On the Release of Proteins from Degrading Dextran Methacrylate Hydrogels and the Correlation with the Rheologic Properties of the Hydrogels

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Purpose. To study the release of macromolecules of different sizes (bovine serum albumin, immunoglobulin G) from degrading (addition of dextranase) dextran methacrylate (dex-MA) hydrogels and to correlate the release with the evolution of the rehologic properties of the hydrogels during degradation.

Methods. The size of the macromolecules, the degree of substitution (i.e., number of methacrylates per 100 glycopyranose residues) of the dex-MA and the dextranase concentration in the hydrogels was varied. The rheologic properties were measured with a controlled stress rheometer.

Results. The release from dex-MA hydrogels without dextranase was very small [7–20% (time frame up to 180 days)] showing that most of the molecules were entrapped within the hydrogel network. The release from degrading dex-MA hydrogels followed zero-order kinetics for all molecules during a substantial period of the release. This was explained by a liberation and an increasing diffusivity of the proteins in the course of the degradation. The total amount released and the release rates could be well correlated with the rheologically observed degradation rates.

Conclusions. It was shown that rheology can be a useful tool to help explain the release from degrading hydrogels.

KEY WORDS: hydrogels; controlled release; rheology; proteins; dextran methacrylate.

INTRODUCTION

Hydrogels are well suited for biomedical applications because of their tissue compatibility, mainly caused by their high water content and their soft, rubbery consistency giving them a certain resemblance to living tissue (1). Although nondegradable hydrogels are used in a number of medical applications (e.g., contact lenses, wound dressings), the clinical use of degradable hydrogels is increasingly explored. The underlying mechanism of the release of macromolecules from hydrogels has been extensively studied by using various approaches. An important contribution to this research was recently made by Amsden (2,3) who aimed at developing a uniformly applicable model. Far fewer studies were performed on degradable hydrogels, and the release is usually explained in terms of swelling data (4).

The degradability of hydrogels is mostly based on hydrolysis of either the cross-links or the polymer backbone. The latter can be achieved by introducing degradable units into the polymer or by bringing the polymer network in contact with a suitable enzyme. In this study, dextran methacrylate (dex-MA) hydrogels are used. Dex-MA hydrogels can be degraded by incorporation of (endo-)dextranase during cross-linking ("dex-MA/dextranase hydrogels"). The dex-MA/dextranase system was designed to modulate the release of proteins initially entrapped in the network of the hydrogel, by the degradation rate of the network. The degradation rate in turn depends on the cross-link density of the hydrogels and the amount of dextranase present (5,6). Franssen *et al.* (6) showed that a delayed release of immunoglobulin G (IgG) could be obtained from dex-MA/dextranase hydrogel slabs with a degree of substitution (DS, i.e., number of methacrylates per 100 glycopyranose residues) of 4.0 and a dex-MA concentration of at least 30%. The delay time could be varied (from 5 up to 120 days) by using different dextranase concentrations. In contrast, Franssen *et al.* also observed that an almost zero-order release from dex-MA/dextranase microspheres could be obtained and that the rate of this release depended on the dextranase concentration. These various results demonstrated that the release of proteins can indeed be tailored by varying the degradation rate of the network.

In this study, the influence of the dextranase concentration, the protein size, and the DS was investigated by using dex-MA hydrogels with a rather high DS (5.0 and 7.2). Knowing that the release is largely determined by the evolution of the network structure of the hydrogels during degradation, we specifically investigated to which extent the release of two model proteins can be interpreted in terms of the degradation of the hydrogels as characterized rheologically.

MATERIALS AND METHODS

Dex-MA Preparation and Characterization

The dex-MA batches were synthesized from dextran T40 in dimethyl sulfoxide in the presence of 4-(*N,N*-dimethylamino)pyridine. Both synthesis and characterization are described in detail elsewhere (7,8). The degree of methacrylate (MA) substitution (DS) was determined by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) in D_2O with a Gemini 300 spectrometer (Varian). The DS of the batches varied between 4.0 and 7.2.

