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SHORT COMMUNICATION

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Self-aggregates of deoxycholic acid-modified chitosan as a novel carrier of adriamycin

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Abstract A novel and simple method for delivery of adriamycin (ADR) was developed using self-aggregates of deoxycholic acid-modified chitosan. Deoxycholic acid was covalently conjugated to chitosan via EDC-mediated reaction to generate self-aggregated chitosan nanoparticles. ADR was physically entrapped inside the self-aggregates and the characteristics of ADR-loaded chitosan self-aggregates were analyzed by dynamic light scattering, fluorescence spectroscopy, and atomic force microscopy (AFM). The maximum amount of entrapped ADR reached 16.5 wt% of chitosan self-aggregates, suggesting a loading efficiency of 49.6 wt%. The size of ADR-loaded self-aggregates increased with increasing the loading content of ADR. AFM images showed spherical shape of ADR-loaded self-aggregates, and ADR was slowly released from chitosan self-aggregates in PBS solution (pH 7.2).

Key words Chitosan · Deoxycholic acid · Self-aggregate · Nanoparticle · Adriamycin

Introduction

Recently, a number of delivery systems for drugs, proteins, antigens, or genes have been developed [1]. The colloidal delivery system is a potent delivery carrier because it may reduce unwanted toxic side effects, prolong circulation time, and reduce uptake by reticuloendothelial system (RES), resulting in an increase in the therapeutic index [2–4]. Among colloidal delivery systems, there has been increasing interest in self-assemblies of polymeric amphiphiles for biotechnological and pharmaceutical applications due to their formation of nanosized vehicles with hydrophobic core [5–7].

Chitosan is a biomaterial well-known for its biocompatibility, biodegradability, and low toxicity, even in in vivo situations [8, 9]. However, there have been few reports on self-assembled systems of chitosan and their utilization as a delivery carrier. We previously reported synthesis and characteristics of self-aggregated nanoparticles from deoxycholic acid-modified chitosan and their potential applicability as a gene delivery system using the cationic character of chitosan [10, 11]. Deoxycholic acid-modified chitosan was considered to form a self-assembled structure in water as deoxycholic acid is known to form micelles in aqueous media [12]. In addition, the hydrophobic core of these self-aggregates was considered to act as a reservoir of hydrophobic drugs. In this report, the initial use of chitosan self-aggregates as anticancer drug delivery carriers was investigated. Adriamycin (ADR) was chosen as a model drug not only due to the easy method of characterization but also due to its wide utilization in the clinical field. ADR was physically entrapped in chitosan self-aggregates, and ADR-loaded self-aggregates were characterized by dynamic light scattering, fluorescence spectroscopy, and atomic force microscopy.

Experimental

Materials

Biomedical grade chitosan ($M_v = 7.0 \times 10^4$, degree of deacetylation = 80%) was supplied from Samchully Pharmaceutical Co.

(Seoul, Korea). Deoxycholic acid with >99% purity and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma (St. Louis, MO). Adriamycin was obtained from Chongkeundang Co. (Seoul, Korea) and used as received. The water was purified by distillation, deionization, and reverse osmosis (MilliQ Plus).

Preparation of chitosan self-aggregates

Chitosan was hydrophobically modified by deoxycholic acid as previously reported [10, 11]. In brief, chitosan was dissolved in 1% acetic acid solution and deoxycholic acid (0.34 mol) was added, followed by the dropwise addition of EDC (0.18 mol) at room temperature. After 24 h, the modified chitosan was precipitated by methanol/ammonia solution (7/3, v/v), filtered off, washed thoroughly, and then dried in a vacuum at room temperature. The number of deoxycholic acid groups per 100 anhydroglucose residues of chitosan was 5.1 as determined by elemental analysis. The modified chitosan was suspended in phosphate-buffered saline (PBS) solution (pH 7.2) at 37 °C for 48 h, and sonicated using a probe type sonifier (Sigma Ultrasonic Processor, GEX-600) at 30 W for 2 min. The sonication was repeated 3 times to get an optically clear solution using pulse function (pulse on, 5.0 s; pulse off, 1.0 s).

