ELECTROCHEMICAL REDUCTION OF THYMINE IN DIMETHYL SULFOXIDE
AUTOPROTONATION OF THE RADICAL ANION AND REDUCED FREE RADICAL

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ABSTRACT

Thymine is reduced in dimethyl sulfoxide ($E_{1/2}$ of —2.4 V); the decrease in apparent faradaic n from one at infinite dilution to two-thirds at concentrations above 2 mM is due to proton transfer by thymine to the initially formed radical anion and the anion formed on reduction of the resulting free radical, which is more easily reduced than the parent compound. The proton transfer, which is more rapid than the radical anion dimerization, deactivates part of the thymine, forming the more difficultly reducible conjugate base. The latter forms insoluble mercury salts, producing up to three oxidation waves ($E_{1/2}$ between —0.08 and —0.3 V). The free radical reduction, which is not observed for uracil or 2-hydroxypyrimidine, occurs due to steric hindrance by the 5-methyl group toward free radical dimerization, thereby increasing the half-life of the free radical. The effect of added base on the electrochemical behavior is described.

INTRODUCTION

Recently, the electrochemical reductions in dimethyl sulfoxide (DMSO) of 2-hydroxypyrimidine (2-HP) [1] and uracil (2,4-dihydroxypyrimidine) [2] were described. The electrochemical reduction of thymine (5-methyl-2,4-dihydroxypyrimidine), in addition to the intrinsic interest in thymine as a constituent of DNA, may aid in clarifying the specific patterns of 2-HP and uracil reduction, since the 5-methyl group of thymine is close to the postulated C(4) site of added electron residence and dimerization in both previously studied compounds [1,2].

In aqueous and non-aqueous media, the keto-enol equilibrium for hydroxypyrimidines very strongly favors the keto form [3]:

(1)
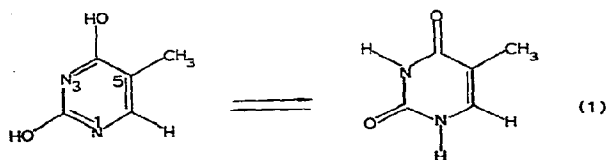
In non-aqueous media (DMSO), thymine, similarly to 2-HP and uracil, is initially reduced in a one-electron ($1e$) step to the radical anion, which reacts with unreduced thymine to produce the neutral free radical and thymine's conjugate base; radical anion dimerization is slower than the competing proton transfer. The reduction mechanism of thymine differs from those for uracil and 2-HP in that, for the latter, the free radical rapidly dimerizes, whereas the thymine free radical is quantitatively reduced to an anion, which also abstracts a proton from thymine to form dihydrothymine and additional conjugate base.

The general nature of the proton transfer, or father-son reaction, and its influence on observed polarographic and voltammetric current and potential response have been discussed [1,2,4].

EXPERIMENTAL

Chemicals

Thymine was obtained from National Biochemicals; aqueous 20% tetraethylammonium hydroxide (TEAH) solution from Aldrich. A 3 M $HClO_4$ solution in DMSO was prepared from aqueous 1 : 1 diluted reagent grade perchloric acid (G. Frederick Smith) (dilution of $HClO_4$ directly with DMSO results in exothermic oxidation of the DMSO). Reagent grade tetra-n-butylammonium perchlorate (TBAP) (G. Frederick Smith) was vacuum dried at 60°C for 48 h. DMSO (Fisher certified ACS; Mallinckrodt analytical reagent; "Baker Analyzed" reagent) was purified by fractional freezing [5]; the cooling bath was H_2O at 12°C to 14°C. Mercury for electrodes was distilled.

Apparatus

A Princeton Applied Research PAR 174 potentiostat was used with a jacketed three-compartment cell thermostatted at 25°C. For rapid cyclic voltammetry, a fast response potentiostat, a single-chamber cell with Luggin capillary, and a faradaic cage were used [6]. The hanging mercury drop electrode was a Metrohm E 410 microfeeder. A Wavetek Model 112 function generator supplied triangular wave forms. Data were acquired on Hewlett-Packard 7005B or Houston 2000 XY recorders, and a Tektronix 502 oscilloscope or a 5103N with 5A15N and 5A18N plug-ins, using a C-5A or C-12 camera. Potentials, monitored with a Hewlett-Packard 3430A or 3440A digital voltmeter, were measured against a modified aqueous saturated calomel electrode (SCE) by the following system: SCE/saturated KCl in water-methylcellulose bridge with asbestos fiber. A Unimetrics 1010 10-μl syringe was used for addition of acid and base solutions.

