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2′,3′-Dideoxyinosine (ddI): its chemical stability and cyclodextrin complexation in aqueous media

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Abstract: The chemical stability of 2′,3′-dideoxyinosine has been studied over a wide pH range (0–12). A stability-indicating HPLC method was used to separate the degradation product from the parent drug. The effects of temperature, ionic strength and buffer components on the degradation kinetics were investigated. Furthermore, the influence of some cyclodextrins (α-, β-, HP-β-, DM-β- and γ-cyclodextrin) on the drug stability have been studied.

Keywords: *2′,3′-Dideoxyinosine; HPLC; chemical stability; cyclodextrin complexation.*

Introduction

2′,3′-Dideoxyinosine (Didanosine, ddI, Videx®) (Fig. 1) is a dideoxypurinenucleoside analogue, which shows high anti-retroviral activity [1]. The drug has been approved by the Food and Drug Administration in 1991 for Human Immunodeficiency Virus (HIV) infected patients who are intolerant or resistant to Zidovudine therapy [2, 3]. ddI is currently studied as monotherapy and as combination therapy with Zidovudine in patients at different stages of HIV disease. The results are encouraging [1–4].

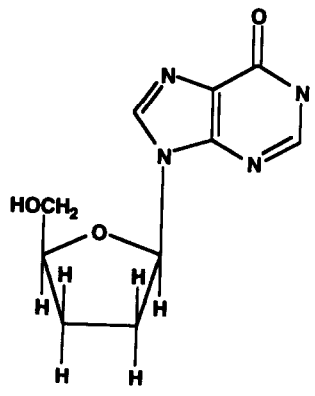

Figure 1
Structure of ddI.

However, there are some relevant problems to be solved before widespread application [5], one being chemical stability. Like other glycosidic compounds [6] ddI is subject to hydrolytic degradation in acidic solutions. It has been reported that at pH 2 the half-life of ddI is only a few minutes [7].

A consequence of the acid lability of ddI is that if ddI is administered orally it will degrade in the acid stomach content. Since degradation is accompanied by loss of activity various oral dosage forms have been studied, including buffered tablets and chewable tablets with antacid [6]. Since it is well known that cyclodextrins (CyDs) can form inclusion complexes with various drug molecules [8–10], and this may improve aqueous solubility, chemical stability and bioavailability, the present project also investigated the influences of CyDs on ddI stability in aqueous media, with the aim to find a starting-point for the design of a new convenient ddI formulation.

In the present investigation a systematic stability study of ddI was undertaken in order to obtain more detailed knowledge on the degradation process and to extend earlier

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kinetic studies with dideoxynucleosides [11]. The influences of various parameters (ionic strength, buffer components, pH, temperature, cyclodextrin complexation) on the degradation process in aqueous media were studied.

Experimental

Chemicals

ddI was generously provided by Bristol–Myers Squibb (Wallingford, CT, USA). The natural CyDs (α-, β- and γ-CyD) originated from Nihon Shokukin Kako Co. Ltd (Tokyo, Japan), heptakis(2,6-di-*O*-methyl)β-CyD (DM-β-CyD) and hydroxypropyl-β-CyD (HP-β-CyD) were both gifts from Dr J. Mesens (Janssen Pharmaceutica, Beerse, Belgium). The CyDs were used as received. All other materials were of analytical grade and deionized water was obtained by filtration through a Milli-Q-Water Purification System (Millipore, Bedford, MA, USA).

Buffer–CyD solutions

For the kinetic studies the following aqueous solutions were used: pH < 3: perchloric acid; pH 3–6: acetate (0.01 M); pH 6–9: phosphate (0.01 M); and pH 9–12: carbonate (0.01 M). The desired pH was adjusted using perchloric acid or sodium hydroxide and was measured with a pH meter (Metrohm, E512, Titriskop, Herisau, Switzerland) at the temperature of study. A constant ionic strength, $\mu = 0.3$, was maintained for each degradation solution, by addition of an appropriate amount of sodium chloride, except for solutions where the influence of ionic strength on the degradation was investigated and where the hydroxyl or proton concentration exceeded 0.3 M.

