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Biodegradable dextran hydrogels crosslinked by stereocomplex formation for the controlled release of pharmaceutical proteins

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Abstract

Hydrogels are based on hydrophilic polymers, which are crosslinked to prevent dissolution in water. Because hydrogels can contain large amounts of water, they are interesting devices for the delivery of proteins. In this contribution a biodegradable dextran hydrogel is described which is based on physical interactions and is particularly suitable for the controlled delivery of pharmaceutically active proteins. The unique feature of our system is that the preparation of the hydrogels takes place in an all-aqueous solution, by which the use of organic solvents is avoided. Furthermore, chemical crosslinking agents are not needed to create the hydrogels, since crosslinking is established physically by stereocomplex formation between enantiomeric oligomeric lactic acid chains. The hydrogel is simply obtained after mixing aqueous solutions of dextran(L)-lactate and dextran(D)-lactate. In this contribution, the formation of the hydrogels as well as their protein release properties and degradation behavior are discussed.

Keywords: Biodegradable dextran; Hydrogels; Cross-linking by stereocomplex; Proteins

1. Introduction

Modern biotechnology has resulted in the production of a great variety of pharmaceutically active proteins (Crommelin and Sindelar, 1997). Their unfavorable biopharmaceutical properties, however, have severely hampered the therapeutic and clinical applications of these proteinaceous drugs. A large number of delivery systems has been designed and evaluated for the release of proteins (Baldwin and Saltzman, 1998; Brannon-Peppas, 1995; Gombotz and Pettit, 1995). A frequently investigated polymer to design controlled release systems is poly(lactic-co-glycolic acid). This polymer, however, has some intrinsic drawbacks as protein-releasing matrix. Organic solvents have to be used to prepare pharmaceutical dosage forms (e.g. microspheres), and a low pH might be generated inside the matrix during degradation. Both factors adversely affect the structural integrity of the protein to be delivered (Van de Weert et al., 2000). Moreover, it is difficult to manipulate the release of a protein from PLGA matrices. As an alternative for these biodegradable polyesters, hydrogels (crosslinked, hydrophilic polymeric networks) have been proposed as protein releasing matrices. In the polymeric network hydrophilic groups or domains are present which are hydrated in an aqueous environment thereby creating the hydrogel structure. As the term 'network' implies, either chemical or physical crosslinks have to be present to avoid dissolution of the

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hydrophilic polymer chains/segments into the aqueous phase.

In a chemically crosslinked hydrogel, dissolution of the hydrophilic polymer is prevented by covalent bonds. Chemical crosslinks can be introduced by reaction of functional groups (e.g. hydroxyl, amines) of a water-soluble polymer with suitable bifunctional reagents, e.g. di-isocyanates, or glutaraldehyde. Alternatively, chemically crosslinked hydrogels can be obtained by derivatization of a water-soluble polymer with e.g. (meth)acryloyl groups followed by radical polymerization of the (meth)acrylate groups after addition of an initiator system. Chemical crosslinking can also be induced by γ-irradiation, for example in PVA or natural polymers, such as gelatin and dextran. An advantage of hydrogels formed by chemical crosslinking is that they have a high mechanical strength. A disadvantage is that the incorporated drug or protein may be affected by the chemical reactions or by the crosslinking agents. This may result in a damage to the protein, which is likely accompanied with loss of its biological activity. To overcome this problem, hydrogels can be prepared first and loaded after crosslinking. However, encapsulation efficiencies are low and the release of the protein is relatively fast.

In physically crosslinked gels, dissolution of the hydrogel is prevented by physical interactions, which exist between the polymer chains. In physically crosslinked hydrogels the use of crosslinking agents is avoided. Physical crosslinking can be established by e.g. ionic, hydrophobic, or coiled-coil interactions. For a recent review in which both chemical and physical crosslinked methods are discussed, the reader is referred to a recent review paper (Hennink and van Nostrum, 2002). A novel physical method to create hydrogel is the use of stereocomplex formation. This method has recently been investigated by us and others (De Jong et al., 2000; Lim et al., 2000; Fujiwara et al., 2001). In this paper the results obtained with these gels are briefly described. For more detailed information we refer to the original papers we published up till now on this system (De Jong et al., 2000, 2001a,b).