Procedures

Solutions (0.1 M TBAP as background electrolyte) were deoxygenated by purging with purified N_2 for ca. 30 min; an N_2 atmosphere was maintained in the cell throughout an experiment. D.c. polarographic data were obtained at a controlled 2.0-s drop-time, using a capillary with open-circuit mercury flow-rate

of 0.575 mg s^{-1}. A new Hg drop (effective area = 0.0153 cm^2 [7]) was used for each cyclic voltammogram.

For controlled potential electrolysis, a 7-cm^2 mercury pool was used. Separate electrolyses were performed on 25.0 ml of background and thymine solutions; N$_2$ was used to continuously stir the solution. Current-time profiles were recorded.

Experiments showed no effect on electrochemical behavior from the amounts of water involved in adding TEAH or HClO$_4$.

Roman numbers are used to identify waves and peaks with appended c or a to indicate cathodic or anodic processes, respectively.

Cyclic voltammetric peak charge calculation

Because of the apparent filming nature of some faradaic peaks, the number of coulombs, Q, passed in faradaic peaks was determined. The peak area was defined by extrapolation of the charging current to form a baseline; integration for peak IIc was performed over the potential span from the peak foot to the potential at which the current returned to the baseline; integration of the total area for peaks Ia, IIa and IIIa was performed from the peak Ia foot to the switching potential for the positive scan and from the switching potential to the point at which the current crossed the baseline for the negative scan. The integrated area (measured by a Gelman Model 39231 planimeter) was multiplied by the read-out device E- and i-axis sensitivities and divided by the scanrate to yield the charge passed in μC.

RESULTS AND DISCUSSION

D.c. polarography

The concentration-dependence of the thymine diffusion current constant $(I_d = \bar{i}_1/c\ m^{2/3}\ t^{1/6})$ is shown in Fig. 1; extrapolation at low c to infinite dilution yields an I_d of 1.3 μA s$^{1/2}$ mM^{-1} mg$^{-2/3}$, which corresponds to a diffusion coefficient, D, of 4.6×10^{-6} cm^2 s^{-1}. Use of the Matsuda equation [8], which considers shielding effects and best describes the i_d–t behavior at controlled drop-times [7], gives a D of 4.0×10^{-6}. The data at c exceeding 1.7 mM yield an I_d of 0.77.

$E_{1/2}$ is -2.41 V at 0.06 mM, -2.39 V at 0.2 mM, and -2.36 V above 1 mM. Although this shift is near experimental uncertainty (0.01 V), it parallels the concentration-dependence of I_d and may, therefore, be real. The $E_{1/2}$–c variation closely parallels that for uracil; $E_{1/2}$ for thymine is ca. 0.07 V negative of that for uracil below 1 mM and ca. 0.05 V above 1 mM.

Thymine and uracil [2] have an infinite dilution I_d of 1.3, which, presumably, represents a 1e reduction; the I_d of 0.77 above 1 mM, which is 15% larger than that for uracil, represents an n of 0.59. Thus, at high c, either 31% of the radical anion formed on 1e reduction dimerizes with 69% being protonated by unreduced thymine or part of the free radical formed on protonation is reduced in an e.c.e. reaction. Dimerization of a significant fraction of radical anion implies a reversal in the relative protonation and dimerization rates as compared
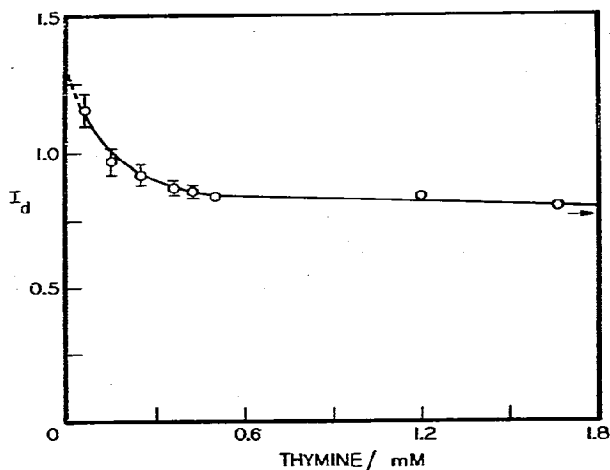
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Fig. 1. Concentration-dependence of the d.c. polarographic diffusion current constant (I_d) for thymine, based on mean currents. The horizontal arrow corresponds to I_d at 2.0 and 2.5 mM. Uncertainties are one standard deviation.

to uracil, for which virtually 100% of the radical anion is protonated. An e.c.e. mechanism requires that the free radical dimerization be considerably slower than that for uracil, which consumes 100% of the free radical.

