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Salt Formation of Lactic Acid Oligomers as Matrix for Sustained Release of Drugs

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Abstract—The calcium and sodium salts of L- and D, L-lactic acid oligomers were obtained by immersion of the oligomer powders (weight average mol. wt 3000–10000) in aqueous solutions of $CaCl_2$ and NaCl, respectively, at room temperature (22°C). The salt formation was analysed by atomic absorption measurement. The thermal properties, including glass transition temperature, melting temperature, and softening temperature, were altered by conversion of the terminal free acid of the oligomer to its salt. An in-vitro release test of an anti-cancer drug from the oligomer beads showed that the drug was released more rapidly from the beads prepared from the non-converted oligomers than those prepared from the salts when the release test was carried out in media containing no calcium and no sodium ions.

Aliphatic polyesters including poly(lactic acid), poly(glycolic acid), their copolymers, and poly(ε-caprolactone) are used as matrices in controlled release systems of pharmaceutical agents (Hutchinson & Furr 1985; Holland et al 1986; Sanders et al 1986; Ogawa et al 1989). Lactic acid oligomers having molecular weights (mol. wt) less than 10000 are also suitable for controlled release systems when the drug release needs to be completed within periods from a week to a month (Ikada et al 1985; Wada et al 1988a, b).

These oligomers become soft around body temperature, a property influencing not only the release rate of drugs, but also the preparation of dosage forms. If the matrix polymer is soft at body temperature, the drug will be rapidly released from the matrix because of the high degradation rate of the matrix and high diffusion of drug from it. However, the drug-matrix composites tend to be sticky, leading to difficulties in the preparation of dosage forms. Recently we found that the softening temperature is increased by conversion of the free carboxyl groups on the end of the oligomer chains to salts. We describe briefly the results on salt formation of the oligomers.

Materials and Methods

Materials

L-Lactic acid(L-LA) and D,L-lactic acid(D,L-LA) oligomers having mol. wt lower than 1×10^4 were synthesized by the conventional condensation polymerization of L-LA and D,L-LA, respectively, at elevated temperatures and reduced pressures (Jamshidi 1984). The oligomers were purified by precipitation with methanol from methylene chloride solution for L-LA oligomers and with water from methanol solution for D,L-LA oligomers. The weight average mol. wt (Mw) was determined by gel permeation chromatography (GPC) using standard polystyrenes for calibration (Van Dijk et al 1983), while the number average mol. wt (Mn) was obtained by determining the number of carboxyl end groups of the oligomers by titration of their acetone solution with NaOH using phenolphthalein as indicator. It was assumed that each of the oligomer chains had one terminal carboxyl group. Poly(L-lactic acid), having Mw of $3{\cdot}4 \times 10^4$, was obtained by ring-opening polymerization of L-lactide in the presence of stannous octoate and lauryl alcohol (Jamshidi 1984; Jamshidi et al 1988). All the solvents and the reagents used were of reagent grade and were used without further purification.

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Conversion of free terminal acids to salts

The size fractions of oligomer powders used ranged from 50 to 300 μm in diameter. Salts were obtained by immersing 1 g of oligomer in 50 mL of calcium chloride or sodium chloride (1·0 M) aqueous solutions and agitating them from 30 min to 24 h. The powders were recovered, washed three times with deionized water, and then dried under reduced pressure.

Characterization of LA oligomer salts

To detect Na and Ca atoms bound to the carboxyl end groups of the oligomers, atomic absorption analysis with a model AA-640-12 atomic absorption spectrometer with a GFA-2 graphite furnace atomizer and U-135 recorder (Shimadzu Corp., Kyoto, Japan) was used. The calcium and sodium hollow cathode lamps were operated at 10 mA and the spectrometer was used to monitor the calcium line at 422·7 nm, and the sodium line at 589·0 nm, with a slit band width of 0·38 nm. The deuterium background was corrected during all the analyses. The furnace was programmed to execute 30 s drying at 200°C, 25 s ashing at 1200°C, and then 10 s atomizing at 2500°C. Argon gas was used to purge the furnace at a flow rate of 2 mL min^{-1}. Five μL of 50 μg mL^{-1} suspension of oligomer salts in deionized water was introduced into the furnace using an Eppendorf pipette.

Solubilities of the oligomers and their salts in different organic solvents were qualitatively determined at 5% (w/v) and 25°C.

The glass transition temperature (Tg) and melting temperature (Tm) were measured with a differential scanning calorimeter (DSC) (Model DT-30, Shimadzu Corp., Kyoto, Japan). Heating was at 5°C min^{-1} under a continuous flow of

helium. The Vicat softening temperature (Ts) was determined by needle penetration into the oligomer specimen using a thermal mechanical analysis (TMA) system (Model DT-30, Shimadzu Corp., Kyoto, Japan).

