The Effect of Thiols on the Dehalogenation of 5-Iodo and 5-Bromouracil

Frank A. Sedor and Eugene G. Sander*

Department of Biochemistry
University of Florida
Gainesville, Florida 32601

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Summary: The 5-iodo- and 5-bromo- analogs of uracil are dehalogenated in the presence of both cysteine and 2-mercaptoethanol to yield uracil. Presumably, the reaction involves the initial addition of the thiol group across the 5,6 double bond of the halopyrimidine to yield the corresponding 5-halo, 5,6-dihydrouracil-6-thioether which then dehalogenates to yield uracil. Under comparable conditions, cysteine causes more rapid dehalogenation of both halouracils than does 2-mercaptoethanol.

Thiol containing compounds catalyze hydrogen-deuterium exchange at carbon five of uracil (1-3) and have been implicated as having a catalytic effect in the deamination of cytosine (4,5). Presumably, these reactions involve the reversible nucleophilic addition of the thiol group across the 5,6 double bond of the pyrimidine to yield the corresponding 5,6 dihydropyrimidine with a substituted thioether group on carbon six. This pathway is supported by comparable reactions involving the addition of bisulfite to the pyrimidine ring system (6-10). Different from the bisulfite addition compounds, the thioether containing dihydropyrimidine adducts have not been isolated and characterized; however, 5'-deoxy-5',6-epithio-5,6-dihydro-2',3'-O-isopropylideneuridine resulting from the intramolecular attack of the 5' thiol group on carbon six of the uracil ring system of 5'-deoxy-5'-thio-2',3'-O-isopropylideneuridine has been isolated and characterized (11).

In a recent communication, we reported that bisulfite buffer systems catalyze the dehalogenation of 5-iodo-, 5-bromo-, and 5-chloro-uracil (12). The object of this work is to demonstrate that cysteine and 2-mercaptoethanol, sulfur nucleophiles with more physiological importance than bisulfite, also cause halopyrimidine dehalogenation under nearly physiological conditions of temperature and pH.

Materials and Methods

Deionized, glass-distilled water was used to prepare all solutions and reaction mixtures. Inorganic reagents were obtained from Fisher Chemical Company and used without further purification. Tris (hydroxymethyl) aminomethane (Tris), 5-bromouracil, 5-iodouracil (Sigma Chemical Company) and cysteine hydrochloride (Aldrich Chemical Company) were used as received. 2-Mercaptoethanol (Type I, Sigma Chemical Company) was redistilled *in vacuo* and stored under argon at 0°. Cellulose thin-layer chromatographic plates containing a fluorescent indicator were obtained from Eastman Organic Chemicals.

Ultraviolet absorption spectra were measured at room temperature with a Cary 14 recording spectrophotometer. Stock solutions of 1.0 M Tris-HCl buffer at the appropriate pH, 1×10^{-3} M EDTA · 2 Na, 4.0 M NaCl and 5.0×10^{-3} M solutions of the halopyrimidines were deaereated with water-saturated argon prior to use. Cysteine hydrochloride solutions (1.0 M) were freshly prepared in the same manner prior to being used. 2-Mercaptoethanol was used neat. Reaction mixtures were prepared under a stream of argon in 25 ml volumetric flasks by adding either the required amount of a 1.0 M cysteine solution or neat 2-mercaptoethanol to solutions containing the required amounts of Tris-HCl buffer, EDTA· 2 Na, and NaCl to maintain ionic strength at 1.0 M. Absorption spectra of the reaction mixtures and their respective blanks which contained all reaction components except the halopyrimidines were measured using 1.0 ml aliquots which were quenched by the addition of 2.0 ml of 1.0 M HCl.

Values of pH of the unquenched reaction mixtures were measured following completion of the reaction using a Radiometer PHM-26 pH meter equipped with a Radiometer GK 2321C combination electrode.

The rates of thiol addition across the 5,6 double bond of the halopyrimidines were spectrophotometrically measured by following the absorbance decrease at 290 nm. Pseudo first-order rate constants (k_{obsd}) were determined from linear semilogarithmic plots of $A_t - A_\infty$ against time and the relationship $k_{obsd} = 0.693/t_{1/2}$.