Cyclic voltammetry

The cyclic voltammetric behavior of thymine (Figs. 2 and 3) is similar to those of 2-HP [1] and uracil [2]. Reversal of the potential scan negative of the thymine reduction produces anodic peaks positive of —0.3 V; when the scan is again reversed, a cathodic peak (IIc) is seen at ca. —0.3 V, which has the characteristic shape of film stripping. At v of 600 to 1200 V s^{-1}, a minute anodic peak, possibly due to radical anion or free radical oxidation, occasionally appears about 0.2 V positive of the thymine reduction peak.

At slow v and thymine anion concentrations above 0.8 mM, three anodic peaks appear. Two unresolved or partially resolved peaks constitute IIc (Fig. 3). The film behavior suggests significant differences in ease of formation and reduction of different salt layers (cf. Nature of thymine-mercury film).

At 0.06 to 0.35 V s^{-1}, E_p shifts linearly with log v (—0.06 V decade^{-1}); at 0.12 V s^{-1}, E_p is —2.44 V, independent of concentration (0.43 to 2.5 mM). Background impurity problems at potentials negative of —2.5 V caused uncertainties in i_p evaluation at higher v.

The peak current function, $i_p/Acv^{1/2}$, is 281 for 0.43 mM thymine at v above 0.1 V s^{-1} and 248 for 2 mM at 0.35 V s^{-1}; the predicted faradaic n is 0.67 at 0.43 mM and 0.59 at 2.0 mM, based on theoretical $i_p/Acv^{1/2}$ of 422 for an irreversible 1e transfer with a transfer coefficient, α, of 0.5 and D of 4×10^{-6}. These n values predict I_d within ±0.01 of those observed. Thus, the $i_p/Acv^{1/2}$ concentration-dependence parallels that of I_d.
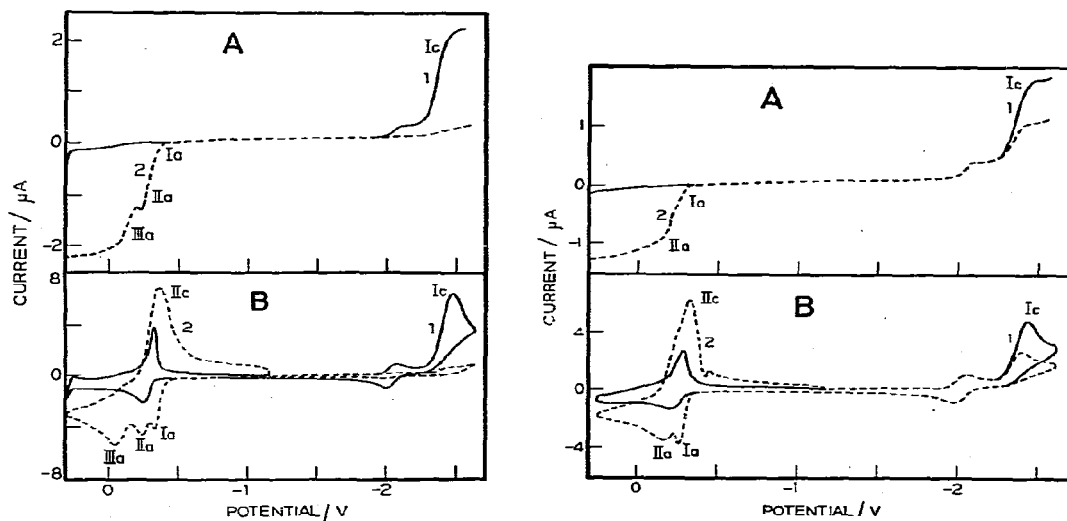
Fig. 2. D.c. polarographic and cyclic voltammetric behavior of a 2.50 mM thymine solution. Curves 1 (solid lines) are before controlled potential electrolysis; curves 2 (dashed lines) are after electrolysis; where curves 1 and 2 coincide, only the dashed line is shown. (A) D.c. polarograms with 2 s drop-time and 0.58 mg s^{-1} mercury flow-rate; (B) cyclic voltammograms with 0.06 V s^{-1} scan-rate and 0.0153 cm^2 electrode area. The unlabelled wave and peaks between -2.0 and -2.1 V are due to a solvent impurity.

