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International Journal of Pharmaceutics 230 (2001) 57–66

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Dextrins as potential carriers for drug targeting: tailored rates of dextrin degradation by introduction of pendant groups

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Received 29 November 2000; received in revised form 10 August 2001; accepted 21 August 2001

Abstract

There is a recognised need to identify new biodegradable polymers suitable for development as targetable drug carriers. The aim of this study was to determine the rate of degradation of two dextrin fractions (Mw 15.5 and 51 KDa) by α-amylase and liver lysosomal enzymes (tritosomes). Also experiments were conducted to discover whether backbone modification by succinolyation (1–34 mol%) or pendant group incorporation (e.g. doxorubicin) could be used to tailor the rate of polymer degradation. Dextrin (α-1,4 polyglucose) is a natural polymer used clinically as a peritoneal dialysis solution and as a controlled drug delivery formulation. Size exclusion chromatography (SEC) showed that dextrin was degraded rapidly (within 20 min) by rat plasma and porcine pancreatic α-amylase. In contrast over 48 h no degradation was observed in the presence of tritosomes. The rate of α-amylase degradation of succinoylated dextrins (Mw ~ 51 KDa) was dependant on the degree of modification (dextrin $> 1 > 5 > 15 > 34$ mol% succinoylation). Dextrin–doxorubicin conjugates were prepared from the 15 and 34 mol% succinoylated intermediates to have a doxorubicin loading of 8 and 12 wt.%, respectively. These doxorubicin conjugates were more stable than their parent intermediates, and SEC showed an apparently higher molecular weight. The drug conjugates did however degrade slowly over 7 days to release oligosaccharide–doxorubicin species. This fundamental study demonstrates the possibility of controlling the rate of dextrin enzymolysis by backbone modification and thus affords the potential to rationally design dextrin–drug conjugates for specific applications as targetable carriers.

Keywords: Dextrins; Drug–polymer conjugates; Succinoylation; Biodegradable polymers

1. Introduction

Polymer therapeutics, including polymeric drugs polymer–drug and polymer–protein conjugates and non-viral vectors for gene delivery are finding increasing clinical use particularly in the

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0378-5173/01/$ - see front matter
PII: S0378-5173(01)00859-6

treatment of cancer (reviewed in Duncan, 1999; Brocchini and Duncan, 1999). Synthetic and natural polymers have been explored as drug carriers (Duncan et al., 1996; Brocchini and Duncan 1999) but almost all polymers used clinically are still non-biodegradable synthetic polymers e.g. poly(ethyleneglycol) (PEG) (Fuertges and Abuchowski, 1990) and *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, (Vasey et al., 1999). Although well tolerated in man, such polymers are not biodegradable in the polymer main chain. Thus in order to ensure renal elimination and to exclude the threat of progressive accumulation after repeated administration, only polymers with a molecular weight below the renal threshold ($\sim$40,000 Da) can be used. This severely limits optimisation of polymer molecular weight to ensure maximal tissue/tumour targeting. Whilst natural polymers (e.g. dextran and poly(aminoacids) are frequently inherently biodegradable, even low levels of chemical modification (to facilitate drug attachment) can lead to the generation of non-degradable adducts (Vercauteren, et al., 1990). In addition, many natural polymers (including dextran) are immunogenic and cannot be given repeatedly.

For these reasons we have begun to explore dextrins as potential biodegradable drug carriers. Dextrins (Fig. 1a) are α-1,4 poly(glucose) polymers obtained by enzymatic hydrolysis of corn starch. They contain few ($<5\%$) α-1,6 links so display minimal branching. Icodextrin, (a polydisperse dextrin with a Mw of $\sim$20 KDa) is already in routine clinical use as peritoneal dialysis solution (Peers and Gokal, 1998). Icodextrin has also been developed as carrier solution for intraperitoneal administraion of 5-fluorouracil (Kerr et al., 1996). The proven clinical tolerability of dextrin and preliminary observations that dextrins are readily degraded by amylases to yield maltose (α-1,4) and isomaltose (α-1,6) (Davies, 1994) suggested that this polymer might be ideal for development as a drug carrier. However, first it was necessary to quantitate the rate of dextrin degradation by α-amylases and also to evaluate the effect of pendant chain incorporation on the biodegradability of the polymer backbone. Succinoylation was chosen previously as the method of choice for introduction of pendant groups into dextrins (Hreczuk-Hirst et al., 1999, 2001a).