Release test

Composites of L-LA oligomer (Mw = 3400) and its Na and Ca salts containing an anti-cancer agent, acularubicin hydrochloride (ACR) (Sanraku Corp., Tokyo, Japan), 10% (w/w), were prepared by a co-melt mixing method and then moulded into beads (each 50 mg, ca. 2 mm in diam.). One bead of the non-converted oligomer was immersed in 5 mL of Tris-HCl, Tris-NaCl, or Tris-$CaCl_2$ buffer medium (50 mM, pH 7·4) in glass vials. Each bead of Na and Ca oligomer salts was immersed in Tris-HCl. The vials were placed in a shaker bath kept at 37°C and the release medium was periodically pipetted out from the vials, the same volume of fresh medium being replaced. The amount of ACR released in the medium was measured with a fluorescence spectrophotometer (Model 650 10-S; Hitachi Corp., Tokyo, Japan) at an excitation wavelength of 444 nm and an emission wavelength of 580 nm.

Results

The atomic absorption profile of an LA oligomer after immersion in 10% $CaCl_2$ aqueous solution for 12 h shows a clear signal which reverts to the baseline when the converted oligomer is immersed in 0·1 M HCl and washed with deionized water (Fig. 1). The Ca mol percentage to the carboxyl end group was calculated using a calcium standard solution ($CaCl_2$ in 1 M HCl, 1000 ppm Ca, Wako Pure Chemical Industries Ltd, Osaka, Japan). Apparently, conversion to the calcium salt is virtually complete at 12 h. Therefore, oligomer salts obtained by the 12 h treatment were used in all experiments. A change of Mw was not detected by GPC assay, indicating that hydrolysis did not occur during this time of immersion in aqueous medium. The results of salt formation for all the oligomers are given in Table 1, together with their average mol. wt and mol. wt distribution. The Ca/COOH values are approximately 100% for all the oligomers examined, and a similar result was obtained for the two Na salts, as shown in Table 1. In contrast, the Ca content of a poly(L-lactic acid), prepared using lauryl alcohol as initiator, having Mw of $3·4 \times 10^4$, was below the detection limit, after immersion in $CaCl_2$ solution for 24 h.

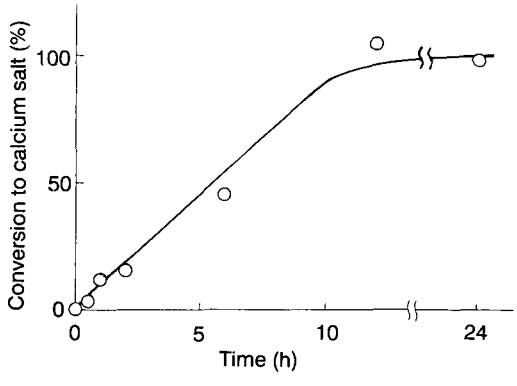

FIG. 1. Time-conversion curve for Ca salt formation of L-LA oligomer (Mw = 3400) in 10% $CaCl_2$ aqueous solution.

Table 2. Thermal properties of lactic acid (LA) oligomers and their salts.

	Mw	Type of salt	Tg (°C)	Tm (°C)	Ts (°C)
L-LA oligomers	3400	acid	43	133	54
		Na	47	137	61
		Ca	47	137	66
	10000	acid	45	135	64
		Na	47	138	69
		Ca	48	138	69
D,L-LA oligomers	3300	acid	27	—	32
		Na	28	—	36
		Ca	—*	—	49
	9600	acid	30	—	46
		Na	—*	—	50
		Ca	—*	—	54

* Tg could not be determined because the DSC charts were too diffuse.

The solubility of LA oligomers and their salts in organic solvents was investigated using chloroform, methylenechloride and benzene (Group A), acetonitrile and acetone (Group B), and the strongly polarizable solvents, *N*,*N*-dimethylformamide (DMF) and dimethylsulphoxide (DMSO) (Group C) as solvents. All the non-converted LA oligomers were soluble in the three groups of organic solvents. On the contrary, all the Na and Ca salts of the L-LA oligomers were insoluble in solvents of Groups A and B at the concentration examined (5%(w/v)) and gave a turbid suspension at room temperature. However, they were soluble in the solvents of Group C to give clear solutions. D,L-LA oligomers had slightly better solubility than L-LA oligomers.

From the DSC scans the Tg and Tm of the non-converted

Table 1. Characteristics of lactic acid (LA) oligomers and their conversion to salts.

	Mw	Mn	Mw/Mn	Ca/COOH*(%)	Na/COOH* (%)
L-LA oligomers	$3·4 \times 10^3$	$1·58 \times 10^3$	2·16	108±8·8	93·3±8·6
	$4·7 \times 10^3$	$2·39 \times 10^3$	1·96	92·3±3·8	—
	$1·0 \times 10^4$	$3·78 \times 10^3$	2·64	94·0±2·1	—
D,L-LA oligomers	$3·3 \times 10^3$	$0·98 \times 10^3$	3·36	92·7±4·6	98·2±3·2
	$6·4 \times 10^3$	$2·04 \times 10^3$	3·14	89·3±5·8	—
	$9·6 \times 10^3$	$3·70 \times 10^3$	2·59	97·0±3·7	—

* Means and s.d. n = 3.

