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Macromolecular prodrugs I. Kinetics and mechanism of hydrolysis of O-benzoyl dextran conjugates in aqueous buffer and in human plasma

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Summary

Benzoate esters of dextran ($M_w = 65{,}600$) with varying degrees of substitution have been synthesized. The kinetics and mechanism of hydrolytic cleavage of the ester bond in aqueous solution over the pH range 3.0–9.5 (60°C) has been investigated. The degradation reactions followed strict first-order kinetics and a rate expression encompassng hydrogen ion-, hydroxide ion- and water-catalyzed hydrolysis of the conjugates is derived. General acid–base catalysis of release of benzoic acid from the conjugates was negligible. No influence of the degree of substitution on the reaction rates was observed. Nearly identical rates of release of benzoic acid from the conjugates at 37°C in 80% human plasma and in 0.05 M phosphate buffer pH 7.40 have been found ($t_{1/2} \sim 190$ h), revealing that hydrolysis in plasma proceeds without enzymatic catalysis. The lack of susceptibility to undergo enzyme-mediated hydrolysis is most likely due to steric hindrance by the dextran backbone. Various chemical methods to attach drug compounds covalently to dextrans and the potential, therapeutic utility of the conjugates are discussed.

Introduction

The linking of drugs covalently to various macromolecular carriers in order to improve the therapeutic effect of the parent compounds has attracted great interest

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in recent years. Especially in the field of cancer chemotherapy, macromolecular drug carrier systems have been developed in an attempt to enhance the selectivity of action of cytotoxic agents by coupling the drugs mainly to carriers with expected affinity to the target tissue (for reviews, see Gregoriadis, 1977; Trouet, 1978; Zaharko et al., 1979; Poznansky and Cleland, 1980; Edwards, 1983; Sezaki and Hashida, 1984). Although most efforts have been directed towards drug targeting, other important objectives may be achieved using drug carrier conjugates, including: (a) stabilization of the therapeutic agent; (b) enhancement of drug solubility; (c) improvement of circulation life time; (d) extended duration of action; and (e) reduction of side-effects including antigenicity of enzyme preparations (Poznansky and Cleland, 1980; Sezaki and Hashida, 1984). The latter objectives may be obtained using macromolecular transport groups devoid of intrinsic target receptor affinities. Numerous polypeptides and polysaccharides have been employed, but also synthetic polymers have been tested for use as macromolecular carriers for various therapeutically active compounds (Duncan and Kopecek, 1984).

Dextrans, which belong to the group of macromolecular carriers without apparent specificity for discrete cell-surface receptors, may serve as one of the most promising carrier candidates due to their excellent physicochemical properties and physiological acceptance (Virnik et al., 1975; Molteni, 1979; Hurwitz, 1983; Takakura et al., 1984). Well-defined dextrans with low polydispersity have been in clinical use as plasma expanders for several decades. In relation to parenteral dextran therapy, rare dextran-induced anaphylactoid reactions do occur (Richter et al., 1981), but this problem may now be circumvented by preinjection of a monovalent hapten dextran preparation, Promiten (Richter and Hedin, 1982). Dextrans possess a high loading capacity due to the presence of the large amount of hydroxy groups available for derivatization and they are satisfactorily stable to chemical manipulations.

Although many conjugates have been evaluated in vivo, only sparse information is available about the chemical and physicochemical characterization of the carrier systems. Realizing that both the onset and duration of action of macromolecular prodrugs are dependent on the rate of release of the active agent from the conjugate, carrier systems which may exert extracellular activities with the drug permanently linked to the carrier should be clearly differentiated from those where the therapeutic effect is achieved after release of the active agent from the conjugate.

The present study was undertaken using benzoylated dextrans as simple model conjugates in order to provide more general information about factors influencing the rate of release of liganded drugs from macromolecular prodrugs. In an associated paper (Johansen and Larsen, 1985) studies pertinent to the physicochemical characterization of benzoyl dextran conjugates with varying average weight molecular weights are reported.

Materials and Methods

Dextran T-70 (M_w = 65,600 and M_n = 34,600) and the Sephadex types, G-10 and G-200, were purchased from Pharmacia, Sweden. The solvents used in the HPLC

procedure were of chromatographic grade. All other chemicals and buffer substances were of reagent grade.

Apparatus

HPLC analysis was carried out using a Waters Associates Model 6000A constant-flow pump equipped with a Pye Unicam PU 4020 variable wavelength detector and a Rheodyne Model 7125 injection valve with a 20-μl loop. Readings of pH were done with a Radiometer Type pH M 26 meter at the temperature of study. Ultraviolet and visible spectral measurements were performed by use of a Shimadzu UV-190 recording spectrophotometer.

Synthesis of benzoyl dextran

Benzoyl dextran conjugates were prepared by reaction of dextrans with benzoyl chloride or benzanhydride in various media including formamide, dimethylsulphoxide and water. The two former solvents should be used when the water solubility of active acyl derivatives is too limited (to be published elsewhere), but in the case of the synthesis of benzoyl dextran, water is the preferred solvent. In short, 0.5 g dextran was dissolved in 10 ml of water at ambient temperature and 0.3–0.9 g benzanhydride in 1–2.5 ml of pyridine was added dropwise under vigorous stirring. pH was maintained at 7.0 by addition of 5 M NaOH until the pH had stabilized (20–40 min). The mixture was desalted and lyophilized. Separation of the low molecular weight substances from the conjugates by dialysis or precipitation with ethanol caused changes in the molecular weight distribution. Trituration of the conjugates with ethanol or acetone resulted in less soluble preparations compared to conjugates obtained from lyophilization.

