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Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

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To cite this Article Ye, Mingyu(1991) 'Determination of Bromide Production in Radiolysis of Nucleobases, Nucleosides, and Nucleotides Using HPLC', *Journal of Liquid Chromatography & Related Technologies*, 14: 19, 3497 – 3511

To link to this Article: DOI: 10.1080/01483919108049406

URL: <http://dx.doi.org/10.1080/01483919108049406>

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DETERMINATION OF BROMIDE PRODUCTION IN RADIOLYSIS OF NUCLEOBASES, NUCLEOSIDES, AND NUCLEOTIDES USING HPLC

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ABSTRACT

Determination of the formation of bromide ions in intermolecular electron transfer in 5-bromouracil (BrUr) and its nucleoside and nucleotide derivatives with nucleobases, nucleosides, and nucleotides was carried out with high performance liquid chromatography (HPLC). Initial electron attachment, at high concentration of nucleobases, nucleosides, or nucleotides, is mainly on these molecules; intermolecular electron transfer then occurs between these molecules and BrUr and the derivatives. The elimination of bromide ions from BrUr and the derivatives then follows. It is concluded that in neutral and basic solution (pH 6 to 10) there is a significant electron transfer from thymine (T), uracil (Ur), thymidine (dT), 2'-deoxyuridine (dU), or 2'-deoxyuridine-5'-monophosphate (dUMP) to BrUr and the derivatives. For example, at a concentration ratio of BrUr and T of 1 : 100, the yield of bromide ions is about 1.6, amounting to 59% of hydrated electron (e_{aq}^-) yield in the radiolysis, in which the pseudo-first-order rate constants predict a bromide yield of less than 0.03.

INTRODUCTION

5-Bromouracil (BrUr) and related compounds have been examined by a number of investigators ^{(1), (2)} because of interest in using

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these compounds to localize the site of radiation damage in radiobiological studies by selective attack of e_{aq}^- on DNA where it had replaced thymine. ⁽¹⁾ A major initial effect of ionizing radiation on DNA is the production of positive and negative ions of DNA bases. ⁽³⁾ These ions may later react to produce biologically significant damage. It has been suggested that BrUr incorporation into DNA increases the number of double strand breaks ⁽⁴⁾ possibly due to the existence of sites of high electron affinity (the bromine atom) in close proximity between strands. Efficient electron transfer to these sites could thus result in an enhanced number of double strand breaks. Evidence for electron transfer along the bases in DNA in the solid state was obtained from electron spin resonance (ESR) measurements after gamma-irradiation. ^{(5), (6)} Study of electron transfer in dinucleoside phosphate anions at low temperature (77 K) by ESR and pulse radiolysis suggested that base-base inter-stacking causes electron transfer to the base with greatest electron affinity. ⁽⁷⁾ However, from the studies of the charge transfer in frozen DNA-water and collagen-water mixtures by means of measurement of the microwave absorption, it was suggested that the charge transfer is due to the unsolvated electron migrating in an ice-like matrix around the biopolymer. ^{(8), (9)} Studies on the luminescence kinetics of adenosine-BrUr ⁽¹⁰⁾ and on BrUr-DNA ⁽¹¹⁾ suggested that the range of electron transfer is 100 ± 20 base pairs. On the other hand, on the basis of optical pulse-radiolysis studies of proflavine intercalated in the DNA helix, ⁽¹²⁾ it was indicated that the maximum migration distance of electrons is five base pairs. One proposed interpretation ⁽¹³⁾ is that the short transfer distance might be typical for the lower energy levels and that the observed long-distance effect may well result from charge migration.

The present experiments were designed to investigate the electron transfer in aqueous solutions of nucleobases, nucleosides, and nucleotides. In these systems, base stacking is not expected at room temperature. ⁽¹⁴⁾ High performance liquid chromatography (HPLC) was used to determine the production of bromide ions formed in the x-ray radiolysis of aqueous solutions of nucleobases, nucleosides, and nucleotides containing BrUr, 5-bromo-2'-deoxyuridine (BrdU), or 5-bromo-2'-deoxyuridine-5'-monophosphate (BrdUMP).

