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How cyclodextrin incorporation affects the properties of protein-loaded PLGA-based microspheres: the case of insulin/hydroxypropyl-β-cyclodextrin system

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Abstract

The aim of this work was to study the influence of cyclodextrin (CD) incorporation on the properties of protein-loaded poly(lactide-co-glycolide) (PLGA) microspheres, with particular regards to protein release kinetics. To this purpose, insulin-loaded microspheres were prepared by spray-drying emulsion or solution formulations, with or without hydroxypropyl-β-cyclodextrin (HPβCD), and fully characterized for encapsulation efficiency and release kinetics of both insulin and cyclodextrin. Homogeneous populations of spherical microparticles entrapping both insulin and HPβCD were obtained. In order to get an insight into insulin/HPβCD interactions occurring inside microspheres, Fourier transform infrared (FTIR) analysis in the Amide I region was performed. FTIR spectra of dried microspheres containing HPβCD showed a change in insulin secondary structure, attributed to the presence of insulin/HPβCD complexes within microspheres. Insulin release was affected by the presence of HPβCD depending on the initial formulation conditions. In the case of microspheres prepared from emulsion, cyclodextrin reduced only insulin burst, whereas in the case of microspheres obtained from solution, the overall insulin release rate was slowed down. Combining the release kinetics of HPβCD with the FTIR results on hydrated microspheres, it was concluded that the formation of insulin/HPβCD complexes inside microspheres is critical to decrease protein diffusivity in the polymer matrix and achieve an effective modulation of protein release rate.

Keywords: Cyclodextrins; Microspheres; FTIR; Release rate; Insulin

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1. Introduction

Poly(lactide-co-glycolide) (PLGA)-based microspheres are biodegradable particulate delivery systems providing both the protection of the drug, encapsulated inside a polymeric matrix, and its release at slow and continuous rate. Depending on microsphere preparation technique, drug molecules are either dispersed within the polymer or deposited inside spherical, or nearly spherical, occlusions (macropores) formed within the particle during processing. Upon immersing microspheres in an aqueous medium, water penetrates toward the centre of the particle (hydration phase) and activate drug diffusion through the innate micropores of PLGA (angstrom- or nanometer-dimension) and the macroporous structure of the particle. In the case of macromolecules, the diffusion in the porous network is highly limited due to the cramped space available and so extremely slow until pores grow in size and/or coalesce because of polymer erosion. Thus, drug release rate from biodegradable PLGA microspheres is mainly controlled by polymer erosion, structure of the porous microenvironment and drug diffusion [1,2]. These factors can be generally regulated by selecting adequate formulation conditions, such as polymer type and preparation method [3]. Actually, the use of PLGAs differing for molecular weight and copolymer composition can change initial hydration and erosion rate of the matrix [4,5]. Much more difficult is to modify the release features of microspheres once a polymer type and a preparation technique have been selected. In this case, control over the release rate could be exerted by either modifying the internal morphology of the system (i.e., internal porosity) or adding a third component that alters drug effective diffusivity in the polymeric matrix.

Cyclodextrins (CDs) have been successfully used to modify the release features of polymeric systems mainly due to their capability of forming noncovalent complexes with drugs, that is a species with a different solubility and/or diffusivity [6,7]. CDs can form complexes also with proteins by including hydrophobic side chains inside their cone-shaped cavity, thus affecting three-dimensional structure and chemical/biological properties of the macromolecule [8]. In the light of these considerations, complexation with CDs can offer an additional tool to modulate protein release rate from PLGA microspheres. Since complex formation is critical to this purpose, a careful investigation of protein/cyclodextrin interactions—that is CD effect on protein conformation—occurring inside microspheres is necessary. Among different experimental and theoretical methods aimed to the recognition of protein secondary structure, Fourier transform infrared (FTIR) spectroscopy has been demonstrated as a useful technique to collect detailed information on protein conformation within microspheres, without altering the controlled release system [9,10].

The aim of this work was to study the influence of hydroxypropyl-β-cyclodextrin (HPβCD) incorporation on the properties of insulin-loaded PLGA microspheres, with particular regard to protein release kinetics. In order to affect the internal morphology of the system, insulin-loaded microspheres were produced by spray-drying different liquids, namely W/O emulsions and solutions. Different microsphere formulations with or without HPβCD were obtained and fully characterized for encapsulation efficiency and release kinetics of both insulin and HPβCD. For a better understanding of the mechanisms by which the coencapsulation of HPβCD affects protein release rate, protein/cyclodextrin interactions within dried and hydrated particles were assessed by FTIR spectroscopy.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (50:50) (PLGA, Resomer RG 504 H; Mw 50 KDa; inherent viscosity 0.5 dl/g) was purchased from Boehringer Ingelheim (Germany). Insulin from bovine pancreas, insulin from bovine pancreas FITC-labeled, trizma base (TRIS), trifluoroacetic acid (TFA), sodium azide and 1,4-diazabicyclo-[2.2.2]octane (DABCO) were obtained from Sigma (USA). Hydroxypropyl-β-cyclodextrin (HPβCD, Mw 1380, molar substitution 0.6), phenolphthalein, polysorbate 80, and polyvinylalcohol (PVA, Mowiol® 40–88) were purchased from Aldrich (USA). Analytical grade methylene chloride (MC), glacial acetic acid and methanol, HPLC grade acetonitrile were supplied by Carlo Erba (Italy).