Desalting procedure

The substituted dextran in the reaction mixture was separated from the low molecular weight compounds, i.e. sodium benzoate and pyridine, by gel filtration. A 10-ml portion of the reaction solution corresponding to 200 mg dextran was applied to a Pharmacia column K 26/70, packed with Sephadex G-10 ($V_t = 154$ ml), and eluted with distilled water. A flow rate of 1.9 ml $\cdot$ min^{-1} was maintained by use of a constant-speed peristaltic pump and the effluent was collected in fractions of 5 ml. The amount of benzoylated dextran in the fractions was determined by the anthrone reaction (according to the Nordic Pharmacopoeia) and by measuring the absorbance at 235 nm, whereas the presence of free sodium benzoate was quantitated by both HPLC and spectral measurement. The eluted pyridine was determined by use of the spectrophotometric method at 235 nm. As shown in Fig. 1, baseline separation of benzoyl dextran from the low molecular weight substances was obtained using this desalting procedure.

HPLC analysis

Quantitation of benzoic acid released in the hydrolysis studies was done by using a reversed-phase high performance liquid chromatographic procedure. A column, 125 $\times$ 4.6 mm, packed with Spherisorb ODS-1 (5 μm particles) was eluted with a

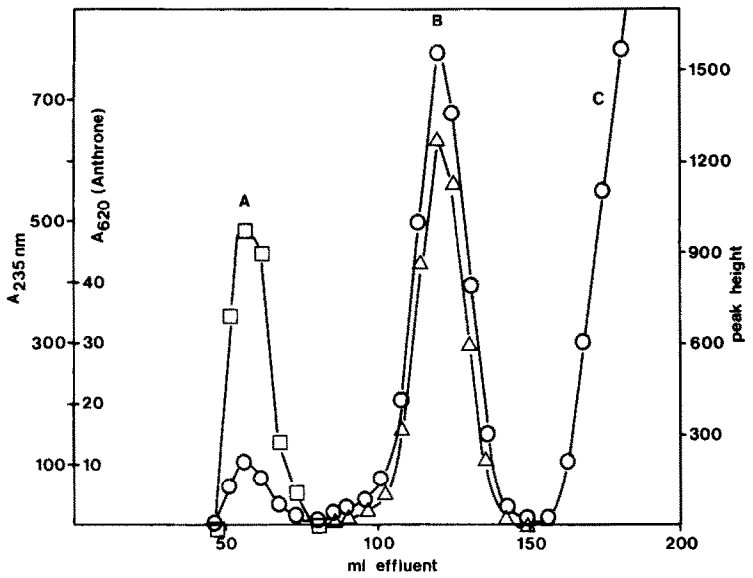


Fig. 1. Separation of benzoyl dextran (A) from benzoate (B) and pyridine (C) by gel permeation chromatography of the reaction solution on Sephadex G-10. The fractions were analyzed by the anthrone method (□), absorbance at 235 nm (○) and HPLC (△).

mobile phase consisting of methanol – 0.02 M citrate buffer pH 2.7 (1 : 1 v/v). The flow rate was 1.0 ml · min^{-1} and the column effluent was monitored at 235 nm. Under these conditions the capacity factor of benzoic acid was 1.5. Quantitation of the compound was done from measurements of the peak heights in relation to those of sodium benzoate standards chromatographed under the same conditions.

Characterization of the conjugates

Various parameters characterizing the conjugates including degree of substitution (DS), polydispersity, solubility and viscosity are reported elsewhere (Johansen and Larsen, 1985). In this study conjugates with DS of 3.4, 9.1 and 15.8% have been used.

Kinetics in aqueous solution

The buffers used were formate, acetate, phosphate, borate and carbonate solutions. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The reaction solutions were kept at constant temperature of 60 ± 0.2°C except in the studies of the influence of temperature on the reaction rates. The reactions were initiated by adding a weighted amount of the conjugate to 25 ml of the appropriate buffer (preheated) to give an initial concentration of about 0.2 mg · ml^{-1}. At suitable intervals aliquots were withdrawn and analyzed immediately.

Pseudo first-order rate constants were calculated from linear plots of log ($p_\infty - p_t$) against time, where p_∞ and p_t are the peak heights at infinity and at time t,

respectively. For the slow reactions in the pH range 3–7.4 the rate constants were derived using the initial rate method (Connors, 1973). At constant pH and temperature the formation of benzoic acid was found to be a linear function of the initial concentration of the substituted dextrans indicating first-order degradation kinetics. In some kinetic runs the observed first-order rate constants were determined by use of both methods and the obtained values were in good agreement.