Preparation of the Dex-MA Based Hydrogels

The hydrogel slabs were prepared by radical polymerization of aqueous solutions of dex-MA. These solutions were prepared by dissolving the dex-MA in buffer (phosphate buffer: 10 mM Na_2HPO_4, 0.02% sodium-azide, adjusted with 1N hydrochloric acid to pH 7.2 or 8.5 for hydrogels with dextranase). Before gelation, the right amount of protein solution (in phosphate buffer 10 mM, pH 7.2) was added to obtain a protein concentration of 2 mg/g hydrogel. Bovine serum albumin (BSA) and IgG (technical grade) were obtained from Sigma (St. Louis, MO). Their molecular weights were 67,000

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ABBREVIATIONS: dex-MA, dextran methacrylate; DS, degree of substitution; BSA, bovine serum albumine; IgG, immunoglobuline G.

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and 150,000 g/mol, respectively, corresponding to a hydrodynamic diameter of 7.2 and 10.7 nm as calculated from the Stokes-Einstein equation (9). The polymerization reagents were N,N,N′,N′-tetramethyleneethylenediamine (TEMED, Fluka, Bornem, Belgium; 20% v/v in deoxygenated phosphate buffer, pH adjusted to 8.5 with hydrochloric acid) and potassium persulfate (KPS, Fluka; 50 mg/mL in deoxygenated phosphate buffer). Adding 50 μL TEMED solution (per gram hydrogel), followed after by stirring in 80 μL KPS solution (per gram hydrogel), started the gelation. All the recipients in which gelation occurred had been coated with a polyethyleneglycol solution (PEG 20,000 g/mol; 10% in phosphate buffer) to simplify the removal of the hydrogel after gelation. Hydrogel slabs were prepared by transferring 9 mL of the gelating mixture into 15-mL polypropylene syringes from which the heads were sawn. After gelation, a part (2–3 mm) of the hydrogel was pushed out of the syringe and sliced off with a thin wire. On average, a complete gelation took 90 min at room temperature. Throughout this work, the dex-MA concentration of the hydrogels was 30% (w/w).

For the preparation of dex-MA/dextranase hydrogels, the enzyme solution (D-1508, Sigma; diluted to 10 U/mL in 5 mM citrate buffer pH 6; one unit will deliver 1 μmol of isomaltose per min at pH 6 at 37°C) was added to the dex-MA solution (cooled to 4°C) before the addition of the gelation reagents, as described above. Because only minute amounts of enzyme solution had to be added, no pH shift was observed in the dex-MA solution. The gelating solution (5 mL) was transferred into the syringes and kept at 4°C for 90 min. Hydrogel slabs were cut and stored overnight in phosphate buffer pH 8.5, to allow swelling under conditions of low dextranase activity (i.e., high pH). Finally, they were transferred to phosphate buffer pH 7 and stored at 37°C to allow degradation.

Release Experiments

For the release experiments, the dex-MA/dextranase hydrogel slabs were transferred to individual containers. To maximize the area for free diffusion from the hydrogels, a mesh was mounted at 7 mm from the bottom of the containers. The hydrogel slabs were put on the mesh, submerged with 20 mL of phosphate buffer (pH 7.2) and stored at 37°C. Samples (4 mL) were taken at regular intervals and replaced by fresh buffer. The protein concentration in the samples was measured by using the Bio-Rad protein assay (Sigma, Bornem, Belgium) [microassay procedure (10)]. Twice a day the containers were slightly shaken.

Rheologic Characterization of the Dex-MA Hydrogels

For the mechanical characterization of the hydrogels, rheologic measurements were performed on an AR1000-N controlled stress rheometer from TA-Instruments (Brussels, Belgium). The rheometer was adapted for the measurement of hydrogel slabs by sticking sandpaper to the geometry. The bottom plate was replaced with a Plexiglas® plate with a roughened surface that was fixed on the rheometer with clamps. This allowed removing the hydrogels together with the bottom plate (to continue the (slow) degradation of the gels outside the rheometer) avoiding manipulation of the degrading hydrogels. All measurements were performed with an acrylic top plate (diameter 2 cm) equipped with a solvent trap to avoid evaporation. Unless described otherwise, all experiments were performed in oscillation mode at 1 Hz by applying a constant strain of 0.5%. Further details on the method used were described in detail by Meyvis *et al.* elsewhere (11).

