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Relationship between physicochemical characteristics and functional properties of chitosan

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Abstract

In order to select an ideal chitosan (CS) species as a material for implantation vehicle to control drug release in the body, the relationship between physicochemical characteristics (including molecular weight, degree of deacetylation, and viscosity) and functional properties (including ability to form spherical gel, control of drug release from CS gel, and biodegradation of CS) was investigated for various CS. The ease of spherical gel formation in aqueous amino acid solution or aqueous solution containing metal ions was affected mainly by viscosity of the CS solution. Drug diffusion rate from the CS gel was controlled by density of the gel matrix structure, which was governed by viscosity of the CS solution prior to gelation. Biodegradation of CS tended to vary with degree of deacetylation. However, linear relationships for these trends were not observed, and the possibility that characteristics other than CS molecular weight, degree of deacetylation, and viscosity of the CS solution, such as distribution of acetamide groups in the CS molecule affect functional properties of CS, was also indicated. These observations demonstrate that CS functions are affected by various CS characteristics and that investigation of individual CS characteristics is important for the selection of the appropriate CS as a material for drug delivery vehicles.

Keywords: Chitosan; Gel; Molecular weight; Deacetylation; Biodegradation; Drug release

1. Introduction

Chitosan (CS) is a plentiful natural polysaccharide that is composed of linearly (1–4)-linked *N*-acetyl glucosamine and glucosamine residues. Various properties of CS, such as non-toxicity, biocompatibility and biodegradability, have been reported [1–3]. CS is also reported to be a useful material for drug delivery. For example, the positive charge of CS might allow peptide and protein drugs to be used pharmaceutically by enhancing absorption of these drugs via mucoadhesion to gastric or nasal mucosa or via opening tight junctions between epithelial cells [4–8]. On the other hand, additional functions of CS, which act on epiphyseal cartilage, may augment articular cartilage wound healing [9]. Inhibition of pro-inflammatory cytokines production [10] and analgesic effects on inflammatory pain [11] have also been demonstrated. These observations suggest that CS vehicles

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may be able to fulfill both wound healing and the drug delivery role. Studies have also demonstrated that CS is an effective and safe implantable vehicle for drug delivery [12–16]. Such systems allow for control of the drug supply with regard to time and quantity, and may minimize side effects while improving efficacy and patient compliance. Therefore, CS is a promising material for biomedical applications [17].

CS is made from chitin, but numerous species of CS exist due to variations in chitin species and differences in deacetylation conditions [18]. Various physicochemical characteristics of CS, such as molecular weight, degree of deacetylation, and distribution of acetamide groups in the CS molecule, influence CS functions [19]. In a previous study, we found that in vitro and in vivo drug release and biodegradative properties of CS gels varied with differing molecular weight and degree of deacetylation of the CS species employed. Control over in vivo drug release from the CS gel was achieved by changing the CS species [20–24]. Therefore, optimizing CS selection as a material for drug delivery vehicles might allow greater control over drug release. However, few studies have investigated the relationship between physiochemical characteristics and functional properties of CS.

In this study, we investigated the relationship between physiochemical characteristics (molecular weight, degree of deacetylation, and viscosity) and functional properties (ability to form spherical gel, drug release behavior, and biodegradation) of CS in order to select an ideal CS species as a material for implantation