Kinetics in human plasma

An accuratey weighed amount of benzoyl dextran was dissolved in 80% human plasma pre-equilibrated at 37°C to give a final concentration of about 5 $mg \cdot ml^{-1}$. The solution was kept in a water bath of 37°C and at appropriate intervals 500-μl samples were removed and deproteinized with 1 500 μl of methanol. The mixture was vortexed and centrifuged for 2 min at $10{,}000 \times g$. The aqueous methanol layer was diluted once with water and assayed for benzoic acid by HPLC. The observed first-order rate constant was determined using the initial rate method.

Results and Discussion

Kinetics and mechanism of hydrolysis of benzoyl dextran conjugates

The kinetics of release of benzoic acid from the conjugates was studied in aqueous buffer solutions over the pH range 3.0–9.5. With the buffer concentration varying from 0.05 to 0.20 M, it was observed that the rates of hydrolysis were not subject to catalysis by any of the buffer substances used in the kinetic studies.

The pH dependence of the first-order rate constant (k_{obs}) for hydrolysis of the dextran benzoic acid esters at 60°C and $\mu = 0.5$ is shown in Fig. 2. At pH < 3.5 and pH > 6.8 the pH–rate profile shows two straight-line portions with slopes −1.0 and 0.97, respectively. The shape of the profile suggests that hydrolysis of the ester linkage is susceptible to specific acid–base catalysis obeying the general rate law:

$$k_{obs} = k_H a_H + k_o + k_{OH} a_{OH} \tag{1}$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. k_H and k_{OH} are the second-order rate constants for specific acid and specific base catalysis, respectively, and k_o is the first-order rate constant for spontaneous or water-catalyzed degradation. The smooth curve in Fig. 2 was calculated from Eqn. 1 and the values for the rate constants are given in Table 1 using the following equations (Harned and Hamer, 1933):

$$pH = 0.15 - \log[H^+] \tag{2}$$

$$pH = 12.87 + \log[OH^-] \tag{3}$$

The good agreement observed between the calculated and the experimental data demonstrates that the rate expression, Eqn. 1, adequately describes the degradation

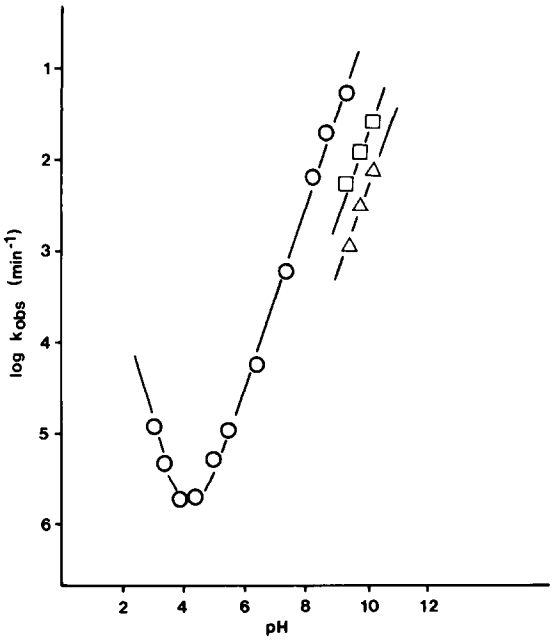


Fig. 2. pH–rate profiles for hydrolysis of benzoyl dextran D.S. 9.1% (○), ethyl benzoate (□) and isopropyl benzoate (△) at 60°C and $\mu = 0.5$. (○) are experimental values and the curve is calculated from Eqn. 1.

kinetics. The effect of temperature on the rate of hydrolysis was determined in 0.05 M phosphate buffer solution (pH 7.40 and $\mu = 0.5$) over the range 37–60°C. From an Arrhenius type plot (Fig. 3) an apparent activation energy, E_a, of 79.9 kJ · mol^{-1} was calculated.

In Fig. 2 the pH–rate profiles for alkaline hydrolysis of ethyl benzoate and isopropyl benzoate are also presented. The calculated second-order rate constants for specific base-catalyzed hydrolysis of the latter compounds (k_{OH}) are given in Table 1 showing a 5-fold increase in rate of alkaline hydrolysis of ethyl benzoate compared to isopropyl benzoate. A similar difference in reactivity has been observed for hydrolysis of the esters in acetonitrile–water mixtures (Washkuhn et al., 1971).

TABLE 1

CATALYTIC RATE CONSTANTS FOR HYDROLYSIS OF BENZOYL DEXTRAN, ETHYL BENZOATE AND ISOPROPYL BENZOATE AT 60°C AND $\mu = 0.5$

	k_H ($M^{-1} \cdot min^{-1}$)	k_{H_2O} ($M^{-1} \cdot min^{-1}$)	k_{OH} ($M^{-1} \cdot min^{-1}$)
Benzoyl dextran (DS 9.1%)	1.37×10^{-2}	2.3×10^{-9}	232
Ethyl benzoate			15.5
Isopropyl benzoate			3.2

