International Journal of Pharmaceutics 531 (2017) 568–576

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Efficient loading of ethionamide in cyclodextrin-based carriers offers enhanced solubility and inhibition of drug crystallization

Jitendra Wankar[a], Giuseppina Salzano[c], Elisabetta Pancani[c], Gabor Benkovics[b], Milo Malanga[b], Francesco Manoli[a], Ruxandra Gref[c], Eva Fenyvesi[b], Ilse Manet[a,*]

[a] Istituto per la Sintesi Organica e la Fotoreattività, ISOF, CNR, via P. Gobetti 101, 40129 Bologna, Italy
[b] CycloLab, Cyclodextrin R&D Ltd., H1097 Budapest, Hungary
[c] Institut des Sciences Moléculaires d'Orsay (ISMO), UMR CNRS 8214, Univ. Paris-Sud, Université Paris-Saclay, 91405 Orsay, France

ARTICLE INFO

Article history:
Received 24 March 2017
Received in revised form 12 May 2017
Accepted 18 May 2017
Available online 22 May 2017

Chemical compounds studied in this article:
Ethionamide (PubChem CID: 2761171)
Isothiocyanatofluorescein (PubChem CID: 18730)
β-Cyclodextrin (PubChem CID: 444041)

Keywords:
Cyclodextrin
Polymers
Ethionamide
Tuberculosis
Delivery
Binding
Fluorescence

ABSTRACT

Ethionamide (ETH) is a second line antitubercular drug suffering from poor solubility in water and strong tendency to crystallize. These drawbacks were addressed by loading ETH in β-cyclodextrin (βCyD)-based carriers. The drug was incorporated in a molecular state avoiding crystallization even for long-term storage and obtaining a tenfold increased solubility up to 25 mM. The binding of ETH to polymeric βCyD nanoparticles (pβCyD NPs) was investigated in neutral aqueous medium by means of solubility phase diagrams, circular dichroism (CD) and UV–vis absorption and compared with the corresponding βCyD monomer. The binding constants and the absolute CD spectra of the drug complexes were determined by global analysis of multiwavelength data from spectroscopic titrations. The spectroscopic and photophysical properties of the complexes evidenced an alcohol-like environment for ETH included in the cavity. Additionally, ETH was found to be located not only in βCyD cavities, but also in confined microdomains inside the crosslinked NPs. This double modality of complexation together with a slightly higher binding constant makes the utilization of pβCyD NPs preferable over the monomeric βCyDs. In order to pave the way to future *in vitro* experiments, fluorescein labeled pβCyDs were synthesized. Interestingly the FITC labeling did not hamper the encapsulation of ETH and the drug improved the fluorescent signal of FITC molecules. The βCyD-based carriers appeared as versatile "green" systems for efficient incorporation and future delivery of ETH.

1. Introduction

Cyclodextrins (CyDs) are water soluble, biocompatible cyclic oligosaccharides, made of α-D-glucopyranose units joined by α (1–4) linkages (Davis and Brewster, 2004). They received considerable attention as carriers/solubilizers for drugs thanks to their ability to host lipophilic guests in their hydrophobic cavity. Various CyD-based nanoassemblies, loading drugs *via* non covalent interactions (Bilensoy and Hincal, 2009; van de Manakker et al., 2009; Zhang and Ma, 2010) or labile covalent bonds (Cheng et al., 2004; Davis, 2009; Haag, 2004; Tanaka et al., 1994) have been synthesized and proposed as delivery platforms and some have been considered for preclinical studies (Davis, 2009). Water soluble CyD polymers have been obtained following several approaches among which the crosslinking method based on polycondensation in the presence of a bifunctional agent has become very popular (Renard et al., 1997). Epichlorohydrin is a very common crosslinking agent allowing to reach a CyD content up to 70% w/w (Renard et al., 1997). Polycondensation using epichlorohydrin allowed to produce in a convenient manner water soluble oligomers as well as polymers by rigorously controlling the initial ratio of the epichlorohydrin/CyD concentrations as well as the reaction time and concentration of NaOH in the mixture (Renard et al., 1997). High molecular weight (MW) polymers can exist under the form of nanoparticles of around 15 nm (Othman et al., 2011) and can further associate with dextran bearing alkyl side chains to produce larger NPs of around 200 nm thanks to the inclusion in the CyD cavities of the dextran hydrophobic alkyl side chains (Gref et al., 2006; Othman et al., 2011). Interestingly, βCyD polymers are able to associate drugs much more efficiently than the monomeric βCyD (Anand et al., 2012; Daoud-Mahammed et al., 2009, 2007a, 2008, 2007b; Gref et al., 2006). So polymeric natural CyDs proved their potential in the drug delivery field by dramatically enhancing the apparent solubility of several guests, and allowing to incorporate more than