Swelling Experiments

To characterize the swelling behavior of the degrading hydrogels, they were weighed immediately after preparation and on several occasions during their degradation. The swelling ratio was calculated by dividing the weight of the hydrogels by their weight after gelation.

RESULTS AND DISCUSSION

Protein Release from Nondegrading Dex-MA Hydrogels

To obtain a degradation-controlled release from an enzymatically degrading hydrogel, the protein has to be initially entrapped in the hydrogel network. To evaluate whether this condition was fulfilled, first the release of the proteins from nondegrading dex-MA hydrogels (i.e., without dextranase) was studied. Figure 1 illustrates that only between 9 and 20% of the total amount of protein in the hydrogels was released while, on average, it took about 100 days. This indicated that most of the proteins were indeed entrapped within the polymer network of the hydrogels. As observed in Fig. 1, it could be expected that the larger the protein, the more likely it was to become entrapped within denser parts of the network.

Because a fraction of the proteins could be released from the nondegrading dex-MA hydrogels, while another fraction seemed completely immobilized, it was suggested that the polymer network was heterogeneous. This was also observed by Kim and Chu (12) by scanning electron microscopy (SEM) and mercury intrusion porosimetry. They found a bimodal pore-size distribution in dex-MA hydrogels with a high DS. As the left part of Fig. 2 shows, although denser regions physically entrap the proteins, other parts of the network ("low-density zones") may allow the movement of proteins through

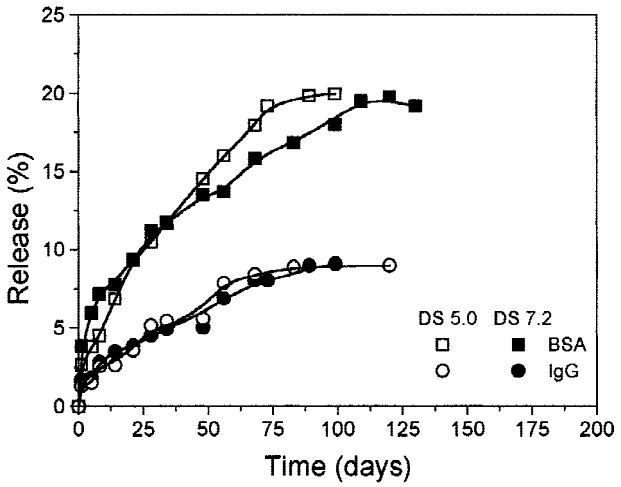

Fig. 1. Cumulative release of various proteins BSA and IgG from dex-MA hydrogels (30%) made from dex-MA with a different DS. The data points are averages of at least three repeats that deviated less than 3% of the total amount of protein in the hydrogels.

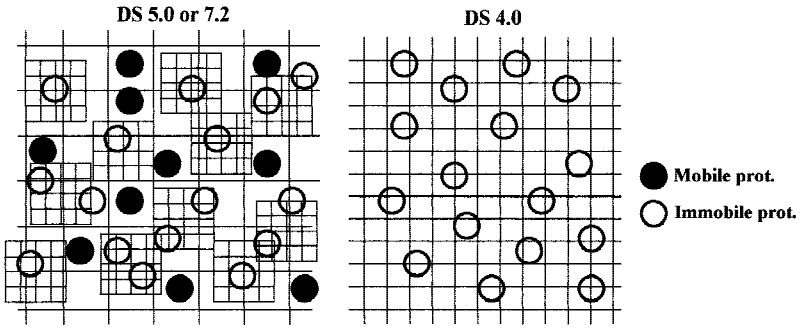

Fig. 2. Schematic representation of the heterogeneous network structure of dex-MA hydrogels with a DS 5.0 or 7.2 (left) and the homogeneous structure of dex-MA hydrogels with a DS 4.0. Both mobile and immobile proteins entrapped by the network structure are shown.

the hydrogel. It could be wondered if the low-density zones were interconnected or if they were completely shielded from each other by the denser zones in the network. Because it took around 100 days to release all the mobile proteins, probably not only proteins in low-density zones, in direct contact with the surface of the hydrogels were released but also proteins from low-density zones in the core of the gels, suggesting that most of the low-density zones were interconnected.