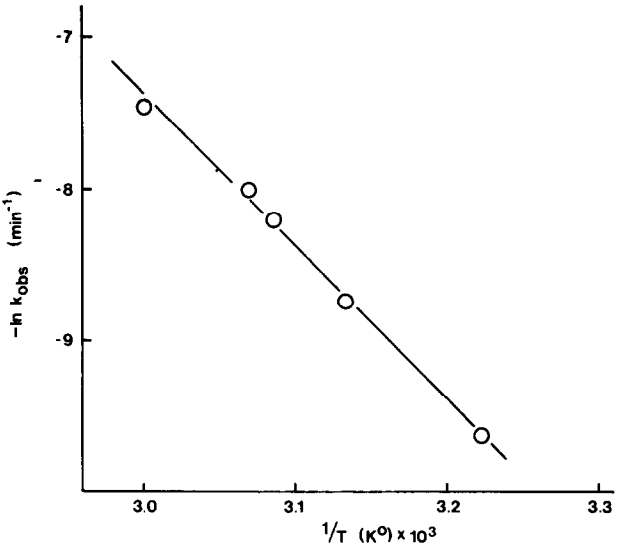


Fig. 3. Arrhenius plot for hydrolysis of benzoyl dextran (DS = 9.1%) in 0.05 M phosphate buffer, pH = 7.4 and $\mu = 0.5$.

In dextrans the main molecular chain consists of anhydro-D-glucopyranose units linked predominantly by α-1,6-glycosidic bonds leaving the secondary alcoholic groups in position 2, 3 and 4 free for derivatization. A comparison of the values for the hydroxide ion catalytic rate constants for hydrolysis of isopropyl benzoate and the dextran benzoic acid esters (Table 1) reveals a 70-fold greater reactivity of benzoyl dextran to undergo specific base-catalyzed hydrolysis. The enhanced rate of alkaline hydrolysis of dextran benzoate esters might be attributed to the basic character of the carbohydrate alkoxide ions. However, in previous studies (Larsen and Bundgaard, 1978; Bundgaard and Larsen, 1978) we have studied the hydrolysis of penicillins in the presence of carbohydrates and polyhydric alcohols. In the pH range 7–11 degradation proceeds through formation of intermediate penicilloyl carbohydrate esters, which subsequently undergo hydrolysis to yield penicilloic acid. By kinetic analysis only little difference was observed for the rates of hydrolysis of the penicilloyl esters derived from glucose and dextran T-40 (M_w 41,000) although the leaving ability of the glucose alkoxide ion, compared to the dextran oxygen anion, is much greater due to a much less basic character of the C-1 hydroxy group of glucose (pK_a 11.81, 35°C and $\mu = 0.1$). Thus, the increased susceptibility of the benzoyl dextran derivatives to undergo hydrolysis in the pH range 6–10 may most likely be attributed to intramolecular catalysis by the neighbouring hydroxy groups. The experiments carried out do not permit discrimination between derivatization at the C-2, C-3 or C-4 position, but it is to be expected that the hydroxy groups exhibit almost equal reactivities to benzoylation at neutral pH. de Belder and Norrman (1968) have studied the distribution of substituents in partially acetylated dextran. The observed similarity in the reactivities of the secondary hydroxy groups during

TABLE 2

HYDROLYSIS OF BENZOYL DEXTRANS WITH VARYING DEGREE OF SUBSTITUTION IN 0.05 M PHOSPHATE BUFFER pH 7.40 AND IN HUMAN PLASMA (37°C)

Benzoyl dextran (DS)	$t_{1/2}$ (h) 0.05 M phosphate pH 7.4	$t_{1/2}$ (h) 80% human plasma
3.4	186	199
9.1	180	183
15.8	182	190

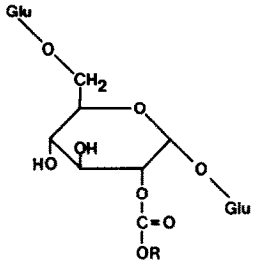
acetylation was suggested to be a result of acyl migration leaving the distribution of substituents to be thermodynamically rather than kinetically controlled.

The rate data for hydrolysis of benzoyl dextrans with variable degree of substitution in 80% human plasma and in aqueous solution (pH 7.4) at 37°C are presented in Table 2. The results show that the rates of hydrolysis are independent of the degree of substitution and that the degradation rates in plasma and in 0.05 M phosphate buffer pH 7.40 is of the same order of magnitude indicating that the hydrolysis reaction in plasma proceeds without enzymatic catalysis. The lack of enzyme-mediated hydrolysis is probably due to steric hindrance by the dextran carrier, which prevents the cleavage of the ester bond by non-specific plasma esterases. Similar lack of susceptibility to undergo enzymatic cleavage has been reported for daunorubicin linked directly to serum albumin (Baurain et al., 1983). These investigators have shown, however, that when a tetrapeptidic spacer arm is incorporated between the carrier and daunorubicin, release of the active drug is provided by action of lysosomal peptidases. Furthermore, steric hindrance of a dextran backbone might be the reason why the receptor binding potency of macromolecular alprenolol is enhanced with increasing length of the incorporated spacer arms (Pitha et al., 1980).