2. Dextran hydrogels crosslinked by stereocomplex formation

2.1. The concept

The novel hydrogel system as under investigation within our department is schematically shown in Fig. 1. Importantly, the hydrogel is formed in an all-aqueous environment by which the use of organic solvents is avoided. Crosslinking is established by stereocomplex formation between enantiomeric lactic acid oligomers grafted to dextran. The hydrogel

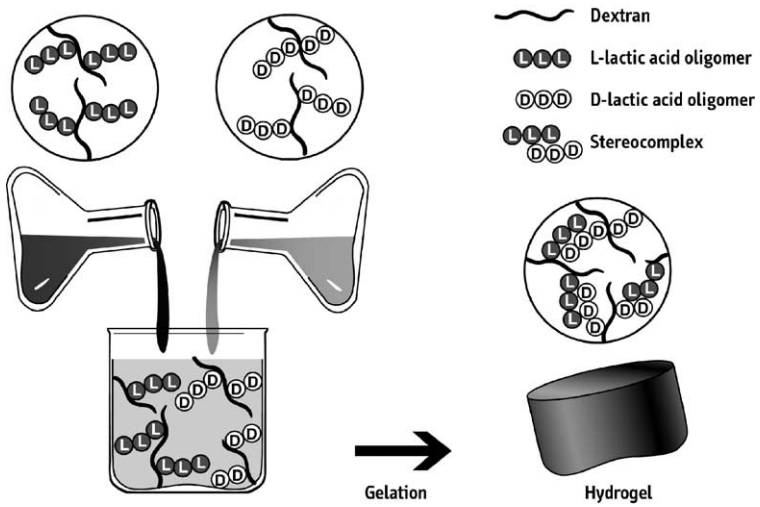

Fig. 1. The concept of the hydrogel system. The hydrogel is simply obtained after mixing aqueous solutions of dex-(L)-lactate (L-lactic acid oligomer grafted to dextran) and dex-(D)-lactate.

system is expected to be fully biodegradable, since the lactic acid oligomers (that will be degraded to lactic acid) are coupled to dextran (a non-toxic water soluble polymer that will be excreted via the kidneys) via a hydrolytically sensitive carbonate ester bond. These features make this hydrogel system very suitable for the controlled release of pharmaceutically active proteins.

To demonstrate the feasibility of this novel system, two requirements have to be fulfilled. First, an operation window should be present where the lactic acid oligomers in either the L- or D-form do not give a crystalline phase (do not associate), whereas in a blend of the L- and D-forms association of oligomers of opposite chirality (stereocomplex formation) occurs. Second, the dextran to which oligolactate chains have been grafted (dex-lactate) has to be soluble in water, and hydrogel formation has to take place by mixing the aqueous solutions of dex-(L)-lactate and dex-(D)-lactate.

2.2. Stereocomplex formation in lactic acid oligomers

PLLA and PDLA, polymers of L-lactic acid and D-lactic acid respectively, are semicrystalline materials with a melting point of around 170 °C. Interestingly, in blends of high molecular weight PLLA and PDLA a phase of a higher melting point (around 230 °C) is observed. This is attributed to the formation of racemic crystallites, also called stereocomplexes, and first described by Ikada et al. (1987) and Tsuji and Ikada (1993).

We investigated whether there is an operation window where lactic acid oligomers in either the D- or L-form do not give a crystalline phase, whereas in a blend of the D- and L-forms stereocomplex formation occurs (De Jong et al., 1998). Lactic acid oligomers with varying molecular weights were synthesized using 2(2-methoxyethoxy)-ethanol (MEE) as initiator and stannous octoate as catalyst (Fig. 2).