In experiments where the influences of various CyDs on the ddI degradation rate were studied, the CyD concentration was 10^{-2} M. For the study of the influence of [HP-β-CyD] on the degradation the concentration varied from 0 to 10^{-1} M. In all these cases the pH of the solution was adjusted to the desired value after the addition of the CyD. The buffer–CyD solutions were always freshly prepared before use. No significant changes of pH were observed throughout the degradations.

Kinetic measurements

The buffer–CyD solutions were stored in polypropylene reaction vessels and were allowed to equilibrate to the temperature of study in a thermostated water bath. Degradation was initiated by adding 40 μl of a ddI stock solution (2.5 mg ml^{-1}) in ethanol to 2 ml of preheated buffer–CyD solutions, yielding an initial concentration of 2×10^{-4} M. At appropriate time intervals 10 μl samples were taken and analysed directly for the content of undegraded ddI with a stability-indicating high-performance liquid chromatography (HPLC) method, described earlier [12]. Samples originating from degradation solutions at pH values <3 were buffered to pH 7 and were analysed immediately.

The kinetic experiments were carried out at 80°C except for the study where the influence of temperature was investigated and in solutions with pH < 3. In these cases the experiments were performed at lower temperature and the results were extrapolated to 80°C using the Arrhenius equation.

Results and Discussion

Analytical methodology

The initial degradation step of ddI is the hydrolysis of the glycosidic bond with the formation of the purinebase hypoxanthine and the residual deoxyribose sugar moiety [13]. When this degradation process is followed spectrophotometrically only slight spectral changes occur which are not suitable for the accurate determination of rate constants. But with the HPLC system used here ddI is well resolved from its aglycone. Figure 2 demonstrates the stability-indicating capability of the assay.

Degradation kinetics

As illustrated in Fig. 2, ddI degrades into its corresponding aglycone. The degradation process follows, at the entire pH-range studied, a pseudo-first order kinetic pattern over at least three half-lives. This indicated by the linearity ($r > 0.99$) of plots of the natural logarithm of the residual ddI concentration versus time. From the slopes of these lines the values for the pseudo-first order rate constants, k_{obs}, can be extracted. The rate equation can be written as:

$$-\mathrm{d[ddI]}/\mathrm{d}t = k_{obs}\,[\mathrm{ddI}]. \qquad (1)$$

The standard deviation in k_{obs} was determined at pH 3 and 25°C. The k_{obs} value and the

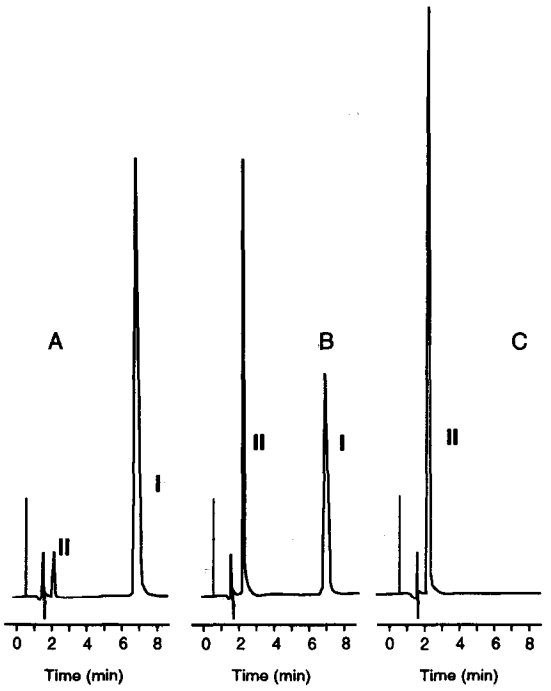


Figure 2
HPLC chromatograms during degradation of ddI at pH 2: (A) t = 0.5 min; (B) t = 15 min; (C) t = 24 h; column: Hypersil ODS 5 μm; flow rate: 1 ml min^{-1}; mobile phase: methanol–phosphate buffer (pH 6.8; 5 mM) (10:90, v/v); detection: 254 nm; I: ddI; II: hypoxanthine.

standard deviation, calculated from eight observations, is 1.63 ± 0.13 × 10^{-4} s^{-1}. All other rate constants are mean values of duplicate measurements.