Chemical fixation of drugs to dextrans

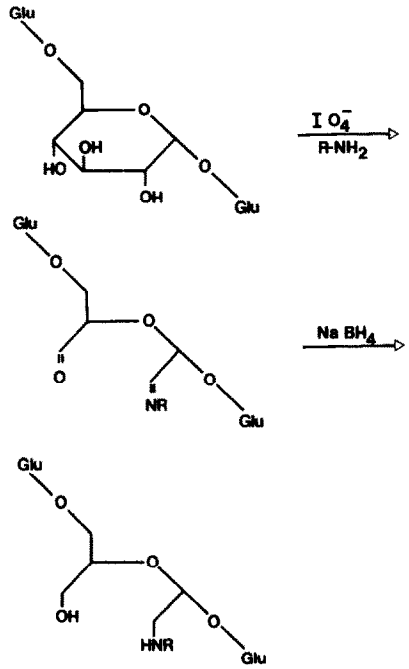
In addition to direct esterification, fixation of drugs with amino or hydroxy functional groups to polysaccharides can be obtained by activation of the polymers or by incorporation of an appropriate spacer arm between the carrier and the drug (to be discussed later). Attachment of drug compounds containing a hydroxy group has been accomplished through carbonate linkages (Scheme 1). Estrone and testosterone (Yolles, 1978; Yolles et al., 1979), salicylate derivatives (Havion et al., 1974) and trypsin (Rudel et al., 1978) have been linked to polysaccharides through carbonate ester linkages, but only sparse information about the stability and the delivery characteristics of the conjugates has been given.

For therapeutic agents with amino groups available for derivatization, covalent conjugation to dextrans have been established most frequently by periodate oxidation (Scheme 2) and by cyanogen bromide activation (Scheme 3). By periodate oxidation antineoplastic antibiotics have been linked to dextrans; for example, daunorubicin (Bernstein et al., 1978; Levi-Schaffer et al., 1982) or dextrans have

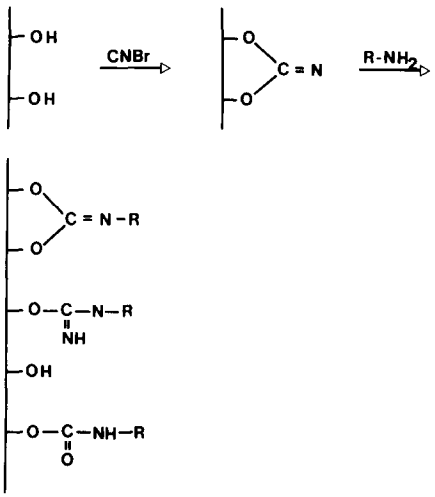


Scheme 1

served as a bridge between the drug and tumor-specific antibodies in the case of bleomycin (Manabe et al., 1983), methotrexate (Manabe et al., 1984) and daunorubicin (Hurwitz et al., 1978; Wilchek, 1979). Among various enzymes linked to macromolecular carriers (for reviews, see Marshall, 1978; Poznansky and Cleland, 1980), for example, the anti-leukemia agent asparaginase has been coupled to periodate-activated dextran (Benbough et al., 1979; Wileman et al., 1983). The feasibility of using the periodate oxidation reaction to provide dextran carrier conjugates may depend on whether the carrier complex, per se, is active or only the released compound is responsible for the therapeutic activity. The latter type of



Scheme 2



Scheme 3

conjugates can be considered as macromolecular prodrugs. Relatively high molecular weight therapeutically active compounds may at least partly retain their activity after conjugation to a macromolecular carrier. This is well documented in studies on immobilization of enzymes (Marshall, 1978), but the percentage of the restored catalytic activity is strongly influenced by the chemical structure of both the enzyme and the polymeric carrier as well as by the type of immobilization chemistry employed (Wingard, 1983). Several enzymes have been coupled to periodate-oxidized dextrans followed by reduction of the resulting Shiff's bases with sodium borohydride. The polyaldehyde structure of oxidized dextran, however, may lock up enzymes in various conformational structures and may additionally lead to crosslinking reactions depending on the nature and location of the enzyme amino groups. It is therefore to be expected that in this case the catalytic activity of the synthesized conjugates is highly sensitive to the reaction conditions.

The potential utility of antitumor antibiotics, daunorubicin and doxorubicin, coupled to several macromolecular carriers has been reviewed by Hurwitz (1983). The experiments have included evaluation of the chemotherapeutic activity of daunorubicin attached to periodate-activated dextran (Bernstein et al., 1978) and to tumor-specific antibodies via a dextran bridge (Hurwitz et al., 1978). It has been demonstrated that the compounds to some extent were able to penetrate the cell membrane of YAC cells in the conjugated form and to accumulate in or on the nuclei. In addition it has been suggested that the therapeutic effect requires release of the drug from the conjugate (Hurwitz et al., 1980), but the concentration of intracellular free daunorubicin has not been determined. By inspection of the chemical structure of the conjugates, the secondary amine bond resulting from periodate activation of dextrans followed by reduction should be stable to chemical hydrolysis. In the light of the present study it is less likely to anticipate extensive

release of the drug mediated by enzymatic disrupture of the C–N linkage. In tumor-bearing mice, however, Bernstein et al. (1978) have observed superior effect of daunorubicin–dextran conjugates compared to the effect of the free drug. Both acute and subacute toxicities of the conjugates were considerably lower than those of free daunorubicin. The efficacy of the daunorubicin conjugate is more explainable from recent reports suggesting that anthracycline antitumor agents, besides having intracellular DNA as a major site of action, can exert cytotoxic activity solely by interaction with the cell surface (Tritton and Lee, 1982; Hickman et al., 1984). Dextran-linked daunorubicin might therefore also be active by extracellular interaction with cell surface receptors. Recognizing the cell membrane of tumor cells as a potential target for antineoplastic agents, carrier-attached drugs may possess several advantages over the free active compound. The conjugates may reach the target area without producing the severe side-effects which normally accompany therapy with cytotoxic agents. In comparison to the free drug, improved circulation life time and stability in plasma may be the result of using drug compounds linked to a macromolecular carrier.