First, two dextrin molecular weight fractions (Mw 51 and 15.5 KDa) were incubated (pH 7.4 and 5.5) with α-amylase (rat plasma or isolated porcine) or isolated rat liver lysosomal enzymes (tritosomes) to determine their basal rates of degradation. Size exclusion chromatography (SEC) was used as an assay method. The higher molecular weight dextrin was then succinoylated (1–34 mol%) (Hreczuk-Hirst et al., 1999) and the rate of porcine pancreatic α-amylase degradation of the modified polymers was determined. As it is well known that addition of pendant drugs can also influence the degradability of polymeric carriers, dextrin–doxorubicin conjugates synthesised from succinoylated dextrin intermediates of Mw $\sim$ 51 KDa (5 and 34 mol%) (Hreczuk-Hirst et al., 2001a) were used to follow the rate of degradation of a putative dextrin–drug conjugate.

2. Materials and methods

2.1. Materials

The dextrin molecular weight fractions (Mw = 15.5 Da; Mn = 5.7 Da and Mw 51.1 Da; Mn 27.8 Da) were supplied by ML Laboratories (Liverpool, UK). Succinoylated dextrins (1–34 mol% modification) were prepared from the dextrin of Mw 51 KDa and the dextrin–doxorubicin conjugates were prepared as previously described (Hreczuk-Hirst et al., 2001a). All solvents were of general reagent grade (unless stated) and were from Sigma–Aldrich Chemical Co. Ltd (Gillingham-Dorset, UK). The substrate for plasma α-amylase determination (Phadebas amylase test) was from Pharmacia AB (Uppsala, Sweden). Dialysis tubing was from BDH Merck (Poole, Dorset UK). Isolated rat liver lysosomal enzymes (tritosomes) were prepared according to a method previously described (Trouet, 1974). Their activity was assessed using the substrate CBz-Phe-Val-Arg-p-nitroanilide (NAp).

Rat plasma was prepared from fresh blood centrifuged at 600 × *g* for 10 min. The α-amylase levels in the isolated plasma was assessed using

the Phadebas amylase assay. Briefly, 4.0 ml of water was added to plasma (200 μl). The samples were pre-incubated for 5 min at 37 °C and reaction started by addition of one tablet of reagent. After vortex mixing, the tubes were incubated at 37 °C for 15 min. The reaction was stopped by adding sodium hydroxide (1.0 ml, 0.5 M). The reaction mixture was centrifuged at 1500 × *g* for 5 min and the supernatant was pipetted in a cuvette and the absorbance read at 620 nm against distilled water. The amylase activity was determined using the calibration curve provided with the assay kit, after subtraction of the absorbance value of the blank.