The products of the reaction of both 5-bromo- and 5-iodouracil with 2-mercaptoethanol were isolated as a solid from reaction mixtures incubated 12 hours at room temperature under argon containing 5 mmoles of the halopyrimidine and 50 mmoles 2-mercaptoethanol in 50 ml of 0.5 M ammonium bicarbonate buffer (pH 8.5). In the case of cysteine, 3 mmoles of each halopyrimidine was dissolved in 500 ml of an argon-purged 0.10 M cysteine solution (pH 8.3). After stirring for 2 hours under argon, the reaction mixture was acidified to pH 2.5 and filtered to remove a small precipitate. The resulting filtrate was poured over an Amberlite MB-3 ion exchange column (3.5 x 30 cm) which was eluted with 500 ml 0.01 M HCl. Uracil precipitated from the pooled fractions after the volume was reduced to 20 ml. The slightly yellow precipitate was recrystallized from water following decolorization with Norit A. All of the pyrimidine products were recrystallized from water, dried *in vacuo* at 105° and qualitatively identified by melting point on a Hoover-Thomas melting point apparatus, ultraviolet absorption spectra in 0.1 M HCl and cellulose thin-layer chromatography using the upper phase of ethylacetate:water:formic acid (65:35:5 v/v) as the developing solvent preceded by equilibration of the plates with the lower phase of the same solvent system (13).

Results and Discussion

Both 2-mercaptoethanol and cysteine react with 5-bromo- and

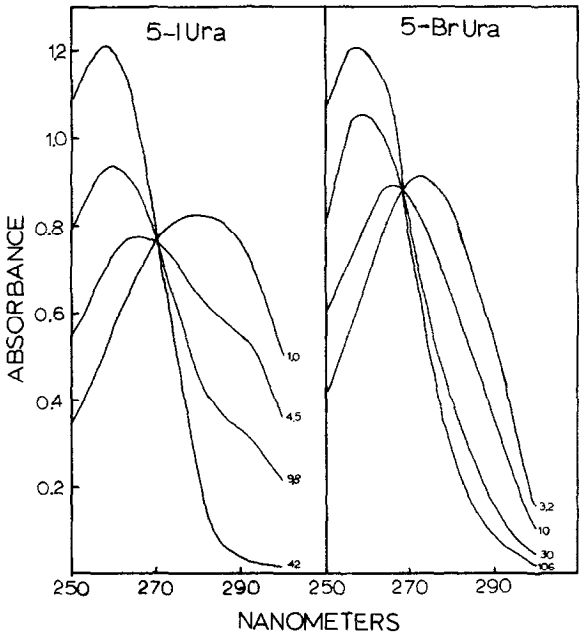
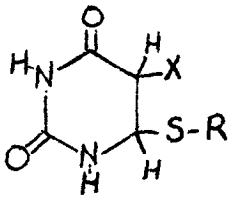


Fig. 1. Ultraviolet absorption spectra of reaction mixtures containing either 4.65×10^{-4} M 5-iodouracil or 4.0×10^{-4} M 5-bromouracil plus 0.20 M cysteine, 0.45 M Tris-HCl buffer and 1.0×10^{-4} M EDTA, pH 7.72, μ = 1.0 M, 23°. Blank solutions contained all reaction components except the halopyrimidines. Prior to recording the spectra, reaction mixtures and blank solutions were quenched by three-fold dilution with 1.0 M HCl. Numbers indicate the time of quenching in minutes.

5-iodouracil causing a rapid decrease in 280-290 nm absorbance which is concomitant with an increase in absorbance at lower wavelengths. Figure 1 shows typical spectra, measured as a function of time, for both 5-bromo- and 5-iodouracil reacting with 0.20 M cysteine at pH 7.73. The results can best be explained in terms of a reaction sequence which involves the initial formation of 5-halo-5,6-dihydropyrimidine with a substituted thioether group at carbon six (I) followed by dehalogenation to yield uracil as the final pyrimidine product. This reaction pathway is similar to that which we have previously proposed for the dehalogenation of 5-bromo-, 5-iodo- and 5-chlorouracil



R = $-CH_2-CH_2-OH$, $-CH_2-CH(NH_3^+)(COO^-)$

X = -I, -Br

I

TABLE I

Pseudo First-Order Rate Constants for the Reaction of 5-Bromo- and 5-Iodouracil with 2-Mercaptoethanol and Cysteine[a/]

Pyrimidine	Thiol Compound	pH	k_{obsd} (min^{-1})
5-Bromouracil	2-Mercaptoethanol	8.30	0.014
	Cysteine	8.27	0.108
5-Iodouracil	2-Mercaptoethanol	8.30	0.028
	Cysteine	8.31	0.143

a/ Reaction mixtures contained 4.5×10^{-4} M halopyrimidine, 0.286 M thiol compound, 1×10^{-4} M EDTA, and 0.60 M Tris-HCl buffer; μ = 1.0 M, 23°. Prior to recording the spectra, the reaction mixtures along with appropriate blank solutions were quenched by three-fold dilution with 1.0 M HCl.

