A MICRO SUSTAINED RELEASE SYSTEM FOR EPIDERMAL GROWTH FACTOR

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SUMMARY

A technique for ensuring the controlled release of microgram and smaller amounts of biologically active epidermal growth factor (EGF) from polymeric delivery systems is described. We show that albumin in milligram quantities can facilitate the sustained release of picogram amounts of EGF for at least 3 wk. The EGF-containing polymer matrix can be placed directly into cell culture and will increase the proliferation rate of serum-starved cells. The method reported here should be suited particularly to the delivery of biologically active growth factors that are obtainable in only microgram or smaller amounts.

Key words: growth factors; controlled release; tissue culture; epidermal growth factor.

INTRODUCTION

Ethylene vinyl acetate copolymer pellets can be used to discharge biologically active macromolecules continuously for several months (1). These pellets have been used as delivery systems for bioactive substances in a number of biological tests. Examples include the use of subcutaneously implanted insulin-containing pellets to normalize blood glucose levels of diabetic rats (2) and the use of pellets containing protamine sulfate to inhibit tumor-induced angiogenesis in the rabbit cornea (3). In these sorts of tests milligram quantities of factors are required for polymer pellet formulation, even for pellets as small as 1 mm³, to ensure the release of physiologically significant amounts during the experimental period (4). The requirement for milligram quantities is a consequence of the mechanism by which macromolecules are released from polymeric matrices. During the formulation process, pores are formed in the macromolecule-polymer matrix due to the presence of particulate macromolecules (4). If small amounts of macromolecules are present, few pores are formed. On the other hand, if large amounts are present pores are numerous and interconnect to form tortuous channels that run through the matrix to the surface through which macromolecules can be released. Because of the requirement for relatively large amounts of macromolecules, these systems have been less suitable for the delivery of growth factors or other substances that are obtainable only in microgram or nanogram amounts. In this report, we show that incorporating milligram amounts of albumin along with microgram amounts of epidermal growth factor (EGF) into polymers allows the sustained release of as little as picograms/day of EGF over a 3 wk period. We also show that polymers containing microgram amounts of EGF are compatible with, and can stimulate the growth of, serum-starved cells in culture.

MATERIALS AND METHODS

General formulation method. The general procedure for making polymer pellets, which release large quantities of macromolecules, has been described in detail elsewhere (5). We used the following modification of that procedure to make

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1To whom reprint orders should be addressed at MIT.
polymer pellets that release small amounts of macromolecules. Briefly, a weighed amount of macromolecule is added to 7 ml of 10% (wt/vol) ethylene-vinyl acetate (EVA) copolymer (Elvax 40P, Dupont Chemical Co., Wilmington, DE) that had been washed with water and alcohol (6), in HPLC grade methylene chloride (Fisher Scientific, Fair Lawn, NJ), in a glass vial. The mixture is vortexed at high speed for 10 s to achieve a uniform suspension. The suspension is poured immediately into a leveled glass mold (4 x 4 x 0.5 cm) precooled on a slab of dry ice. The polymer slab is allowed to rest in the mold on the slab of dry ice for at least 10 min, after which it is removed to a –20°C freezer. The slab is kept in the freezer for 48 h and then dried for 48 h at room temperature under 600 mtorr vacuum. The dried polymer slab is cut into 1 x 1 cm squares before use.

**Experimental Polymer Pellets**

Polymer pellets were made containing bovine serum albumin (BSA), methyl [14C]BSA, EGF, and [125I]EGF in various combinations.

**Radiolabeled EGF polymer pellets.** [125I]EGF was obtained from Collaborative Research (Waltham, MA) at an initial 212 μCi/μg sp act. [14C]BSA (New England Nuclear, Boston, MA) was obtained at a 0.016 mCi/mg sp act and diluted to a final 1.40 nCi/mg sp act. Three polymer slabs were made. The first was made with 8.7 ng [125I]EGF and 460 mg [14C]BSA. One centimeter by one centimeter squares from this slab weighed on the average of 76.21 mg and contained 0.5 ng [125I] EGF and 30.48 mg [14C]BSA. The second was made with approximately 8.7 ng [125I]EGF and 175 mg [14C]BSA. One centimeter by one centimeter squares from this slab weighed on the average 57.29 mg and contained 0.5 ng [125I]EGF and 11.46 mg [14C]BSA. The third slab was made with 8.7 ng [125I]EGF and no BSA. The resultant squares weighed 50.12 mg on average and contained 0.5 ng [125I]EGF.

**Unlabeled EGF polymer pellets.** One slab containing 460 mg BSA (Sigma, St. Louis, MO) and 50 μg mouse EGF (Collaborative Research, Waltham, MA) was made. One centimeter by one centimeter squares were excised from this slab and weighed on average 100.0 mg and contained 4.3 μg EGF and 39.8 mg BSA.

**Measurement of Release**

**Release from polymer squares containing radiolabeled macromolecules.** One centimeter by one centimeter squares were placed in glass scintillation counting vials containing 750 μl sterile phosphate buffered saline (PBS) (pH 7.4) and 0.02% sodium azide at 37°C. At various times the saline was removed for scintillation counting and replaced with fresh saline. Ten microliters Instagel (Packard Instruments, Downers Grove, IL) was added to each sample before counting. Samples were counted in a Packard tri-carb liquid scintillation spectrometer Model 3220 set up for double isotope analysis for 125I and 14C (7). Data were corrected to disintegrations per minute using appropriate quench curves. Data from experiments using 125I were corrected for decay of that isotope. At the end of the experimental period (3 wk) the PBS was removed from each vial and 1 ml of xylene was added to each square to dissolve the polymer; then 1 ml of distilled water was added to solubilize the remaining macromolecule. This procedure allowed us to recover unreleased macromolecules for counting so that mass balances could be conducted (8). The counting efficiencies of standards containing 1 ml xylene and 1 ml water were determined in order to correct for any quenching. Data from these experiments were plotted as a function of Time 1/2 (square root of time). Time 1/2 is used to linearize the data (9). The duration of the experiment was 23 h 1/2, which is the same as 529 h or 22 d.

**Release from polymer squares containing non-radiolabeled EGF.** One centimeter by one centimeter polymer squares containing 4.3 μg EGF and 39.8 mg BSA were placed in sterile scintillation vials and irradiated overnight with a General Electric 15 watt ultraviolet lamp placed 6 cm from the squares. One milliliter of sterile, distilled water containing 50 U/ml penicillin and 50 μg/ml streptomycin was placed in each vial with the polymer square. Squares were incubated at 37°C, and every 48 h the water was removed from the vial, lyophilized, and stored at –20°C. One milliliter sterile distilled H2O was added to replace that which had been removed. The experiment was carried out for 2 wk. Sterile technique was observed throughout the experiment. A representative lyophilized sample of H2O that had been incubated with a square during the period from Days 10 to 12 was resuspended in water; various volumes were assayed for the ability to stimulate DNA synthesis in confluent monolayers of quiescent BALB/c 3T3 cells.

**DNA Synthesis in 3T3 Cells**

A detailed description of the growth of BALB/c 3T3 cells, the preparation of confluent quiescent