Oligomers with different degrees of polymerization (DP) were obtained by varying the M/I (monomer/initiator) ratio. HPLC analysis showed the presence of at least 17 oligomers. The individual peaks were collected to obtain monodisperse products. Stereocomplex formation was studied by differential scanning calorimetry. Fig. 3 summarizes the results.

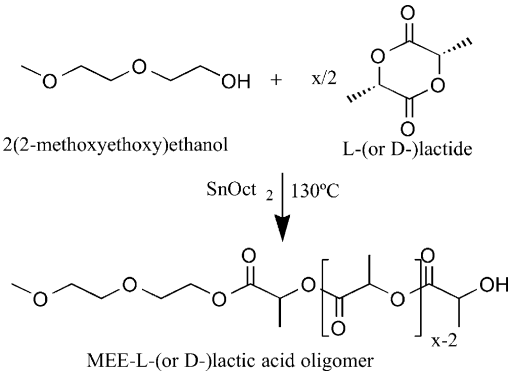

Fig. 2. Synthesis of MEE-lactic acid oligomer.

From Fig. 3 it can be concluded that enantiomeric oligomers can crystallize at DP $\geq$ 11. On the other hand the products with a DP $<$ 11 were completely amorphous. As expected, the T_g increases with DP. From Fig. 3 it appears that the blends of the D- and L-oligomers show the formation of a crystalline phase at a DP $\geq$ 7, which is attributed to the formation of stereocomplexes. It can therefore be concluded that indeed an operation window (namely the degree of polymerization should be between 7 and 11) is present where the lactic acid oligomers in either the L- or D-form do not give a crystalline phase, whereas in a blend of the L- and D-forms association of oligomers of opposite chirality (stereocomplex formation) occurs.

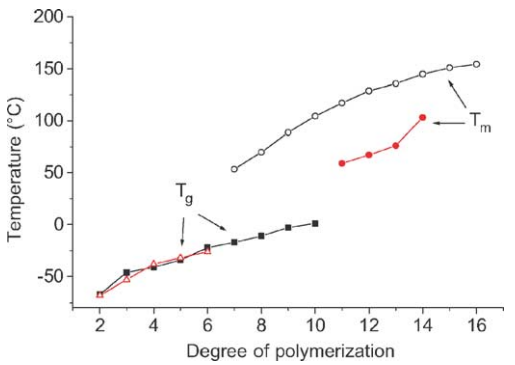

Fig. 3. T_g and T_m of monodisperse lactic acid oligomers (filled symbols) and the blends of L- and D-forms (open symbols) as function of the degree of polymerization. Adapted from De Jong et al. (1998).

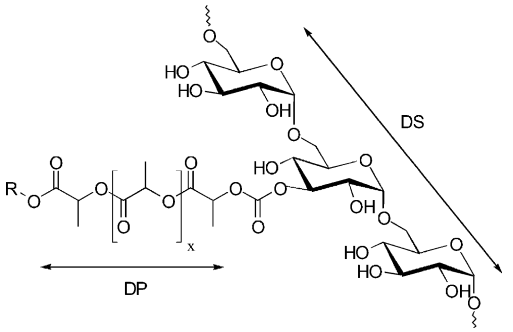

Fig. 4. Dex-lactate product with DP = $x + 2$ and DS (degree of substitution; the number of lactate grafts per 100 glucopyranose units).

2.3. Hydrogels crosslinked by stereocomplexes

To create hydrogels crosslinked by stereocomplex formation, lactic acid oligomers were coupled to a water soluble polymer (dextran) (De Jong et al., 2000). Fig. 4 shows the chemical structure of the synthesized dex-lactate products characterized by their degree of polymerization of the lactate graft (DP) and degree of substitution (DS).

