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Determination of the composition, encapsulation efficiency and loading capacity in protein drug delivery systems using circular dichroism spectroscopy

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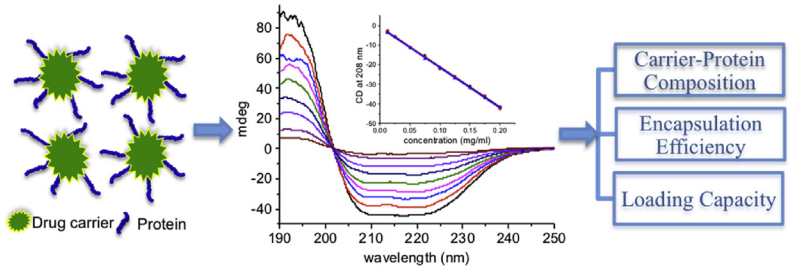
HIGHLIGHTS

- CD spectra of different concentrations of α_1-antitrypsin, hemoglobin, human serum albumin, human transferrin and r-globulin were obtained.
- The Five above mentioned proteins were chemically conjugated to carbon dots and polymer O-(2-carboxyethyl) polyethylene glycol.
- A simple yet convenient method based on CD spectroscopy was developed to determine the composition of various protein-carrier conjugates.

GRAPHICAL ABSTRACT



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ABSTRACT

Peptides and proteins have become very promising drug candidates in recent decades due to their unique properties. However, the application of these drugs has been limited by their high enzymatic susceptibility, low membrane permeability and poor bioavailability when administered orally. Considerable efforts have been made to design and develop drug delivery systems that could transport peptides and proteins to targeted area. Although it is of great importance to determine the composition after loading a drug to the carrier, the ability to do so is significantly limited by current analytical methods. In this letter, five important proteins, α_1-antitrypsin, hemoglobin human, human serum albumin, human transferrin and r-globulin were chemically conjugated to two model drug carriers, namely carbon dots and polymer O-(2-carboxyethyl) polyethylene glycol. A simple yet convenient method based on circular dichroism spectroscopy was developed to determine the compositions of the various protein-carrier conjugates.

1. Introduction

One of the most promising fields in pharmaceutical industry is the selective preparation of macromolecules such as peptides and proteins to be used as drug candidates for disease treatments [1,2].

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Compared to the traditional low-molecular-weight compounds (i.e., less than 500 g/mol), peptides and proteins are highly selective due to their rich structure features that allow the multiple points of contact with their target. The increased selectivity normally leads to better therapeutic activity and relatively fewer side effects. The market for peptide and protein drugs was estimated to be about 10% of the pharmaceutical market in 2013, and it continues growing much faster than the counterpart of small molecules [3]. Targeted drug delivery [4,5] by drug delivery system (DDS) has been the main strategy to overcome the problems (i.e., high enzymatic susceptibility and poor bioavailability) associated with peptide and protein drugs [6,7]. A properly designed DDS would provide alternative transportation mechanism and thus increase the ability to pass the bio-barriers. Thanks to the specific targeted transport, a DDS could improve the bio-distribution of peptide and protein drugs while minimizing their toxicity [8].

In targeted drug delivery, a drug is entrapped in or chemically linked to a DDS (i.e., nanoparticles or biodegradable polymers) that is designed to selectively deliver it to the site of interest. There are two main strategies to load peptide and protein drugs to a carrier: one is passive adsorption through non-covalent interactions and the other is via labile covalent bonding between the drug and the carrier [9]. In both strategies, one important aspect to consider when designing a DDS is the drug-to-carrier ratio since the use of high quantity of carrier could potentially cause a series of problems, such as carrier-related toxicity due to the poor metabolism and biodegradability of the carriers [10]. Also, the use of unreasonably high amount of carrier would increase the possibility of immune reactions against the carrier in the patient body [11]. Thus, the development of convenient and reliable analytical methods for the determination of drug-to-carrier ratio and the study of the detailed composition of a DDS is of great importance.

Traditionally, to determine the composition of a DDS, the protein-carrier conjugate is separated from the unbound protein by centrifugation after the drug loading process is completed. The amount of unbound protein (free drug) in the supernatant or bounded protein (loaded drug) after centrifugation is then determined by either UV–vis absorbance or dye-binding assays based on a known extinction coefficient or a standard calibration curve [12–14]. However, this method sometimes suffers from low reliability [15] presumably because of the possible interference from the drug carriers (i.e., nanoparticles). Most of nanoparticle drug carriers demonstrate strong UV–vis absorbance and often overlap with the ones of protein drugs, making the UV–vis quantification of protein drugs extremely difficult [16]. Also, nano drug carrier (<10 nm) usually has a strong fluorescence, complicating the fluorescence-based determination. Another shortcoming associated with this traditional method is that the determination of encapsulation efficiency and loading capacity of a DDS is based on bulk analysis [17,18], it does not exclude the contribution from the free carriers (i.e., carriers that do not carry any drugs).

