-only vesicles are endowed with membrane potential and its periodic discharges. They are also able to sustain potassium concentration gradient during at least several months (ability to keep across-

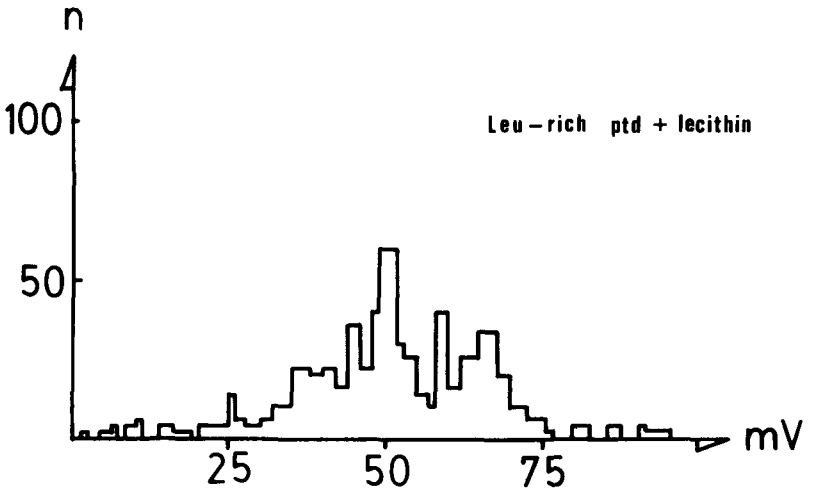

Fig. 4. The frequency distribution of the amplitude of the membrane potential of the 2:2:1 proteinoid–lecithin vesicles.

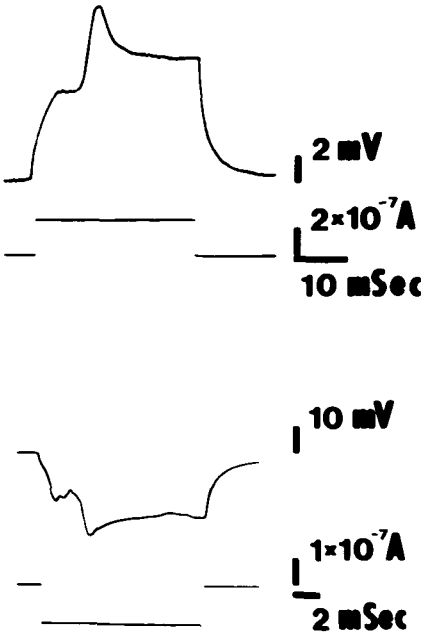

Fig. 5. The electrical current induced potentials of the 2:2:1 proteinoid–lecithin vesicle.

membrane potential and its diminution followed after an external addition of potassium ions to the medium). This means also that the encapsulation capacity of such electrically active vesicles last longer, and can be varied at will by changing the amino acid composition of the vesicles. Chlorophyll-containing vesicles are efficient light converters (*9*).

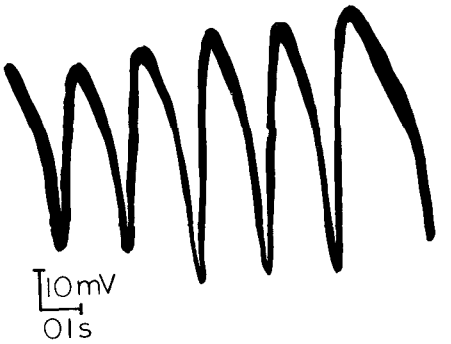

Fig. 6. The light induced oscillations of the poly(Asp, Glu) proteinoid–lecithin vesicle with chlorophyll.

Additionally, by measuring the membrane potential by means of conventional electrophysiological technique or potential-sensitive probes (*10*), this capacity can be tested easily.

Because of their surface potential, the coalescence of the proteinoid vesicles may be diminished considerably.

Taking into account the fact that pathologically altered cells and tissues differ in their electrical potential compared to normal cells, we may assume that the delivery of drugs to such cells can be enhanced by means of electrically charged particles. The self-resealing ability of the membranes is a further advantage of such vesicles. They can hold the same charge until there is complete depletion of the substance being carried by the vesicles. Nothing like this is possible, for instance, in the case of nylon encapsulation of hemoglobin (*11*). Electrical behavior is thus a possible means for following the course of permeation through microcapsules.

In the literature on microencapsulation, there are attempts to approach cell simulation (*12–15*). This article seems to be closely related to such papers.

ACKNOWLEDGMENTS

This work was supported by the National Aeronautics and Space Administration, Grant No. NGR-10-007-008. Contribution No. 361 of the Institute for Molecular and Cellular Evolution.

REFERENCES

1. Fox, S. W., and Dose, K. (1977), *Molecular Evolution and the Origin of Life*, rev. ed., Dekker, New York.
2. Fox, S. W. (1976), *Origins of Life* **7**, 49.
3. Brooke, S., and Fox, S. W. (1977), *BioSystems* **9**, 1.
4. Fox, S. W., and Brooke, S. (1979), in *Microencapsulation*, Kondo, T., ed., Techno Books, Tokyo.

5. Fox, S. W. (1965), *The Origins of Prebiological Systems and of Their Molecular Matrices,* Academic Press, New York, pp. 361–382.
6. Vegotsky, A. (1972), in *Molecular Evolution,* Rohlfing, D. L., and Oparin, A. I., eds., Plenum Press, New York.
7. Ishima, Y., Przybylski, A. T., and Fox, S. W. (1981), *BioSystems* **13,** 243.
8. Przybylski, A. T., Stratten, W. P., Syren, R. M., and Fox, S. W. (1982), *Naturwissenschaften* **69,** 561.
9. Przybylski, A. T., and Fox, S. W. (1982), in *Alternative Energy Sources IV,* Vol. 3, Veziroglu, T. N., ed., Ann Arbor Science Press, Ann Arbor, pp. 95–102.
10. Tasaki, I., and Warashina, A. (1976), *Photochem. Photobiol.* **24,** 191.
11. Jay, A. W. L., and Burton, A. C. (1969), *Biophys. J.* **9,** 115.
12. Kondo, T., Arakawa, M., and Tamamushi, B. (1974), in Microencapsulation, Nixon, J. R., ed., Dekker, New York, pp. 163–172.
13. Kondo, A., and van Valkenburg, J. W. (1979), *Microcapsule Processing and Technology,* Dekker, New York, pp. 106–120.
14. Chang, T. M. S. (1974), in *Microencapsulation,* Vandegaer, J. E., ed., Plenum, New York, pp. 95–102.
15. Chang, T. M. S. (1979), in *Microencapsulation,* Kondo, T., ed., Techno Books, Tokyo, pp. 369–375.