The gelation kinetics, (thermo)reversibility and the effect of different variables (degree of polymerization, degree of substitution, water content) on the rheological properties of the hydrogel were studied. Fig. 5 shows that the rheological characteristics (G′ and tan δ) of a dex-(L)lactate solution did not change in time. In contrast, the dex-(L)lactate and dex-(D)lactate mixture

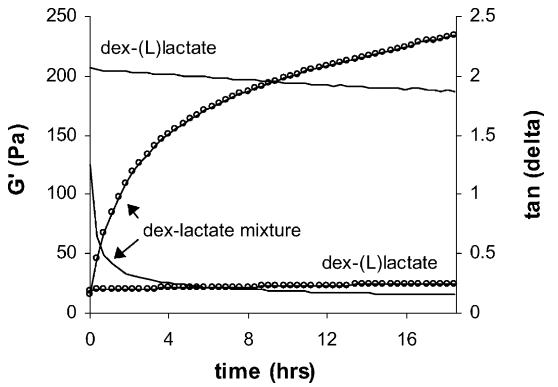

Fig. 5. Storage modulus (G′, ○) and tan δ (—) as function of time of dex-(L)lactate and of a mixture of dex-(L)lactate and dex-(D)lactate (DP_{av} 9, DS 3 and 80% water).

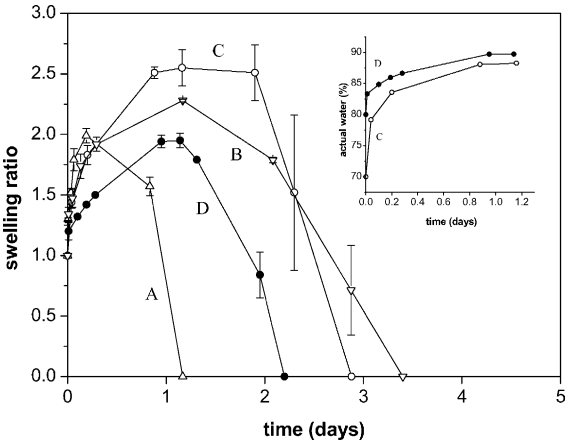

Fig. 6. Swelling behavior of dex-lactate hydrogel with 70% initial water content, DP_{av} 9, DS 3 (A); idem DS 12 (B); DP_{av} 12, DS 6, 70% initial water content (C); idem 80% initial water content (D) (pH 7, 37 °C) (average ± S.D., $n = 3$). The inset shows the actual water content of the hydrogels (C and D) vs. time. Adapted from De Jong et al. (2001b).

showed an increase in G′ and a dramatic decrease in tan δ in time. This demonstrates that an elastic polymeric network is formed.

The network formation can be attributed to self-assembling of the enantiomeric lactic acid chains (stereocomplex formation). It was further shown that the storage modulus of the obtained hydrogel strongly decreased upon heating to 80 °C, while it was restored upon cooling to 20 °C. This demonstrates the thermo-reversibility of the hydrogel and the physical nature of the cross-links (De Jong et al., 2000).

2.4. Hydrogel swelling and degradation

Fig. 6 shows the swelling behavior of dex-lactate hydrogels at 37 °C in phosphate buffer (100 mM) (pH 7). The dex-lactate hydrogels absorbed an amount of water two to three times their initial mass (swelling ratio (Z) of 2–3) during the swelling phase. Thereafter, a dissolution phase followed in which the gel fully dissolved.

The swelling behavior was also investigated in a medium with pH 4, in which both the lactic acid oligomers and the carbonate ester were shown to be stable. At pH 4, the hydrogel was stable for more than 1 month despite comparable swelling, indicating that degradation of the hydrogel at physiological pH