Herein, we report a new approach to study the properties (i.e., composition, encapsulation efficiency and loading capacity) of chemically linked protein-carrier conjugates based on our previously developed circular dichroism (CD) spectroscopy determination method [19]. Since the approach is based on CD spectroscopy, it could well avoid the aforementioned problems associated with the traditional analytical techniques.

2. Materials and methods

2.1. Reagents and instruments

The chemicals of human serum albumin (HSA) and human transferrin (iron saturated, HT) were obtained from MP Biomedicals, LLC (Solon, OH). Carbon powder (99.99%) was purchased from Strem Chemicals Inc. (Newburyport, MA). ACS grade sulfuric acid (98%) and nitric acid (68–70%) were purchased from ARISTAR (distributed by VWR, Radnor, PA). O-(2-carboxyethyl) polyethylene glycol (PEG), r-Globulins from human blood (rGb), Hemoglobin human (Hb), α1-Antitrypsin from human plasma (A1AT), carbon nanopowder and other chemicals were purchased from Sigma–Aldrich (St. Louis, MO). All of the chemicals above were used as received without any further purification. Dialysis tubing with a molecular weight cutoff of 3500 was purchased from Thermo Scientific (Rockford, IL). Size exclusion chromatography was performed using GE Healthcare Sephacryl S-300 (Uppsala, Sweden). Purified water was obtained from a Modulab 2020 water purification system (San Antonio, TX). The water has a surface tension of 72.6 mN m^{-1} and a resistivity of >18 MΩ cm at 20.0 ± 0.5 °C.

2.2. Synthesis and characterization of carbon dots

Carbon dots (C-Dots) used in this study were synthesized and characterized according to a previously developed procedure [20]. The titration of C-Dots: 6.0 mg of C-Dots were dissolved in 60.0 ml of water and titrated by 0.00055 M NaOH solution (the NaOH solution used in titration was freshly prepared and standardized by KHP). When reaching the end point, 63.42 ml of this NaOH solution were used, which is about 0.03488 mmol of NaOH. Assuming only carboxylic group on C-Dots reacted with NaOH, it is estimated that there are 5.8 mmol of −COOH available for each gram of C-Dots.

2.3. Syntheses and characterization of protein-C-Dots conjugates

A typical procedure is as follows (A1AT-C-Dots conjugates): To a phosphate-buffered saline (PBS) solution (1.0 ml, pH = 7.4) of carbon dots (2.0 mg) at ambient temperature was added a PBS solution (1.0 mL) of A1AT (5.0 mg). The resulted solution was stirred for 10 min before a PBS solution (0.5 mL) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 5.0 mg) was added dropwise. The mixture was then allowed to stir at the same temperature for 2 h before it was subjected to a size-exclusion column for separation and purification. The elution from the size-exclusion column was collected into small vials and then characterized by fluorescence spectroscopy (Horiba Jobin Yvon Fluorolog–3) before used in the circular dichroism spectroscopy study (JASCO J–810 spectropolarimeter). After lyophilization, 6.05 mg of A1AT-C-Dots conjugate were obtained, resulting in an 86.4% yield. The conjugates of C-Dots with other four proteins were synthesized following the same procedures: for Hb-C-Dots conjugate, 15.0 mg of Hb were reacted with 6.0 mg of C-Dots; after purification and lyophilization, 15.29 mg of conjugate were obtained, resulting in a 72.8% yield. For rGb-C-Dots conjugate, 15.0 mg of rGb were reacted with 6.0 mg of C-Dots; after purification and lyophilization, 17.46 mg of conjugate were obtained, resulting in an 83.1% yield. For HT-C-Dots conjugate, 15.0 mg of HT were reacted with 6.0 mg of C-Dots; after purification and lyophilization, 17.8 mg of conjugate were obtained, resulting in an 84.8% yield. For HSA-C-Dots conjugate, 15.0 mg of HSA were reacted with 6.0 mg of C-Dots; after purification and lyophilization, 18.2 mg of conjugate were obtained, resulting in an 86.7% yield.

2.4. Syntheses and characterization of protein-PEG conjugates

A typical procedure is as follows (A1AT-PEG conjugates): To a phosphate-buffered saline (PBS) solution (1.0 mL, pH = 7.4) of PEG (10.0 mg) at ambient temperature was added a PBS solution (1.0 mL) of A1AT (5.0 mg). The resulted solution was stirred for 10 min before a PBS solution (0.5 mL) of 1-ethyl-3-(3-