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Macromolecular prodrugs. IV. Alginate-chitosan microspheres of PHEA-L-dopa adduct

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Abstract

A polymeric prodrug α,β-poly(*N*-hydroxyethyl)-DL-aspartamide-L-dopa adduct (PHEA-L-dopa) was microencapsulated in alginate-chitosan microspheres in order to achieve drug release from a complex reservoir device. The gel/matrix material of alginate-chitosan complex protects the adduct from hydrolysis by the surrounding medium. On the basis of a 10^3-fold difference between the drug released from microspheres as the adduct and that released in the unbound form, a model of the microencapsulated system was proposed.

Keywords: PHEA-L-dopa adduct; Alginate; Chitosan; Microsphere; Prodrug

1. Introduction

Prodrugs in which drugs are covalently linked to polymeric carriers have been suggested as an effective way to prolong the pharmacological activity, minimize unfavorable side effects and toxicity, decrease the required dose and increase the solubility of the drug, as well as alter the body distribution and ensure adequate drug delivery to target cells or tissues (Duncan and Kopeček, 1984). The chemical bond of a polymer-drug adduct should be of limited stability in biological environments and the polymeric prodrugs should release the active form of the drug in therapeutically efficacious quantities over a given span of time (Ferruti, 1977).

PHEA (α,β-poly(*N*-hydroxyethyl)-DL-aspartamide) is an interesting and suitable drug carrier, since it is hydrosoluble, nontoxic, nonantigenic, biodegradable and is easily and inexpensively prepared. Hydroxyl groups of PHEA permit the attachment of selected carboxylic and amino acid drugs to the polymer by ester bonds (Zorc et al., 1993a,b; Giammona et al., 1994). In our previous paper we described the preparation of PHEA-L-dopa (Scheme 1), a polymer-drug adduct in which L-dopa is linked to polyhydroxy drug carrier by ester linkage. Release of active substance based on hydrolysis of the PHEA adduct was studied in vitro, and a (pseudo) first-order release rate constant of 1.06×10^{-3} min^{-1}

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for L-dopa was obtained, the half-time in vitro being 10.9 h (Zorc et al., 1993b).

It can be speculated that the polymer-drug adduct might be a candidate for microencapsulation into a biocompatible and biodegradable matrix/gel forming material with the aim of achieving a double reservoir device. The model drug (L-dopa) would in such a manner be protected from its biological surroundings by a two-fold protection.

For this purpose, a polyelectrolyte complex of alginate and chitosan was chosen. Chitosan [(1,4)-2-acetamido-2-deoxy-β-D-glucan] is a deacetylated derivative of the polysaccharide chitin (Li et al., 1992), while alginate is a random copolymer of D-mannuronic acid and D-guluronic acid (Smidsrod and Skjak-Break, 1990). Both of these polysaccharides are biocompatible and biodegradable. The electrostatic interaction of carboxyl groups of alginate with the amine groups of chitosan and the resulting entanglement of the polymers form a membrane that encloses the active material (Polk et al. 1994). The model should be also suitable for evaluation of the release pattern from such a complicate system.

