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Kinetics of Degradation of Levothyroxine in Aqueous Solution and in Solid State

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9

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The kinetics of the deiodination of levothyroxine in aqueous solution were studied over the pH range 1 to 12. Temperature dependence of the reaction was also studied. The log k - pH profile indicated that the kinetics of deiodination include proton attack on the anion and dianion in acidic solution and water attack on the anion and dianion in acidic solution. A possible mechanism of the deiodination was discussed. The solid-state stability of levothyroxine sodium was studied at elevated temperatures; and the composition follows biphasic first-order kinetics, with the most rapid decomposition solutor courring at the beginning of heating.

KEY WORDS: levothyroxine; liothyronine; degradation, deiodination; pH effect; solid-state stability.

INTRODUCTION

Levothyroxine sodium is the sodium salt of the levoisomer of thyroxine (T₄), an active physiological compound found in the thyroid gland. Preparations of the synthetic hormone are indicated in replacement or supplemental therapy for patients with hypothyroidism and as pituitary thyroid stimulating hormone suppressants in the treatment of euthyroid goiters (1).

Deiodination occurs as a result of ultraviolet irradiation in aqueous media, and the deiodination process was proportional to the decrease in the pH of the aqueous solution (2). When T_4 labeled with ¹³¹I was administered intravenously to normal humans; most labeled iodine eventually appeared in the urine as free inorganic iodide (3). The possible physiological significance of the deiodination of the thyroid hormone caused many investigators to study the process both *in vitro* and *in vivo*. The deiodination of T_4 occurred in kidney, brain, liver, and muscle homogenate preparations. There was, however, only limited evidence for liothyronine (T_3) formation (4).

Despite a long history of its use, the *in vitro* degradation studies of T_a appearing in the above literature were of descriptive nature, providing limited kinetic information. The purpose of this study was to obtain kinetic data on the degradation of T_4 in solution and in solid state and to investigate possible mechanisms of the degradation processes. This study was not intended to predict the *in vivo* transformation of the drug.

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MATERIALS AND METHODS

Materials Levothyroxine sodium and liothyronine were obtained from Biochemie (Austria) and Aldrich Chemical (Milwaukee, WI), respectively. Water used for kinetics was deionized and distilled. Acetonitrile used was HPLC grade. All

HPLC Analysis

ark, DE).

The chromatography system consisted of an isocratic pump (Beckman Model 110A), an automatic injector (IBM LC/9504), a variable-wavelength UV detector (LDC Spectromonitor III), and a computing integrator (Hitachi D-2000). The HPLC method employed a 250×4.6 -mm-i.d., 5-µmparticle size, cyano-bonded silica column (Phenomenex) and a mobile phase consisting of water:acetonitrile:phosphoric acid (600:400:1, v/v). The flow rate was 1.5 ml/min and the detector wavelength for ultraviolet absorbance detection was 225 nm.

the chemicals used were reagent grade and were used as received. TLC plates were obtained from Analtech (New-

Solution Degradation Product

Approximately 500 mg of levothyroxine sodium was dissolved in 70 ml of 0.01 N NaOH and refluxed for 40 hr. The solution was then neutralized by adding concentrated HCI dropwise and evaporated to dryness using a rotary evaporator. The residue was extracted with 50 ml of acetonitrile. The acetonitrile solution was concentrated, streaked across a 1-mm-thick silica gel GF preparative TLC plate, and developed with a solvent system consisting of methylene chloride: acetonitrile:formic acid (80:10:10). Using the TLC system, a T_4 spot showed at R_7 0.27 and a major degradate spot at R_7 0.18. The band corresponding to R_7 0.18 was removed from the plate and eluted with 50 ml of 0.01 N methanolic NaOH solution. The eluent was acidified by adding concentrated HCI dropwise and the resultant precipitate was centrifuged. The supernatant was decanted and the solid was dried under vacuum at 60°C for 2 hr.

The electron impact mass spectrum of the degradate was run on a VG 7070 SE mass spectrometer. The spectrum was obtained at 70 eV with the source temperature at 190°C. The low-resolution mass spectrum exhibited a protonated molecular ion at m/z 651 corresponding to $C_{13}H_{12}I_3NO_4$, loss of water (m/z 633), and loss of carbon dioxide (m/z 607). The proton NMR spectrum of the isolated degradate was recorded on a Varian VXR 200 NMR spectrometer using deuterated DMSO as the solvent. The proton NMR spectrum of the degradate showed a doublet (6.91 and 6.95 ppm) corresponding to the hydrogen atom which had replaced iodine at either the 3' or the 5' carbon, a doublet (7.02 and 7.03 ppm), and a quadruplet (6.60, 6.62, 6.65 and 6.66 ppm), corresponding to the hydrogens at 2' and 6' carbons. The MS and NMR spectra were identical to those of authentic T₃. The major degradation peak seen in an HPLC chromatogram of T₄ which had been partially degraded in acidic solution had a retention time identical to that of authentic T₃. The coin-

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131

jection of an acid-degraded T_4 with authentic T_3 resulted in one peak (chromatogram not shown).