Table 1
Molecular weight and degree of deacetylation of various CS species

Maker	Brand name	DA (%)	$M_w \times 10^{-4}$ (Da)	$M_n \times 10^{-4}$ (Da)	$M_t \times 10^{-4}$ (Da)	M_w/M_n
Ajinomoto	PC-100	53	135.0	25.3	99.8	5.3
Dainichiseika	Daichitosan 100D	100	19.0	6.2	45.0	3.0
Dainichiseika	Daichitosan 100D (VL)	100	2.6	0.38	5.1	6.8
Dainichiseika	Daichitosan H	83	101.0	30.6	160.0	3.3
Dainichiseika	Daichitosan M	86	77.9	26.6	116.2	2.9
Dainichiseika	Daichitosan VL	92	3.6	0.45	5.3	8.0
Katokichi	Chitosan 7B	70	282.6	68.4	146.2	4.1
Katokichi	Chitosan 8B	82	221.7	40.8	122.2	5.4
Katokichi	Chitosan 9B	91	98.0	10.5	84.7	9.3
Katokichi	Chitosan 10B	100	16.2	1.3	24.5	12.0
Kimica	Kimitsuchitosan B	100	49.0	7.2	47.9	6.8
Kimica	Kimitsuchitosan F	88	21.3	2.7	7.4	8.0
Kimica	Kimitsuchitosan H	99	143.1	30.3	99.5	4.7
Kimica	Kimitsuchitosan L	89	90.6	17.0	54.3	5.3
Kimica	Kimithuchitosan M	87	148.5	32.3	87.5	4.6
Koyo Chemical	Koyochitosan FH-80	87	213.6	38.4	119.5	5.6
Koyo Chemical	Koyochitosan FM-80	85	52.5	7.0	28.2	7.5
Kyowa Tecnos	Flonac C	90	10.8	1.7	7.8	6.3
Kyowa Tecnos	Flonac H	96	75.0	16.5	64.9	4.5
Kyowa Tecnos	Flonac LV	87	3.1	0.73	2.4	4.2
Kyowa Tecnos	Flonac N	90	110.6	39.3	81.1	2.8
Kyowa Tecnos	Flonac S	28	0.13	0.05	0.08	2.7
Kyowa Tecnos	Flonac W	93	66.5	18.5	59.0	3.6
Nipponkayaku	Chitosamine	95	84.2	10.6	52.3	7.9
Nipponkayaku	Chitosamine for food	88	129.8	5.2	73.0	25.2
Yaegaki	Y.H. Chitosan HD-200	99	5.7	1.4	5.8	4.0
Yaegaki	Y.H. Chitosan HD-80A	97	6.6	1.6	5.5	4.0
Yaegaki	Y.H. Chitosan HD-SP	96	4.4	1.2	3.4	3.6
Yaegaki	Y.H. Chitosan K-200	84	44.8	4.1	25.2	10.9
Yaegaki	Y.H. Chitosan KII	93	17.9	2.6	14.7	6.8
Yaizu Suisankagaku	Chitosan LL	84	8.3	0.81	4.3	10.2
Yaizu Suisankagaku	Chitosan LL-40	80	8.2	0.81	4.4	10.0
Yaizu Suisankagaku	Chitosan PSH-80	86	150.6	18.9	118.8	8.0
Yaizu Suisankagaku	High Molecular Chitosan	89	123.1	9.2	80.0	13.4
Wako	Chitosan 1000	87	103.6	36.0	95.5	2.9
Wako	Chitosan 500	88	51.4	17.8	53.3	2.9

DA: degree of deacetylation; M_w: weight-average molecular weight; M_n: number-average molecular weight; M_t: molecular weight at peak retention time; M_w/M_n: molecular weight distribution.

vehicles into the body and to achieve greater control over drug release.

2. Experimental

2.1. Materials

Chitosan7B, Chitosan8B, Chitosan9B, and Chitosan10B were purchased from Katokichi Co., Ltd. (Japan). Chitosan500 and Chitosan1000 were purchased from Wako Pure Chemical Industries, Ltd. (Japan). All other species of CS listed in Table 1 were generous gifts from Ajinomoto Co., Inc. (Japan), Dainichiseika Color & Chemicals Mfg. Co., Ltd. (Japan), Kimica Co., Ltd. (Japan), Koyo Chemical Co., Ltd. (Japan), Kyowa Tecnos Co., Ltd. (Japan), Nipponkayaku Foodtechno Inc. (Japan), Yaegaki Bio-industry, Inc. (Japan), and Yaizu Suisankagaku Industry Co., Ltd. (Japan). All other chemicals were of reagent grade.

2.2. Determination of degree of CS deacetylation

Degree of CS deacetylation was determined by colloidal titration. CS (0.5% w/w) was dissolved in aqueous acetic acid solution (5% v/v). One gram of the CS solution was added in a flask, and was diluted to 30 ml with distilled/demineralized water. After adding 100 μl of 0.1% w/v toluidine blue indicator solution, the sample solution was titrated against 1/400 N potassium polyvinyl sulfate solution. A single molecule of potassium polyvinyl sulfate reacts with each deacetylated amino group in the CS molecule. The degree of deacetylation was then calculated from the molar ratio of deacetylated amino groups in the CS molecule, which was estimated from the volume of potassium polyvinyl sulfate solution consumed.