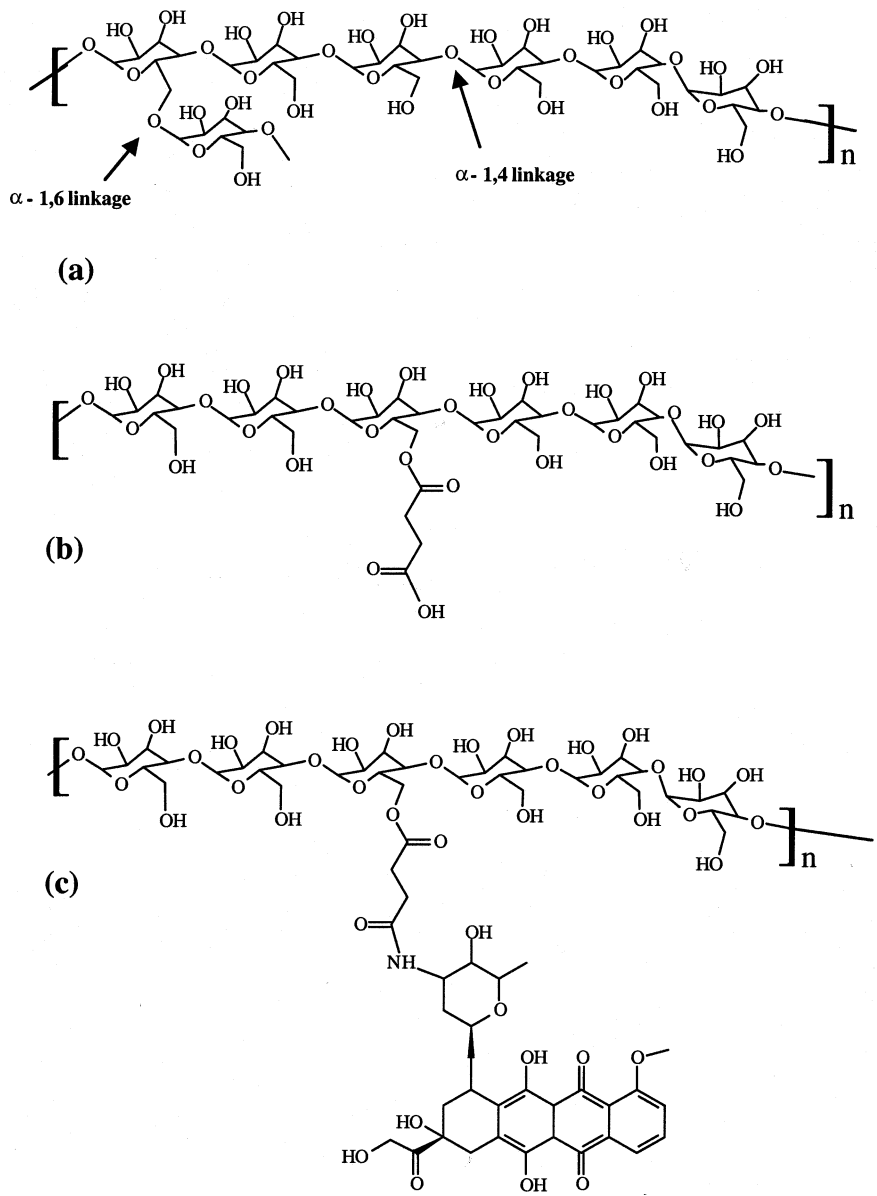

Fig. 1. Structure of: (a) dextrin; (b) succinoylated dextrin; and (c) dextrin–doxorubicin.

2.2. Evaluation of the degradation of dextrins during incubation with rat plasma and isolated rat liver lysosomal enzymes

Dextrins (10 mg/ml) were made up in either phosphate buffered saline (PBS) at pH 7.4 or citrate phosphate buffer at pH 5.5 (0.5 ml). To start the experiment rat plasma (diluted 1/20 in PBS, at pH 7.4) (0.4 ml) or tritosomes in citrate phosphate buffer at pH 5.5 (containing 0.2% Triton-X100) (0.5 ml) were added (in triplicate), thoroughly mixed, and incubated for 1–48 h at 37 °C. At each time point samples (100 μl) were withdrawn and placed for 3 min in a boiling water bath, to stop the reaction and precipitate the enzyme.

The supernatant was analysed by SEC using a TSK-GEL G 4000 PW analytical column (7.5×300 mm^2) with a Pregel TSK PWH guard column (7.5×75 mm^2). The respective incubation media was used as eluant (flow rate 1 ml/min) with detection using a refractive index detector (Gilson 133 RI). Signal intergration was carried out using Powerchrom software (ADI Instruments). The column was calibrated using pullulan standards of defined molecular weights from 5.8 to 853 KDa.

2.3. Effect of the degree of succinolyation on dextrin degradation by isolated porcine pancreatic α-amylase

Initially rat plasma amylase was used in vitro to study dextrin degradation to allow comparison with measurements of dextrin degradation in vivo during studies in the rat. However, to avoid preparation of rat plasma, commercially available porcine pancreatic α-amylase was used as a model to conduct these studies.

Dextrin (Mw ~ 51 Da), succinolylated dextrins (5, 15 and 34 mol% succinoylation) and dextrin–doxorubicin conjugates containing 6.3 or 11.2 wt.% doxorubicin (all 15 mg) was dissolved in PBS (4 ml, pH 7.4) in triplicate. To each was added porcine pancreatic α-amylase (1.0 unit in 400 μl; PBS) and thoroughly mixed. As a control a sample of each (100 μl) was taken immediately and frozen in liquid nitrogen. Incubations were carried at 37 °C and samples (100 μl) taken at various times, usually over 2 h but in some experiments samples were taken over 7 days.

All samples were immediately frozen and stored at −20 °C before analysis. Before assay, samples were placed in boiling water for 5 min to denature enzyme activity and precipitate the protein. The supernatant (20 μl) was then analysed by SEC as described above except that a TSK 2000 PW column (30×0.75 cm^2) was used with Polymer Laboratories Caliber™ software to analyse the results. PBS pH 7.4.was used as eluent.

