Heterogeneous hydrolytic behaviour of dextran–α-naphthylacetic and dextran–naproxen adducts

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(Received 30 March 1994; revised 13 June 1994)

This work deals with the bioactive agent release from dextran–α-naphthylacetic and dextran–naproxen adducts, in which the bioactive agent is linked to the dextran carrier through a spacer. Water absorption characteristics of these adducts have been studied. The hydrolysis in the heterogeneous phase showed autocatalytic effects and the release of the active compound is dependent upon the hydrophilic character of the adduct as well as the pH value of the medium.

(Keywords: spacer; hydrophilic character; equilibrium water content)

Introduction

The macromolecular carrier–bioactive compound approach has attracted considerable interest in recent years in order to optimize the action of the bioactive compound. Polysaccharides are often selected as carriers for the preparation of polymer–bioactive agent adducts. These polymers possess a high loading capacity due to the presence of a large number of alcoholic groups available for derivatization. In this connection, we chose dextran as carrier because of its advantageous properties such as hydrophilicity, water solubility, biocompatibility and degradability[1,2]. However, direct covalent coupling of bioactive compounds to dextran is limited to those molecules that have an appropriate functionality.

The release of the bioactive compound from a polymeric matrix can be achieved by hydrolytic or enzymatic cleavage of the linking bond. It may be noticed that hydrolysis near the polymeric backbone is difficult due to steric and/or hydrophobic considerations, thus it seems probable that the use of a spacer would enhance release rates. In a previous paper we reported the synthesis of dextran–α-naphthylacetic and dextran–6-methoxy-α-methyl-2-naphthaleneacetic (naproxen) adducts from chloroacetylated dextran[3], a reactive derivative of dextran, which facilitates the attachment of bioactive acid compounds as well as the incorporation of a suitable spacer between the carrier and the bioactive compound. In the present communication, we report the preliminary results on the release pattern of α-naphthylacetic and naproxen linked to dextran carriers through a spacer arm.

Experimental

Materials. α-Naphthylacetic and naproxen acids were attached to chloroacetylated dextran as described earlier[3]. Then, potassium salts of α-naphthylacetic or naproxen acids were reacted with chloroacetylated dextran [degree of substitution (DS) = 0.74, 1.28 and 2.16] using dimethylsulfoxide as solvent. The resulting adducts were isolated by precipitation in water. All samples were purified by reprecipitation using tetrahydrofuran (THF) as solvent and water as precipitant. Characterization of the modified polymers was carried out by i.r. and ^{1}H and ^{13}C n.m.r. techniques. The DS was determined by means of alkaline hydrolysis using a standard solution of sodium hydroxide. The amount of bioactive compound resulting from the alkaline hydrolysis was quantitatively determined by u.v. spectroscopy (water as solvent) at the absorption wavelength of the α-naphthylacetic or naproxen acid [281 nm ($\varepsilon = 6.32 \times 10^3$ l mol^{-1} cm^{-1}) and 271 nm ($\varepsilon = 5.12 \times 10^3$ l mol^{-1} cm^{-1}), respectively], using calibration curves previously determined.

Water absorption. Films of dextran–α-naphthylacetic and dextran–naproxen adducts were obtained by casting from THF solutions. After evaporation of nearly all the solvent at room temperature, the resulting film was detached and discs (13 mm diameter) were cut and dried to constant weight in a vacuum oven at room temperature. Dynamic swelling experiments were performed by placing the disc in distilled water at 37°C. At various times the swollen discs were removed, surface dried with filter paper, weighed in a tared stoppered bottle and then quickly replaced in the water. These films were treated with water at 37°C until equilibrium was reached. The equilibrium water content (EWC) is given by[4]:

$$EWC = 100h/(h+m)$$

where h is the amount of the water retained in the disc and m is the weight of the dry sample.

Release experiments. For this study we used dextran–α-naphthylacetic and dextran–naproxen adducts which were insoluble in water, but did swell on standing in the medium used. Polymer samples in disc form were obtained as indicated above. The resulting discs were introduced into a small wire basket, which was entirely permeable to water. This device was placed in Pyrex stoppered test tubes, each containing 25 ml of the aqueous buffer solution. The tubes were then immersed at the desired temperature in a thermostatically controlled bath.

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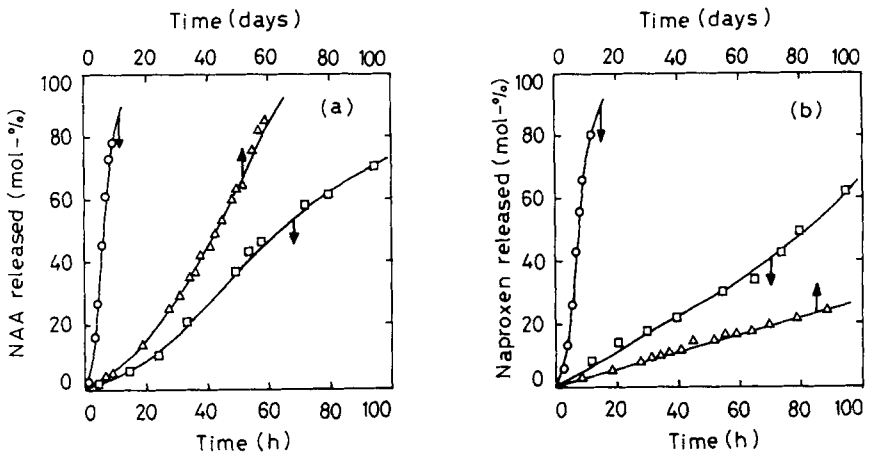