Solid-State Degradation Products

132

Approximately 500 mg of levothyroxine sodium in an open vial was kept in an oven at 60°C for 7 weeks. The degraded levothyroxine sodium was suspended in 150 ml of a methanol and water mixture (30:70). The sample was completely dissolved by dropwise addition of conc. NH4OH and subsequently acidified by dropwise addition of conc. HCl. The resultant precipitate was filtered, dissolved in methanol, streaked across a 1-mm-thick silica gel GF plate, and developed with a solvent system consisting of toluene:ethylacetate:acetic acid (75:22:3). Using the TLC system, a T₄ spot showed at $R_f 0.05$ and two major degradate spots at $R_f 0.89$ and 0.06. The bands corresponding to $R_f 0.89$ and $R_f 0.60$ were separately removed from the plate and eluted with methanol. The eluent was evaporated to dryness and the residue was dried at 60°C for 2 hr under vacuum. Highresolution EI-MS of the $R_f 0.89$ product showed a molecular ion at m/z 717.6488 corresponding to C13H6O3I4 (calculated m/z 717.6494) and ions resulting from the two subsequent losses of iodine (m/z 591 + H, 464). The other product $(R_f 0.60)$ showed a protonated molecular ion at m/z 760.6638 corresponding to C15HoldO4 (calculated m/z 760.6677) and ions resulting from loss of water (m/z 742) and loss of carbon dioxide (m/z 716).

Solubility-pH Profile

The solubility of levothyroxine sodium in aqueous buffer solution was determined as a function of pH ranging from 1 to 11. The buffers used were glycine (pH 1.0-3.7), actiate (pH 4.5-4.7), phosphate (pH 5.8-8.0), and carbonate (pH 9.3-11.0) buffers. The ionic strength was adjusted to 0.5. An excess amount of levothyroxine sodium was placed in a screw-cap test tube containing the aqueous buffer solutions. The solution was shaken at 25°C on a rotary shaker until replicate analyses of the solution sampled at 4-hr intervals indicated that a steady concentration had been reached. Eighteen hours of shaking sufficied for the equilibration. The undissolved solid was then separated by centrifugation. The pH of the solution was measured, and after appropriate dilutions with the mobile phase, the solution was analyzed by HPLC.

Kinetic Method

The buffers used for kinetics were citrate (pH 3.2), acetate (pH 4.4-5.4), phosphate (pH 6.3-7.0), borate (pH 8.8), and carbonate (pH 9.6-10.7) buffers. Buffer stock solutions of 0.2 M were prepared. Aliquots of the buffer stock solutions and 1 M NaCl solution were diluted so that the final total buffer concentration was 0.02 M and the ionic strength was at 0.1. Low buffer concentrations were used to minimize the possible general acid-base catalysis by the buffer species. The pH of each solution was measured at the reaction temperature. For strongly acidic and basic solutions, aqueous HCl or NaOH solutions were used to obtain the desired pH. The pH values of these solutions at the reaction temperatures were calculated from the published activity coef-



ficients (5). In a typical kinetic experiment, 0.5 ml of 1 mg/ml levothyroxine sodium stock solution in methanol, an appropriate amount of buffer stock solution, HCl or NaOH, and an appropriate amount of 1 *M* NaCl were transferred into a 100-ml volumetric flask and filled to volume with water. The final concentration of levothyroxine sodium was 5 μ g/ml. The reaction flask was kept in a constant/temperature water bath at 50.0, 60.0, 70.0, and 80.0°C (±0.5°C). Aliquots of the sample were taken at appropriate time intervals and analyzed by HPLC.