Fig. 3. Effect of added base on the d.c. polarographic and cyclic voltammetric behavior of 1.67 mM thymine solution. Curves 1 (solid lines) are without added base; curves 2 (dashed lines) are with 0.92 mM TEAH present; where curves 1 and 2 coincide, only the dashed line is shown. (A) D.c. polarograms with 2 s drop-time and 0.58 mg s^{-1} mercury flow-rate; (B) cyclic voltammograms with 0.06 V s^{-1} scan-rate and 0.0153 cm^2 electrode area. The unlabelled wave and peaks between -2.0 and -2.1 V are due to a solvent impurity.

Effect of added acid

The only available protonation sites on thymine are the acidic exocyclic oxygens. Since added acid could only serve as a proton donor, replacing thymine in the protonation steps following reduction, the effect of added acid was not investigated.

Effect of added base

Addition of base (TEAH) at 1 : 1 ratio for 1.67 mM thymine decreases wave Ic and produces waves Ia to IIIa ($E_{1/2} = -0.3, -0.23, -0.08$; Fig. 3; Table 1); polarographic and voltammetric behavior of the electrolyzed solution (Fig. 2) are equivalent to that of 1.67 mM thymine with 1 : 1 added TEAH (cf. c.p.e. discussion). $E_{1/2}$ shifts slightly negatively with increasing added base, e.g., -0.03 and -0.04 V for Ia and IIa, respectively, for a two-fold increase; $E_{1/2}$ for IIIa is independent of anion concentration. E_p shifts parallel those of $E_{1/2}$. At the potential wave Ia reaches an i_1 density of ca. 45 μA cm^{-2} without a discernable plateau, IIa begins to appear; distinction between the two waves is evident from

TABLE 1

Variation of the DME waveheights for 1.67 mM thymine in DMSO on addition of tetraethyl-ammonium hydroxide

[TEAH] mM	i_1/μA for wave			I_d [a]
	Ic	Ia + IIa	IIIa	
0.0	1.22	0	0	0.80
0.43	0.89	0.51	0	0.92
0.92	0.58	1.08	0	1.09
1.34	0.24	1.14	0.44	1.19
1.67	≤0.05	1.10	0.84	≤1.31

[a] Summation diffusion current constant for waves Ic, Ia, IIa and IIIa.

an inflection in the i—E profile and the obvious filming nature of single-drop i—t profiles on Ia, which is not evident in the IIa process. Wave IIIa does not appear until the height of Ia plus IIa reaches its limiting value of 120 μA cm^{-2}. At high anion concentration, IIa shows a slight maximum (Figs. 2 and 3).

Controlled potential electrolysis

Subtraction of background current, i_b, from total current, i_t, on CPE at —2.42 V of 2.50 mM thymine gave the current due to thymine reduction, i; the ln i vs. t plot is linear only after 2400 s (Fig. 4). The i—t data, planimeter integrated over the first 2500 s and mathematically integrated from 2500 s to infinity, gave an n of 0.73 ± 0.02.

Polarographic and voltammetric patterns for the thymine solution before and after c.p.e. are shown in Fig. 2. Because peaks Ia to IIIa and IIc are not ob-
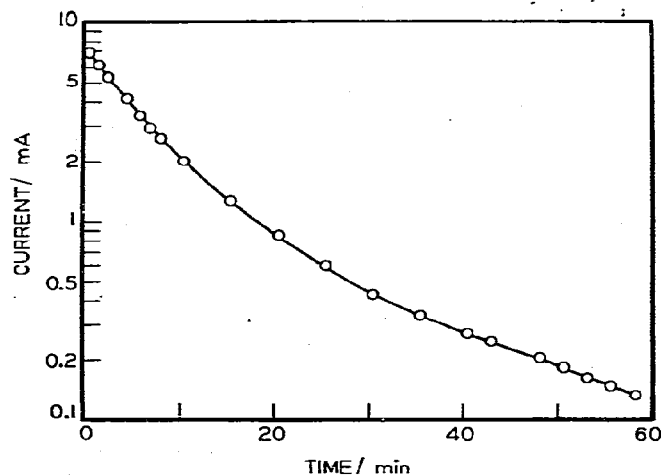