Zero-Order Protein Release from Degrading Dex-MA Hydrogels

Degradable dex-MA hydrogels were made by adding dextranase to the dex-MA solution just before gelation. Consequently, the dex-MA hydrogels were bulk degraded by the entrapped enzyme. The release of BSA and IgG from hydrogels made from dex-MA with a different DS (5.0 and 7.2) and with different concentrations of dextranase (0.03 and 0.1 U/g gel, respectively) was studied. The results are represetned in Figs. 3a–d. Some general observations were made. First, the total amount released from degrading dex-MA hydrogels was always larger compared to the nondegrading analogues. Second, the release lasted up to 150 days, which was significantly longer than the release from the nondegrading dex-MA hydrogels. However, a 100% release of the proteins was never obtained. Finally, in contrast with a delayed protein release reported by Franssen *et al.* (6), all the degrading dex-MA gels showed zero-order protein release during a substantial period.

To explain the release results, first the degradation of the polymer network has to be considered. During degradation, the dextranase hydrolyzes network chains. Because the dextranase was added to the dex-MA solution before cross-linking, the enzyme was homogeneously distributed in the dex-MA hydrogel and, consequently, both chains in low- and high-density zones of the network could be degraded by the enzyme. As chains are hydrolyzed, the hindrance of the network chains on the movement of the proteins may reduce, which can lead to both an increase in the diffusivity of the proteins within the network and a liberation of entrapped proteins from the denser zones. The latter is clearly illustrated by the always-larger release of proteins from degrading dex-MA hydrogels compared to the release from nondegrading analogues. None of the hydrogels could be fully degraded by the enzyme explaining why a 100% release was never obtained.

To explain the observed zero-order release, the basic mechanisms governing the release of proteins from hydrogels have to be considered. The release rate of a protein from a hydrogel is strongly determined by the protein concentration gradient between the hydrogel and the release medium as well as the protein diffusivity within the hydrogel (13). As a protein diffuses from a hydrogel the concentration gradient decreases, which gradually slows down the release rate. On one hand, if all the proteins in a hydrogel are mobile, the decreasing release rate can be compensated by an increasing diffusivity of the proteins due to the degradation of the hydrogen network. On the other hand, if a major part of the proteins is immobile, the concentration gradient, and thereby the release rate, can be kept constant by a gradual liberation of entrapped proteins upon hydrolysis of network chains. Chang and Himmelstein (14) showed that a combination of dissolution and diffusion of drugs from nonswellable, nonerodable matrices could indeed result in a zero-order release. Such systems are very comparable to the degrading dex-MA hydrogels because through degradation both the amount of "dissolved" drug (i.e., the amount of mobile proteins) as well as the porosity of the matrix, which determines the diffusivity, is increased. A zero-order release of proteins from dextran hydroxymethacrylate hydrogels was also found by Van Dijk-Wolthuis *et al.* (15). In these hydrogels, hydrolysis of the cross-links is accompanied by an increased swelling, which may also increase the diffusivity of the proteins.

To study the influence of parameters such as protein size, DS, and dextranase concentration, the zero-order release rates (% release/day), as calculated from the slopes of the fitted curves, were summarized in Table I. When comparing dex-MA hydrogels of the same composition (DS and dextranase concentration), the release of IgG was always 40–50% slower than that of BSA. This difference roughly corresponds to the relative size difference of both proteins. Because of its smaller hydrodynamic size, fewer network chains had to be hydrolyzed both to liberate a BSA molecule from denser zones and to increase its diffusivity. In previous studies, it was shown that the release of proteins from nondegrading dex-MA hydrogels strongly depended on the size of the proteins (9,16). The current data indicate that also in degrading hydrogels the release of proteins is size dependent. Increasing the DS only slightly decreased the zero-order release rates. However, because of the higher DS, a higher cross-link density and associated concentration of network chains is ex-

