Microencapsulation of Methylglyoxal and Two Derivatives

YASUO NOZAWA and SIDNEY W. FOX ×

Received August 10, 1979, from the *Laboratory of the National Foundation for Cancer Research, Institute for Molecular and Cellular Evolution, University of Miami, Coral Gables, FL 33134.* Accepted for publication January 22, 1980.

Abstract □ Microcapsules of methylglyoxal, methylglyoxal bis(guanylhydrazone), and methylglyoxal–ascorbic acid condensation complex were prepared and release curves were determined. The effect of various concentrations of hydrochloric acid on the decomposition of the ascorbic acid complex was investigated.

Keyphrases □ Methylglyoxal—preparation of microcapsules of methylglyoxal, methylglyoxal bis(guanylhydrazone), or methylglyoxal–ascorbic acid complex, decomposition in aqueous solution □ Release—of microcapsules of methylglyoxal, methylglyoxal bis(guanylhydrazone), or methylglyoxal–ascorbic acid complex, decomposition in aqueous solution □ Microencapsulation—symposium, preparation of microcapsules of methylglyoxal and derivatives for gradual release in aqueous solution

In Szent-Györgyi's model for tumorgenesis, the sequence of reactions is: (*a*) methylglyoxal (I) or a related compound interferes with cell division, (*b*) release of glyoxalase I by an oncogenic agent reduces the I concentration, and (*c*) cell division then proceeds unhindered (1). Aside from using inhibitors of glyoxalase I (2–5), one may attempt to reestablish control of cell division by administering I or suitable derivatives.

Compound I, the bis(guanylhydrazone) of I (II) (6), and the methylglyoxal–ascorbate condensation product (III) (7) inhibited the growth of Ehrlich ascites tumor cells in mice[1]. Since the toxicity of I and that of complexes with I generally is high (8), such compounds have been microencapsulated for gradual release.

Although the prospects for gradual release are not promising due to the high solubility of each compound in water, gradual release from such microencapsulated products has been observed.

EXPERIMENTAL

Production of Microcapsules—A 20% methylene chloride solution of polymethyl methacrylate[2] (very high molecular weight) was emulsified (5:1) with an aqueous solution of I or either derivative by stirring on an homogenizer[3] at a setting of 2.5 (2–5 min). To this solution was added 10 parts of a 1% solution of gelatin[4] (or polyaminoacid), and the original water/oil emulsion was converted to a (water/oil)water emulsion by stirring at a setting of 4 (~10 min). This emulsion was stirred magnetically for 5–6 hr at room temperature. Compound II was purchased[2].

In each case, the product was sifted through a 500-mesh sieve[5] and then centrifuged at 4000 rpm to a paste.

Stability of III—Compound III[6] was unstable under all conditions but less so at high acidity. Its decomposition rate was tested in various concentrations of hydrochloric acid. Compound III (3.9 mg) was allowed to stand for various periods in 50 ml of each hydrochloric acid concentration (Fig. 1). The entire experiment was shielded from light.

Release Experiments—For release of I, 8 g of the moist paste of I microcapsules was stirred magnetically in 500 ml of 4.6% HCl. Release was complete at ~175 hr. Samples of the clarified fluid were monitored at 270 nm.

For release of II, 1 g of paste was used. The receiving solution was 500 ml of water adjusted to pH 3 with hydrochloric acid. The wavelength for analysis was 280 nm.

For III, 6 g of paste was stirred into 500 ml of 4.6% HCl; the release was monitored at 240 nm.

The 4.6% HCl was used for I because high acidity is required to inhibit the rapid polymerization that occurs otherwise[7]. The decomposition observed with III (Fig. 1), which is also attenuated by concentrated hydrochloric acid, is perhaps related mechanistically to the decomposition of I. Concentrated hydrochloric acid was unnecessary to stabilize II, in which both carbonyl groups are derivatized.

No control experiments on the relative rate of solution of I or II in the absence of microcapsules are reported; I and II are highly soluble in the receiving solution, and they dissolve momentarily. Compound III is more slowly soluble; its rate of solution is approximately twice as great as its rate of release.