Influence of ionic strength

The influence of the ionic strength was investigated at pH values in the acidic range, since the degradation of ddI in this medium is most prominent. The ionic strength was varied by adding different amounts of sodium chloride to the degradation solution, while the buffer concentration (0.01 M) was kept constant. Within the range investigated (μ = 0.2–1.0) at pH 2 (T = 25°C) and pH 6 (T = 80°C) no influence of the ionic strength on the degradation rate of ddI was observed under these conditions.

Influence of buffer components

Buffer components, such as acetate and phosphate ions, may exert catalytic effects on the degradation reactions of many drugs. The influence of an acetate buffer at pH 3 (T = 25°C) and a phosphate buffer at pH 6 (T = 80°C), both in the concentration range 5–100 mM, on the degradation of ddI has been studied. It was found that the concentration of both buffer components show no effect on the observed degradation constant of ddI.

Influence of pH

The influence of pH has been studied in the range 0.3–12. During these experiments ionic strength (μ = 0.3) and buffer concentration (0.01 M) were maintained constant. The temperature was 80°C, except at pH < 3.2 stability tests at low temperatures were carried out since the degradation of ddI at these pH values is too fast to follow with the methods used here. By application of the Arrhenius equation [equation (2)] the k_{obs} values were extrapolated to 80°C. The log k_{obs} versus pH profile (Fig. 3) shows that the ddI degradation is acid catalysed. In alkaline medium ddI is very stable.

Temperature dependence

The temperature dependence of the hydrolytic ddI degradation was investigated over the temperature range 10–30°C at pH 3.2 and 3.6. The relation between k_{obs} and the reciprocal of the absolute temperature fulfils the Arrhenius equation:

$$\ln k_{obs} = \ln A - (E_a/RT), \qquad (2)$$

in which A represents the frequency factor, E_a the activation energy, R the molar gas constant and T the absolute temperature. By plotting

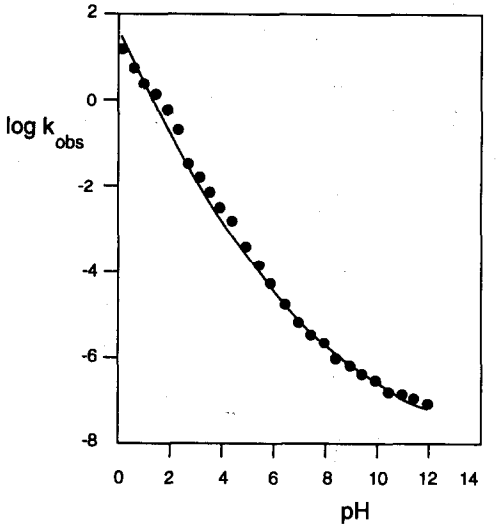


Figure 3
log k_{obs} (s^{-1})–pH profile for the degradation of ddI at 80°C.

the reciprocal of T versus ln k_{obs} the activation energy and the frequency factor can be calculated. The results are summarized in Table 1. Extrapolation to 80°C of the k_{obs}-values at pH 3.2 and 3.6, agrees with the values measured at this temperature and indicates that the Arrhenius equation is valid over a wide temperature range.

Table 1
Influence of temperature on the degradation of ddI in terms of activation energies and frequency factors (μ = 0.3).

pH	E_a (kJ mol^{-1})	A (s^{-1})
3.2	82.0	2.9×10^{10}
3.6	87.3	8.8×10^{10}

Influence of CyDs

The effects of various CyDs on the degradation of ddI have been studied in acidic medium. In the presence of CyDs, ddI can also be analysed by using the HPLC system. The retention times of ddI and its degradation product are unaffected by the presence of CyDs. At the pH of study the kinetic behaviour of ddI remains pseudo-first order in the presence of CyDs.

