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Macromolecular prodrugs. XIII. Determination of the ionization constant of dextran by potentiometric titration and from kinetic analysis of the hydrolysis of dextran indomethacin ester conjugates

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Summary

The kinetics of hydrolysis of dextran indomethacin ester conjugates in aqueous buffer solutions in the pH range 6.81–9.13 at 37 °C was studied. As demonstrated by HPLC, the degradation of the dextran ester derivatives proceeded through parallel formation of 4-chlorobenzoic acid and indomethacin, per se. The pH dependence of the pseudo-first-order rate constants for regeneration of the latter compounds showed parallel straight line portions with slopes close to unity, indicating that the hydrolysis reactions were subject to specific base catalysis. A ratio k_{OH}(Indomethacin)/k_{OH}(4-chlorobenzoic acid) of 3.25 was found, revealing that approximately 25% of the attached indomethacin is degraded while bound to dextran. Compared to the dextran indomethacin ester conjugate, the 2,2,2-trifluoroethyl ester of indomethacin exhibited an almost equal susceptibility towards base-catalyzed hydrolysis. The rate data indicate that the pK_a values of the two hydroxy compounds (trifluoroethanol: 12.3 at 25 °C) are of comparable size. Thus the results of the kinetic experiments are in favourable agreement with the obtained ionization constant for dextran of $10^{-11.78}$ (37 °C) as determined by potentiometric titration.

Introduction

In the macromolecular prodrug approach, drug attachment to dextrans has been accomplished through a variety of chemical bonds (Molteni, 1979; Poznansky and Cleland, 1980; Sezaki and Hashida, 1984; Larsen and Johansen, 1985a; Friend and Pangburn, 1987; Larsen, 1989).

In a series of studies, we have investigated the release kinetics of carboxylic acid compounds and alcohols from dextran ester conjugates in aqueous buffer and biological media: benzoic acid derivatives (Larsen and Johansen, 1985b; Johansen and Larsen, 1985; Larsen et al., 1986), NSAID compounds (Harboe et al., 1988; Larsen and Johansen, 1989) and metronidazole (Larsen and Johansen, 1987; Larsen, 1986; Larsen et al., 1987, 1988). During these investigations, several indications have emerged suggesting that the enhanced reactivity of dextran esters compared to simple aliphatic esters might be attributed to a lower pK_a value of the dextran hydroxy groups in proportion to those of aliphatic alcohols.

As part of our evaluation of the potential utility of dextran prodrugs to provide parenteral pro-

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longed duration of action formulations of NSAID compounds, the present study was undertaken in order to determine the kinetics of hydrolysis of dextran indomethacin ester conjugates in neutral to alkaline solution. Furthermore, the aim of this study was to determine the ionization constant of dextran. Since the structure of the polysaccharide is not totally elucidated, it was deemed necessary to design kinetic experiments which could substantiate the results obtained from the potentiometric titration of the dextrans.

Materials and Methods

Indomethacin was kindly supplied by Dumex A/S (Copenhagen, Denmark). 5-Methoxy-2-methyl-indole-3-acetic acid was purchased from Sigma (St. Louis, U.S.A.). The dextran fractions T-70 (M_w 74,300; M_n 36,000) and T-500 (M_w 488,000; M_n 184,800) were obtained from Pharmacia (Uppsala, Sweden). Acetonitrile used in the mobile phases was of chromatographic grade. All other chemicals and buffer substances were of analytical or reagent grade.

The dextran indomethacin ester prodrugs were prepared according to a method of Harboe et al. (1988). The acid chloride of indomethacin was synthesized as previously reported (de Martiis et al., 1975). The 2,2,2-trifluoroethyl ester of indomethacin (**II**) was prepared by adding dropwise a solution of the acid chloride of indomethacin (1.0 g, 2.6 mmol) in 15 ml of tetrahydrofuran to a solution of trifluoroethanol (2.0 ml, 28 mmol) and pyridine (800 μl, 10 mmol) in 5 ml of tetrahydrofuran. After stirring overnight the solution was evaporated in vacuo. The residue was taken up in 30 ml of ethyl acetate, washed with water (30 ml), 5% $NaHCO_3$ (30 ml) and water (30 ml). The dried solution (Na_2SO_4) was evaporated to dryness. Recrystallization of the crude product from toluene–petroleum ether gave crystals (m.p. 93–95 °C). Anal.: Calcd. for $C_{21}H_{17}F_3ClNO_4$: C, 57.35; H, 3.90; F, 12.96; Cl, 8.06; N, 3.18. Found: C, 57.24; H, 3.85; Cl, 8.02; N, 3.21.