The cyanogen bromide procedure (Axén et al., 1967) has probably been the most widely used reaction to achieve covalent attachment of various compounds to polysaccharides, including incorporation of ligands for affinity chromatography (Cuatrecasas and Anfinsen, 1971) and coupling of drugs and enzymes to, for example, dextrans (Poznansky and Cleland, 1980). As seen from Scheme 3, cyanogen bromide activation leads to formation of three different covalent linkages, where the proportion of the individual chemical structures is highly dependent on the reaction conditions (pH) and the basicity of the compound amino group to be attached (Schnaar et al., 1977). Therefore the cyanogen bromide method might not be optimal to provide drug–dextran conjugates in the case where the therapeutic effect of the conjugate has to be established through a predictable and reproducible release rate of the drug from the conjugate in vivo. Furthermore the CNBr-reaction introduces cationic charge into the conjugate primarily due to formation of N-substituted isoureas and N-substituted cycloiminocarbonates which are expected to be protonated at physiological pH (Jost et al., 1974). The presence of these cationic sites in affinity gels have caused significant non-specific adsorption of proteins (Nishikawa and Bailon, 1975) suggesting that charged dextran conjugates may influence the action of hydrolases in vivo. Similarly, in the case of enzyme-mediated hydrolysis of low molecular weight ester derivatives, it has been reported that charged ester compounds are not attacked by or are only poor substrates for non-specific plasma esterases (Krisch, 1971; Johansen and Larsen, 1984).

Alternative to the direct drug–polymer linkage is the incorporation of a spacer between the active compound and the carrier. In affinity chromatography it is well demonstrated that the use of spacer arm-linked ligands improve protein purification compared to gels where ligands are attached directly to the polymer backbone. The successful application of the method requires that the essential group on the ligand for interaction with the molecules to be purified must be sufficiently distant from the matrix surface in order to overcome steric restrictions (Cuatrecasas and Anfinsen, 1971). Various potential spacer arm preparations have been proposed by Wilchek

(1974). Surprisingly, the exploitation of the spacer arm technique in the macromolecular drug delivery approach has received little attention (for reviews see Poznansky and Cleland, 1980; Baurain et al., 1983). Only few reports have dealt with the release characteristics of the conjugates in vitro as well as in vivo. Sezaki and coworkers have synthesized a series of mitomycin C–dextran conjugates using ϵ-aminocaproic acid as an intercalating agent (Kojima et al., 1980; Kato et al., 1982; Hashida et al., 1983). Regeneration of mitomycin C from the conjugates was studied in aqueous buffer and in homogenates. In all cases the release of the antitumor agent followed first-order kinetics, but liver homogenates did not accelerate the liberation of mitomycin c from the carrier. The authors suggest that high molecular weight dextran-mediated delivery of mitomycin c may offer a potentially effective method in cancer chemotherapy by sustaining the supply of the drug in vivo (Hashida et al., 1984). Furthermore the usefulness of the dextran conjugates as lymphotropic delivery systems for preventing lymphatic metastasis has been suggested (Takakura et al., 1984). Enzyme-catalyzed liberation of daunorubicin has been achieved by intercalating between the drug and the carrier protein an oligopeptidic spacer arm (Baurain et al., 1983). The efficacy of enzymatic hydrolysis was enhanced with increased length of the spacer. In addition the authors have shown that by proper selection of the chemical drug–spacer linkage selective activation of daunorubicin by lysosomal peptidases can be obtained.

Several factors determine the potential therapeutic utility of macromolecular prodrugs. Among these are both the chemical nature of the carrier drug linkage and the position of the drug in relation to the polymeric matrix. The two parameters will dictate the sensitivity of the conjugates to undergo chemical- and/or enzyme-mediated hydrolysis. With the above discussion in mind, a means to design improved controlled release preparations will be the incorporation of an appropriate spacer arm between the drug and the carrier. By proper selection of the spacer to drug bond a localized effect may be obtained even in the case where the macromolecular carrier has no specificity for discrete cell-surface receptors, as for example dextrans, if the drug to carrier link is sensitive to enzymes excreted selectively by the target pathological cells. With focus on dextran carriers, one obvious advantage of using spacers is that the number of drugs with different functional groups to be attached, can be expanded. Various spacer arm types using dextran as a model carrier are presently under investigation in this laboratory.

References

Axén, R., Porath, J. and Ernback, S., Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. Nature (London), 214 (1967) 1302–1304.