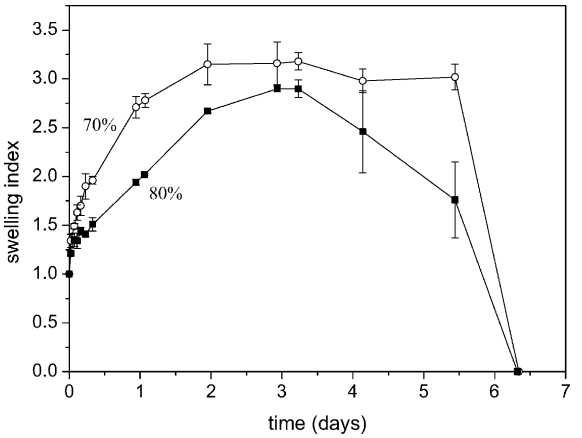

Fig. 7. Swelling behavior of low polydisperse (DP 11–14; $M_w/M_n = 1.01$) dex-lactate hydrogels (DS 6, pH 7, 37 °C) with 70% (open symbols) and 80% (filled symbols) initial water content (pH 7, 37 °C) (average ± S.D., $n = 4$).

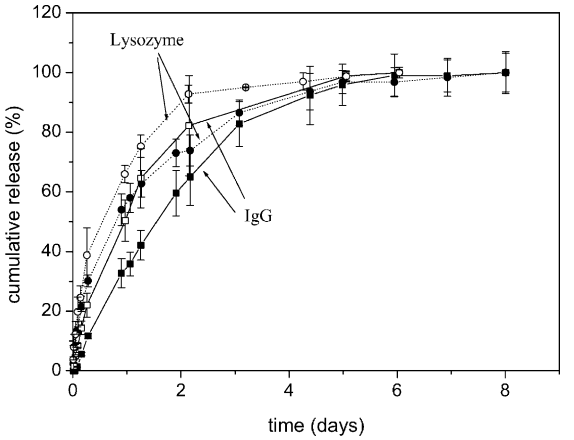

Fig. 8. Release profiles of lysozyme (circles, dotted line) and IgG (squares, solid line) from dex-lactate hydrogel with high (open symbols) and low (filled symbols) polydispersity of the lactate grafts (70% initial water content, pH 7, 37 °C). From De Jong et al. (2001b).

is due to hydrolysis and not by e.g. strong swelling forces.

Lactic acid oligomers with low polydispersity were prepared by preparative HPLC to remove short oligomers (DP < 11), which do not participate in gel formation, and long oligomers (DP ≥ 15) which reduce the water solubility of dex-lactate because of their hydrophobicity. The low polydisperse lactic acid oligomers (DP 11–14) were grafted onto dextran to study the influence of polydispersity on hydrogel formation and degradation.

Fig. 7 shows that longer degradation times (approximately 6 days) were obtained for dex-lactate hydrogels with low polydisperse lactate grafts than for hydrogels with high polydisperse (DP 1 to approximately 30) lactate grafts (1–3 days, Fig. 6).

2.5. Protein release from stereocomplex hydrogels

Fig. 8 shows the release profiles of two model proteins (lysozyme and IgG) from the dex-lactate hydrogel with DP_{av} 12 (high and low (DP 11–14) polydispersity), DS 6 and an initial water content of 70%.

Both hydrogels showed a faster release for lysozyme than for IgG, due to the larger hydrodynamic diameter for the latter protein. The hydrogels with lower polydispersity of the lactate grafts showed a slightly retarded release compared to those with high polydispersity.

Importantly, the proteins were quantitatively released from the gels and with full preservation of the enzymatic activity of lysozyme, emphasizing the protein-friendly preparation method of the protein-loaded stereocomplex hydrogel.

3. Conclusions

A novel hydrogel system based on stereocomplex formation between enantiomeric lactic acid oligomers is described. Lactic acid oligomers of opposite chirality were coupled to dextran in two separate batches: one yields dextran with oligo-(L)-lactate grafts (dex-(L)-lactate) and the other results in dex-(D)-lactate. Hydrogel formation occurs upon mixing aqueous solutions of dex-(L)-lactate and dex-(D)-lactate. Since for the gel formation no organic solvents and/or crosslinking agents are used, this system is very suitable as controlled release matrix for pharmaceutically active proteins.

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