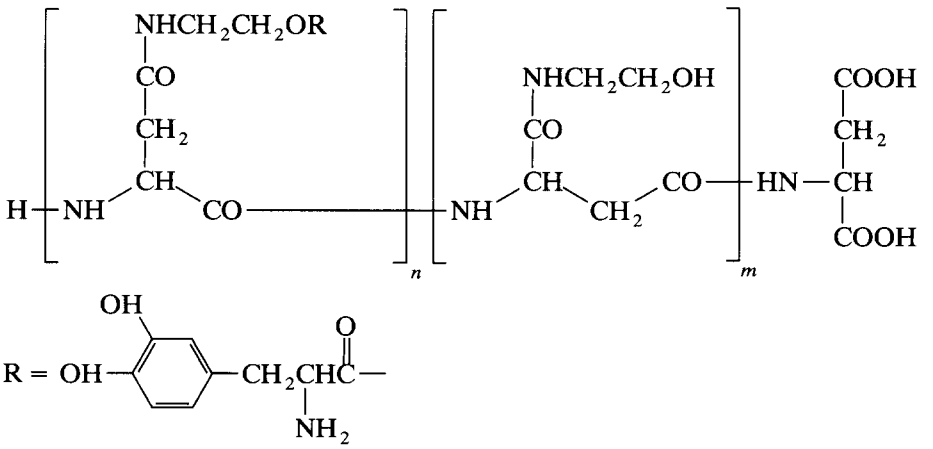
2. Materials and methods

2.1. Materials

Sodium alginate (Pronova LVG sodium alginate) and three types of chitosan of varying viscosity (Seacure CL 210, CL 211 and CL 311) were obtained from Protan Laboratories Inc. (Drammen, Norway). L-Aspartic acid was purchased from Kemika (Zagreb), and L-dopa from Merck (Darmstadt). All solvents were of analytical grade quality and were dried and distilled prior to use. All solutions for HPLC were prepared with HPLC-grade solvents (Fisher Scientific).

2.2. Chemistry

The outline of the procedure for the preparation of PHEA-L-dopa (Scheme 1) was the same as the method described previously (Zorc et al., 1993b). In short, to a solution of 0.50 g PHEA in 20 ml DMF (nitrogen atmosphere) 0.34 g (0.001 mol) 1-benzotriazolylcarbonyl-L-dopa (Btc-L-dopa) was added and thereafter a solution of 0.71 g (0.007 mol) triethylamine (TEA) in 12 ml *N,N*-dimethylformamide (DMF) was added dropwise. The reaction was run at room temperature for 24 h under a nitrogen atmosphere. Solvent was evaporated in vacuo to a small volume and PHEA-L-dopa precipitated by adding acetone/glacial acetic acid mixture. The product was filtered off and washed several times with a small amount of acetone. The load of L-dopa in conjugate was 17.4%. Btc-L-dopa was prepared from L-dopa and 1-benzotriazolecarboxylic acid chloride (BtcCl). PHEA with a weight-average molecular weight of $M_r = 54\,850$ was prepared by thermal polycondensation of L-aspartic acid at 160°C in the presence of phosphoric acid and



Scheme 1.

subsequent aminolysis of polysuccinimide with ethanolamine (Neri et al., 1973; Zorc et al., 1993b).

UV spectra were recorded on Pharmacia LKB Ultrospec Plus and Pye Unicam SP-100 spectrophotometers. An HPLC system with an Applied Biosystems ABI 783 programmable UV absorbance detector was used. Polymer solutions were dialyzed against several changes of deionized water using Visking dialysis tubing (18/22 inch, Serva) with a molecular weight cut-off of 12 000–14 000.

2.3. Microsphere formation

The encapsulation solutions were prepared as follows. Alginate solution (3%, w/v) was prepared by dissolving sodium alginate in distilled water. Chitosan stock solution (1%, w/v) was prepared by adding the chitosan to distilled water. The flasks were covered with parafilm and stirred for 1 h at 60°C. The solutions were filtered to remove particulates. Chitosan stock solution was diluted to 0.1% (w/v) and calcium chloride was added to 1.5% or 8% (w/v). A weighed amount of PHEA-L-dopa adduct was dissolved in artificial cerebrospinal fluid (CSF), pH 6 and the resulting solution was added to alginate solution. The ratio of PHEA-L-dopa adduct to dry alginate was 1:1.

Alginate solution was loaded into a 1 ml syringe to which an adapted microdialysis probe (4 mm, CMA 10, Carnegie Medicine AB, Stocholm) was then attached. An air-jet droplet generator was attached to the probe and the entire apparatus was mounted onto a microdialysis syringe pump (CMA 100, Carnegie Medicine AB, Stocholm). 2 ml of 0.1% chitosan in calcium chloride solution was put into a test tube and stirred gently using a magnetic stirrer. Alginate was extruded at a fixed rate of 0.1 ml/min. The microspheres were formed by gently stirring with a magnetic stirrer for 10 min. The microspheres were allowed to settle and the chitosan/$CaCl_2$ was removed by aspiration. The microspheres were then rinsed with 1.5 ml of artificial CSF.