2.3. Determination of CS molecular weight

CS was subjected to gel permeation chromatography (GPC). CS (0.1% w/w) was dissolved in 0.1 M acetate buffer (pH 4.5). A 20-μl aliquot of the sample was loaded onto a column for GPC (Shodex SB-806M HQ; 300 mm × 8.0 mm) and was eluted with 0.1 M acetate buffer (pH 4.5) as a mobile phase at a flow rate of 0.5 ml/min (Shimadzu LC-10AS). CS in the effluent was detected with a refractive index detector (Shimadzu RID-10A). Molecular weight standards were 8 types of pullulan (Shodex STANDARD P-82). Molecular weight was estimated from a calibration curve produced using these standards.

2.4. Viscosity determination of CS solution

CS (0.5–2.0% w/w) was dissolved in 0.1 M acetate buffer (pH 4.5). Viscosity of the solution was measured at 37 °C using a vibration type viscometer (Viscomaite VM-1G, CBC Materials).

2.5. Spherical gel preparation

CS spherical gel was prepared as follows. CS (0.5–2% w/w) was dissolved in 0.1 M acetate buffer (pH 4.5). This solution was slowly added dropwise into 20 ml of 10% w/v aqueous glycine solution (pH 9.0) or 40 mM aqueous $CuSO_4$ solution using a pipette and left to stand at room temperature for 25 min. CS spherical gel formed spontaneously and was retrieved from the preparative medium to confirmed that its shape was maintained.

2.6. Dissolution test

CS spherical gels for dissolution test were prepared as follows. CS (1–2% w/w) was dissolved in 0.1 M acetate buffer (pH 4.5). Model drug, 1% w/w prednisolone (PS), was then added to the CS solution. Ten drops of this suspension was slowly added dropwise into 20 ml of 10% w/v aqueous glycine solution (pH 9.0) using a pipette and left to stand at room temperature for 25 min. CS spherical gels formed spontaneously and were dried at 37 °C for 24 h in a dish before desiccating under vacuum in the presence of P_2O_5. The amount of PS in the gel was calculated by subtracting the amount of PS detected in the preparative medium after spherical gel formation from the theoretical total amount of PS added to the initial CS solution. The dried CS gels with diameters of about 3.5 mm retained more than 99.5% of the theoretical total amount of PS. The rate of PS release from the CS gels into 0.1 M phosphate buffer (pH 7.2) was determined. Dried gels were added to 500 ml of dissolution medium in a dissolution test apparatus (paddle method, 100 rpm, 37 °C) following Japanese

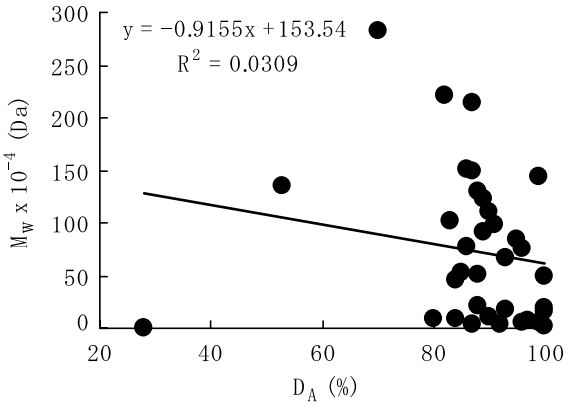

Fig. 1. Relationship between molecular weight and degree of deacetylation. DA: degree of deacetylation; M_w: weight-average molecular weight; R^2: correlation coefficients (subjected to least-squares fitting).

Pharmacopoeia Fourteen Edition (JP XIV). Aliquots of 4 ml were periodically removed for analysis and replaced with 4 ml of the dissolution medium (pre-warmed to 37 °C) in order to maintain a constant volume. The absorbance of the test solution was determined with a spectrophotometer (Shimadzu UV-1200) at 246 nm. The amount of PS released from the CS gels was estimated from a standard curve produced in advance. All dissolution tests were performed in triplicate.