3. Results and discussion

Dextrins showed no significant degradation by SEC when incubated in buffer alone at pH 5.5 and 7.4 and neither were low molecular weight oligosaccharides detectable biochemically (results not shown). The SEC profile of dextrins of Mw 15.5 and 51.1 KDa is shown in Fig. 2a. Throughout these experiments it should be noted that a denatured rat plasma product eluted at fraction 6 (Fig. 2b). On the addition of diluted rat plasma the main polymer peak quickly decreases in height (Fig. 2b). Simultaneously peaks corresponding to low molecular weight degradation products began to appear and they increased in height with time. Analysis of the profiles showed that both dextrins were degraded rapidly (Fig. 3a, b) with $>50\%$ degradation within 1–5 min. The amylase activity in rat plasma (as determined by Phadebas amylase test) was always in the range 7000–10,000 U/l. Over the time course of the degradation study activity dropped slightly to 6000–8000 U/l). In contrast, neither dextrin molecular weight fraction showed any evidence of degradation in the presence of isolated rat liver lysosomal enzymes (Fig. 4). Porcine pancreatic α-amylase also degraded dextrin (Mw 51.1 KDa) rapidly with a $t_{1/2}$ (time for mass to reach half of its original) of 2 min (Fig. 5). The pancreatic α-amylase proved a useful model for studying the effect of dextrin modification on amylase degradation without the need to obtain rat plasma. Succinoylated dextrins (5 and 15 mol%) and dextrin–doxorubicin (produced from the 5 mol% succinoylated intermediate) showed a biphasic pattern of degradation (Figs. 5