In this manner 200–250 μm diameter microspheres were formed. The viscosity of chitosan varied from experiment to experiment. For drying the microspheres isopropanol was chosen due to its known use as a drying agent. After removal of the artificial CSF, isopropanol was added to the microspheres. The spheres were allowed to settle and the isopropanol was removed by aspiration. After repeating the procedure, the spheres were dried for an additional 2 h in air. The size and shape of the microspheres were determined using a microscope (Olympus BH-2, Japan) connected to an image analyser (Optomax V, Cambridge).

2.4. Release of L-dopa from alginate-chitosan microspheres

The microspheres obtained were eluted with artificial CSF at pH 6.0. Microspheres were placed in vials and in contact with 0.3 ml of CSF. At suitable time intervals over 10 days the elution solution was replaced with fresh solution, and analysis of the solution was performed by HPLC. Each determination was carried out in triplicate and the release results were plotted as the amount of cumulative L-dopa release into the dissolution medium from the microspheres vs time.

2.5. Determination of L-dopa and conjugated L-dopa

20-μl aliquots of elution samples were directly injected into a reverse-phase ion-pair HPLC system with electrochemical detection. L-dopa was well separated from the peaks of PHEA-L-dopa adduct on a 250 × 4.6 mm i.d. column prepacked with ultrasphere ODS 5 μm (Altex). The mobile phase consisted of 0.15 mol l^{-1} sodium dihydrogen orthophosphate containing 0.1 mmol l^{-1} EDTA, 0.65 mmol l^{-1} sodium octyl sulfate and 10% methanol. The pH was set at 3.8 using concentrated phosphoric acid. The flow rate was 1.0 ml/min. The amounts of L-dopa and conjugated L-dopa in samples were calculated using L-dopa and PHEA-L-dopa adduct as an external standard by determining the peak height ratios. The limit of determination of L-dopa in samples was 0.2 μmol l^{-1}.

3. Results and discussion

Preliminary experiments showed that the necessary conditions for successful encapsulation of the PHEA-L-dopa adduct into microspheres were met. The alginate and chitosan concentrations were kept constant, as well as the reaction time for counterion polymers to form a stable gel matrix. The $CaCl_2$ concentration was varied (1.5 and 8%) and the type of chitosan used (Seacure CL 210, 60 mPa s; Seacure CL 211, 70 mPa s; Seacure CL 311, 580 mPa s) in order to study the influence of Ca^{2+} and the viscosity of the chitosan sample on the release behaviour of the encapsulated substance.

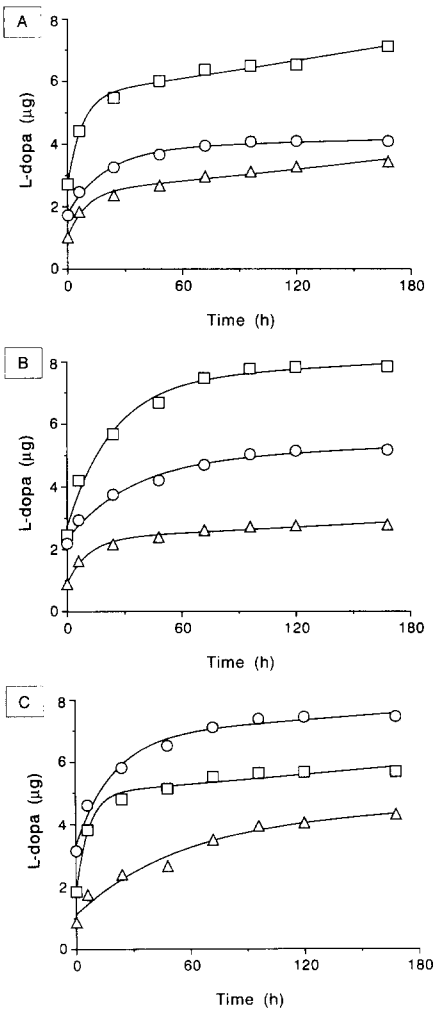


Fig. 1. Release of L-dopa from alginate-chitosan microspheres in CSF (pH 6.0) prepared with: (A) Seacure CL 210; (B) Seacure CL 211; (C) Seacure CL 311. Points are experimental and curves are the best fit for first-order kinetics of the biphasic type: (○—○) 1.5% $CaCl_2$; $r^2 = 0.993$, 0.987 and 0.985 for A, B and C, respectively; (□--□) 8% $CaCl_2$; $r^2 = 0.992$, 0.987 and 0.988 for A, B and C, respectively; (△—△) 8% $CaCl_2$ dried microspheres; $r^2 = 0.987$, 0.987 and 0.971 for A, B and C, respectively.