2.7. Enzymatic degradation of CS

0.5% w/w CS solution dissolved in 0.1 M acetate buffer (pH 4.5) containing 0.2 M sodium ion was prepared and incubated at 37 °C. Pre-incubated lysozyme solution (about 4 mg/ml) in physiological saline was added into 180 g of the CS solution to make up to a final lysozyme concentration of 20 μg/g. Viscosity of the solution was measured periodically at 37 °C using a B type viscometer (Tokyokeiki).

3. Results and discussion

3.1. Molecular weight and deacetylation degree of CS

Molecular weight determined by GPC and degree of deacetylation determined by colloidal titration for various CS species are summarized in Table 1. Generally, CS was obtained by deacetylation of chitin under strong

Table 2
Viscosity of CS solution dissolved in 0.1 M acetate buffer (pH 4.5)

Maker	Brand name	CS concentration (%)			
		0.5	1	1.5	2.0
Ajinomoto	PC-100	13	40	68	175
Dainichiseika	Daichitosan 100D	14	38*	58*	92*
Dainichiseika	Daichitosan 100D (VL)	<10	<10	<10*	<10*
Dainichiseika	Daichitosan H	25	72	220*	400*
Dainichiseika	Daichitosan M	28	85	180	310*
Dainichiseika	Daichitosan VL	<10	<10	<10	13*
Katokichi	Chitosan 7B	23	65	160	290
Katokichi	Chitosan 8B	22	49	120	280
Katokichi	Chitosan 9B	19	48	110	190*
Katokichi	Chitosan 10B	10	23*	30*	37*
Kimica	Kimitsuchitosan B	13	28*	38*	58*
Kimica	Kimitsuchitosan F	<10*	<10*	11*	15*
Kimica	Kimitsuchitosan H	19	48*	82*	180*
Kimica	Kimitsuchitosan L	13	29*	64*	120*
Kimica	Kimithuchitosan M	18	45*	82*	180*
Koyo Chemical	Koyochitosan FH-80	31	100	190	380
Koyo Chemical	Koyochitosan FM-80	15	35	88	210
Kyowa Tecnos	Flonac C	<10	<10	13	20
Kyowa Tecnos	Flonac H	16	39	80	170
Kyowa Tecnos	Flonac LV	<10	<10	<10	<10
Kyowa Tecnos	Flonac N	19	50	140*	200*
Kyowa Tecnos	Flonac S	<10	<10	<10	<10
Kyowa Tecnos	Flonac W	15	39	88*	175*
Nipponkayaku	Chitosamine	17	49	110	200
Nipponkayaku	Chitosamine for food	14	39	82*	200*
Yaegaki	Y.H. Chitosan HD-200	<10	<10	<10*	<10*
Yaegaki	Y.H. Chitosan HD-80A	<10	<10	<10	<10*
Yaegaki	Y.H. Chitosan HD-SP	<10	<10	<10	<10*
Yaegaki	Y.H. Chitosan K-200	<10	19	41	64
Yaegaki	Y.H. Chitosan KII	<10	<10	14	23*
Yaizu Suisankagaku	Chitosan LL	<10	<10	<10	13
Yaizu Suisankagaku	Chitosan LL-40	<10	<10	<10	12
Yaizu Suisankagaku	Chitosan PSH-80	19	49	110	200
Yaizu Suisankagaku	High Molecular Chitosan	22	58	120	210
Wako	Chitosan 1000	28	75	200*	290*
Wako	Chitosan 500	24	70	170	200*

* CS is not completely dissolved.

alkaline conditions. Molecular weight decreases during the progression of deacetylation, and thus, CS species with a high degree of deacetylation were expected to have a low molecular weight. However, these properties were not predictably associated and, as shown in Fig. 1, a linear relationship between weight-average molecular weight and deacetylation degree of CS was not always observed. Rege et al. [25] reported that the preparative methods of CS from chitin, particularly reaction temperature and processing time, influenced the physicochemical characteristics of CS. Furthermore, Cho et al. [19] investigated the physicochemical characteristics and the functional properties of five chitin species and five CS species. They demonstrated that the functional properties, such as dye-binding capacity, water-binding capacity, fat-binding capacity, and emulsifying capacity, differed, as did the physicochemical characteristics. Thus, differing physicochemical characteristics of CS would be observed from different source crustacean species and by using different preparative methods, thereby influencing the functional properties of CS.