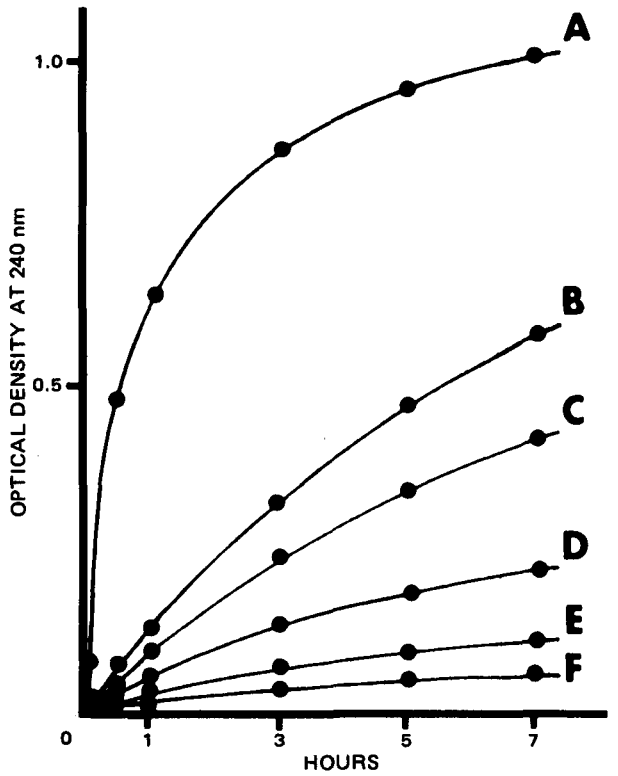

Figure 1—*Decomposition of methylglyoxal–ascorbic acid complex (III). Key: A, pH 3; B, 0.95% HCl; C, 1.6% HCl; D, 2.3% HCl; E, 6.9% HCl; and F, 11.5% HCl.*

RESULTS AND DISCUSSION

The decomposition of III is shown in Fig. 1. The high acidities required to stabilize III suggest the need for extensive study of its physical properties should this compound prove to have pharmacological activity.

Figure 2 shows microcapsules of I and indicates that they are typical

[1] T. F. Slater, Brunel University, Brunel, England, personal communication.
[2] Aldrich Chemical Co., Metuchen, N.J.
[3] Sorvall Omni-Mixer, Newtown, Conn.
[4] Swine skin type 1, ~300 bloom, Sigma Chemical Co., St. Louis, Mo.
[5] W. S. Tyler Co., Mentor, Ohio.
[6] Gift from Dr. Gabor Fodor, West Virginia University, Morgantown, W.Va.
[7] P. Gascoyne, Marine Biological Laboratory, Woods Hole, Mass., personal communication.