Baurain, R., Masquelier, M., Deprez-De Campeneere, D. and Trouet, A. Targeting of daunorubicin by covalent and reversible linkage to carrier proteins. Lysosomal hydrolysis and antitumor activity of conjugates prepared with peptidic spacer arms. Drugs Exp. Clin. Res., 9 (1983) 303–311.

de Belder, A.N. and Norrman, B. The distribution of substituents in partially acetylated dextran. Carbohydr. Res., 8 (1968) 1–6.

Benbough, J.E., Wiblin, C.N., Rafter, T.N.A. and Lee, J. The effect of chemical modification of L-asparaginase on its persistence in circulating blood of animals. Biochem. Pharmacol., 28 (1979) 833–839.

Bernstein, A, Hurwitz, E., Maron, R., Arnon, R., Sela, M. and Wilchek, M., Higher antitumor efficacy of daunomycin when linked to dextran: in vivo and in vitro studies. J. Natl. Cancer Inst., 60 (1978) 379–384.

Bundgaard, H. and Larsen, C. Kinetics and mechanism of reaction of benzylpenicillin and ampicillin with carbohydrates and polyhydric alcohols in aqueous solution. Arch. Pharm. Chemi, Sci. Edn., 6 (1978) 184–200.

Connors, K.A., Reaction mechanisms in organic analytical chemistry, Wiley, New York, 1973, pp. 41–110.

Cuatrecasas, P. and Anfinsen, C.B., Affinity chromatography. Annu. Rev. Biochem., 40 (1971) 259–278.

Duncan, R. and Kopecek, J., Soluble synthetic polymers as potential drug carriers. In Dusek, K. (Ed.), Advances in Polymer Science, Vol. 57, Springer-Verlag, Berlin-Heidelberg, 1984, pp. 51–101.

Edwards, D.C., Targeting potential of antibody conjugates. Pharmacol. Ther., 23 (1983) 147–177.

Gregoriadis, G., Targeting of drugs. Nature (London), 265 (1977) 407–411.

Harned, H.S. and Hamer, W.J., The ionization constant of water and the dissociation of water in potassium chloride solutions from electromotive forces of cells without liquid function. J. Am. Chem. Soc., 55 (1933) 2194–2206.

Hashida, M., Kato, A., Takakura, Y. and Sezaki, H., Disposition and pharmacokinetics of a polymeric prodrug of mitomycin C, mitomycin C–dextran conjugate in the rat. Drug Metab. Dispos., 12 (1984) 492–499.

Hashida, M., Takakura, Y., Matsumoto, S., Sasaki, H., Kato, A., Kojima, T., Muranishi, S. and Sezaki, H., Regeneration characteristics of mitomycin C-dextran conjugate in relation to its activity. Chem. Pharm. Bull., 31 (1983) 2055–2063.

Havion, A., Weiner, B. and Zilkha, A., Polymeric salicylate derivatives. J. Med. Chem., 17 (1974) 770–772.

Hickman, J.A., Scanlon, K.J. and Tritton, T.R., Membrane targets in cancer chemotherapy. Trends Pharmacol. Sci., 6 (1984) 15–17.

Hurwitz, E., Specific and nonspecific macromolecule-drug conjugates for the improvement of cancer chemotherapy. Biopolymers, 22 (1983) 557–567.

Hurwitz, E., Maron, R., Arnon, R., Wilchek, M. and Sela, M., Daunomycin-immunoglobulin conjugates, uptake and activity in vitro. Eur. J. Cancer, 14 (1978) 1213–1220.

Hurwitz, E., Wilchek, M. and Pita, J., Soluble macromolecules as carriers for daunorubicin. J. Appl. Biochem., 2 (1980) 25–35.

Jost, R., Miron, T. and Wilchek, M., The mode of adsorption of proteins to aliphatic and aromatic amines coupled to cyanogen bromide-activated agarose. Biochim. Biophys. Acta, 362 (1974) 75–82.

Johansen, M. and Larsen, C, Stability and kinetics of hydrolysis of metronidazole monosuccinate in aqueous solution and in plasma. Int. J. Pharm., 21 (1984) 201–209.

Johansen, M. and Larsen, C., Macromolecular prodrugs II. Influence of variation in molecular weight and degree of substitution of O-benzoyl dextran conjugates on their physicochemical properties and stability in aqueous buffer and in plasma. Int. J. Pharm., 27 (1985) 219–231.

Kato, A., Takakura, Y., Hashida, M., Kimura, T. and Sezaki, H., Physico-chemical and antitumor characteristics of high molecular weight prodrugs of mitomycin C. Chem. Pharm. Bull., 30 (1982) 2951–2957.

Kojima, T., Hashida, M., Muranishi, S. and Sezaki, J., Mitomycin C–dextran conjugate: a novel high molecular weight pro-drug of mitomycin C. J. Pharm. Pharmacol., 32 (1980) 30–34.

Krisch, K., Carboxylic ester hydrolases. In Boyer, P.D. (Ed.), The Enzymes, Vol. 5., 3rd edn., Academic Press, New York, 1971, pp. 43–69.

Larsen, C. and Bundgaard, H., Penicilloyl ester intermediates in glucose- and fructose-accelerated degradation of benzylpenicillin in aqueous solution. Arch. Pharm. Chemi, Sci. Edn., 6 (1978) 33–40.