Table 2 shows the influence of α-CyD to be of little significance, γ-CyD has some influence, whereas β-, HP-β- and DM-β-CyD have the largest stabilizing effects. Apparently, the lipophilic moiety in ddI fits best in the β-CyD cavity. Since HP-β-CyD has the greatest stabilizing effect and is a powerful solubilizer suitable to use also, contrarily to β- and DM-β-CyD, in parenteral formulations [14], the influence of the HP-β-CyD concentration on the stability of ddI was investigated in more detail. Figure 4 illustrates the influence of the [HP-β-CyD] on the k_{obs} for the ddI degradation. It is obvious that in the concentration range 0–6 × 10^{-2} M the stabilizing effect of

Table 2
Influence of various CyDs ([CyD] = 1.0×10^{-2} M) on the degradation of ddI at pH 2; μ = 0.3; T = 25°C

CyD	k_{obs} (s^{-1})
–	1.73×10^{-3}
α	1.61×10^{-3}
β	1.18×10^{-3}
γ	1.42×10^{-3}
HP-β	1.13×10^{-3}
DM-β	1.20×10^{-3}

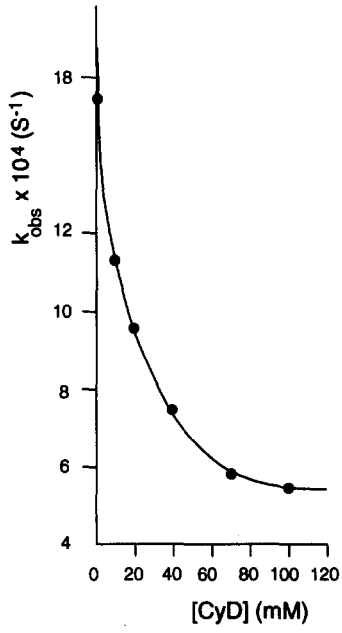
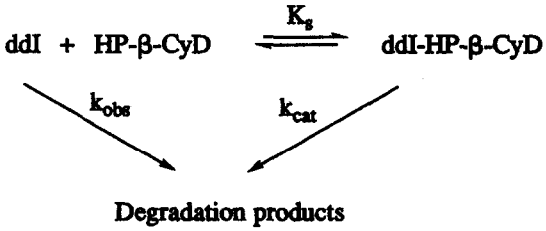


Figure 4
Influence of the HP-β-CyD concentration on the degradation of ddI; pH 2 and T = 25°C.

HP-β-CyD is most prominent. On increasing the [HP-β-CyD] above the level of 6 × 10^{-2} M, only a slight effect on the stability of ddI is observed. This observation can be explained by assuming that after addition of a sufficient HP-β-CyD concentration almost all ddI has been included in the CyD cavity.

The complex formation constant, K_s, of the ddI–HP-β-CyD complex has been determined by using the Lineweaver–Burk equation [15, 16] [equation (3)]. The complexation of ddI with HP-β-CyD and the degradation reactions of both free and complexed drug are shown in Scheme 1.



k_{obs} is the pseudo-first order constant for degradation of the free drug and k_{cat} the pseudo-first order rate constant for the degradation of ddI trapped in the CyD cavity. These constants can be extracted from:

$$[\text{HP-}\beta\text{-CyD}]/(k_o - k_{obs}) = 1/(k_o - k_{cat})\cdot[\text{HP-}\beta\text{-CyD}] + 1/K_s(k_o - k_{cat}). \quad (3)$$

Table 3
Complex formation constant (K_s) and degradation rate constant (k_{cat}) of the ddI–HP-β-CyD complex at pH 2 and T = 25°C

k_o* (s^{-1})	k_{cat} (s^{-1})	K_s (M^{-1})	k_o/k_{cat}
1.73×10^{-3}	3.34×10^{-4}	70	5.1

* Rate constant for degradation of the free drug.