0022-3549/81/0400-0385$01.00/0

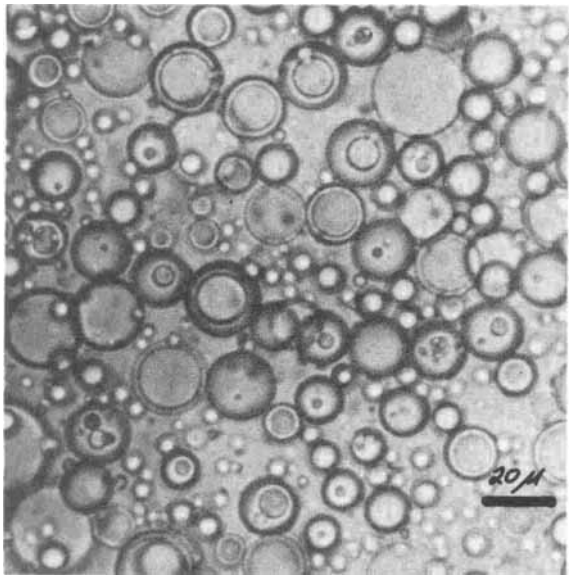

Figure 2—*Microcapsules containing methylglyoxal (I).*

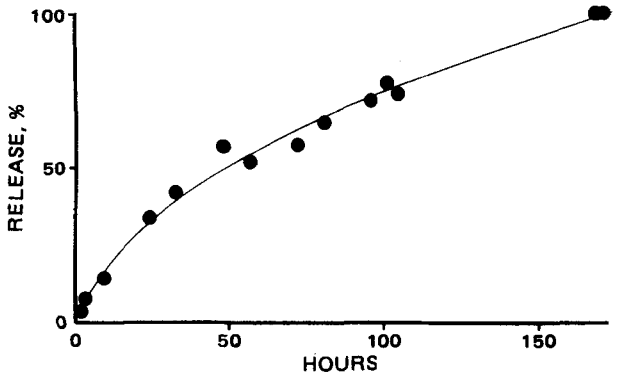

Figure 3—*Release of methylglyoxal (I).*

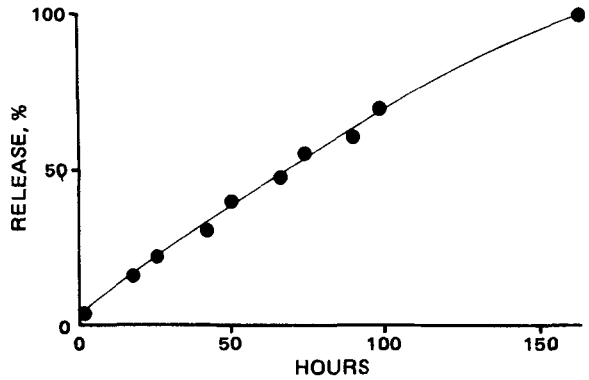

Figure 4—*Release of methylglyoxal bis(guanylhydrazone) (II).*

microcapsules. The microcapsules of II and III are visibly indistinguishable from those of I.

The release curve for I is shown in Fig. 3. The release curves for II and III are given in Figs. 4 and 5, respectively.

Microencapsulation of I, II, or III was accomplished, despite their considerable aqueous solubility. There are several possible explanations for the capture of these compounds.

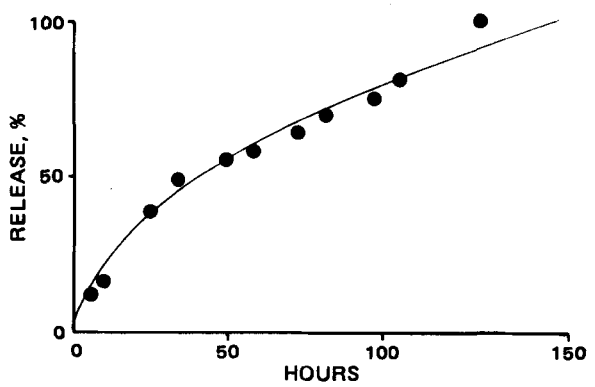

Figure 5—*Release of methylglyoxal–ascorbic acid condensation product (III).*

One inferred influence is gross physical entrapment of the type common to all microencapsulation. However, this entrapment is insufficient to delay release of many water-soluble compounds.

The encapsulated material may be caught within the meshwork resulting from the interdigitation of branched macromolecules, such as with amylopectin (9). Such a structure also was proposed for collagen (10), from which is obtained gelatin, which is often used in microcapsules.

Another explanation depends on a loose kind of bond that could be anticipated from the interaction of a dicarbonyl compound with special groups in the gelatin. Since II and III do not have free carbonyl groups, the meshwork explanation is more general and is in accord with the similar release curves observed for I–III. Moreover, the leaching of solid from a network structure is easily visualized. The probable mechanism thus includes physical entrapment by walls of microcapsules and entrapment within the lacunae provided by a meshwork of branched macromolecules.

REFERENCES

(1) A. Szent-Györgyi, *Int. J. Quantum Chem. Quantum Biol. Symp.*, **4**, 179 (1977).

(2) S. W. Fox, R. M. Syren, and C. R. Windsor, in "Submolecular Biology and Cancer," G. E. W. Wostenholme, D. W. Fitzsimons, and J. Whelan, Eds., Ciba Foundation Press, London, England, 1979, p. 175.

(3) R. Vince and W. B. Wadd, *Biochem. Biophys. Res. Commun.*, **35**, 593 (1969).

(4) R. Vince, S. Daluge, and W. B. Wadd, *J. Med. Chem.*, **14**, 402 (1971).

(5) C. Silipo and C. Hansch, *Farmaco, Ed. Sci.*, **34**, 3 (1979).

(6) S. N. Pathak, C. W. Porter, and C. Dave, *Cancer Res.*, **37**, 2246 (1977).

(7) G. Fodor, R. Mujumdar, and J. Butterick, in "Submolecular Biology and Cancer," G. E. W. Wostenholme, D. W. Fitzsimons, and J. Whelan, Eds., Ciba Foundation Press, London, England, 1979, p. 165.

(8) T. F. Slater, in *ibid.*, p. 169.

(9) K. H. Meyer, "Natural and Synthetic High Polymers," Interscience, New York, N.Y., 1950, p. 468.

(10) N. P. Stimler and M. L. Tanzer, *Adv. Exp. Biol. Med.*, **86B**, 675 (1977).

ACKNOWLEDGMENTS

Supported by Grant NGR-10-007-008 of the National Aeronautics and Space Administration.

The authors thank Mr. Charles R. Windsor for experimental repetitions.

This report is Contribution 333 of the Institute for Molecular and Cellular Evolution.

Y. Nozawa was on leave from Shizoku College of Pharmacy.