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer, using 1-cm quartz cuvettes. Infrared spectra were recorded on a Unicam SP 200 spectrophotometer using the potassium chloride disc technique. ^{1}H-NMR spectra were run on a Jeol C-60-HL instrument. Melting points were taken in capillary tubes and are not corrected. Readings of pH were done on a Radiometer Type pH M26 meter at the temperature of study. Two HPLC systems were employed: (A) a Waters Assoc. Model 6000A constant-flow pump, a Waters Assoc. Model 450 variable wavelength detector and a Rheodyne Model 7125 injection valve with a 20 μl loop; and (B) a Hitachi Model 655A-11 solvent delivery pump equipped with a variable wavelength Hitachi L4000 UV detector and a Rheodyne Model 7125 injection valve with a 20 μl loop.

HPLC analysis

The concentration of intact Dex-Indo conjugate in the reaction mixtures was determined by using a HP(SEC) procedure. The column, 50 × 8 mm, was packed with spherically shaped Nucleosil Diol 7-OH particles (7 μm) (Macherey-Nagel, Düren, F.R.G.). By employing a mobile phase composed of 0.05 M phosphoric acid–acetonitrile (7 : 3 v/v) at a flow rate of 1.2 ml · min^{-1} separation of the conjugate from parent indomethacin was achieved within 4 min (Fig. 1). The eluting compounds were monitored at 340 nm. A reversed-phase HPLC procedure was used for the determination of the initial rates of indomethacin and 4-chlorobenzoic acid formation. The column, 250 × 4 mm, was packed with Nucleosil C-18 (10 μm particles, Macherey-Nagel) and was equipped with a small pre-column containing Perisorb RP-8 particles (30–40 μm) (Merck, F.R.G.). The eluent consisted of acetonitrile–0.05 M citrate buffer pH 2.5 (55 : 45 v/v). The flow was 1 ml · min^{-1} and the column effluent was detected at 245 nm. The latter procedure was also used to follow the degradation of the indomethacin trifluoroethyl ester (**II**). However, the analytical wavelength was changed to 270 nm and the flow rate to 3 ml · min^{-1}.

The indomethacin degradation products, 4-chlorobenzoic acid and 5-methoxy-2-methyl-indole-3-acetic acid were separated on the Nucleosil C_{18} column. The compounds were eluted by using

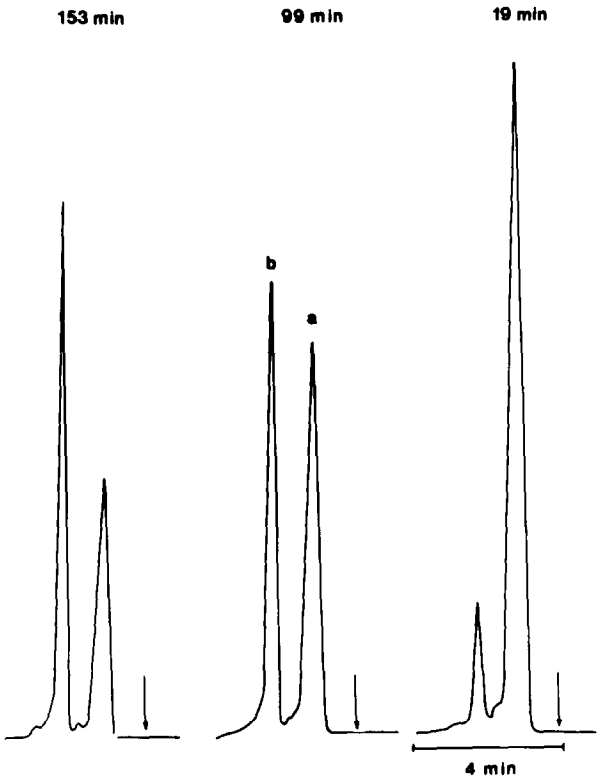


Fig. 1. Hydrolysis of a dextran T-70–indomethacin ester conjugate (DS 6.5) (a) in 0.05 M borate buffer pH 9.13 (37 °C) as followed by the HP(SEC) procedure. 20 μl samples were chromatographed at the times indicated. (b) parent indomethacin.

an acetonitrile–0.05 M citrate buffer pH 2.5 (60 : 40 v/v) mobile phase and were detected at 245 and 280 nm, respectively.