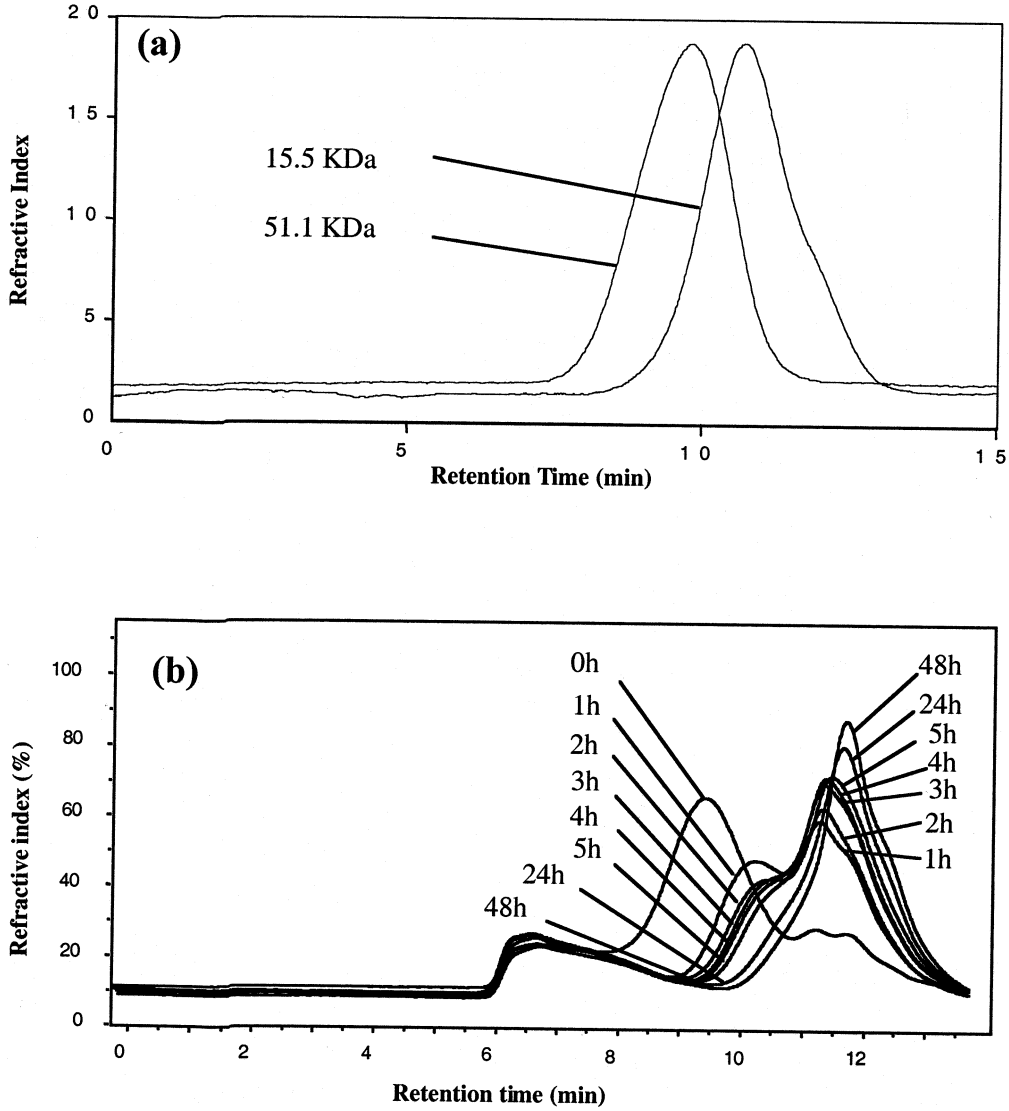

Fig. 2. Degradation of unmodified dextrins by isolated rat plasma. Panel (a) shows the SEC profile of dextrins (Mw 15,100 and 51,100 Da). Panel (b) shows the time-dependant change in SEC profile of dextrin (Mw 51,100 Da) during incubation with isolated rat plasma (1–48 h).

and 6). Rapid initial hydrolysis produced fragments of Mw 10,000–20,000 Da within 30 min (Fig. 5a and Fig. 6). The $t_{1/2}$ for degradation of the succinoylated dextrin modified to a level of 5 and 15 mol% was approximately 15 min.

The dextrin having the highest degree of modification (34 mol%) showed little evidence of degradation by porcine pancreatic α-amylase over the 180 min incubation period. The relative peak mass (referred to the pullulan standards) fell from 51,100 to 40,600 Da (Fig. 5a). It can be seen that the dextrin–doxorubicin adduct synthesised from the 34 mol% intermediate (12 wt.% doxorubicin) had an apparent molecular weight much higher than expected and showed evidence of aggregation on SEC (Fig. 6) and this could not be due to an increase in mass alone. It is partly due to aggregation and also in part change in shape of

the polymer coil—distortion to the polymer backbone as it becomes more heavily modified. Degradation of the dextrin–doxorubicin conjugate was dependent on the doxorubicin loading. The conjugate containing 12 wt.% doxorubicin was not degraded by α-amylase over the 180 min incubation period (Fig. 5a), but it did degrade over longer incubation periods (Fig. 5b). The relative molecular mass fell from 84,000 to 54,000 and 21,000 Da after 24 h and 1 week, respectively. The conjugate with the lower doxorubicin loading (6.3 wt.%) was degraded, but more slowly that the parent succinoylated dextrin precursor (Fig. 5a). Even after 180 min (Fig. 6b) there is significant conjugate remaining.

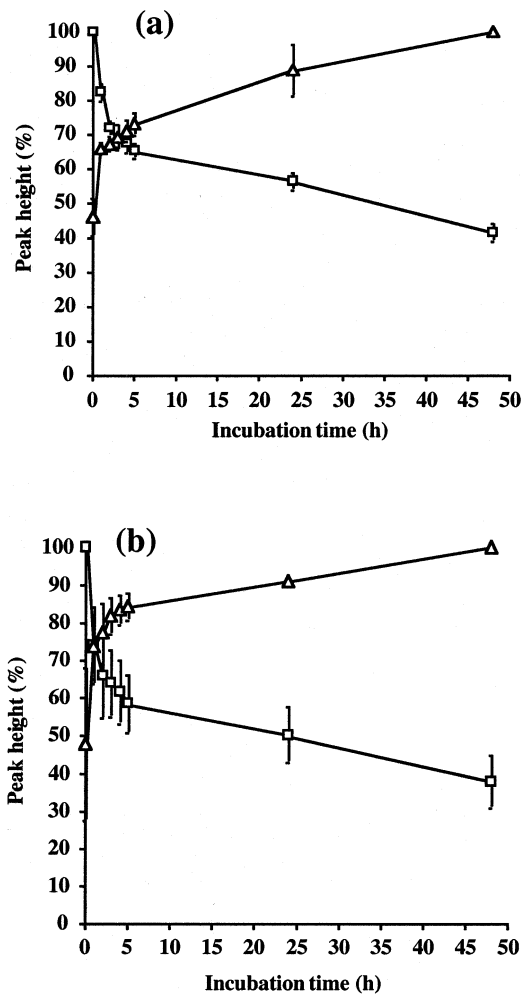

Fig. 3. Quantitation of the degradation of unmodified dextrins by isolated rat plasma. Panel (a) Mw 15,500 Da and panel (b) Mw 51,100 Da. The results are expressed as percentage reduction in dextrin peak height (□) and percentage increase in low molecular weight products (△) ($n = 3$; mean ± S.D.).