In Table 3 the characteristics of the ddI–HP-β-CyD complex are summarized. The complex constant for the ddI–HP-β-CyD complex is 70 M^{-1}, indicating that at a concentration of 10^{-1} M HP-β-CyD, and assuming that the [ddI] ≪ [HP-β-CyD], the ratio complexed:free ddI is 700:1. From Table 3 it appears that the complex degrades with lower velocity (factor 5.1) compared to the free drug, indicating that complexation with HP-β-CyD protects the labile glycosidic band of ddI for the acid-catalysed hydrolysis.

Conclusions

This study demonstrates that ddI is very susceptible to acid hydrolysis. The degradation process involves hydrolysis of the glycosidic bond. The decomposition rate is mainly affected by acidity and temperature. Buffer ions and ionic strength have no influence on the degradation rate of the drug. The half-life at 37°C, extrapolated with the Arrhenius equation, is about 1 min at pH 1, indicating the importance for adequate control of stomach pH in order to prevent extensive degradation of the drug before it is absorbed from the gastro-intestinal tract. Complexation with CyDs may be an alternative approach to investigate further ways to circumvent biodegradation of ddI.

References

[1] R. Yarchoan, H. Mitsuya, C.E. Meyers and S. Broder, *N. Engl. J. Med.* **312**, 726–738 (1989).
[2] M.J. Shelton, A.M. O'Donnel and G.D. Morse, *Ann. Pharmacother.* **26**, 660–670 (1992).
[3] D. Faults and R.N. Brogden, *Drugs* **44**, 94–116 (1992).
[4] R. Yarchoan, J.M. Pluda, R.V. Thomas, H. Mitsuy, P. Brouwer, K.M. Wyvill, N. Hartman, D.G. Johns and S. Broder, *Lancet* **336**, 526–529 (1990).
[5] N. Hartman, R. Yarchoan, J.M. Pluda, R.V. Thomas, K.M. Wyvill, K.P. Flora, S. Broder and D.G. Johns, *Clin. Pharmacol. Ther.* **50**, 278–285 (1991).
[6] J.H. Beijnen, G. Wiese and W.J.M. Underberg, *Pharm. Weekbl. Sci. Ed.* **7**, 109–116 (1985).
[7] V.E. Marquez, C.K.H. Tseng, J.A. Kelley, H. Mitsuya, S. Broder, J.S. Roth and J.S. Priscoll, *Biochem. Pharmacol.* **36**, 2719–2722 (1987).
[8] J. Szejtli, *Cyclodextrin Technology*, pp. 79–306. Kluwer Academic, Dordrecht (1988).
[9] K. Uekama and M. Otagiri, *Crit. Rev. Ther. Drug Carrier Syst.* **3**, 1–40 (1987).
[10] O. Bekers, E.V. Uijtendaal, J.H. Beijnen, A. Bult and W.J.M. Underberg, *Drug Dev. Ind. Pharm.* **17**, 1503–1549 (1991).
[11] B.D. Anderson, M.B. Wygant, T.-X. Xiang, W.A. Waugh and V.J. Stella, *Int. J. Pharm.* **45**, 27–37 (1988).
[12] J.H. Beijnen, P.L. Meenhorst, H. Rosing, R. van Gijn, G. Los and W.J.M. Underberg, *J. Drug Dev.* **3**, 127–133 (1990).
[13] J.A. Zoltewicz, D.F. Clark and T.W. Sharpless, *J. Am. Chem. Soc.* **92**, 1741–1749 (1970).
[14] M.E. Brewster, J.W. Simpkins, M. Sing Hora, W.C. Stern and N. Bodor, *J. Parent. Sci. Technol.* **43**, 231–240 (1989).
[15] H. Lineweaver and D. Burk, *J. Am. Chem. Soc.* **56**, 658–666 (1934).
[16] O. Bekers, J.H. Beijnen, M.J.T. klein Tank, A. Bult and W.J.M. Underberg, *J. Pharm. Biomed. Anal.* **9**, 1055–1060 (1991).

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