Fig. 4. Variation of the current with time during the controlled potential electrolysis of 2.50 mM thymine.

served before or after electrolysis of background alone, these are due to products of the thymine reduction. Addition of $HClO_4$ to the electrolyzed solution decreased Ia and IIa, and caused Ic to reappear; addition of sufficient acid to eliminate Ia and IIa gave the Ic wave height expected for 1.67 mM thymine. Addition of TEAH in a 1 : 1 ratio for 1.67 mM thymine produced polarographic and voltammetric results identical to those obtained after c.p.e.; I_d for the thymine anion is 1.29.

The latter data indicate that two-thirds of the thymine is consumed by a non-faradaic process, e.g., deprotonation. Since the n of 0.73 corresponds to slightly more than 2e process for one-third of the thymine, all of the free radical formed on protonation of the initial 1e reduction product is reduced in a second 1e process whose product reacts with thymine to generate thymine anion and dihydrothymine. Thus, the anion formed on free radical reduction is more basic than thymine itself.

The correspondence between cathodic and anodic I_d for both thymine and uracil confirms the 1e nature for an I_d of 1.3. Due to the dependence of the e.c.e. mechanism on supply rate of proton donor, i.e., unreduced thymine, the larger n obtained on c.p.e. compared to d.c. polarography at the same concentration is reasonable because of the convective stirring.

Although c.p.e. n might be expected to reflect some weighted average of the d.c. polarographic concentration-dependent n (Fig. 1), i.e., the bulk concentration of reactants decrease as electrolysis proceeds, the 1.67 mM thymine anion in electrolyzed solution clearly indicates that the entire electrolysis proceeds via the expected high concentration mechanism.

Product characterization

It was not feasible to isolate individual dimetric and dihydrothymine products and to determine their structure, e.g., chromatography of electrolyzed solutions could not yield definitive results due to product instability and, even if successful, would not allow differentiation between anionic products and their protonated forms. Product recovery by freeze-drying was impractical due to background electrolyte present and likelihood of alteration during evaporation. It was not possible to locate a solvent immiscible with DMSO, which would completely extract the reaction products without removing background electrolyte and from which the unaltered products could be recovered. Spectral identification of reduction products was only partially successful due to absence of reference spectra and instability of the likely products [9].

Consequently, the products have been characterized largely on the basis of faradaic patterns, behavior of solutions resulting from preparative electrolysis, well-characterized products obtained from related pyrimidines, reported products on radiolysis, and deductions based on electronic structure.

Nature of mercury-thymidine film

The mercury-thymine salt presumably involves Hg(I), as do the 2-HP and uracil anions [1,2]. The peak IIa shape suggests that the film deposited by Ia is not passivating in nature, i.e., IIa does not show a drawn-out leading edge

(Figs. 2 and 3). The drawn-out nature of IIIa indicates that the film deposited by Ia plus IIa is passivating.

The amount of salt reduced in peak IIc, Q_c, is less than that deposited, Q_a, in peaks Ia, IIa plus IIIa. At 0.12 V s^{-1}, Q_c/Q_a is 0.5, independent of Q_a/A magnitude (150 to 979 μC cm^{-2}); it is 0.7 at 0.36 V s^{-1}, independent of Q_a/A (98 to 436 μC cm^{-2}).

Three possible magnitudes for the electrode area occupied by a thymine anion can be conceived, based on an anion diameter of 0.85 nm, parallel to the ring plane and thickness of 0.4 nm, assuming free methyl group rotation (CPK molecular models [10] were used to estimate molecular dimensions). For a flat orientation, the molecular swept area is 0.572 nm^2 (2.90 $\times$ 10^{-10} mol cm^{-2}; 28.0 μC cm^{-2}); for a perpendicular orientation, the molecular area for close packing is 0.806 nm^2 (5.4 $\times$ 10^{-10} mol cm^{-2}; 52 μC cm^{-2}) if N(1), N(3) or O(4) lies flat on the surface, or 0.277 nm^2 (6.0 $\times$ 10^{-10} mol cm^{-2}; 58 μC cm^{-2}) if O(2) lies flat on the surface.