Levi-Schaffer, F., Bernstein, A., Meshour, A. and Arnon, R., Reduced toxicity of daunorubicin by conjugation to dextran. Cancer Treat. Rep., 66 (1982) 107–114.

Manabe, Y., Tsubota, T., Haruta, Y., Kataoka, K., Okazaki, M., Haisa, S., Nakamura, K. and Kimura, I.,

Production of a monoclonal antibody–methotrexate conjugate utilizing dextran T-40 and its biological activity. J. Lab. Clin. Med., 104 (1984) 445–454.

Manabe, Y., Tsubota, T., Haruta, Y., Okazaki, M., Haisa, S., Nakamura, K. and Kimura, I., Production of a monoclonal antibody–bleomycin conjugate utilizing dextran T-40 and the antigen-targeting cytotoxicity of the conjugate. Biochem. Biophys. Res. Commun., 115 (1983) 1009–1014.

Marshall, J.J., Manipulations of the properties of enzymes by covalent attachment of carbohydrates. Trends Biochem. Sci., 3 (1978) 79–83.

Molteni, L., Dextrans as drug carriers. In Gregoriadis, G. (Ed.), Drug Carriers in Biology and Medicine, Academic Press, London, 1979, pp. 107–125.

Nishikawa, A.H. and Bailon, P., Affinity purification methods. Nonspecific adsorption of proteins due to ionic groups in cyanogen bromide treated agarose. Arch. Biochem. Biophys., 168 (1975) 576–584.

Pitha, J., Zjawiony, J. Lefkowitz, R.J. and Caron, M.G., Macromolecular β-adrenergic antagonists discriminating between receptor and antibody. Proc. Natl. Acad. Sci. U.S.A., 77 (1980) 2219–2223.

Poznansky, M.J. and Cleland, L.G., Biological macromolecules as carriers of drugs and enzymes. In Juliano, R.L. (Ed.), Drug Delivery Systems, Oxford University Press, New York, 1980, pp. 253–320.

Richter, W. and Hedin, H., Dextran hypersensitivity. Immunol. Today, 3 (1982) 132–138.

Richter, W., Hedin, H., Messmer, K. and Ljungström, K.G., Hapten inhibition in the dextran antidextran system and its application to prevent dextran anaphylaxis in man. Int. Arch. Allergy Appl. Immunol., 66 (1981) 288–290.

Rudel, M., Gabert, A. and Möbius, G., Carbonatgruppenhaltiges vernetztes Dextran — ein Trägermaterial für kovalente Immobilisierung von Trypsin. Z. Chem., 18 (1978) 178–179.

Schnaar, R.L., Sparks, T.F. and Roseman, S., Cyanogen bromide activation of polysaccharides. Effects of reaction conditions on cationic charge and ligand content. Anal. Biochem., 79 (1977) 513–525.

Sezaki, H. and Hashida, M., Macromolecule-drug conjugates in targeted cancer chemotherapy. CRC Critical Reviews in Therapeutic Drug Carrier Systems, 1 (1984) 1–38.

Takakura, Y., Matsumoto, S., Hashida, M. and Sezaki, H., Enhanced lymphatic delivery of mitomycin C conjugated with dextran. Cancer Res., 44 (1984) 2505–2510.

Tritton, T.R. and Lee, G., The anticancer agent adriamycin can be actively cytotoxic without entering cells. Science, 217 (1982) 248–250.

Trouet, A., Increased selectivity of drugs by linking to carriers. Eur. J. Cancer, 14 (1978) 105–111.

Virnik, A.D., Khomyakov, K.P. and Skokova, I.F., Dextran and its derivatives. Russian Chem. Rev., 7 (1975) 1280–1307.

Washkuhn, R.J., Patel, V.K. and Robinson, J.R. Linear free energy models for ester solvolysis with a critical examination of the alcohol and phenol dissociation model. J. Pharm. Sci., 60 (1971) 736–744.

Wilchek, M., Affinity therapy and polymer bound drugs. Makromol. Chem., Suppl. 2 (1979) 207–214.

Wilchek, M., Affinity Chromatography. New approaches for the preparation of spacer containing derivatives and for specific isolation of peptides. Adv. Exp. Med. Biol., 42 (1974) 15–31.

Wileman, T., Bennett, M. and Lilleymann, J., Potential use of an asparaginase–dextran conjugate in acute lymphoblastic leukaemia. J. Pharm. Pharmacol., 35 (1983) 762–765.

Wingard, L.B., Jr., Immobilized drugs and enzymes in biochemical pharmacology. Biochem. Pharmacol., 32 (1983) 2647–2652.

Yolles, S., Time-release depot for anticancer drugs. Release of drugs covalently bonded to polymers. J. Parent. Drug. Assoc., 32 (1978) 188–191.

Yolles, S., Morton, J.F. and Sartori, M.F., Preparation of steroid esters of hydroxypropyl cellulose. J. Polym. Sci. Polym. Chem. Eds., 17 (1979) 4111–4113.

Zaharko, D.S., Przybulski, M. and Oliverio, V.T. Binding of anticancer drugs to carrier molecules. In DeVita, Jr., V.T. and Busch, H. (Eds.), Methods in Cancer Research, Vol. XVI, Academic Press, New York, 1979, pp. 347–380.