RESULTS AND DISCUSSION

Solubility-pH Profile

 T_4 has three ionizable moieties and it can exist as the cation (C), zwitterion (Z), anion (A), and dianion (DA), depending on the pH of the solution (Scheme I). The solubility-pH profile (Fig. 1) was used to determine the ionization con-



Kinetics of Degradation of Levothyroxine

stants (6). The equation for the solubility (S) of T_4 as a function of hydrogen ion activity up to pH 9.8 is given by

$$S = S_{o} \left(1 + [H^{+}]/K_{a1} + K_{a2}/[H^{+}] \right)$$
(1)

solubility of A. As the pH increases above 10, the solubility increases as more DA is formed, in accordance with

where S_o' is the maximum solubility of A. The S_o' (120 µg/

 $S = S_o + S_o' (1 + K_{a3}/[H^+])$ (2)

133

where S_0 is the intrinsic solubility of Z. The value of S_0 (0.25 µg/ml) was estimated from the best fit of the solubility-pH profile between pH 3 and pH 6. The p K_a values that gave the best fit to Eq. (1) are $pK_{a1} = 2.40$ for the carboxyl group and $pK_{a2} = 6.87$ for the phenolic OH group. The solubility-pH profile shows a break around pH 10 indicating a saturation





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Fig. 3. log k - pH profiles for the degradation of T_4 in aqueous solution at 50°C (\diamond), 60°C (\triangle), 70°C \square), and 80°C (\bigcirc) and of T_5 at 80°C (\bigcirc).

Log k - pH Profile

134

For all the kinetic experiments, the first-order plots for loss of T_4 were linear (correlation coefficient >0.998) for two or more half-lives. Typical first-order plots are shown in Fig. 2. The log k - pH profiles for the deiodination of T_4 are shown in Fig. 3. The rate of deiodination shows a plateau in the acidic pH region, drops off sigmoidally in the neutral pH region, and shows another plateau in the alkaline pH region. The log k - pH profile shows a sigmoidal change only when the phenolic OH group is ionized ($pK_{a2} = 6.87$). No change was observed when the carboxyl group ($pK_{a1} = 2.40$) or

Table I. Catalytic Rate Constants, " Arrhenius Parameters, " and Entropies of Activation for Deiodination of T_4

:	Temperature (°C)	$k_{\rm H} (\sec^{-1} M^{-1})$		$10^8 k_{o'}$ (sec ⁻¹)
	80.0 ± 0.5	14.3	•	34.3
	70.0 ± 0.5	7.00	1. A.	20.8
	60.0 ± 0.5	3.16		13.0
÷.	50.0 ± 0.5	1.56	1.1.1	6.95
	25.0°	0.165	1.1	1.36
2	E_{\star} (kcal \cdot mol ⁻¹)	16.8	1.1	12.1
•	log A	11.5		1.0
	ΔS^{\ddagger} (cal, mol ⁻¹ K ⁻¹)	-7.8		- 55.9

^a Where $k = k_{\rm H} [{\rm H}^+] f_A + k_0' f_A$. ^b Where log $k = -E_{\rm e}/2.303 {\rm RT} + \log A$. ^c Extrapolated from $E_{\rm e}$ and log A.

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(3)

(4)

Fig. 4. Typical HPLC chromatograms of partially degraded samples. (a) T_4 in acidic solution. (b) T_4 in alkaline solution. (c) T_3 in acidic solution. (d) T_3 in alkaline solution. T_4 (A); T_3 (B); T_2 (C); unknown (D).

amino group $(pK_{a3} = 9.96)$ was ionized. The carboxyl and amino groups are too far away from the reaction center so that the ionization of these groups appears to have little resonance or inductive effect on the deiodination rate.

As shown in the log k - pH profile, the rate constant does not increase with increasing acidity in the strongly acidic region, indicating that there is no contribution to the rate constant from the proton attack on C and Z. The overall deiodination of T₄ can be explained by spontaneous or water-catalyzed reactions (k_{o}) of C and Z and spontaneous or water-catalyzed reactions (k_{o}) of C and DA. The deiodination rate constant can be formulated as

$$k = k_0 f_{HA} + k_0' f_A$$

where f_{HA} and f_A are the sum of the fractions of C and Z and of A and DA, respectively. Equation (3) is kinetically equivalent to

$$k = k_{\rm H} \left[{\rm H}^+ \right] f_{\rm A} + k_{\rm o}' f_{\rm A}$$

where $k_o = k_H K_{a2}$ and k_H is the catalytic rate constant for proton attack on A and DA. Substituting $f_A = K_{a2}/([H^+] + K_{a2})$ in Eq. (4),



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Kinetics of Degradation of Levothyroxine

 $k = K_{a2} (k_{H} [H^{+}] + k_{o}')/([H^{+}] + K_{a2})$ · (5)

In the acidic pH region,
$$[H^+] \ge K_{n2}$$
, and Eq. (5) reduces to
 $k = k_{\rm H} K_{n2}$ (6)

In the alkaline pH region, $[H^+] \ll K_{a2}$, and Eq. (5) reduces to $k = k_{o}'$

The catalytic rate constants, $k_{\rm H}$ and $k_{\rm o}'$, were estimated from the best fit of the $\log k$ - pH profiles (Table I). The Arrhenius parameters of the rate constants are included in Table I.