Preparation of adriamycin-loaded chitosan self-aggregates

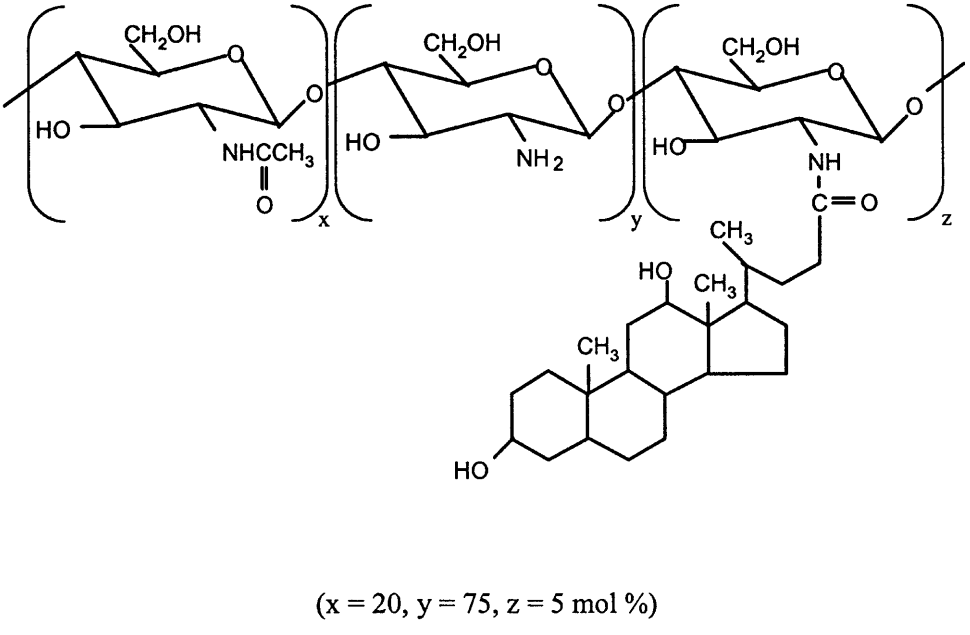
Different amount of adriamycin (ADR) was added to 0.2% solution of chitosan self-aggregates while stirring and stored in a dark place for 24 h. The excess ADR, which was not incorporated into the self-aggregates, was removed by ultrafiltration using a membrane filter with molecular weight cut-off of 1.0×10^4 (Amicon, USA). Ultrafiltration was repeated until the fluorescence intensity of the filtrate was negligible, and the self-aggregates were freeze-dried for 3 days. To calculate loading content (drug/carrier, mg/mg) and loading efficiency (loaded drug/initially added drug, mg/mg), ADR-loaded self-aggregates were dissolved in 1% acetic acid solution and fluorescence intensity of ADR was measured.

Measurements

Fluorescence intensity of ADR was measured by an ISS K2 fluorometer (ISS, Champaign, IL). The excitation wavelength (λ_{ex}) and the emission wavelength (λ_{em}) were set at 470 nm and 595 nm, respectively. A He-Ne ion laser was operated at a wavelength of 623.8 nm for dynamic light scattering measurement (Malvern Instrument). The intensity autocorrelation was measured at a scattering angle of 90° and a second cumulative analysis method was used to obtain the distribution of decay function. Mean diameter (d) was estimated by the Stokes-Einstein equation. Atomic force microscopic (AFM) images were observed by an Autoprobe CP system (Park Science, Sunnyvale, CA) using the contact mode under ambient condition. A silicon nitride tip on a cantilever with spring constant of 0.12 N/m was used.

Results and discussion

Deoxycholic acid was covalently coupled to an amino group of chitosan using EDC, a water-soluble carbodiimide (Fig. 1). Conjugation was confirmed by the existence of an amide band at 1655 cm^{-1} from the infrared spectrum, and the degree of substitution per 100 anhydroglucose residues was 5.1 as determined by elemental analysis [11]. The critical aggregation concentration (cac), a threshold concentration self-aggregated behavior, in PBS solution (pH 7.2) was 1.7×10^{-2} mg/ml [11]. This value is much lower than the critical micelle concentration of deoxycholic acid in water (1.0 mg/ml) [12]. The lower cac value of polymeric micelles compared to those of low molecular weight surfactants is an important characteristic suggesting stability of polymeric self-assemblies in dilute conditions. The hydrodynamic diameter of chitosan self-aggregates and its distribution



(x = 20, y = 75, z = 5 mol %)

Fig. 1 Chemical structure of deoxycholic acid-modified chitosan

in PBS solution (pH 7.2) were measured by dynamic light scattering (225.0 ± 22.5 nm).

It has already been reported that ADR could be physically entrapped into polymeric self-assemblies [13]. Although ADR is known to be a weak base (pKa = 8.22) [14], ADR can be physically loaded into chitosan self-aggregates because ADR has amphiphilic character due to the hydrophobic anthracylcline and the hydrophilic sugar moiety [15]. The characteristics of ADR-load chitosan self-aggregates are listed in Table 1. Loading content and loading efficiency were increased with an increase in drug to carrier ratio as expected. In the case of a 1/2 ratio, almost 50% of the initially added drug was physically loaded into chitosan self-aggregates and it reached 16.5% of its own carrier weight. The size of chitosan self-aggregates increased as the amount of loaded ADR increased (Table 1). The spherical shape of ADR-loaded self-aggregates was observed by atomic force microscopy after lyophilization (Fig. 2).