The probable reason for Q_c/Q_a less than one is the number of layers of salt deposited by Ia to IIIa (Table 2); the minimum number of layers stripped by IIc at 1.67 mM added TEAH is five. Such a large number of layers, which corresponds to a film thickness of 4—5 nm clearly suggests that small Q_c/Q_a are caused by mechanical film detachment due to non-adherence, whose extent would be expected to increase with both the amount of film deposited and the

TABLE 2

Number of layers of thymine anion deposited by cyclic peaks Ia, IIa and IIIa (m_a) and stripped from the electrode surface by peak IIc (m_c) as functions of scan-rate, amount of added base and assumed orientation [a]

[TEAH] mM	Parallel [b]		Perpendicular [b,c]		Perpendicular [b,d]	
	m_a	m_c	m_a	m_c	m_a	m_c
0 [e]	5.4	2.8	2.9	1.5	2.6	1.3
0.43 [e]	11.6	6.0	6.2	3.2	5.6	2.9
0.92 [e]	19.7	10.4	10.5	5.5	9.6	5.0
1.34 [e]	24.2	14.0	13.0	7.5	11.8	6.8
1.67 [e]	34.9	16.7	18.7	8.9	16.9	8.1
0 [f]	3.5	2.5	1.9	1.4	1.7	1.2
0.43 [f]	8.0	5.6	4.3	3.0	3.9	2.7
0.92 [f]	12.7	8.1	6.8	4.4	6.1	3.9
1.34 [f]	11.8	9.7	6.3	5.2	5.7	4.7
1.67 [f]	15.5	11.1	8.3	5.9	7.5	5.4

[a] Data are based on integrated area of cyclic voltammetric peaks for 1.67 mM thymine (cf. Experimental section for method of peak area evaluation); assumed molecular dimensions for conversion from total charge under peaks to m_a or m_c are given in the discussion of mercury-thymine salt nature; electrode area was 0.0153 cm^2.

[b] Assumed orientation of plane of the thymine ring relative to the electrode surface.

[c] N(1), N(3) or O(4) is assumed to be oriented toward the surface.

[d] O(2) is assumed to be oriented toward the surface.

[e] Scan-rate was 0.12 V s^{-1}.

[f] Scan-rate was 0.35 V s^{-1}.

time from deposit to stripping; the observed results show both dependencies.

The large number of layers deposited and stripped indicates strong binding of the Hg(I)-thymine salt to the Hg surface and strong cohesive forces between salt layers. Such strong cohesion was not observed for the Hg(I)-uracil salt [2]. The larger salt deposit attainable with thymine, which is indicative of lower monolayer passivation, may be due to the less closely packed pyrimidine rings for a perpendicular orientation (due to space required by the methyl groups), thereby facilitating diffusion of Hg(I) through the deposit to form additional layers. The larger fraction of salt stripped on reduction at fast v indicates that the major source of film loss is mechanical detachment preceding or during film reduction. The shape of the film reduction peak on slow v and its partial resolution into two peaks for high film deposits indicate a difference in ease of reduction between layers. At faster v and high anion c, only one peak appears at a potential corresponding to the more difficultly reducible layers; hence, the layers closest to the electrode surface seem to be the most difficult to reduce.