Although biodegradable polymers e.g. poly(lactide-co-glycolide) and poly(anhydrides) have found wide use as controlled release depot formulations, it has proved difficult to identify biodegradable, water-soluble polymers with all the necessary attributes for clinical use as targetable drug carriers. Polysaccharides are obvious candidates and numerous investigations have studied dextran (Nishikawa et al., 1996), pullulan (Nogusa et al., 1995) and chitosan (Richardson et al., 1999) as potential carriers. So far clinical studies with conjugates based on polysaccharides have been disappointing and a Phase I study involving dextran–doxorubicin showed evidence of hepatotoxicity (Danauser-Reidl et al., 1993). In addition, it has been clearly shown that even low levels of dextran derivatisation creates an essentially non-degradable polymer (Vercauteren et al., 1990).

In contrast, dextrins would seem to be ideal candidates for development as targetable carriers. Their clinical tolerance is proven. Pendant groups can be reproducibly introduced by succinoylation (Hreczuk-Hirst et al., 2001a) and data presented here show that both in their native form and also after limited modification (< 15 mol%) dextrins are rapidly degraded by plasma α-amylase. If longer plasma circulation times are required, a higher degree of dextrin succinoylation can be used to slow the rate of degradation to hours or days as required. This provides the opportunity to design conjugates with greater capacity for tissue targeting. This is particularly important in the context of passive tumour targeting by the enhanced permeability and retention (EPR) effect (Maeda and Matsumura, 1989). We have previously shown the tumour levels attained are primarily driven by the plasma concentration of a long-circulating polymer conjugate or liposome (Seymour et al., 1995; Sat et al., 1999). As the dextrin–doxorubicin conjugates described can be synthesised to have a Mw greater than the renal threshold (e.g. 51 KDa), and additionally with a

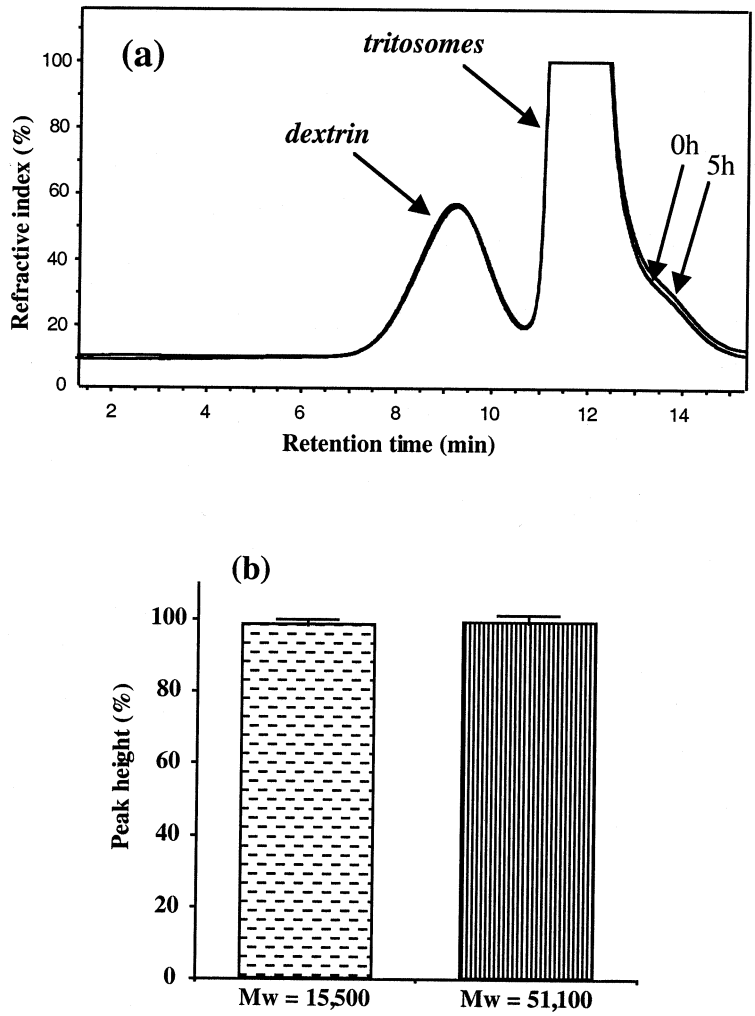

Fig. 4. Degradation of unmodified dextrins by isolated rat liver lysosomal enzymes. Panel (a) shows the SEC profiles obtained for the dextrin Mw 51,100 Da after 0 and 5 h. Panel (b) shows the percentage change in dextrin peak height after 5 h seen following incubation of both dextrin Mw fractions with lysosomal enzymes.

drug loading (~ 10 wt.%) that will ensure circulation for prolonged periods of time, it would be predicted that this could facilitate improved tumour targeting. Preliminary experiments comparing s.c. B16F10 tumour levels of dextrin–doxorubicin conjugates with 15 and 34 mol% modification support this hypothesis (Hreczuk-Hirst et al., 2001b)

It was surprising that dextrins did not show any sign of degradation when incubated with isolated rat liver lysosomal enzymes over 5 h. Whereas, it is known that dextrans are degraded very slowly by lysosomal enzymes, it was expected that dextrins might be degraded more readily. If specific dextrin–drug conjugates are being developed for clinical application it would be important to study their lysosomal degradation over longer time periods.