Mechanism of Deiodination

9

The HPLC chromatogram (Fig. 4a) of T₄ partially degraded in acidic solution contains a peak with a retention time corresponding to that of T_3 . The HPLC chromatogram (Fig. 4b) of T₄ partially degraded in alkaline solution contains a peak with a retention time of 4.0 min (Peak D) of an unknown degradate but no peak corresponding to that of T₃. When the initial concentration of T_4 was increased above 5 mg/ml in alkaline degradation, T_3 was found to be the major degradate. The chromatogram (Fig. 4c) of T₃ partially degraded in acidic solution contains a peak with a retention time corresponding to that of 3,5-diiodothyronine (T₂). The chromatogram (Fig. 4d) of T_3 partially degraded in alkaline solution contains Peak D.

When degraded under the same conditions as T_4 at 80°C, T₃ was found to be more stable than T₄ in acidic solution and less stable in alkaline solution (Fig. 3). In alkaline solution, because T_3 was found to be less stable than T_4 and because T₃ was the major degradate at higher T₄ concentration, it can be assumed that T₄ degrades to T₃ and T₃ degrades further to the unknown degradate (Peak D) as soon as it is produced. At higher T₄ concentrations, the high concentration of free iodonium ion in solution may play an important role in the deiodination rate (8). At higher iodonium ion concentrations, the deiodination may follow a different rate law, and T₁ may become relatively stable.

The usual mechanism of the iodination of phenol is a two-step process involving an addition of an iodonium ion to the carbon, followed by a loss of the hydrogen atom being replaced (9). According to the principle of microscopic reversibility, the reverse sequence of these steps constitutes the favored mechanism for the reverse reaction, deiodination

Among the four iodines in the T₄ molecule, those at the 3' and 5' positions are more labile than those at the 3 and 5 positions (3). The substituted phenoxide ion moiety gives 3 and 5' carbons carbanionic character by resonance so that these carbons are favored for electrophilic attack by proton or other electrophiles (Scheme II).

Even though Eqs. (3) and (4) are kinetically equivalent, they imply different mechanisms for T4 deiodination. A simple pH dependency study does not allow differentiation of the two equations and the possible mechanisms consistent with these equations. However, because a proton should be a far more powerful electrophile than water and because the substituted phenoxide ion should be vastly more reactive than the substituted phenol, it is very likely that the proton-



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ation is due to proton attack on the anion and dianion in acidic solution rather than water attack on the cation and zwitterion. The protonation in alkaline solution may be due to water attack on the anion and dianion. Therefore, the reaction scheme consistent with Eq. (4) is a more likely mechanism than that defined by Eq. (3).

Solid-State Degradation

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Low assay values are a concern of most manufacturers of levothyroxine sodium products, and in all probability, it might be attributable to solid-state instability of the drug substance (10).

In contrast to its solution degradation, levothyroxine sodium does not deiodinate in solid state. Rather, the isolated degradation products indicate a deamination reaction (Scheme III). Plots showing the rates of solid-state degradation are presented in Fig. 5. The solid-state degradation exhibited biphasic first-order degradation profiles indicating the possibility of complex degradation pathways. Correlation coefficients of no less than 0.994 were obtained for linear portions of the first-order plots. The initial rate of degradation is much greater than that obtained in the later part of the degradation curve at all temperatures. The rapid degradation phase is more pronounced at higher temperatures. As the temperature is lowered, the initial phase becomes shorter compared to the later phase. The initial phase is almost nonexistent at 50°C. It appears that there is a threshold temperature between 50 and 60°C where levothyroxine sodium degrades rapidly. The biphasic degradation kinetics of levothyroxine sodium can be described by

$$D = D_{o} (A e^{-k_{0}t} + B e^{-k_{0}t})$$

Table II. First-Order Rate Constant for Degradation of T₄ in Solid State in Accordance with $D = D_{1} (A e^{-k_{0}t} + B e^{-t})$

l'emperature (°C)	$10^6 k_a (sec^{-1})$	$10^7 k_{\rm b} ({\rm sec}^{-1})$
50 ± 2 60 ± 2	· · _	171
70 ± 2 80 ± 2	7.19	3.97



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