The microspheres were in the size range of 200–250 μm and of roughly spherical shape. They appear to have a smooth surface structure without microscopically visible pores or crevices. The internal structure appeared to be less smooth and microporous. It would be reasonable to suppose that the internal structure of the spheres was not homogeneous, since Ca alginate and alginate-chitosan complexes should be entangled with the regions of the PHEA-L-dopa conjugate. Also, Ca^{2+} is supposed to penetrate alginate droplets more readily than the chitosan molecule; the smooth surface can be attributed to a higher concentration of chitosan at the surface forming a denser matrix structure. As the Ca^{2+} concentration is essential for optimal cross-linking of the alginate polymer chain (Burns et al., 1985) two sets of spheres were prepared, i.e., with more standard (1.5% $CaCl_2$) and with higher concentration (8% $CaCl_2$). It will be shown later that the higher Ca^{2+} concentration applied during the preparative phase produced spheres with the highest release rates of the encapsulated substances.

A set of dry microspheres was prepared in order to observe the difference in the release of the dried and later resuspended microspheres. As noted previously (Polk et al. 1994), on drying, the microspheres shrank in size and the particles obtained were of irregular shape. After the dried product has been resuspended in aqueous medium (artificial cerebrospinal fluid), capsules swelled rapidly (in less then 1 h) up to about 70% of their original wet size.

The release from alginate-chitosan microspheres is shown in Fig. 1 and 2. Fig. 1 presents the unbound L-dopa release while Fig. 2 corresponds to bound L-dopa release, i.e., the drug released still in the form of PHEA adduct. Contrary to expectations, the release of PHEA-L-

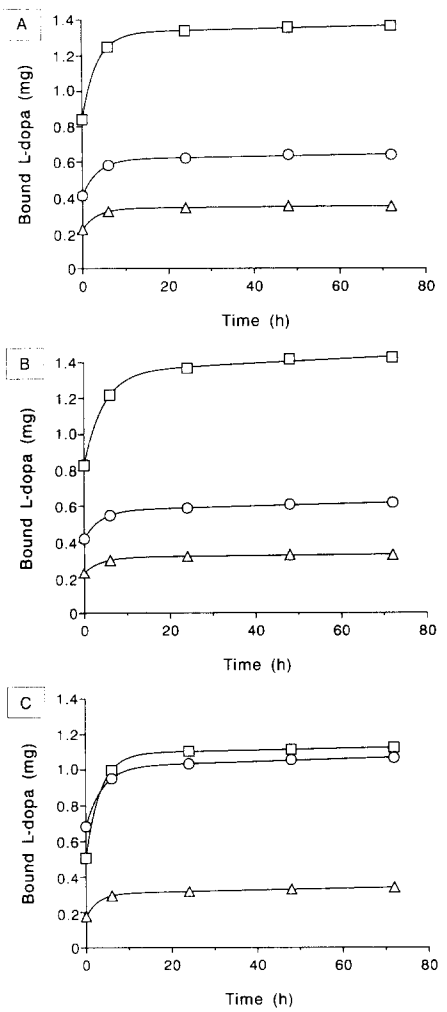


Fig. 2. Release of L-dopa from alginate-chitosan microspheres in CSF (pH 6.0) prepared with: (A) Seacure CL 210; (B) Seacure CL 211; (C) Seacure CL 311. Points are experimental and curves are the best fit for first-order kinetics of the biphasic type: (○—○) 1.5% $CaCl_2$; $r^2 = 0.999$, 0.998 and 0.999 for A, B and C, respectively; (□--□) 8% $CaCl_2$; $r^2 = 0.999$, 0.999 and 0.999 for A, B and C, respectively; (△—△) 8% $CaCl_2$ dried microspheres; $r^2 = 0.998$, 0.999 and 0.999 for A, B and C, respectively.

dopa adduct vs that of unbound L-dopa is strongly shifted towards the former. It is evident that there is a 10^3-fold difference in the amounts of L-dopa released: while the bound drug is released in milligram quantities the unbound drug is available in microgram quantities. Also, the time span for complete release is prolonged in the case of unbound drug. For instance, it takes about 60–80 h and up to 20 h for bound and unbound drug release, respectively. This finding contrasts with previous observations that the diffusion of small molecules seems to be very little affected by the alginate gel matrix whereas transport of larger molecules may be restricted (Smidsrod and Skjak-Break, 1990). Taking these differences into consideration it should be possible to speculate upon the mechanism of release.