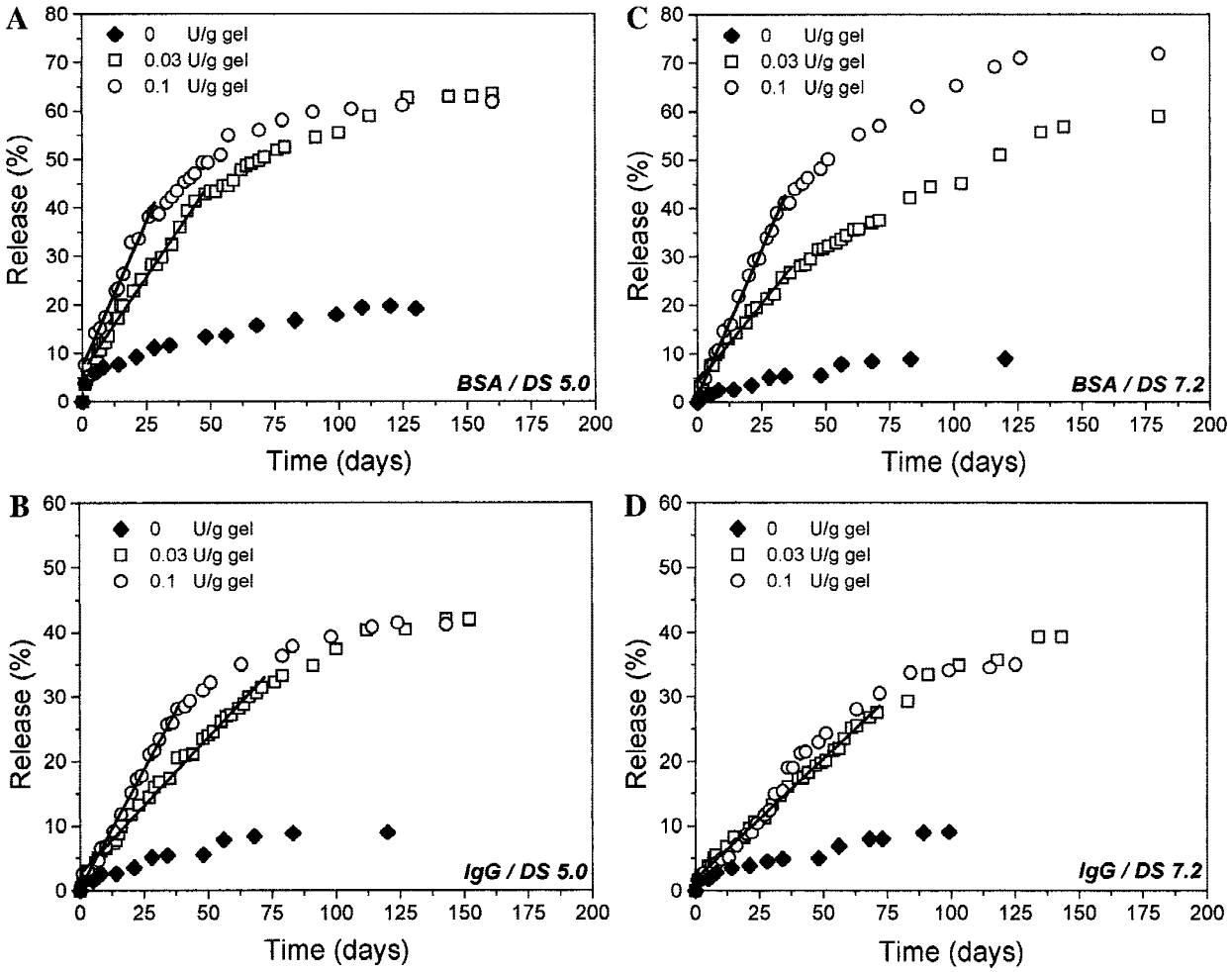

Fig. 3. Cumulative release of proteins from dex-MA/dextranase hydrogels (30%) with different dextranase concentrations made from dex-MA with different DS: (a) BSA/DS 5.0; (b) IgG/DS 5.0; (c) BSA/DS 7.2, and (d) IgG/DS 7.2. The fitted zero-order release curves are also shown [only one (0.03 U/g gel) in part d.]. The data points are averages of at least three repeats that deviated less than 5% of the total amount of protein in the hydrogels.

pected to be present in the network of these hydrogels (is proven in Fig. 4 by the much higher G' of DS 7.2 hydrogels). This high cross-link density can result in very small network chains. Next to being hardly degradable by the dextranase (17), these chains probably did not contribute to the direct entrapment of protein as they formed very small cages. The latter is also supported by the almost identical release profiles observed for each protein from DS 5.0 and 7.2 nondegrading dex-MA hydrogels.