MECHANISM

Several aspects of the behavior expected for the mechanism, which best fits the information available (Fig. 5), can be specified. Reduction of $\dot{R}H_2$ is generally easier than that of RH. Based on studies of similar compounds [2,11], k_3
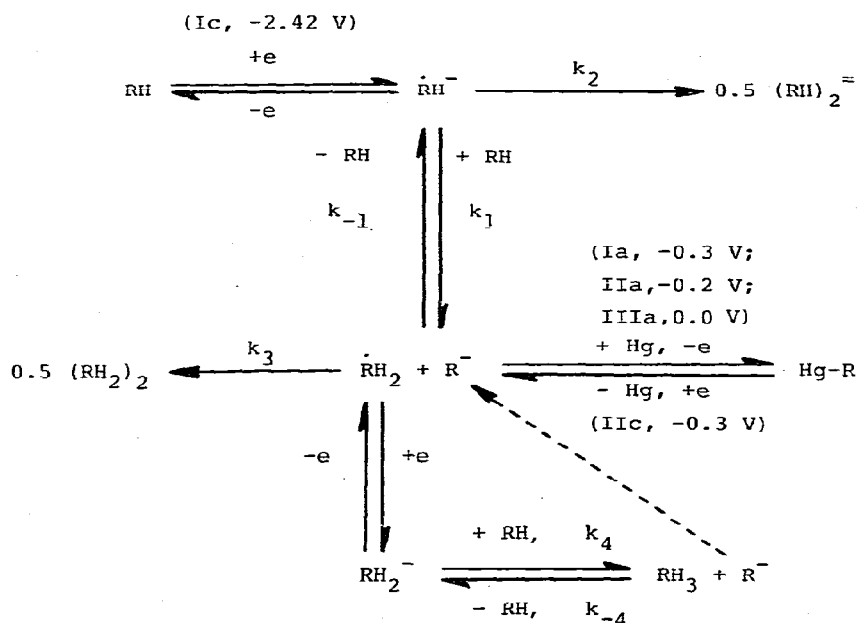
Fig. 5. Reduction mechanism for thymine in DMSO. Symbols: RH, thymine; $\dot{R}H^-$, radical anion, $\dot{R}H_2$, free radical; RH_2^-, reduced free radical; RH_3, dihydrothymine; R^-, thymine anion. The k represent rate constants; the Roman numbers are wave and peak designations (cf. Fig. 3). Potentials are E_p for 1.67 mM thymine; for Ia, IIa, IIIa and IIc, solution is also 1.67 mM in TEAH.

for $\dot{R}H_2$ dimerization may be 10^6 to 10^8 M^{-1} s^{-1}; because of electrostatic repulsion, $\dot{R}H^-$ dimerization should be slower, e.g., k_2 of 10^4 to 10^6 M^{-1} s^{-1}. Because the protonation does not involve reaction between similarly charged molecules with concomitant repulsion and radical anions are very basic, protonations of $\dot{R}H^-$ should be more rapid than its dimerization, i.e., k_1 is greater than k_2; however, since the protonation does not involve two molecules with the strong reaction tendency of free radicals, e.g., dimerization where charge repulsion is not involved, the protonation is expected to be slower than dimerization of $\dot{R}H_2$, i.e., k_1 less than k_3.

These kinetic relations may not be valid for thymine in respect to the $\dot{R}H_2$ dimerization; at high c where dimerization should be favored, all of the initially reduced thymine undergoes a second $1e$ transfer. This presence of an e.c.e. process, which is not observed for uracil [2], suggests that $\dot{R}H_2$ dimerization for thymine is slower than that of uracil; this is reasonable, if dimerization occurs at C(4), C(5) or C(6), since the 5-methyl group on thymine might sterically hinder dimerization.

The thymine anion, as expected, is much more difficultly reducible than thymine itself; its reduction is not observed within the available potential range.

Calculated and experimental ease of reduction

Published calculations of molecular orbital energy levels are fewer for thymine than for uracil (cf. ref. 2 and references cited therein.) The calculated 0.03 V difference in electron affinities between uracil [12] and thymine [13] is in good agreement with the difference in $E_{1/2}$ (0.07 V at 0.2 mM to 0.05 V above 1 mM); the small difference is within the uncertainty of the calculated electron affinities. Thus, thymine reduction appears to be kinetically hindered, similarly to uracil [2], due to a combination of heterogeneous and homogeneous kinetic effects. This is supported by the agreement between $i_p/Acv^{1/2}$ at 0.25 V s^{-1} and I_d, assuming an α of 0.5. The latter, which is larger than the α of 0.39 observed for uracil [2], may be due to involvement of the second step.

Sites of electron entrance and residence

Since rapid electron rearrangement may occur after initial addition to pyrimidine [14], the structure of detectable intermediates and final products is not conclusive regarding the site of electron injection.