* Corresponding author.
E-mail address: ilse.manet@isof.cnr.it (I. Manet).

http://dx.doi.org/10.1016/j.ijpharm.2017.05.041
0378-5173/

one drug contemporarily (Fraix et al., 2013; Gref and Duchene, 2012; Monti and Manet, 2016). A βCyD polymer of relatively high MW (200 kDa) under the form of 15 nm nanoparticles (NPs), was synthesized and used here to address the challenges of solubilizing ETH (Scheme 1a), an important antitubercular drug. First line drugs like isoniazid, pyrazinamide and rifampicin are facing problems because of increased microbial resistance (Laxminarayan et al., 2013; Pham et al., 2015; Zumla et al., 2014). ETH is a second line drug gaining a lot of interest due to its potential against multidrug resistant strains of *Mycobacterium tuberculosis* (Flipo et al., 2012; Thee et al., 2016; Wolff and Nguyen, 2012). However, this drug suffers from poor water solubility (Tuberculosis, 2008) and strong tendency to crystallize, making its administration particularly challenging. To address the difficulties of delivering the sparingly soluble ETH in a more efficient way, various carrier systems made of poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) have been investigated (Kumar et al., 2011; Vale et al., 2012). However, the preparation of these systems required the use of toxic organic solvents such as methanol and dichloromethane. Moreover, concerns were raised on the possible crystallization of the drug at the NP surfaces (Kumar et al., 2011). In this context, we investigated the use of CyD based carriers, as alternatives to PLA/PLGA ones in an attempt to avoid ETH crystallization during particle preparation and storage. Indeed, one of the major application of CyDs is the encapsulation of drugs at a molecular level, so that the drug can no longer self-assemble in crystals (Uekama et al., 1998, 1992). This property is fundamental as crystallization of drugs during storage or administration hampers their possible *in vivo* applications. We explored here the possibility of incorporating ETH in a molecular state in CyD polymers which do not require the use of organic solvents. Moreover, to further investigate the delivery system, a mixture of fluorescein labeled βCyD (FITC-βCyD) and native βCyDs was crosslinked with epichlorohydrin in order to obtain a fluorescent polymer (FITC-pβCyD – Scheme 1b).

ETH inclusion in monomeric βCyD as well as in pβCyD NPs and FITC-pβCyD was thoroughly studied by means of circular dichroism (CD) and UV–vis absorption titrations, solubility phase diagrams and fluorescence measurements. We have evidenced that the drug is incorporated in βCyD where it experiences a chiral environment resulting in an induced CD signal of the drug. Next we determined the apparent binding constants of ETH to the βCyD polymers by means of global analysis of multiwavelength CD data. The drug was associated with pβCyD where it experienced likely two environments, the βCyD cavity and other confined spaces inside the crosslinked polymer. Moreover, we found that the fluorescence features of the labeled polymer were improved after complexation with ETH. Finally, the ETH loaded formulations were stable upon storage up to six months and ETH crystallization was totally avoided. These results allow envisaging further development of polymeric carriers for the delivery of ETH. In the future we plan to evaluate the activity of the optimized pβCyD/ETH NPs in a mouse model of tuberculosis by administering the particles intrapulmonary. In this context, nanoparticles with particle size of few tens of nanometers are suited for pulmonary delivery by means of inhalation (Haque et al., 2012; Yang et al., 2008).

