Amphiphilic Cyclodextrins as Nanocarriers of Genistein: A Spectroscopic Investigation Pointing Out the Structural Properties of the Host/Drug Complex System

ROSANNA STANCANELLI,[1] MARTA GUARDO,[1] CARMELA CANNAVÀ,[1] GIOVANNI GUGLIELMO,[1] PAOLA FICARRA,[1] VALENTINA VILLARI,[2] NORBERTO MICALI,[2] ANTONINO MAZZAGLIA[3]

[1]Dipartimento Farmaco-Chimico, Università di Messina, V.le Annunziata, 98168 Messina, Italy

[2]CNR-Istituto per i Processi Chimico-Fisici, sede di Messina, Contrada Papardo, Salita Sperone, Faro Superiore, 98158 Messina, Italy

[3]CNR-Istituto per lo studio dei Materiali Nanostrutturati, c/o Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, Università di Messina, Salita Sperone 31, 98166 Messina, Italy

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ABSTRACT: Nanoggregates of nonionic amphiphilic cyclodextrin (ACyD) modified with hydrophobic chains of intermediate length [(2-oligo-ethyleneoxide-6-hexylthio)-β-CyD, SC6OH] were prepared by emulsification–diffusion method. They are able to entrap an isoflavone, genistein (Gen), and the complexed species are studied at different host/guest molar ratio. The increased isoflavone solubility in the presence of the aggregates of SC6OH is investigated by UV–Vis spectroscopy, whereas size, charge, and structure of aggregates and their complexes with Gen are measured by means of static and quasi-elastic light scattering, and electrophoretic mobility measurements. On the other hand, preparing samples by the conventional method used for liposomes (hydration of an organic film of SC6OH and sonication) gives rise to aggregates with different sizes and lower colloidal stability. It is shown that the improved stability in water of ACyD aggregates both in the absence and in the presence of Gen, obtained by emulsification–diffusion is due to the existence of nanodomains of organic solvent ($R_H \cong 120$ nm) which cannot be completely removed by evaporation and freeze-drying and in which host/guest complexes are contained. This result shows that residues of organic solvent from preparation step favor the colloidal stability of the aggregate, but their presence must be taken into account in designing systems for drug delivery. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:3141–3149, 2010

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INTRODUCTION

One of the main goals of the pharmaceutical science is to design carriers which can increase the solubility of the encapsulated guest and modulate the release in a specific cellular target.[1–3] Low solubility of drug in water is usually associated with poor absorption and bioavailability, hence the drug molecule must possess sufficient aqueous solubility to be successfully delivered to the site of action *in vivo*. Conventional ways which address poor solubility of drugs and increase their bioavailability make use of excipients such as ethanol and some other organic solvents and/or certain surfactants.[1] The formation of salts or pH adjustment in some cases facilitates the dissolution of poorly soluble drugs if they contain ionizable groups. Nowadays, successful approaches include the use of lipid derivatives,[1] polymeric micelles,[4] microemulsions,[5] and cyclodextrins (CyDs).[6]

In particular, cyclodextrins are a group of cyclic oligosaccharides composed of α(1,4)-linked glucopyranose units which form hydrophobic nanocavities. This cavity enables them to act as hosts to lipophilic

Correspondence to: Valentina Villari (Telephone: +39 090 39762 219, Fax: +39 090 3974130; E-mail: villari@me.cnr.it)

Correspondence to: Antonino Mazzaglia (Telephone: 0903974108; Fax: 0903974108; E-mail: antonino.mazzaglia@ismn.cnr.it)

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molecules making them excellent candidates for variety of applications from drug delivery to molecular machines.[7] Due to their biocompatibility and to well-known capability to host molecules in water, CyDs may find useful application in biology, nanomedicine, and pharmaceutics.[8,9] CyD molecules are relatively large with a number of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes.[10]