Determination of degree of substitution

The degree of substitution (*DS*) was determined by alkaline hydrolysis of the Dex-Indo conjugates as previously reported (Johansen and Larsen, 1985). However, due to the instability of indomethacin, per se, the released degradation products of the drug were quantitated by the HPLC procedure described above. On a molar basis equal quantities of 4-chlorobenzoic acid and 5-methoxy-2-methyl-indole-3-acetic acid were formed, revealing that attached indomethacin was not degraded during the purification of the conjugates. The *DS* has been expressed as the percentage of mg indomethacin released per mg of the conjugate.

Kinetic measurements

The kinetic studies in aqueous solution were conducted in the pH range 6.81–9.13 employing phosphate and borate buffers. The various buffers were adjusted to an ionic strength of 0.5 by the addition of a calculated amount of potassium chloride. Samples were maintained at 37 ± 0.2 °C in a constant-temperature water bath.

For pH above 8, the rate of disappearance of the Dex-Indo ester and the indomethacin trifluoroethyl ester was monitored after adding the compounds to 10 ml of preheated buffer solution to give initial concentrations of about 0.6 mg · ml^{-1} and 0.2 μg · ml^{-1}, respectively. At suitable intervals, aliquots were withdrawn and analyzed immediately. Pseudo-first-order rate constants were

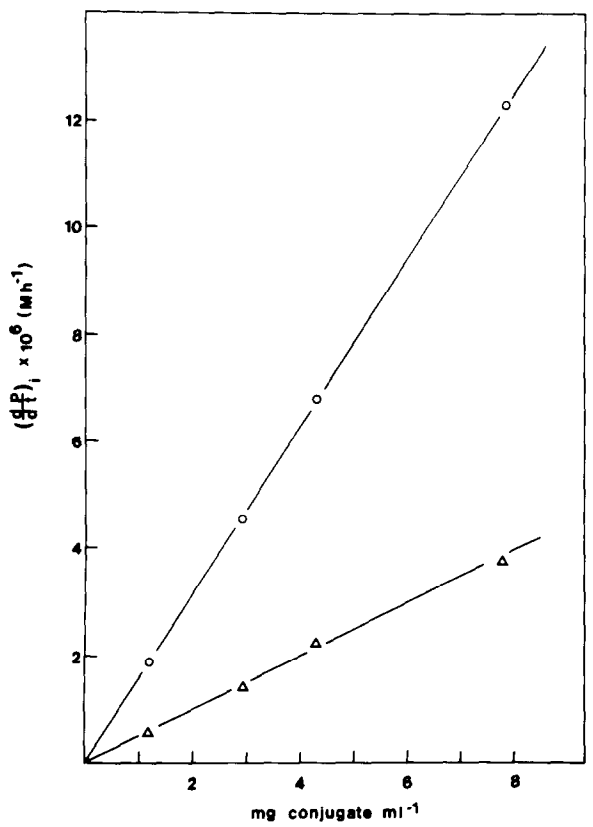


Fig. 2. The influence of the concentration of a dextran T-70–indomethacin ester conjugate (DS 6.5) on the initial rates of product formation $(dP/dt)_i$ in 0.05 M phosphate buffer pH 7.43 (37 °C and $\mu = 0.5$). ○, indomethacin; △, 4-chlorobenzoic acid.

calculated from the slopes of the logarithm of the concentration of intact ester derivative versus time plots using linear regression.

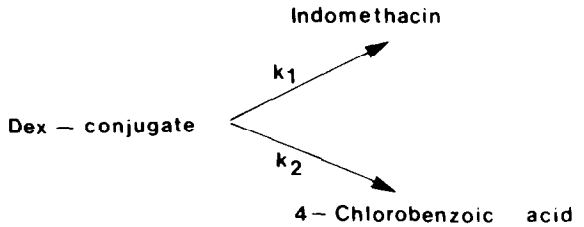
Initial rates of product formation were determined from reaction mixtures after adding an accurately weighed amount of the conjugate to the appropriate buffer to give an initial concentration of the dextran derivative corresponding to 1.5–9 $mg \cdot ml^{-1}$. The simultaneous appearance of indomethacin and 4-chlorobenzoic acid were monitored versus time up to a total percentage of no more than 2% of the initial reactant concentration. The presence of pseudo-first-order hydrolysis kinetics was confirmed by linear plots of the initial rates of product formation versus the concentration of Dex-Indo (Connors, 1973) (Fig. 2).