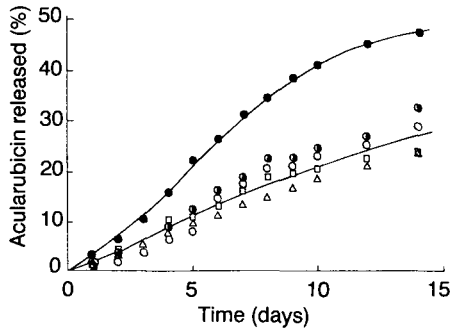

FIG. 2. Release profiles of acularubicin (ACR) from D,L-LA oligomer (mol. wt = 3300) beads: closed circles, non-converted in Tris-HCl buffer; open circles, non-converted in Tris-$CaCl_2$ buffer; half-closed circles, non-converted in Tris-NaCl buffer; open triangles, Ca salt bead in Tris-HCl; open squares, Na salt in Tris-HCl. (ACR loading = 10%, diameter of bead = 2 mm, indicated as average of two measurements.)

oligomers were found to increase with conversion to the salt (Table 2). Thermal mechanical analysis showed the onset temperature of needle penetration into a specimen, defined here as the softening temperature (Ts) of the oligomer, was higher for salts than for the non-converted oligomers (Table 2). There was no significant difference in transition temperature between the Ca and Na salts.

Release profiles of drug from beads of a D,L-oligomer (Mw = 3300) and its salt are shown in Fig. 2. The release from the bead of non-converted oligomer in Tris-HCl buffer medium was faster than from the same bead in Tris-NaCl or Tris-$CaCl_2$ buffer and than from the bead made of Ca and Na oligomer salts in Tris-HCl buffer. The difference among the latter four was not significant.

Discussion

Calcium, as well as sodium ions, forms salts with the carboxyl end group of the LA oligomers by simple immersion of the oligomer powder in aqueous solutions containing the ions. As the carboxyl groups must be located throughout the oligomer powder matrix, it is probable that ions diffuse into the matrix to react with the carboxyl end groups. The rate of water diffusion through the D,L-LA oligomer with Mw of 9600, was found to be 1·0 to 1·2 mm day^{-1} (40 to 50 $\mu m\ h^{-1}$, unpublished data), suggesting that the rate of water penetration is the prime factor for the reaction of ions with the carboxyl end group of the oligomer.

Salt formation with Ca and Na ions seems to be possible only for the LA oligomers synthesized by condensation polymerization in the absence of any catalyst. Salt formation of poly(L-lactic acid), having Mw of $3{\cdot}4 \times 10^4$, obtained by ring-opening polymerization using lauryl alcohol as initiator could not be detected by the atomic absorption analysis. This is probably because lauryl ester is formed at the end of the polymer chain as a result of attack of the initiator on the carboxyl group of the LA monomer.

The salt formation of condensation oligomers enables us to modify the physical properties of LA oligomers as matrix material for sustained release systems of drugs. In our previous studies (Ikada et al 1985; Wada et al 1988a,b, 1990), we have found that the LA oligomers are suitable for short-term drug release (weeks), because they have higher rates of degradation than the conventional, high-molecular-weight poly(lactic acid) synthesized by ring-opening polymerization. However, Tg and Ts of LA oligomers are much lower than those of poly(lactic acid) having higher Mw (Jamshidi et al 1988), giving rise to some difficulties in preparing sustained release formulations such as microspheres and beads. The microspheres often aggregate when prepared using D,L-oligomers as a wall matrix, because they are sticky at preparation temperatures (40 to 50°C). As shown in Table 2, their thermal properties can be modified to some extent by their conversion to salts. In particular, Ts, which may affect the preparation of dosage forms, is shifted to higher temperatures thereby avoiding problems such as aggregation. This modification by salt formation is more effective for oligomers of lower Mw, as they exhibit more difficulties in handling. For example, Ts of non-converted L- and D,L-oligomers having Mw around 3×10^3 is close to room temperature and body temperature, while that of the salts is 12–17°C higher thereby improving their handling at room temperature.

Drug diffusion through the matrix of LA oligomers may be affected by the salt formation. Drug release from the D,L-oligomer salt was much slower than that from the non-converted D,L-LA oligomer having the same Mw of 3300, when the release test was performed using Tris-HCl buffer as release medium (Fig. 2). However, when Tris buffer containing Na^+ or Ca^{2+} was used as release medium, the difference between the free acid oligomer and its salt was not significant. This suggests that the buffer medium containing Na^+ or Ca^{2+} will be able to diffuse into the non-converted oligomer bead during release of drug, resulting in salt formation with the carboxyl group existing inside the oligomer matrix. Thus, usage of the oligomer salts instead of the non-converted oligomers will not give any significant effect on the release of drugs from the LA oligomer devices when they are administered in-vivo, because the free acid oligomers will be converted to their salts by ions existing in body fluid.

In conclusion, the lactic acid oligomers synthesized by condensation polymerization without any initiator have an ability to form a sodium or calcium salt, which changes the thermal properties of the oligomer to improve the handling, without modifying drug release.

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