3.2. Viscosity of CS solution

The various CS species listed in Table 1 were dissolved in 0.1 M acetate buffer (pH 4.5), and the viscosities of these solutions were determined (Table 2). Fig. 2 shows the relationship between viscosity and weight-average molecular weight of CS. Viscosity is commonly proportional to molecular weight, particularly for materials with a high molecular weight. As shown in Fig. 2, the viscosity of CS solution with a higher molecular weight tended to be higher. However, a linear relationship was not observed. This discrepancy between viscosity and molecular weight might be attributed to differences in the distribution of acetamide groups in the CS molecule, which results from differences in deacetylation conditions [26]. This influences the viscosity of CS solution by changing the inter- or intra-molecular repulsion forces.

3.3. Spherical gel formation in preparative aqueous medium

In a previous study, we were able to prepare CS gel for drug delivery under aqueous conditions (e.g. 10% aqueous amino acid solution (pH 9.0) or aqueous solution containing metal ions) [20–24]. When CS solution dissolved in acetic acid, which forms salts with the amino groups of CS, was added dropwise into the preparative medium, CS-acetic acid salts dissociated rapidly with inhibiting the diffusion of CS within the preparative medium, leading to spherical gel formation. If this process occurs more rapidly after CS is added into the preparative medium, drug encapsulation is more efficient as escape of the drug to the preparative medium is minimized. The ease of CS spherical gel preparation was found to depend on CS characteristics such as viscosity, molecular weight and degree of deacetylation. Fig. 3 shows CS spherical gel formation in 10% aqueous glycine solution (pH 9.0). High concentration of CS species with a high molecular weight could not be used to prepare CS spherical gel; its high viscosity in solution prevents it from being dropped by pipette into preparative medium. The use of very low concentration of CS did not result in instantaneous spherical gel formation because the diffusion of CS within the preparative medium was too rapid. Thus, the ease of spherical gel formation was mainly affected by the viscosity of the CS solution. With regard to gelation of CS by chelation with metal ions (i.e. CS gel prepared in 40 mM aqueous $CuSO_4$

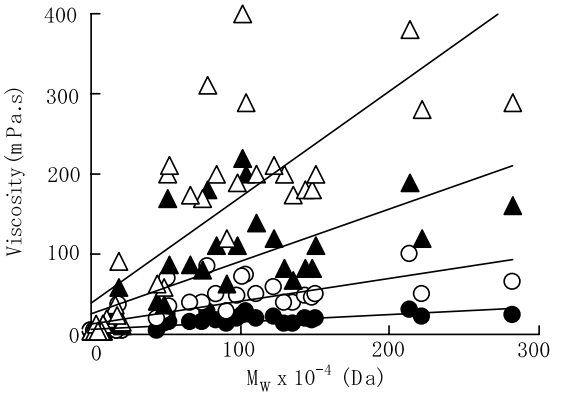

Fig. 2. Effect of molecular weight of CS on the viscosity of CS solution. CS concentration (%): △, 2.0 ($R^2 = 0.6420$); ▲, 1.5 ($R^2 = 0.4990$); ○, 1.0 ($R^2 = 0.5520$); ●, 0.5 ($R^2 = 0.5741$). R^2: correlation coefficients (subjected to least-squares fitting), M_w: weight-average molecular weight.