MATERIALS AND METHODS

Irradiation was carried out with a Norelco MG 300 x-ray unit (Philips Electronic Instruments) at absorbed dose rates of 1 Gy min⁻¹.

HPLC determination of bromide ion production Samples were analyzed by HPLC using a VVDAC column 3040L54. Detection was with a Linear 206 PHD optical detector at wavelength of 198 nm. Elution was with an aqueous buffer of 0.02 and 0.04 M NaH₂PO₄ in 0.3% acetic acid. The detector sensitivities were calibrated with known concentrations of reference samples. All quantitative results of HPLC were from comparison of peak area with reference samples run under identical chromatographic conditions.

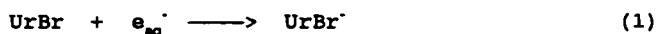
Solutions were prepared in triply distilled water purified by a millipore Milli-Q Reagent system. The pH was adjusted by addition of Becker Analysis KOH and determined with a Corning 610A pH meter calibrated with VWR buffers. All nucleobases, nucleosides and nucleotides were from Sigma, 2-propanol, tert-butyl alcohol (t-BuOH) and NaOH from Fisher. The chemicals used in the mobile phases of HPLC were acetic acid from Fisher and Na₂HPO₄ from Aldrich. Solutions were saturated with nitrogen to purge oxygen.

Irradiated samples were covered with aluminum foil to avoid UV exposure and introduced into the HPLC system in a few minutes after completion of irradiation. All experiments were carried out at room temperature (22°C).

RESULTS AND DISCUSSION

Production of Bromide Ions

It is known from the studies of radiation chemistry of BrUr⁽²⁾ that e_{aq}^- reacts diffusion-controlled with BrUr ($k = 1.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$) to produce a radical anion (BrUr^{•-}).



The radical anion is very unstable and decomposes within a few nanoseconds into the bromide ion and the uracilyl radical (Ur[•]).⁽²⁾



Because thymine and uracil also react with e_{aq}^- very rapidly ($k = 1.7$ and $1.5 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, Table I), in a solution containing both thymine(T), or uracil(Ur) and BrUr, they are expected to compete with BrUr to react with e_{aq}^- . In particular, when T and Ur are present in a much higher concentration than BrUr, the electron attachment to T and Ur is expected to be the dominant reaction.

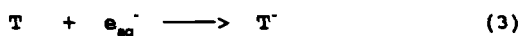


TABLE I

RATE CONSTANTS ⁽¹⁾ OF THE REACTIONS OF e_{aq}^- WITH
NUCLEOBASES, NUCLEOSIDES AND NUCLEOTIDES

	k ($10^{10} \text{ M}^{-1} \text{ s}^{-1}$)
Thymine	1.7
Uracil	1.5
5-Bromouracil	1.6
Uridine	1.4
Thymidine-5'-monophosphate	0.15
Uridine-5'-monophosphate	0.5

(1) Buxton, G.V.; Greenstock, C.L.; Helman, W.P.; Ross, A.B., J. Phys. & Chem. Ref. Data, 17, 513-635, 1988.

T and Ur anions will then undergo electron transfer with BrUr,



this is followed by elimination of bromide ion from BrUr anion.



Table I gives the rate constants for the reactions of e_{aq}^- with nucleobases, nucleosides, and nucleotides.