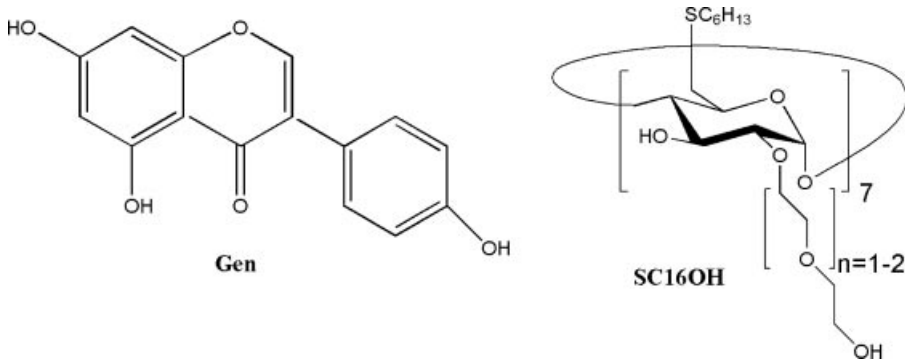
Amphiphilic cyclodextrins (ACyDs) represent a new perspective in the use of these oligosaccharides in pharmaceutical science. Among CyD derivatives, hydrophilic ACyDs have been widely used as excipients although their surface hydrophilicity can represent a drawback for cell internalization and result in a lack of affinity of the included molecule for biological membranes. This is one of the reasons why researchers have been interested in developing CyD derivatives with a modulated external hydrophobicity. However, the potential of these molecules in engineering nanocarriers able to deliver and specifically target a drug is largely unexplored. In fact, supramolecular aggregates, either spontaneously obtained or structured through a specific preparation method, have a size compatible with i.v. injection and could be used to optimize drug distribution in the body.

Furthermore, ACyDs were reported to be nonhemolytic and noncytotoxic according to studies on human blood samples and L929 mouse fibroblast cells regardless of their different chemical structures.[11] In the recent past, some of us demonstrated that amphiphilic β-CyDs grafted with oligoethylene glycol moieties[12–14] are capable of forming nanoaggregates (micelles, micellar aggregates, or vesicles)[15,16] of potentially low immunogenicity according to the hydrophobic–hydrophilic balance. ACyDs are good candidates for encapsulating molecules more efficiently than a single CyD[16] and for delivering a photosensitizer drug into cancer cells[17–19] since there is superior guest molecule retention by these aggregates.

ACyDs can be conveniently tailored by covalently appending receptor-targeting glycosyl groups[20,21] in order to build nanocarriers able to deliver drugs with increased selectivity toward specific cell lines. Supramolecular aggregates of ACyDs are thereby versatile systems toward the encapsulation of both hydrophobic and hydrophilic guests.[15,22,23] These supramolecular aggregates and their complexes with drugs were usually prepared by dissolution of CyD molecule in organic solvent, slow evaporation, rehydration of organic film, and sonication according to the conventional method used for liposomes.[12,15] Generally, manufacturing techniques of polymeric nanoparticles also include the salting out, emulsification–diffusion, and nanoprecipitation. These techniques can allow a modulation both of the structure of the nanoparticles and of their drug-loading and release properties.[24]

It has been established that genistein (4′,5,7-trihydroxyisoflavone, Gen, Scheme 1) present in soya beans are efficient in cancer chemoprevention. Gen and its related substances can act as inhibitors of cervical, prostate, skin cancer, etc. These and other findings explain the great interest that has been paid to the phytoestrogen compounds.[25,26] Recently, we investigated the improvement of isoflavones bioavailability by complexation with (2-hydroxypropyl)-β-cyclodextrin (HP-β-CyD) at different host/guest molar ratios.[27] Moreover, nanoparticles made of the ACyD heptakis (2-*O*-oligo(ethyleneoxide)-6-hexadecylthio-)-β-CD (SC16OH) entrapping docetaxel (Doc) were developed and established their *in vivo* potential. Doc-loaded SC16OH nanoparticles were prepared by the emulsification–diffusion method and fully characterized.[28]

In view of these recent results, in this article Gen was studied in aqueous medium and in presence of a shorter hydrophobically substituted ACyD [(2-oligo-(ethyleneoxide)-6-hexylthio)-β-CyD, SC6OH, Scheme 1]. The modulation of the amphiphilic properties of ACyD, aimed to use a more water-soluble nanocarrier, can offer the following advantages:[4] (i) the formation of aqueous dispersions of ACyD nanocar-



Scheme 1. Molecular structure of the investigated 4′,5,7-trihydroxyisoflavone (Gen) and (2-oligo(ethyleneoxide)-6-hexylthio)-β-CD (SC6OH).

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