2. Experimental

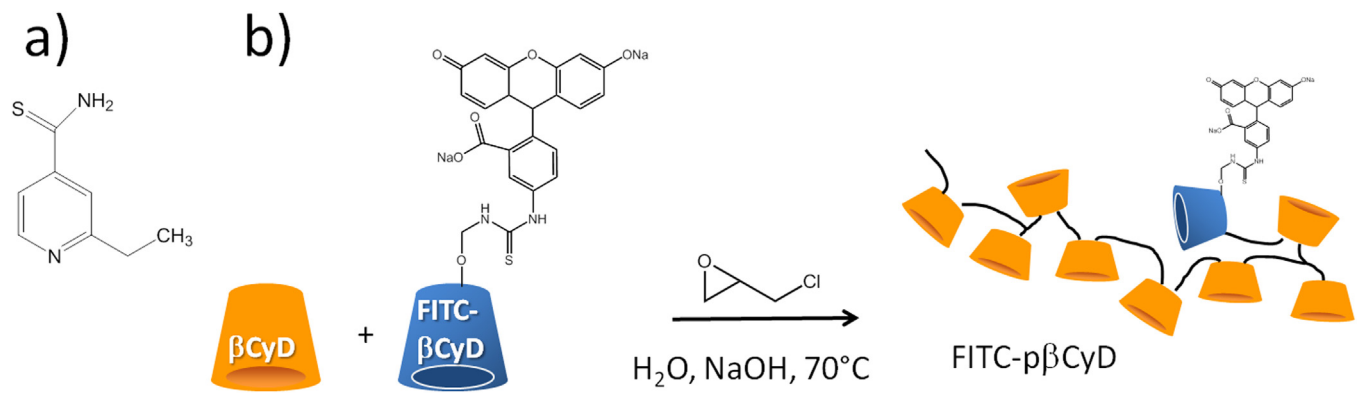
2.1. Materials

Ethionamide (purity 99.9%) and fluorescein sodium salt (analytical reference material) were obtained from Sigma Aldrich and βCyD from Serva and used as received. The drug has a molecular weight of 166.244 g/mol. Water was purified by passage through a Millipore MilliQ system. ETH was dissolved at concentration of 2×10^{-4} M in MeOH or EtOH (Merck, UVASOL for spectroscopy).

pβCyDs, forming NPs of 10–15 nm in water, were produced as previously described by crosslinking βCyD under strongly alkaline conditions in the presence of epichlorohydrin (Gref et al., 2006; Othman et al., 2011). Briefly, anhydrous βCyD (100 g) was dissolved in 160 ml sodium hydroxide 33% w/w solution under magnetic stirring. Then, epichlorohydrin (molar ratio βCyD/epichlorohydrin = 10) was rapidly added to the solution heated at 30 °C. The reaction was stopped in the proximity of the gelation point by adding acetone (Gref et al., 2006; Malanga et al., 2014; Renard et al., 1997). After neutralization with hydrochloric acid (6 N), the obtained polymer was ultrafiltered using membranes with a cut-off of 100,000 g/mol. The βCyD polymer was finally recovered by freeze-drying.

Monomeric fluorescein labeled βCyD (FITC-βCyD), was prepared as follow: fluorescein isothiocyanate (FITC) (210 mg) was dissolved in pyridine (6 ml) and added to a stirred solution of 6-monodeoxy-6-monamino-βCyD free base (306 mg) in pyridine (6 ml) (See Fig. S1). The reaction mixture was heated at 60 °C for 3 h, the solvent was evaporated, the crude was washed with acetone (3 × 10 ml) under sonication. The mixture was filtered, the solid was dissolved in water (50 ml), extensively dialyzed and complete evaporation of the retentate yielded FITC-βCyD as yellow powder (0.38 g). Next, FITC-pβCyD was prepared by crosslinking a mixture of βCyD and FITC-βCyD with epichlorohydrin according to a procedure already described (Gref et al., 2006; Malanga et al., 2014; Renard et al., 1997).

In both cases, the βCyD content in the polymer was of 70% w/w as determined by ^{1}H NMR investigations (See Fig. S1 and S2 for FITC-βCyD spectra) and the average molecular weight determined by static light scattering and aqueous gel permeation



Scheme 1. Structure of Ethionamide (a) and cartoon representation for the preparation of FITC-pβCyD (b).