There have been many attempts to incorporate physically ADR into the hydrophobic core of colloidal delivery systems. However, due to the low efficiency ADR was chemically conjugated to the delivery carrier [5, 15, 16]. Although the chemical conjugation of ADR into the core of polymeric micelles suggested the high loading content, only the physically entrapped ADR played a major role in anti-tumor activity in vivo [13]. Chitosan self-aggregated nanoparticles were able to load ADR effectively and easily without any chemical conjugation.

Release behavior of ADR from chitosan self-aggregates in PBS solution (pH 7.2) was monitored (Fig. 3). Curve (d) in Fig. 3 displays a characteristic spectrum of ADR. However, when ADR was entrapped in chitosan self-aggregates, the fluorescence intensity decreased due to self-quenching between ADR molecules (Fig. 3, curve (a)). Physically entrapped ADR was released from chitosan self-aggregates very slowly as shown in Fig. 3, curves (b) and (c). Since micelles of low molecular weight surfactants have a liquid-like core, the molecules inside this core can move freely and would not be constrained to their core [5]. However, polymeric micelles have a very rigid core and the slow release of drug could be considered to result from a solid-like hydrophobic core. In the case of chitosan self-aggregates, they had a rigid and hydrophobic core due to deoxycholic acid moieties as previously reported [10]. Therefore, the migration of ADR was strongly restricted and slow release behavior from the core was observed.

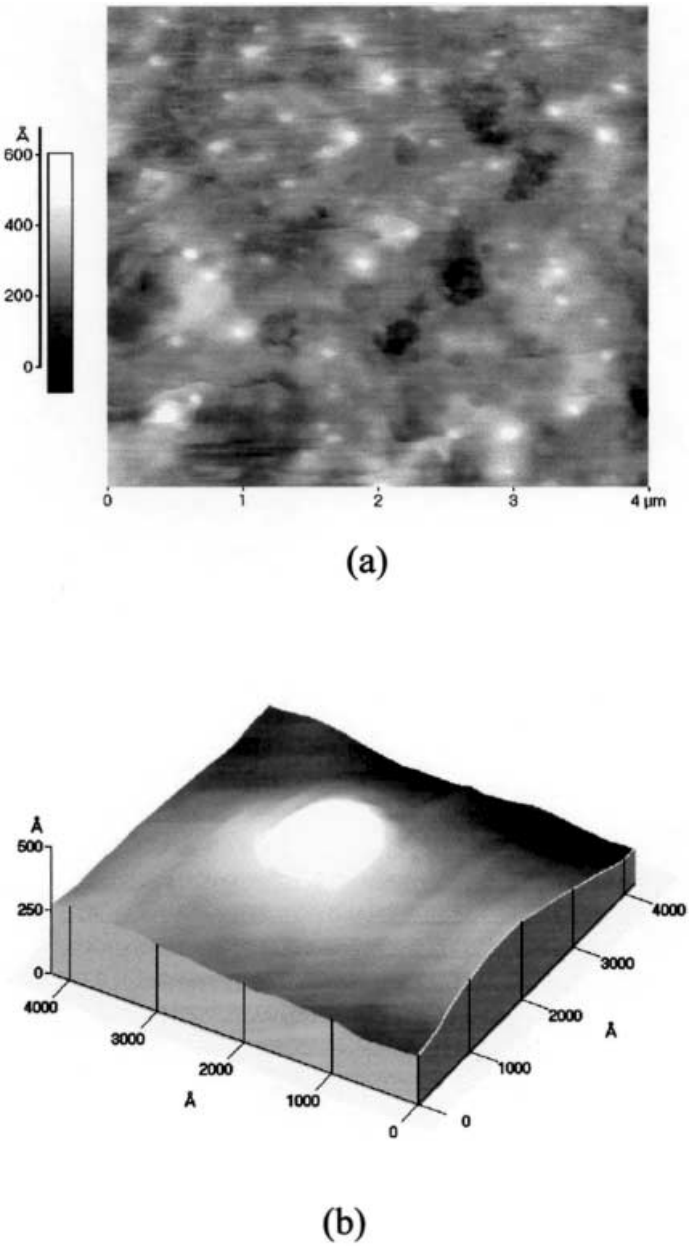


Fig. 2a, b Atomic force microscopic images of ADR-loaded self-aggregates prepared from deoxycholic-acid modified chitosan: **a** X-Y scan on 4 × 4 μm scale; **b** close-up of a particle on 0.4 × 0.4 μm scale