Looking at the influence of the dextranase concentration, a threefold increase only resulted in a 1.5–2-fold increase of the zero-order release rate. Both the nonideal substrate (17) (presence of nondegradable and probably also nonaccessible dextran chains) and the fact that the hydrolysis of the chains can influence protein diffusivity as well as the protein liberation can explain this discrepancy. The result was consistent for all conditions studied except for the release of IgG, which was found to be independent of the dextranase concentration. As illustrated in Fig. 3d, only a slight increase (although statistically not significant) in the release of IgG from DS 7.2 dex-MA hydrogels with the highest dextranase concentration could be observed. Probably the presence of more nondegradable chains in the network in combination with the size of the IgG molecules limited the increase in diffusivity during degradation, thereby masking the influence of the liberation speed of the entrapped proteins on the release.

From these results it can be concluded that the release of proteins from degrading dex-MA hydrogels with a rather high DS is mainly controlled by the size of the protein molecules, the enzyme concentration, and to a minor extent the DS. For larger molecules, the influence of the enzyme concentration may be cancelled out because of a minor increase in diffusivity during degradation.

Table I. Fitted Zero-Order Release Rates of BSA and IgG from Degrading Dex-MA Hydrogels of Various Compositions and Apparent Rheologic Degradation Rates of These Hydrogels

		Release rate (%/day)		
DS	U/g gel	BSA	IgG	Rheologic degradation rate (Pa/day)
5	0.03	**0.78**	**0.42**	**3000**
	0.1	**1.2**	**0.73**	**5900**
7.2	0.03	**0.64**	**0.37**	**3700**
	0.1	**1.13**	**(>0.37)**	**8200**

Correlation of the Protein Release with the Rheology of the Degrading Dex-MA Hydrogels

Because the release of the proteins was clearly influenced by the degradation of the dex-MA hydrogels it was questioned to what extent the release data could be correlated with the evolution of the rheologic properties of these hydrogels. The storage modulus (G'; Fig. 4) of a hydrogel is proportional to the amount of elastic chains within the polymer network (18). Dex-MA with a higher DS has more possible cross-link sites, which results in a denser mesh structure, more network chains, and an associated higher G' (Fig. 4). The initial part of the rheologic profiles could be approximately fitted with a straight line to calculate an apparent degradation rate (Table I). As shown in Table I, increasing the dextranase concentration doubled the degradation rates, which was roughly comparable to the 1.5–2-fold increase of the zero-order release rates. This confirms that the degradation of the hydrogels strongly controlled the release. In contrast to the release rates, the degradation rates of the DS 7.2 dex-MA hydrogels were higher than those of the DS 5.0. As indicated by the higher G' (Fig. 4) of DS 7.2 dex-MA hydrogels, the concentration of network chains is higher compared to DS 5.0 dex-MA hydrogels. This finding, on one hand, means that more chains have to be degraded to create a similar increase in diffusivity and, on the other hand, that relatively fewer chains are involved in the entrapment of proteins. Both phenomena explain why a faster degradation did not result in a faster release.

Comparing the release profiles with the degradation, it can be seen that already after about 15–20 days, the rheologically observed degradation rate slowed down while the zero-order release generally lasted far longer (between 30 and 80 days). To explain this time discrepancy, a closer look should be taken at the work of Franssen *et al.* (17) who recently suggested that the degradation of dex-MA hydrogels by dextranase could be described by a two-substrate model. The "primary substrate," with a high affinity for dextranase, was identified as dextran segments of at least 18 unsubstituted glucopyranose residues. The "secondary substrate," with a much lower affinity for dextranase, consisted of dextran segments of 6–18 unsubstituted glucopyranose residues. Moreover, they showed that dextran segments of less than 6 unsubstituted glucopyranose residues were not degraded in dex-MA hydrogels. During the initial period of the degradation, mostly primary (and some secondary) substrate is degraded. Because the primary substrate consists of fewer cross-linked chains, it probably represents the low-density zones and, consequently, has less chance to be involved in the entrapment of the proteins within the network (Fig. 2). In the course of degradation, the amount of primary substrate decreases, and more secondary substrate becomes degraded by the enzyme. Consequently, although the rheologically observed degradation rate slows down (as dextranase shows a lower affinity for this secondary substrate), the effect of the hydrolysis of a network chain on the liberation of entrapped proteins increases, which may prolong the zero-order release.