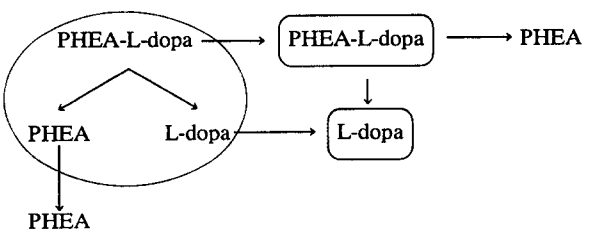
Other characteristics of the release pattern were similar. In all cases, a burst effect at the onset of measurement was noted, resulting in a sudden rise in the amount of drug released. It has been reported that calcium may form ionic bonds between the hydroxyl groups on the polysaccharide molecules and this can toughen the starch granules and can provide the textural characteristics (Kost and Shefer, 1990). Thus, it seems that the textural layer of chitosan can swell very rapidly in the buffer, causing burst release of drugs.

The effect of applied $CaCl_2$ concentration is straightforward; the spheres prepared with a 8% solution gave the best drug release, except for the sample prepared with high-viscosity chitosan (Seacure CL 311) for unbound drug release, and the dry microspheres always gave the poorest release for a given type of chitosan used. It would appear that the incomplete swellability up to the original microsphere wet volume is the source of the phenomenon.

The influence of chitosan type (viscosity) used in preparations on the release is not clear, although the high-viscosity chitosan (Seacure CL 311) gave the fastest release in the set of dried spheres, as well as spheres prepared with a 1.5% $CaCl_2$ solution. The molecular weight of chitosan employed in the encapsulation procedure was determined to be a key factor in the capsule strength and flexibility. For example, low molecular weight chitosan produced the strongest capsules (Polk et al., 1994). Due to insufficient data this cannot be explained at present. Also, the data on the possible influence of the third polymer in the system (PHEA) at this stage of investigation are still not clear, since no macroscopic differences between the spheres prepared were noted. However, the PHEA effect should not be

excluded in future work due to its inherent/functional groups present. An analysis of finer details of interplaying effects between various physicochemical parameters of the substances used should be the purpose of future investigation.

The release profiles were analyzed kinetically. All the curves for bound or unbound L-dopa release followed first-order kinetics of the biphasic type (Fig. 1 and 2). According to the kinetic data of release, it appears that L-dopa originally present in the capsules in the form of adduct can be liberated via two pathways, i.e., (i) it may be firstly hydrolyzed into unbound L-dopa and the PHEA polymer, and then the free drug will have permeated out of the sphere or (ii) the unchanged PHEA conjugate permeates out of spheres and is consecutively subjected to hydrolysis in the outer medium. The relative amounts of L-dopa detected in the outer medium and the 10^3-fold difference in amounts suggest the second pathway to be dominant (Scheme 2). It may be speculated that the internal microsphere matrix space protects the L-dopa adduct from hydrolysis and the principal amount of the drug is released in the form of the PHEA adduct. In our previous paper (Zorc et al. 1993b) $t_{50\%}$ in vitro for L-dopa hydrolysis from the PHEA adduct was determined to be about 10.9 h. From the results presented here, it is clear that the release even after several times the $t_{50\%}$ value is very small, and that the hydrolysis is restricted. On the other hand, PHEA-L-dopa conjugate permeates out of spheres mainly unchanged and the drug is available for further release in the outer medium.



Scheme 2.

The microsphere-polymer-drug adduct system investigated here may provide some additional features over either the conventional microsphere system or conventional drug polymer conjugate, and we are of the opinion that further investigation in similar systems can be fruitful.

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