

PII: S0040-4039(96)01241-5

Polar Substituent and Solvent Effects on the Kinetics of Radical Reactions with Thiols

Christopher Tronche, Felix N. Martinez, John H. Horner, and Martin Newcomb*

Department of Chemistry, Wayne State University, Detroit, Michigan 48202, USA

Martin Senn and Bernd Giese*

Department of Chemistry, University of Basel, St. Johanns Ring 19, CH-4056 Basel, Switzerland

Abstract: The rates of thiol trapping of radicals depend upon polar substituents (Table 1) and solvent effects (Table 2). The rate accelerating effect of water on the reactions of biologically relevant radicals is of special importance. Copyright © 1996 Elsevier Science Ltd

Radical reactions in biological processes have been an area of growing interest in recent years, and the chemistry of DNA radicals is an especially highly targeted area of study because a number of anti-cancer agents are known to act by destroying DNA via formation of radical intermediates.¹ 4'-DNA radicals are of particular interest because they undergo spontaneous strand cleavage in competition with trapping reactions by H-donors or by O_2 .² Although qualitative observations concerning product formation and quantitative relative rate constants from competition studies can be determined, absolute rate constants for these radical reactions are not known because an absolute kinetic scale for 4'-DNA radicals does not yet exist. The significant questions to address in the development of such a rate scale are (1) what are the general consequences of multiple oxygenated substituents on radical kinetics, and (2) how are radical kinetics affected by the highly polar aqueous media of biological systems? In this Letter, we report preliminary results of the kinetics of alkoxy substituted radical clocks and thiol trapping that address both points.

The series of isostructural radicals 1a-d was used to evaluate the general effects of single and double alkoxy substitution on radical kinetics. These radicals, produced by laser flash photolysis (LFP, Nd-YAG, 355 nm) of the corresponding PTOC esters precursors,³ cyclize in a 6-*exo* fashion to give diphenylalkyl radical products 2a-d that are readily monitored by UV spectroscopy. The method and cyclization rate constants for radicals 1a and 1b in THF were reported previously.⁴ Arrhenius functions for the cyclizations of radicals 1 were determined in various solvents over the temperature range of 0-40 °C, and the results are given in Table 1. The data demonstrate that the rate of cyclization is enhanced only slightly by increasing the solvent polarity.⁵



a: R = R' = H; **b**: R = OCH₃, R' = H; **c**: R = H, R' = OCH₃; **d**: R = R' = OCH₃

Radical	Solvent	Arrhenius function ^a	$k_{\rm c}^{\rm b}$ (25 °C) s ⁻¹	$k_{\rm T}^{\rm c}$ (30 °C) M ⁻¹ s ⁻¹
1a	THF CH₃CN CH₃CN/H₂O (50:50)	9.52 - 5.27/ 0 9.61 - 5.18/ 0 10.12 - 5.67/ 0	4.5×10^{5} 6.4×10^{5} 9.1×10^{5}	$(1.9 \pm 0.3) \times 10^7$
1 b	THF CH₃CN	8.76 – 4.87/ 0 9.56 – 5.68/ 0	1.5×10^{5} 2.4×10^{5}	$(2.0 \pm 0.3) \times 10^7$
1 c	THF CH₃CN	9.47 4.38/ 0 9.79 4.74/ 0	1.8×10^{6} 2.0×10^{6}	$(1.0 \pm 0.1) \times 10^7$
1 d	THF CH₃CN CH₃CN/H₂O (50:50)	9.75 – 6.07/0 9.18 – 5.12/0 10.19 – 6.38/0	2.0×10^{5} 2.6×10^{5} 3.2×10^{5}	$(2.1 \pm 0.4) \times 10^6$

Table 1. Rate constants for cyclization reactions (k_c) and trapping reactions by octadecanehiol (k_T) of radicals 1.

a $\theta = 2.3RT$ in kcal/mol; ^b rate constant at 25 °C from Arrhenius function; ^c bimolecular rate constant for reaction of radicals 1 with octadecanethiol in THF at 30 °C; errors are 2 σ .

Second order rate constants for reactions of radicals 1 with octadecanethiol in THF at 30 °C were measured directly, and the results are also listed in Table 1. Unfortunately, the PTOC precursors for 1 were unstable in aqueous solutions containing thiols precluding solvent effect studies. Therefore alkyl radical 3 was used to study the effect of H_2O on the rate of H-abstraction from thiols.



Radical 3 was produced from the corresponding PTOC ester at 30 °C in the presence of various concentrations of *t*-BuSH or glutathione (GSH) diethyl ester.⁶ Rate constants for cyclization (k_c) of 3 determined by competitive trapping by Bu₃SnH have been reported,^{7a} but we recalibrated the cyclization of 3 at 30 °C by competition against PhSH^{7b} trapping; at 30 °C in cyclohexane the rate constant was 1.6×10^5 s⁻¹. With the assumption that the cyclization is nearly insensitive to solvent effects (see Table 1), rate values for the H-transfer (k_T) were measured and are collected in Table 2.