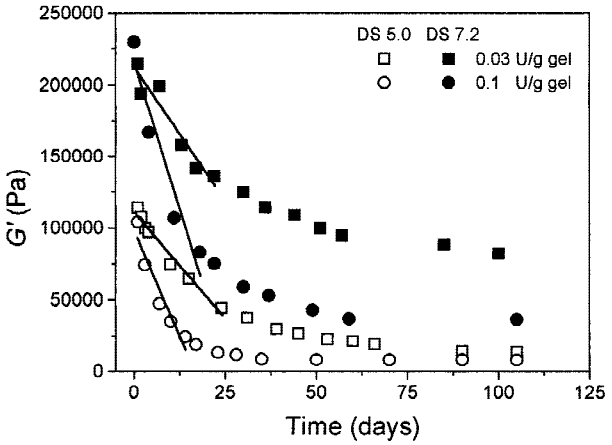

Fig. 4. G' of degrading dex-MA/dextranase hydrogels (30%) as a function of time. The data points are averages of at least three repeats that deviated less than 10%.

Finally, the total release of both BSA and IgG correlated well with the G' of the dex-MA hydrogels at the end of the degradation. DS 5.0 hydrogels (Fig. 4) had a similar G' at the end of the degradation, resulting in similar amounts of total release protein (Figs. 3a–b). DS 7.2 dex-MA hydrogels, on the contrary, evolved toward a clearly different G', which was reflected in the dependence of the total release of BSA on the dextranase concentration. For IgG, however, the difference in G' did not have an effect on the total released amount. Apparently, the extra chains that were hydrolyzed in the dex-MA hydrogels with 0.1 U dextranase/g gel did not result in an extra liberation of entrapped IgG.

From these results, it could be concluded that a good correlation exists between the influence of the dextranase concentration on the rheologic degradation rates and the zero-order release rates. This finding shows the large dependence of the release on the structural changes within the network during degradation. By knowing the degradation mechanism, rheologic data can help to interpret release profiled of proteins from degrading hydrogels.

Delayed Protein Release from Degrading Dex-MA Hydrogels

In a recent study, Franssen *et al.* reported a delayed IgG release from 30%, DS 4.0 dex-MA hydrogels containing 0.03 U dextranase/g gel. Although the dex-MA hydrogels used in this study had a denser network structure [i.e., higher G' (11)], no delayed release was observed (Figs. 3a–d). The difference in release behavior must be sought in a comparison of the network structure of DS 4.0 and DS 5.0 and 7.2 dex-MA hydrogels. In a previous study, the rheologic and swelling properties of dex-MA hydrogels synthesized from dex-MA varying in DS (between 1.2 and 8) were investigated (11). Several features indicated that DS 4.0 hydrogels were by far more homogeneous than hydrogels synthesized from dex-MA with a higher DS. First, G' of DS 4 hydrogels was directly proportional to the concentration of dex-MA at cross-linking, indicating that few network imperfections were present (G' of highly substituted dex-MA hydrogels leveled off upon increasing the dex-MA concentration). Second, as shown in Fig. 5, DS 4.0 hydrogels swelled significantly upon submersion in water and continued to swell during degradation, which indicated that the network chains were rather long and that most of the chains had similar lengths (19). In contrast, DS 5.0 and 7.2 hydrogels hardly swelled and showed no extra swelling during degradation (Fig. 5). Finally, DS 4.0 hydrogels were