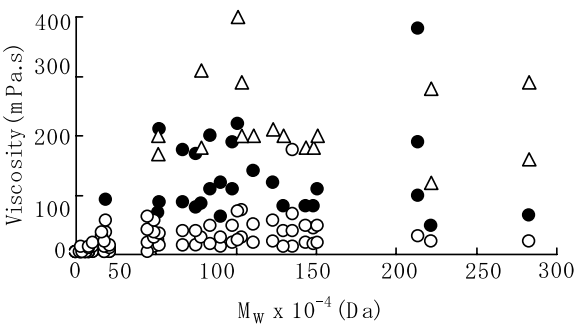

Fig. 3. Effect of viscosity of CS solution prior to gel formation and molecular weight of CS on the spherical gel formation. Gel formation: ○, no gelation; ●, spherical gel formation; △, CS could not be dropped into the preparative medium on account of high viscosity. CS solution dissolved in 0.1 M acetate buffer (pH 4.5) was dropped into 10% aqueous glycine solution (pH 9.0). M_w: weight-average molecular weight.

solution), the extent of deacetylation was also related to spherical gel formation. CS species with a high extent of deacetylation was able to form spherical gel by chelation with metal ions despite using CS species with low viscosity that did not form spherical gel in 10% aqueous glycine solution (pH 9.0). This is attributed to the increased availability of CS amino groups in highly deacetylated CS because amino groups of CS facilitate chelation with metal ions. Thus, instantaneous formation of spherical gels with metal ions was achieved because the proximity of a sufficient number of CS amino groups allowed for spherical gel formation.

3.4. Drug release rate from CS spherical gel

CS gel has been investigated as a vehicle for drug delivery. In a previous study, the rate of drug release from CS gel varied with CS species, gel preparative method and drug species [20–24]. The relationship between drug release behaviors and CS characteristics was thus examined. Fig. 4 shows the percentage of PS released after 6 h from CS spherical gel prepared in 10% aqueous glycine solution (pH 9.0). CS which completely dissolved in 0.1 M acetate buffer (pH 4.5) was used in this experiment. PS release from the CS gel tended to be inhibited as viscosity of the CS solution prior to gel formation increased. However, the percentage of PS released after 6 h did not depend on the weight-average molecular weight of CS ($R^2 = 0.0101$) or the deacetylation degree of CS ($R^2 = 0.00001$). PS did not interact with CS, and in vitro release of PS from CS gel prepared in 10% aqueous glycine solution was controlled by diffusion of PS from the gel matrix. Thus, PS diffusion rate from the CS gel was governed by the structure of the gel matrix. Inhibition of drug release might have resulted

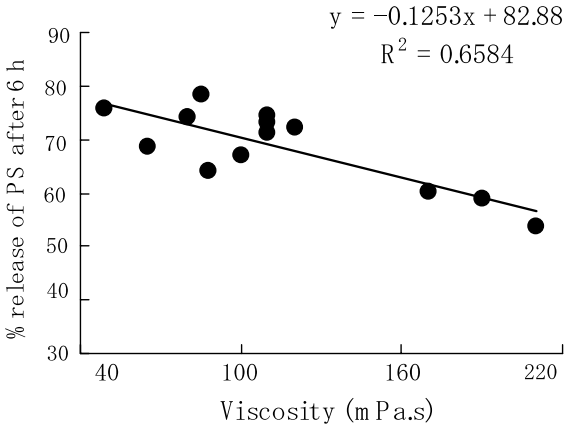

Fig. 4. Effect of viscosity of CS solution prior to gel formation on PS released after 6 h from CS spherical gel prepared in 10% aqueous glycine solution (pH 9.0). Model drug: Prednisolone (PS). R^2: correlation coefficients (subjected to least-squares fitting).