Thiol	Solvent	$k_{\rm T}/k_{\rm c}$	$k_{\rm T}^{\rm a}$ (30 °C) ${\rm M}^{\rm -1} {\rm s}^{\rm -1}$
t-BuSH	CH ₃ CN CH ₃ CN/H ₂ O (50:50)	21.7 83.4	$(3.5 \pm 0.3) \times 10^{6}$ $(1.3 \pm 0.1) \times 10^{7}$
glutathione diethyl ester	CH ₂ Cl ₂ CH ₃ OH CH ₃ CN CH ₃ CN/H ₂ O (75:25) CH ₃ CN/H ₂ O (50:50) CH ₃ CN/H ₂ O (20:80)	22.9 53.6 41.6 71.4 147 372	$\begin{array}{c} (3.7 \pm 0.2) \times 10^6 \\ (8.6 \pm 0.4) \times 10^6 \\ (6.7 \pm 0.4) \times 10^6 \\ (1.1 \pm 0.1) \times 10^7 \\ (2.4 \pm 0.1) \times 10^7 \\ (6.0 \pm 0.5) \times 10^7 \end{array}$

Table 2. Rate constants for thiol trapping of radical 3 at 30 °C.

^a Bimolecular rate constant using a cyclization rate k_T (3->4) of 1.6×10^5 s⁻¹ (cyclohexane, 30 °C).

The data of Tables 1 and 2 demonstrate that the rates of H-transfer from alkyl thiols to alkyl radicals depend upon the radical substituents and the polarity of the solvent. Thus, the α,β -dimethoxy substituted radical 1d reacts about 10 times slower than the unsubstituted radical 1a (Table 1). This rate decrease is caused by polar and stability effects of the methoxy substituents at the radical. A rate decreasing polar effect of the β -methoxy substituent is demonstrated by radical 1c which reacts two times slower than the unsubstituted alkyl radical 1a. A more extreme example for this polar effect is the reaction of an electron deficient perfluoroalkyl radical with PhSH which is a factor of 500 less rapid than reaction of an alkyl radical.⁸ Because of polar effects, one could expect a rate increase with α -methoxy substituted radical 1b compared to the unsubstituted radical 1a. However, both radicals abstract a H-atom from octadecanethiol with the same rate. Presumably the influence of the polar effect is compensated by the radical stabilizing effect of the α -methoxy group. In radical 1d this α -methoxy stabilizing effect is even more pronounced because of the synergistic effect of the two methoxy substituents.⁹ The same trend has been observed in pulse radiolysis studies in water where the α -hydroxy radical formed from ethanol reacts with dithiothreitol about four times faster than does the α,β -dihydroxy radical produced from ethylene glycol.¹⁰

The kinetic measurements listed in Table 2 show that the thiol trapping rate constants increase as the water content of the solution is increased. For the glutathione diethyl ester reaction with 3, the rate enhancement upon proceeding from CH₃CN to a 4:1 H₂O/CH₃CN mixture is about an order of magnitude. Replacing the solvent CH₂Cl₂ by the more polar CH₃CN or CH₃OH increases rates of the hydrogen abstraction by a factor of 1.8 and 2.3, respectively.¹¹ From these kinetic measurements in different solvents, a highly polarized transition state 6 for hydrogen transfer from thiols is apparent.¹²

$$R^{\bullet} + H - SR' \longrightarrow \left[\begin{matrix} \delta_{+} \\ R^{\dagger} \\ R^{\dagger} \end{matrix} \right]^{\bullet} R^{\bullet} R - H + R'S^{\bullet} \\ 6 \\ 6 \\ R^{\bullet} \\ R^{\bullet}$$

In conclusion, polar substituent effects and the rate accelerating effect of water on the kinetics of thiol reactions with radicals must be taken into consideration for reactions occurring under biochemical conditions.¹³

Acknowledgment: This work was supported by grants from the National Science Foundation (CHE-9917929) and the Swiss National Science Foundation.