Table 1 Characteristics of ADR-loaded chitosan self-aggregates

Drug/carrier[a]	d[b], nm	Loading content, %	Loading efficiency, %
1/5	270.5 ± 24.5	4.6	27.5
1/2	432.8 ± 42.6	16.5	49.6

[a] ADR/chitosan self-aggregates (mg/mg)
[b] Mean diameter ± standard deviation from dynamic light scattering method

Conclusion

Deoxycholic acid-modified chitosan formed stable self-aggregates in aqueous media and showed feasibility as a

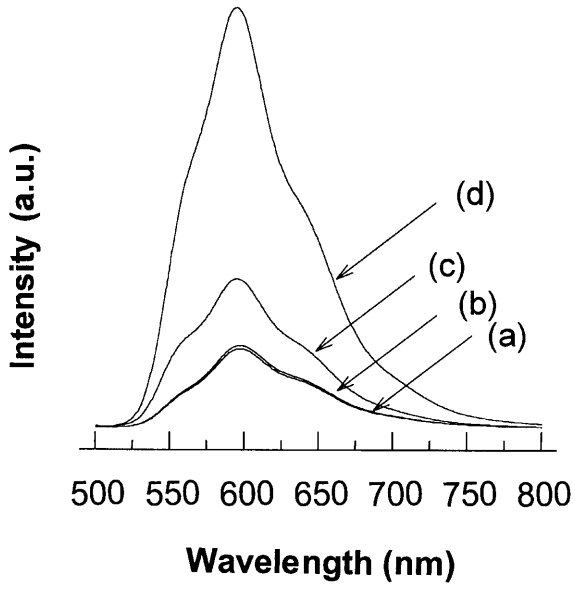


Fig. 3 Fluorescence emission spectra of (a) ADR-loaded chitosan self-aggregates (ADR/self aggregates = 1/2, w/w), (b) ADR-loaded chitosan self-aggregates incubated for 3 days, (c) incubated for 7 days, and (d) ADR only in PBS solution (pH 7.2)

potential carrier of adriamycin. These chitosan self-aggregates may be useful in the delivery of anticancer drugs that can be physically entrapped in the core, in the in vivo situation as well as in vitro, perhaps due to its enzymatically degradable properties.

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References

1. Langer R (1998) Nature 392(suppl):5
2. Muller RH (1991) In: Colloidal carriers for controlled drug delivery and targeting: modification, characterization and in vivo distribution. CRC Press, Florida
3. Kwon GS, Kataoka K (1995) Adv Drug Deliv Rev 16:295
4. Trubetskoy VS, Torchilin VP (1995) Adv Drug Deliv Rev 16:311
5. Kwon GS, Naito M, Yokoyama M, Okano T, Sakurai Y, Kataoka K (1995) Pharm Res 12:192
6. Akiyoshi K, Kobayashi S, Shichibe S, Mix D, Baudys M, Kim SW, Sunamoto J (1998) J Control Rel 54:313
7. Maruyama A, Watanabe H, Ferdous A, Katoh M, Ishihara T, Akaike T (1998) Bioconjugate Chem 9:292
8. Lee KY, Ha WS, Park WH (1995) Biomaterials 16:1211
9. Hirano S, Seino H, Akiyama H, Nonaka I (1990) Chitosan – a biocompatible material for oral and intravenous administration. In: Gebelein CG, Dunn RS (eds) Progress in biomedical polymers. Plenum Press: New York; pp 283–290
10. Lee KY, Jo WH, Kwon IC, Kim YH, Jeong SY (1998) Macromolecules 31:378
11. Lee KY, Kwon IC, Kim YH, Jo WH, Jeong SY (1998) J Control Rel 51: 213
12. Coello A, Meijide F, Nunez ER, Tato JV (1996) J Pharm Sci 85:9
13. Yokoyama M, Fukushima S, Uehara R, Okamoto K, Kataoka K, Sakurai Y, Okano T (1998) J Control Rel 50:79
14. Goldman R, Facchinetti T, Bach D, Raz A, Shinizky MA (1978) Biochim Biophys Acta 512:254
15. Akiyoshi K, Taniguchi I, Fukui H, Sunamoto J (1996) Eur J Pharm Biopharm 42:286
16. Yokoyama M, Okano T, Sakurai Y, Ekimoto H, Shibazaki C, Kataoka K (1991) Cancer Res 51:3229