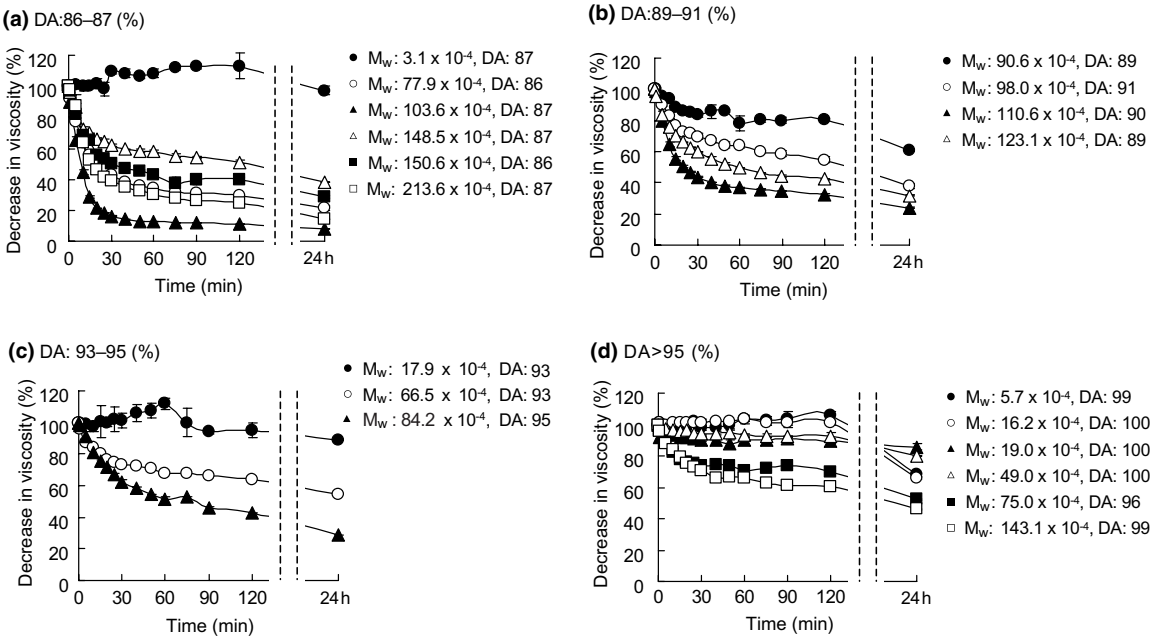

Fig. 5. The enzymatic degradation behaviors of CS observed by changes in the viscosity of CS solution in the presence of lysozyme. DA: degree of deacetylation; M_w: weight-average molecular weight. Viscosity was determined at 37 °C using a B type viscometer (Tokyokeiki).

from the dense structure of matrices composed of high viscosity CS. In the case of CS gel prepared by chelation with metal ions, the extent of deacetylation and the strength of interaction between CS and drug, as well as drug species (e.g. protein drugs), were also related to drug release.

3.5. Biodegradation of CS

CS is known to be enzymatically degraded primarily by lysozyme in the human body [27]. For drug delivery, it is necessary for the vehicle to remain at the implantation site for the duration of treatment period, and to disappear after completion of drug delivery. Degradation of CS has been shown to increase as degree of deacetylation decreases [28–30]. This trend was also observed when CS was processed as a vehicle (e.g. film prepared by solution casting method [31], or conjugate with drug [32]). Furthermore, the biodegradation rate of CS was also controlled by extent of deacetylation in vivo [33]. Similar results were obtained in our previous study, in which the rate of enzymatic degradation by lysozyme reflected the degree of biodegradation after CS vehicle implantation in mice [23]. Thus, the enzymatic degradation behaviors of various CS species were investigated by observing changes in the viscosity of CS solution in the presence of lysozyme.

Fig. 5 shows the changes in the viscosity of CS solutions having similar degree of deacetylation. CS with a low degree of deacetylation tended to be degraded more rapidly. However, the enzymatic degradation of CS species differed, even in CS species with similar extents of deacetylation. Nakamura et al. [32] reported that there is a limit to the enzymatic degradation of CS species with low molecular weights. Aiba et al. [34] suggested that the differences in degradation are due to variations in the different distribution of acetamide groups in the CS molecule. Furthermore, Shigemasa et al. [35] concluded that the degradation of CS is influenced by aggregation of CS inter- and intra-molecular forces as a result of random distribution of acetamide groups in the CS molecule. Therefore, it is impossible to estimate biodegradation rate from the degree of deacetylation alone.

4. Conclusion

Control of biodegradation and drug release of CS gel in vivo can be achieved by manipulating the species of CS. However, numerous investigations have studied only one or a few species of CS. Selection of ideal CS species based on various characteristics may be promising for identifying vehicles that allow sustained drug delivery, prolong the duration of drug activity, improve therapeutic efficacy, and reduce side effects. However, CS functions could not be estimated based only on one characteristic of the CS. CS functions were affected by various characteristics of the CS, such as molecular weight, degree of deacetylation, viscosity of the solution, and distribution of acetamide groups. Therefore, investigation of individual CS characteristics is important for selection of ideal CS species as a material for drug delivery vehicles.

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