References and Notes

- 1. a) Leading reviews: Stubbe, J.; Kozarich, J. W. Chem. Rev. 1987, 87, 1107-1136; b) Nicolaou, K. C.; Dai, W.-M. Angew. Chem. Int. Ed. Engl. 1991, 30, 1387-1416; c) Pratviel, G.; Bernadou, J.; Meunier, B. Angew. Chem. Int. Ed. Engl. 1995, 34, 746-770.
- Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Petretta, M.; Schwitter, U. Chem. & Biol. 1995, 2, 367-375.
 The PTOC precursors to 1a and 1b have been reported,⁴ and the PTOC precursors to radicals 1c and 1d were prepared from the corresponding carboxylic acids by conversion to the acid chlorides (CO₂Cl₂) and reaction with the sodium salt of N-hydroxypyridine-2-thione by the reported method.⁴ All PTOC precursors had appropriate ¹H and ¹³C NMR spectra. Reaction of two equivalents of PhMgBr with 5-bromopentanoyl chloride, acid-catalyzed dehydration of the resulting alcohol, reaction of the resulting bromoalkene with NaCN, and hydrolysis of the nitrile gave 6,6-diphenyl-5-hexenoic acid. The corresponding acid chloride was allowed to react with the anions from methyl acetate (for 1c) and methyl methoxyacetate (for 1d). The resulting β-keto esters were reduced with NaBH4, the alcohols were methylated, and the esters were saponified to give 3-methoxy-8,8-diphenyl-7-octenoic acid and 2,3-dimethoxy-8,8-diphenyl-7-octenoic acid, respectively. All intermediates were characterized by NMR spectroscopy, and the carboxylic acids were characterized by ¹H and ¹³C NMR spectroscopy and high resolution mass spectrometry.
- 4. a) Newcomb, M.; Horner, J. H.; Filipkowski, M. A.; Ha, C.; Park, S. U. J. Am. Chem. Soc. 1995, 117, 3674-3684; b) Johnson, C. C.; Horner, J. H.; Tronche, C.; Newcomb, M. J. Am. Chem. Soc. 1995, 117, 1684-1687.
- 5. In early experiments K. U. Ingold and coworkers have observed that hexenyl radicals cyclize in hydrocarbons and THF with similar rates, see: Maeda, Y.; Schmid, P.; Griller, D.; Ingold, K. U. J. Chem. Soc., Chem. Commun. 1978, 525-526.
- 6. About 0.040 mmol of the PTOC ester (Lusztyk, J.; Maillard, D.; Deycard S.; Lindsay, D. A.; Ingold, K. U. J. Org. Chem. 1987, 52, 3509-3514) was dissolved in degassed solvent (20 mL) under argon in a thermostated irradiation flask (Pyrex glass). After addition of the thiol (0.182 to 0.667 mmol), the reaction mixture was immediately irradiated with a 100 W tungsten filament bulb (Phillips Spotline R95) for 5 min at 30 °C. The products were detected by GC and identified by comparison with literature data (Walborsky, H. M.; Topolski, M.; Hamdouchi, C.; Pankowski, J. J. Org. Chem. 1992, 57, 6188-6191). The yield was nearly quantitative.
- 7. a) Chatgilialoglu, C.; Ingold, K. U.; Scaiano, J. C. J. Am. Chem. Soc. 1981, 103, 7739-7742. b) Franz, J. A.; Bushaw, B. A.; Alnajjar, M. S. J. Am. Chem. Soc. 1989, 111, 268-275.
- 8. Rong, X. X.; Pan, H. Q.; Dolbier, W. R.; Smart, B. E. J. Am. Chem. Soc. 1994, 116, 4521-4522.
- 9. Korth, H.-G.; Sustmann, R.; Giese, B.; Rückert, B.; Gröninger, K. S. Chem. Ber. 1990, 123, 1891-1898. In contrast to the stabilizing effect of α , β -dialkoxy substituents, a β -alkoxy substituent does not stabilize a radical; see: Crich, D.; Beckwith, A. L. J.; Chen, C.; Yao, Q.; Davison, I. G. E.; Longmore, R. W.; de Parrodi, C. A.; Quintero-Cortes, L.; Sandoval-Ramirez, J. J. Am Chem. Soc. 1995, 117, 8757-8768.
- 10. Akhlaq, M. S.; Al-Baghdadi, S.; von Sonntag, C. Carbohydr. Res. 1987, 164, 71-83.
- 11. Since the rates of cyclization reactions are slightly increased by increasing the polarity of the solvent (Table 1), the thiol trapping rates of Table 2 are the minimum values.
- 12. An inverse solvent effect in H-transfer from the OH-group of phenols and hydroperoxides to alkoxyl radicals was observed by Lusztyk and coworkers: Avila, D. V.; Ingold, K. U.; Lusztyk, J.; Green, W. H.; Procopio, D. R. J. Am. Chem. Soc. 1995, 117, 2929-2930. The inverse solvent effect was explained by hydrogen bond donor effects of the solvent towards the OH group of the educts.
- 13. According to the data of Tables 1 and 2, the 4'-DNA radicals should be trapped by thiols such as glutathione diethyl ester with a rate of ca. $10^7 \text{ M}^{-1} \text{ s}^{-1}$ at 30 °C in water as solvent (see also ref 10).

(Received in USA 13 May 1996; accepted 18 June 1996)