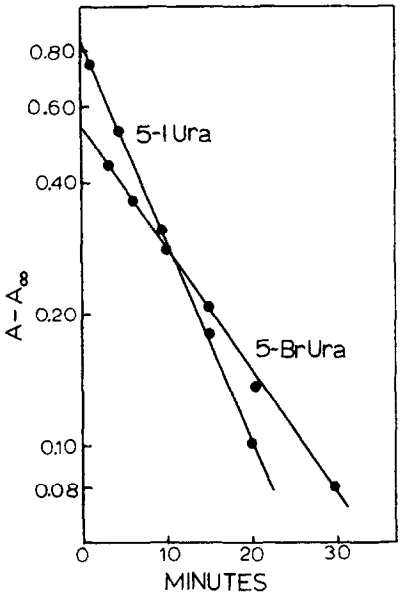


Fig. 2. Semilogarithmic relationship between the 290 nm absorbance decrease and time which occurs when either 5-iodo- or 5-bromouracil reacts with 0.20 M cysteine in 0.45 M Tris-HCl buffer and 1.0×10^{-4} M EDTA, pH 7.72, μ = 1.0 M, 23°. Reaction mixtures and blank solutions were quenched by three-fold dilution with 1.0 M HCl prior to recording the spectra.

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in the presence of sodium bisulfite buffers (12); however, due to the unfavorable equilibrium which exists between thiol compounds and pyrimidines, uracil and not the 6-thioether derivative of 5,6-dihydrouracil is the final pyrimidine product. As in the case of bisulfite, the actual dehalogenation step probably involves the formation of the very unstable halonium ion as a reactive intermediate. Since both thiol compounds and bisulfite are strong reducing agents, it is likely that halide and not halonium ion is the final halogen product. The detailed mechanism of the dehalogenation step is currently under investigation.

The kinetics of thiol addition to both 5-iodo and 5-bromouracil were spectrophotometrically followed at 290 nm, a wavelength where absorbance by uracil would not be observed. Semilogarithmic plots of

extent reaction ($A - A_\infty$) versus time were constructed. Typical results with both 5-iodo- and 5-bromouracil are shown in Figure 2. In all cases, the reactions followed strict first-order kinetics for at least two and in most cases three half-lives. The pseudo first-order rate constants which were obtained for the reaction of both halopyrimidines with both thiol compounds under exactly the same experimental conditions are shown in Table I. Under these experimental conditions, dehalogenation in cysteine containing solutions is about 6-8 times more rapid than in 2-mercaptoethanol containing solutions. This result is not unexpected since the pK_a of cysteine's thiol group is about 1.30 units lower than the corresponding pK_a of 2-mercaptoethanol.

To confirm that uracil was the final product of the reaction of 5-bromo- and 5-iodouracil with both 2-mercaptoethanol and cysteine, the final pyrimidine products were isolated, recrystallized from water, and dried *in vacuo*. The melting points in all cases were 333-335° which is within the range obtained with authentic uracil (lit 335°). Ultraviolet absorbance spectra and cellulose thin-layer chromatography also show that uracil is the final pyrimidine product. Table II shows the absorbance ratios of the reaction products and authentic uracil at 240, 250, 270, and 280 nm relative to 260 nm absorbance. These data indicate that products of the reactions of 5-bromo- and 5-iodouracil with either cysteine or 2-mercaptoethanol have the same ultraviolet absorption spectrum as authentic uracil. The R_f values (Table III) obtained from cellulose thin-layer chromatography of the reaction products and the authentic pyrimidines again support the final conclusion that both 2-mercaptoethanol and cysteine cause the rapid dehalogenation of 5-iodo- and 5-bromouracil.

Acknowledgements

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TABLE II

Ultraviolet Spectral Data for the Products of Either 5-Bromo- or 5-Iodouracil Reacting with both 2-Mercaptoethanol and Cysteine.[a]

Pyrimidine	λ_{max} (nm)	λ_{min} (nm)	Ratio $A/A_{260\ nm}$			
			240 nm	250 nm	270 nm	280 nm
Uracil	258	228	0.50	0.85	0.69	0.17
5-Bromouracil	276	241	0.33	0.51	1.49	1.52
+ 2-Mercaptoethanol	258	228	0.48	0.85	0.67	0.16
+ Cysteine	258	228	0.49	0.85	0.67	0.16
5-Iodouracil	283	245	0.59	0.58	1.49	1.74
+2-Mercaptoethanol	258	228	0.46	0.84	0.68	0.17
+Cysteine	258	228	0.49	0.85	0.68	0.18

[a] Difference spectra were measured in 0.10 M HCl at room temperature.

TABLE III

R_f Values for Cellulose Thin-Layer Chromatography of the Products of the Reaction of 5-Bromo- and 5-Iodouracil with Cysteine and 2-Mercaptoethanol[a]

Pyrimidine	R_f		
	Authentic	Plus 2-Mercaptoethanol	Plus Cysteine
Uracil	0.31	---	---
5-Bromouracil	0.67	0.31	0.30
5-Iodouracil	0.75	0.31	0.31

[a] Developing solvent was upper phase of ethylacetate:water:formic acid (65:35:5 v/v). Plates were pre-equilibrated overnight in lower phase of the same solvent system (13).

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