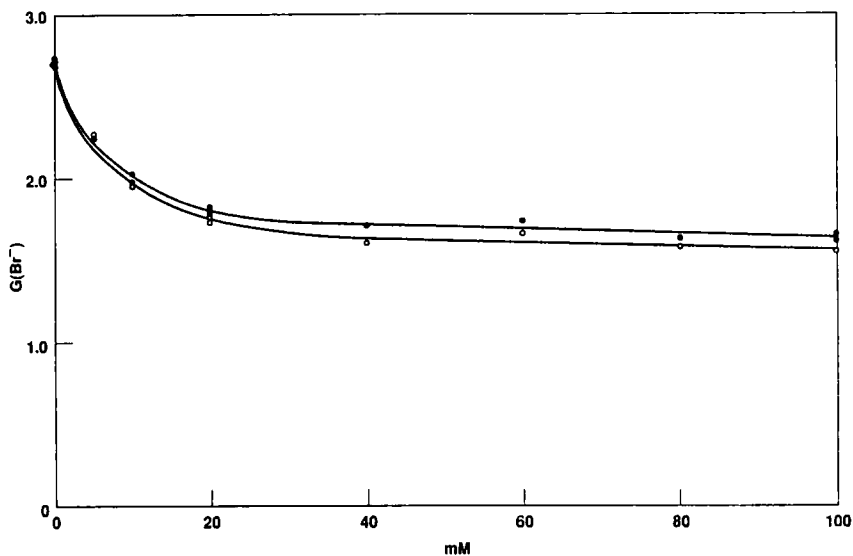


Fig. 1 Production of Bromide Ions in the Radiolysis of 1 mM BrUr Solution with T (●), and with Ur (○) Saturated with N₂ with 0.4 M t-BuOH at pH ~ 6.4. Radiation doses: 20 Gray

1. Nucleobases

a. BrUr-T system The yields of bromide produced from the electron transfer reaction were determined at Ph 6.5 and 10 for 1 mM BrUr solutions containing T at concentrations from 0 to 100 mM. These solutions also contained 0.4 M t-BuOH which should, from the relative pseudo-first-order rate constants, remove ~ 99% of the OH radicals. Figure 1 shows the dependence of bromide yield on the thymine (solid circles) and uracil (open circles) concentration. From the treatment of Balkas et al. ⁽¹⁵⁾ one expects, taking the rate constants typically as $5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, that the radiation yield of products at millimolar solute concentrations should be ~ 2.7. The

bromide yield in the solution without T is 2.68, indicating that the reduction quantitatively eliminates bromide ions. The bromide yields of 1.62 was found in the solution containing 100 mM thymine.

b. Bromouracil-uracil system Similar studies were carried out in 1 mM bromouracil solutions containing 0 to 100 mM uracil. In the solution with 100 mM uracil, the yield of bromide is 1.54. From the relative pseudo-first-order rate constants, the bromide yield should be less than 0.03. The dependence of bromide yield on the uracil concentration (open circles) is shown in Figure 1.

The results indicate that there is a very efficient electron transfer between BrUr and T or Ur in aqueous solution.

2. Nucleosides

Studies of BrdU and thymidine (dT) (BrdU-dT system), and BrdU and 2-deoxyuridine (dU) (BrdU-dU system) reported in Table II, show that in both cases at the concentration ratio of 1 : 100 of both BrdU to dT, and BrdU to dU there are appreciable yields of bromide (1.52, and 1.61, respectively). The chromatograms obtained from 1 mM BrdU solution with 0.4 M t-BuOH, containing 100 mM dT, or 100 mM dU are shown in Figures 2a and 2b.

3. Nucleotides

The bromide yields were obtained from the 1 mM 5-bromo-2'-deoxyuridine-5'-monophosphate (BrdUMP) BrdUMP with 0 to 20 mM dUMP (Table III). The yield of bromide in the absence of dUMP is 2.64, although the rate constants for the reactions of e_{aq}^- with nucleotides are almost one order lower than that with nucleobases (Table I). The decrease of the rate constant, which is ascribed to electrostatic repulsion between the negatively charged reactants