The probable site of the added electron on uracil reduction in DMSO is C(4), once the generated anion is protonated [2]. The radical anion in basic aqueous media is predicted to be the 3-H lactim with the unpaired electron delocalized [15]. The electron first added to thymine likely also resides at C(4) after protonation. The electron density, bond order and free valence in the thymine C(5)=C(6) region are high, in agreement with observed chemical oxidation at 5,6 C=C [13]; the latter, consequently, is unlikely to be the site of initial reduction, in as much as the most easily oxidized site is rarely also the most easily reduced site.

The strongest argument for initial $1e$ reduction at C(4) rather that at the

other C=O site, C(2), is the fact that the thymine free radical is reduced while the uracil free radical dimerizes before it can be reduced [2]. The considerably slower thymine free radical dimerization suggests that the 5-methyl group might influence the dimerization rate through steric hindrance, a likely effect only if the dimerization site were C(4). Reduction at C(4) is also supported by Hayon's observation that on pulse radiolysis of thymine the ketyl radical at C(4) is formed [9]. Thus, the free radical formed on initial 1e reduction appears to be species I. The second electron likely also goes onto C(4), followed by proton abstraction to form species II.

(I)   (II)

Concentration and kinetic dependence of reduction process

The high-concentration n of 0.59 seen on d.c. polarography indicates that species I is not quantitatively reduced to II. This lower yield of II, as compared to c.p.e., is likely due to the small unreduced thymine concentration near the electrode surface on polarography and to the relative acidity of I and thymine. The free radical pK_a of 7.2 ± 0.3 [9] makes I more acidic by ca. two pK units than thymine. Thus, although the electrochemical results clearly indicate that thymine is chemically consumed to yield the conjugate base, from which formation of the free radical can be inferred, the protonation reaction must be explained by kinetic, rather than equilibrium, arguments.

Any reaction consuming the free radical, e.g., dimerization or reduction, should shift the proton transfer equilibrium to favor free radical formation. At sufficiently low proton donor (thymine) concentration near the electrode surface, e.g., d.c. polarography on the i_l plateau, the radical anion must diffuse from the electrode into a region of higher thymine concentration to acquire a proton. Under such circumstances, appreciable dimerization of free radical occurs before it can diffuse back to the electrode and be reduced. At high thymine concentration near the electrode, e.g., c.p.e. with convective stirring, the radical anion will be protonated near the electrode surface and the free radical will be reduced more rapidly than it is consumed by the sterically hindered dimerization.

The high-concentration mechanistic process differs from that of uracil [2] principally in terms of free radical dimerization rate. The pK_a of 7.3 ± 0.3 for the uracil free radical [9] indicates that kinetic, rather than equilibrium, arguments must also explain radical anion protonation by unreduced uracil. However, the uracil free radical dimerization is not sterically hindered and is extremely rapid; thus, even on c.p.e., the free radical dimerizes before it can be reduced.

The low-concentration results, i.e., n approaching one, agree with the u.v.

248

spectral behavior of thymine on adding TEAH. The inability of TEAH at a
3 : 1 ratio to deprotonate 0.047 mM thymine indicates that the thymine to
radical anion proton-transfer reaction is unlikely at low thymine concentration.
The 0.35 mM TEAH concentration necessary to convert all thymine to its con-
jugate base at this thymine level is only slightly below the thymine concentra-
tion at which the I_d—c relation plateaus, suggesting that TEAH and the thy-
mine radical anion have equivalent basic strength in DMSO, possibly due to
ion-pairing of OH⁻. The possibility that a larger than 1 : 1 TEAH: thymine
ratio is generally required for complete thymine conversion to its conjugate
base is precluded by behavior at 1.67 mM thymine, for which wave Ic dis-
appears at 1 : 1 added TEAH.

At low concentration, e.g., below 0.4 mM, the biomolecular protonation
reaction is slowed under d.c. polarographic conditions to the point that the
radical anion must diffuse far from the electrode before the protonation rate
becomes appreciable. Although the fate of the radical anion under these condi-
tions is unclear, dimerization seems more likely than protonation followed by
dimerization of the resulting free radical. Since the pK_a of thymine and its free
radical indicate that protonation is not favored, and since free radical forma-
tion far from the electrode results in a low free radical concentration, its dimer-
ization will be slow; without the driving force of a rapid, irreversible following
reaction, the proton transfer reaction will not proceed appreciably, and the
ultimate fate of the radical anion will be dimerization. Thus, very little thymine
is consumed by the protonation reaction and the observed n approaches one.

ACKNOWLEDGMENTS

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