4. Conclusions

Dextrins are readily degraded by α-amylase. The rate of degradation decreases with increased

modification of the polymer chain. However, even with high degrees of modification (~ 34 mol%) conjugates degrade slowly over several days. Dextrin constructs can therefore be tailored to satisfy a variety of drug delivery objectives. Dextrin conjugation might be used to solubilise hydrophobic drugs. This has been demonstrated for doxorubicin and amphotericin B (Hreczuk-Hirst et al., 2001a; German et al., 2000). If conjugates were synthesised with low levels of substitution rapid plasma hydrolysis would allow immediate systemic bioavailability of the drug bound. In con-

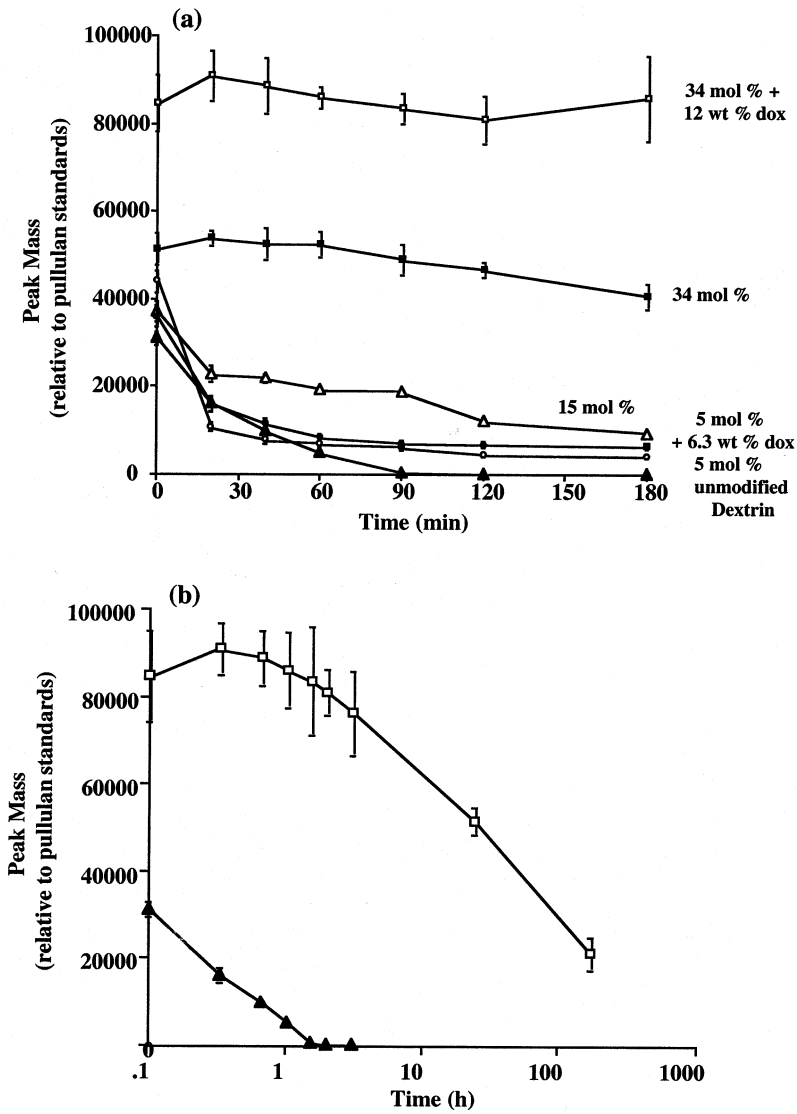

Fig. 5. Effect of dextrin (Mw = 51,100 Da) modification on degradation by porcine pancreatic *a*-amylase. Panel (a) shows the change of relative (pullulan standards) peak mass over 180 min. Panel (b) shows degradation of dextrin–doxorubicin (34 mol%; 12 wt.% doxorubicin) measured over 1 week. Key: unmodified dextrin (▲), 5 mol% succinoylated dextrin (○), dextrin–doxorubicin (5 mol% succinoylation; 6.3 wt.% doxorubicin (●), 15 mol% succinoylated dextrin (△), 34 mol% succinoylated dextrin (■),dextrin–doxorubicin (34 mol% succinoylation; 12 wt.% doxorubicin (□). ($n = 3$, results are mean ± S.D.)