Determination of ionization constant

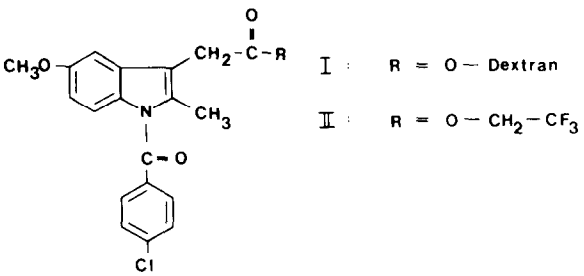
Dextran T-70 and T-500 samples were dried to constant weight. 0.01 M solutions (calculated as anhydroglucose) were titrated with 0.1000 N NaOH at 37 °C and $\mu = 0.5$. Identical ionization constants, derived according to Albert and Serjeant (1971), of $10^{-11.78 \pm 0.02}$ were found.

Results and Discussion

The kinetics of hydrolysis of the dextran indomethacin ester derivatives (Dex-Indo) (**I**) and the indomethacin trifluoroethyl ester (Indo-Flu) (**II**) were studied in aqueous buffer solutions in the pH range 6.81–9.13 at 37 °C. At constant temperature and pH, the decomposition rates followed strict first-order kinetics.



Scheme 1.



Both the dextran drug ester bond and the indole amide linkage are susceptible to hydrolytic cleavage. Consequently, the hydrolysis of Dex-Indo is accompanied by parallel formation of parent indomethacin and 4-chlorobenzoic acid (Scheme 1). The overall pseudo-first-order rate constant, k_{obs}, for hydrolysis of the conjugates was determined directly by following the disappearance of the conjugates by means of the HP(SEC) procedure on Nucleosil Diol. An analytical wavelength of 340 nm was chosen, at which the formed dextran 5-methoxy-2-methyl-indole-3-

TABLE 1

Pseudo-first-order rate constants for hydrolysis of dextran indomethacin ester conjugates in 0.05 M aqueous buffer solutions at 37 °C and an ionic strength of 0.5

Indomethacin conjugate [a]	pH	k_1 (h^{-1}) $\times 10^3$	k_2 (h^{-1}) $\times 10^3$	k_{obs} (h^{-1}) $\times 10^3$	k_1/k_2
Dex-T-70	9.13	–	–	450	–
Dex-T-70	7.73	17.6	5.47	23.1	3.22
Dex-T-70	7.43	7.43	2.29	9.72	3.24
Dex-T-500	7.38	7.26	2.31	9.57	3.14
Dex-T-70	7.11	4.71	1.40	6.11	3.36
Dex-T-70	6.81	2.34	0.71	3.05	3.30

[a] The DS of the Dex-T-70 and Dex-T-500 derivatives were 7.5 and 6.9, respectively.

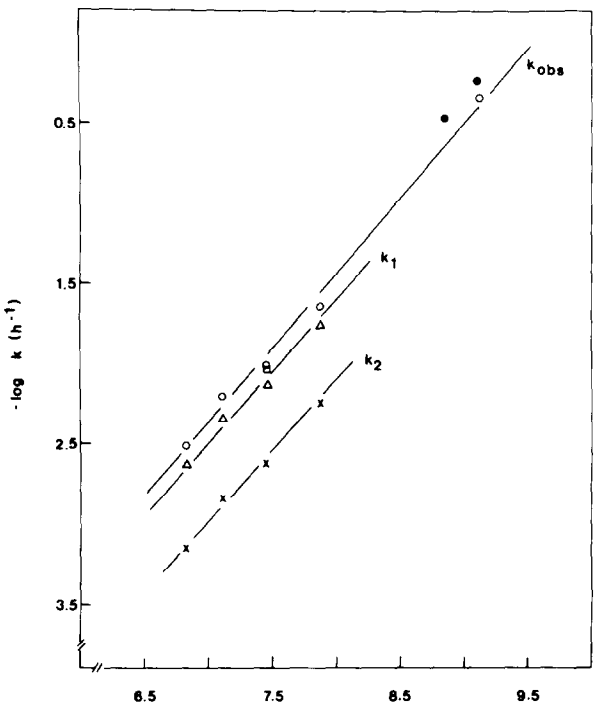


Fig. 3. pH dependence of the first-order rate constants involved in the degradation of a dextran T-70–indomethacin ester conjugate (DS 6.5) in 0.05 M aqueous buffers (37 °C and $\mu = 0.5$). Overall first-order rate constants, k_{obs}, for hydrolysis of a dextran T-500–indomethacin ester conjugate (DS 6.73) (□) and the 2,2,2-trifluoroethyl ester of indomethacin (●) are further included.