TABLE II

YIELDS ⁽¹⁾ OF BROMIDE IN THE RADIOLYSIS OF BrdU-dT SOLUTIONS

Solutes ⁽²⁾	pH	G	pH	G
1 mM BrdU	6.6	2.62	10.1	2.63
1 mM BrdU, 5 mM dT	6.1	2.24	10.2	2.30
1 mM BrUr, 10 mM dT	6.4	2.05	10.4	2.10
1 mM BrUr, 20 mM dT	6.3	1.7	10.1	1.72
1 mM BrdU, 40 mM dT	6.4	1.73	9.9	1.69
1 mM BrdU, 60 mM dT	6.0	1.69	10.2	1.70
1 mM BrdU, 80 mM dT	6.3	1.61	10.3	1.59
1 mM BrdU, 100 mM dT	6.8	1.59	10.5	1.55

1. Radiation yields in molecules/100 eV. Yields were averaged from at least three experiments.

2. All solutions contained 0.4 M t-BuOH and were saturated with N₂.

(e_{aq}⁻ and nucleotides) ⁽¹⁶⁾ apparently does not result in a decrease of bromide yield. Figure 3 shows the chromatogram obtained from 1 mM BrdUMP solution with 0.4 M t-BuOH at pH 6.2. Bromide ion and dUMP were produced in the radiolysis. Presumably, BrdUMP reacts with e_{aq}⁻ similarly to BrUr, as shown in reaction 1.



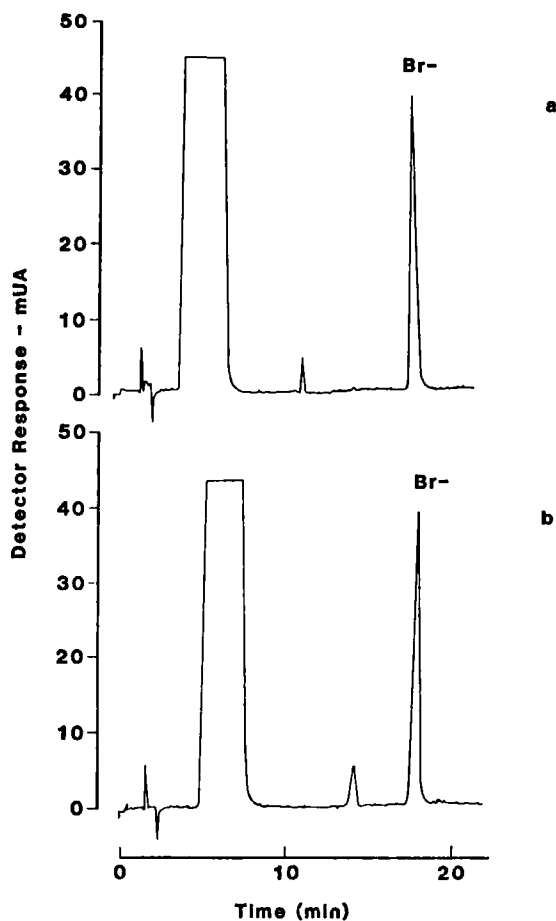


Fig. 2 Formation of Bromide Ions in Radiolysis of 1 mM BrdU Solutions with 0.4 M t-BuOH in Neutral pH (a) with 100 mM dT; (b) with 100 mM dU;

Irradiation dose: 20 Gray

Detector: Linear 206 PHD optical at wavelength of 200 nm.

Column: VYDAC 3040L54

Mobil Phase: 0.02 M NaH_2PO_4 in 0.3% acetic acid

Flow Rate: 1.6 ml/min.

TABLE III

YIELDS ⁽¹⁾ OF BROMIDE IN THE RADIOLYSIS OF BrdU-dU SOLUTIONS

Solutes ⁽²⁾	pH	G
1 mM BrdU	6.8	2.67
1 mM BrdU, 5 mM dU	6.4	2.26
1 mM BrUr, 10 mM dU	6.3	2.00
1 mM BrUr, 20 mM dU	6.5	1.80
1 mM BrdU, 40 mM dU	6.6	1.74
1 mM BrdU, 60 mM dU	6.7	1.70
1 mM BrdU, 80 mM dU	6.1	1.63
1 mM BrdU, 100 mM dU	6.5	1.55

1. Yields were averaged from at least three experiments.
2. All solutions contained 0.4 M t-BuOH and were saturated with N₂.