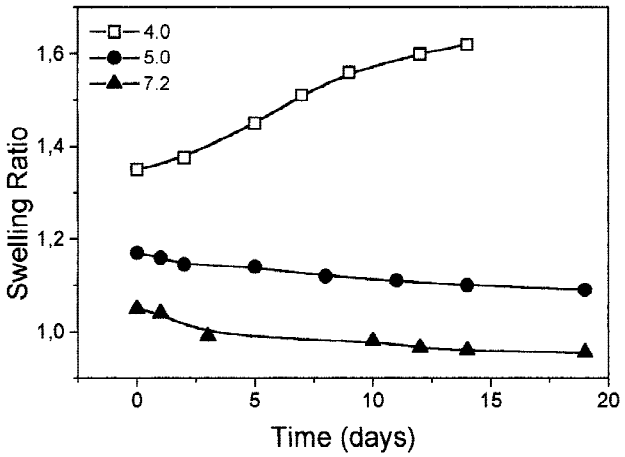

Fig. 5. Evolution of the swelling ratio of dex-MA/dextranase hydrogels (30%; 0.1 U/g gel) made of dex-MA with various DS.

fully transparent, whereas DS 5.0 and 7.2 hydrogels were slightly opaque. Because the DS 4.0 hydrogels showed a delayed delivery of IgG, the whole network should be able to entrap IgG (as schematically represented in the right part of Fig. 2). Therefore, to start the release of IgG, the cage formed by the network around the molecule has to be sufficiently degraded (i.e., the creation of low-density zones). In addition, to allow transport of IgG located in the middle of a hydrogel to the surface, newly created less-dense zones should be interconnected. In contrast, because of the bimodal pore-size distribution of DS 5.0 and 7.2 hydrogels, high-density zones throughout the hydrogels are perfused by low-density zones (left part Fig. 2), allowing the diffusion of liberated protein to the surface of the hydrogels right from the start of the degradation. This resulted in a zero order instead of a delayed release.

CONCLUSIONS

In this work, the release of two different proteins from degrading dex-MA hydrogels made of dex-MA with a rather high DS was studied. The release of BSA and IgG from the nondegrading analogues was low (9–20%, in 100 days), indicating that most of the protein molecules were entrapped in the hydrogel matrix. The total amount released was related to the size of the molecules. Based on these findings, a model was proposed for the structure of the hydrogels. The network of the hydrogels probably consisted of dense zones, which entrapped the proteins, perfused by interconnected less dense zones, through which the molecules could diffuse.

The total amount of protein released from degrading dex-MA hydrogels was always larger compared to the release from the nondegrading analogues and followed zero-order kinetics for all molecules during a substantial period of the release. The latter could be explained by a liberation of entrapped protein possibly also accompained by an increasing diffusivity of the molecules in the course of the degradation. By comparison of the fitted zero-order release rates of the various conditions studied, it could be concluded that the release of proteins from degrading dex-MA hydrogels (with a rather high DS) is mainly controlled by the size of the protein molecules, the dextranase concentration, and to a minor extent the DS. For larger molecules, the influence of the dextranase concentration may even be canceled out because of a minor increase in diffusivity during degradation.

The evolution of the dex-MA hydrogels during degradation was also studied rheologically. From these results it could be concluded that a good correlation exists between the influence of the dextranase concentration on the rheologic degradation rates and the zero-order release rates. This shows that the release of proteins from degrading dex-MA hydrogels is largely controlled by the evolution of the network structure of the hydrogels during degradation.

Finally, a comparison was made between the DS 4.0 dex-MA/dextranase hydrogels [studied by Franssen *et al.* (6)] that showed a delayed release of IgG and DS 5.0 and 7.2 dex-ma/dextranase hydrogels that released IgG with zero-order kinetics. Based on rheologic and swelling data, it was concluded that DS 4.0 hydrogels probably had a largely homogeneous network structure with rather large network chains. Both conditions are prerequisites for a delayed release of an entrapped molecule upon degradation of the network.

Generally, it was shown is this study that rheologic analysis can be a useful tool to explain the release from degrading hydrogels. However, the effect of the degradation of the network strongly depends on the dimensions of the solute and especially the topography of the network. It was shown for dex-MA that small changes in the degree of substitution can result in totally different release profiles of the small molecule.

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