acetic acid ester compound did not contribute to the detector response. Employing the initial rate method, the rate constant k_{obs} was also derived from the equation:

$$k_{obs} = k_1 + k_2 \qquad (1)$$

where k_1 and k_2 are the pseudo-first-order rate constants associated with the rates of release of indomethacin and 4-chloro-benzoic acid from the conjugates, respectively. In Table 1 are presented the values of the latter rate constants determined at various pH values together with those of the overall degradation rate constant k_{obs}, calculated from Eqn. 1.

The effect of pH on the individual rate constants is shown in Fig. 3. In the investigated pH range, the pH–rate profiles exhibit almost parallel straight line portions with slopes varying from 0.92 to 0.94, indicating that the hydrolysis reactions are subject to specific base catalysis. Using the values of the hydroxide ion activity, calculated according to Harned and Hamer (1933):

$$\log a_{OH} = pH - 13.62 \qquad (2)$$

the value of the overall second-order rate constant for specific base catalysis (k_{OH}) was found to be 1.56×10^4 $M^{-1} \cdot h^{-1}$. A value of 3.85×10^3 $M^{-1} \cdot h^{-1}$ of the second-order rate constant for the base-catalyzed hydrolysis of the conjugate indole amide bond was calculated. Thus, approximately 25% of the attached indomethacin is degraded while bound to the carrier. This is also apparent from the almost constant ratio of k_1/k_2 which amounts to about 3.25 (Table 1). Data for alkaline hydrolysis of indomethacin, per se (Krasowska, 1974; Hepatwala and Dawson, 1977; Cipiciani et al., 1983), is of the same order of magnitude as the k_2 values of the present study, suggesting that the stability of indomethacin is largely unchanged after conjugation to dextran.

The pseudo-first-order rate constants for hydrolysis of the Indo-Flu ester at pH 8.85 (0.33 h^{-1}) and pH 9.13 (0.58 h^{-1}) are also incorporated in the pH–rate profile (Fig. 3). From the latter rate data, a k_{OH} value of 1.86×10^4 $M^{-1} \cdot h^{-1}$ was calculated. The ratio k_{OH}(Indo-Flux)/k_{OH} (Dex-Indo) is 1.2, demonstrating an almost equal susceptibility of the two indomethacin esters to undergo base-catalyzed hydrolysis. This observation was to be expected, since the pK_a values of dextran (11.78 at 37 °C) and 2,2,2-trifluoroethanol (12.37 at 25 °C (Ballinger and Long, 1960)) are of comparable size. In alkaline ester hydrolysis increasing degradation rates are generally observed with decreasing pK_a of the leaving alcoholic group (Humffray and Ryan, 1967; Ryan and Humffray, 1966; Washkuhn et al., 1971), but also steric factors have to be taken into consideration. A 5-fold increase in the rate of hydrolysis of ethyl esters compared to isopropyl esters of various acids for pH above 7 have been reported (Jones and Thomas, 1966; Washkuhn et al., 1971; Larsen and Johansen, 1985b). This difference in reactivity of esters derived from primary and secondary alcohols probably accounts for the k_{OH} ratio ex-

ceeding 1 for the base-catalyzed hydrolysis of Indo-Flu and Dex-Indo. Thus, through kinetic analysis and potentiometric titration, it has been established that dextran hydroxy groups possess a relatively low pK_a compared to those of simple aliphatic alcohols. Compared to dextran, a nearly identical pK_a of 11.81 at 37°C for glucose has been found (Bundgaard and Larsen, 1978). The polysaccharide is built of glucose units linked together predominantly in the form of 1,6-linkages. The acidity of such carbohydrates might therefore be ascribed to the OH groups at the positions C-2, C-3 or C-4. Stabilization of the carbohydrate alkoxide ion through intramolecular hydrogen-bonding effected by a neighbouring hydroxy group seems to be a plausible explanation for this acidic site (Haines, 1976). In keeping with the latter proposal, relatively low pK_a values are reported for a variety of polyhydroxy compounds: sorbitol: 13.57 at 18°C (Thamsen, 1952); cyclohexaamylose: 12.36 at 30°C (Gelb et al., 1980); adenosine: 12.35 at 25°C (Izatt et al., 1965).

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