Figure 1 Heterogeneous hydrolysis at 37°C of (a) a dextran–α-naphthylacetic adduct ($DS=0.64$) and (b) a dextran–naproxen adduct ($DS=0.64$) at various pH values: (○) 9.0; (□) 8.1; (△) 1.0. NAA = α-naphthylacetic acid

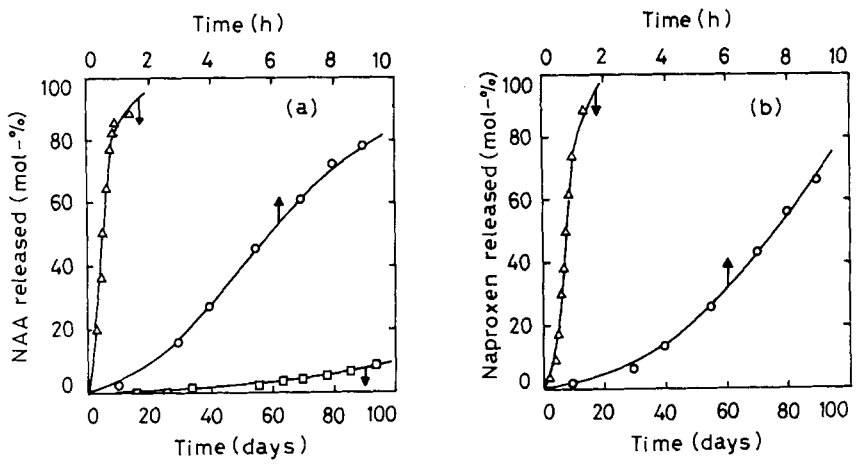

Figure 2 Heterogeneous hydrolysis at 37°C and pH 9.0 of (a) dextran–α-naphthylacetic adducts with *DS* equal to: (○) 0.64; (△) 1.22; (□) 2.05 and (b) dextran–naproxen adducts with *DS* equal to: (○) 0.64; (△) 1.14

Table 1 Variation of the equilibrium water content (*EWC*) with the degree of substitution (*DS*) of dextran–α-naphthylacetic and dextran–naproxen adducts

Adduct	*DS*	*EWC* (wt%)
α-Naphthylacetic	0.64	68
	1.22	55
	2.04	39
Naproxen	0.64	69
	1.14	59

A periodic assay of samples was obtained by removing the wire basket, stirring the solution and pipetting a 1 ml sample. The wire basket was quickly re-inserted, making sure that the disc remained completely immersed throughout the hydrolysis study.

The volume pipetted for each sample was replaced by an equivalent volume of fresh solvent, with corrections being applied in the calculations. The released bioactive compound was measured by u.v. spectroscopy at 281 nm (α-naphthylacetic acid) and at 271 nm (naproxen).

Results and discussion

The release of bioactive compounds from polymer–bioactive compound adducts by a hydrolysis process in the heterogeneous phase is often dependent on the hydrophilic character of the adduct[5,6]. Thus, we have studied the water swelling of dextran–α-naphthylacetic and dextran–naproxen adducts with different chemical compositions.

Table 1 shows the *EWC* values of some dextran–α-naphthylacetic and dextran–naproxen adducts. As can be seen, the *EWC* value was found to be a function of the bioactive compound content, according to other dextran derivatives[7]. It decreases with increasing bioactive compound content.

Figure 1 shows typical profiles of the heterogeneous hydrolysis at 37°C of dextran–α-naphthylacetic ($DS=0.64$) and dextran–naproxen ($DS=0.64$) adducts, in acidic or alkaline medium. It may be noteworthy that under acidic conditions (pH 1.0) dextran adducts show a slow rate of hydrolysis. Greatly enhanced release rates are observed at pH 9.0 and 8.1. The analysis of the kinetics, when the hydrolysis reaction is carried out at higher pH values, revealed the occurrence of autocatalytic effects.

The interpretation of apparent released bioactive agent concentrations as a function of the original polymer structure and the percentage of bioactive agent attached is difficult probably because of the several competing mechanisms involved. Hydrophilicity and distance of the labile bond from the polymer backbone often have large

effects. Polymers with pendent bioactive agents appear to hydrolyse by a mechanism in which the degradation occurs uniformly, and it would be expected that the release rate decreases with time as the number of hydrolysable bonds decreases. However, the shapes of the curves in *Figure 1* suggest the existence of autocatalytic effects.

According to the literature, only a small amount of data exist concerning this behaviour and no conclusive explanation has been given[8,9]. Nevertheless, the autocatalytic effects have been tentatively assumed to be related to an increase of hydrophilicity, particularly when the product of the hydrolysis reaction is a very hydrophilic group that causes the remaining polymer–bioactive agent to swell, thereby facilitating the approach of nucleophilic species to the active sites. This appears to be the cause of the increase in the release rate with time, as shown for dextran–α-naphthylacetic and dextran–naproxen adducts in *Figure 1*.

Figure 2 shows the release behaviour at 37°C and pH 9.0 of some dextran–α-naphthylacetic and dextran–naproxen adducts with different *DS* values. Two general trends are apparent from the inspection of *Figure 2*. First, all the kinetic curves show autocatalytic effects. Second, the total release of the active compound was reached faster in the case of dextran adducts modified with lower *DS*. As indicated above, these two features may be explained in terms of the interaction of the polymer with water. Decreasing the content of α-naphthylacetic or naproxen groups renders the polymer more hydrophilic and, therefore, facilitates the entry of nucleophilic species to the active sites, effectively increasing the relative hydrolysis rates. Further investigations of the influence of other spacer groups of different size and nature are now in progress.

Acknowledgements

The financial support provided by the Comision Interministerial de Ciencia y Tecnología is gratefully acknowledged.

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