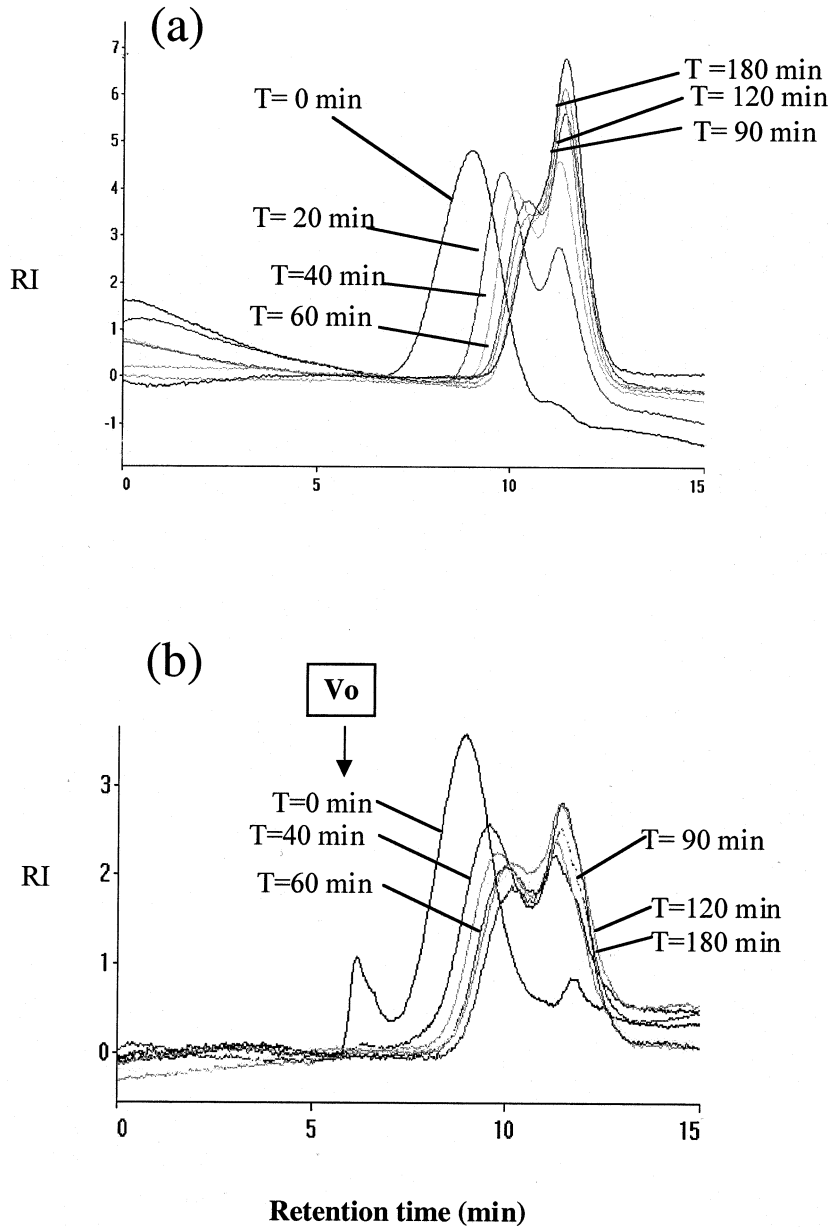

Fig. 6. SEC showing the time-dependent degradation of dextrin and a dextrin–doxorubicin conjugate by porcine pancreatic *a*-amylase. Panel (a) shows the SEC profiles obtained for the dextrin (Mw 51,100 Da; 15 mol% modification) and panel (b) shows the shows the SEC profiles obtained for dextrin–doxorubicin (5 mol% succinoylation; 6.3 wt.% doxorubicin).

trast, synthesis of dextrin–drug conjugates (Mw > 50 KDa) with higher levels of substitution could be used to prolong plasma residence time and assist passive or receptor-mediated (with use of the appropriate ligand) tissue targeting.

Acknowledgements

The authors wish to thank ML Laboratories PLC for financial support and Dr S. Dimitrijevic and Professor H. Ringsdorf for helpful discus-

sions. LG was supported by a BBSRC Case Studentship.

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