The radical (dUMP[•]) can abstract a hydrogen atom from t-BuOH to form the dUMP molecule. We were unable to determine the bromide yield at the concentration of dUMP above 20 mM because of the band overlap of dUMP and bromide ion.

General Discussion

Electron transfer from thymine radical anion to BrUr is known to occur rapidly ($k_{\text{trans}} = 1.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) ⁽¹⁶⁾ and appears to

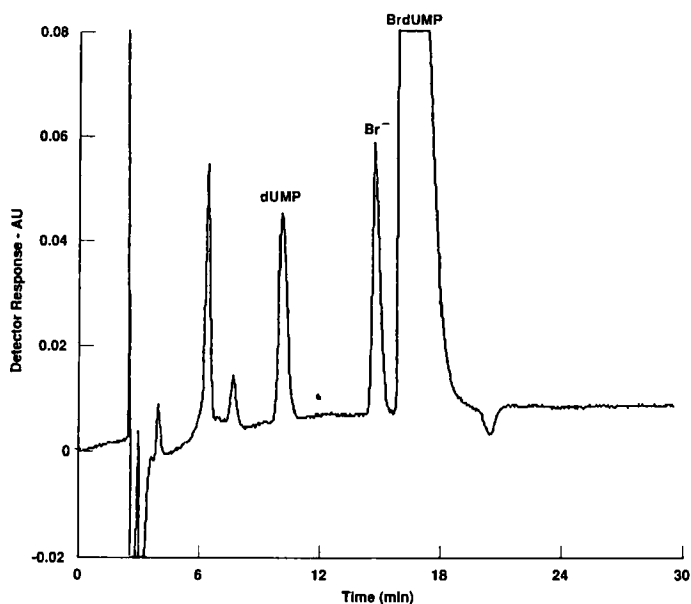


Fig. 3 Chromatogram of dUMP Produced in X-Ray Radiolysis of
 1 mM BrdUMP Solutions in Neutral pH with 0.4 M 2-Propanol
 Irradiation dose: 20 Gray
 Detector: Linear 206 PHD optical at wavelength of
 200 nm.
 Column: VYDAC 304OL54
 Mobil Phase: 0.04 M NaH_2PO_4 in 0.3% acetic acid
 Flow Rate: 1.6 ml/min.

account for the high yields of bromide ion produced from BrUr
 solutions containing relatively higher concentrations of thymine
 (Figure 1). Because oxygen has a greater electron affinity than
 carbon, it is reasonable to assume that the negative charge in the
 radical anion resides mainly on the oxygen (O(4)). ^{(17), (18)} As a
 result of the high charge density at the oxygen, the radical anions

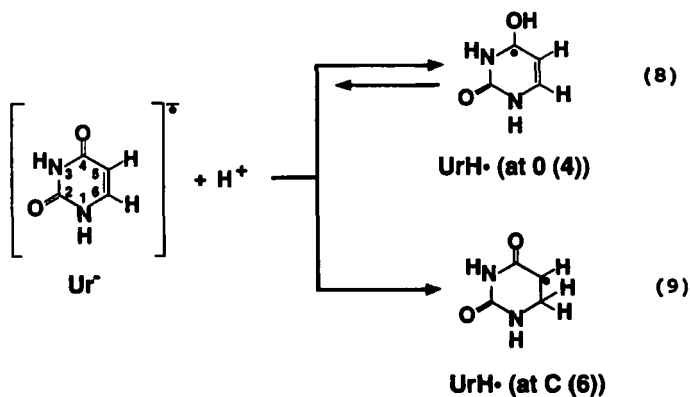
TABLE IV

YIELDS ⁽¹⁾ OF BROMIDE IN THE RADIOLYSIS OF BrdUMP-dUMP SOLUTIONS

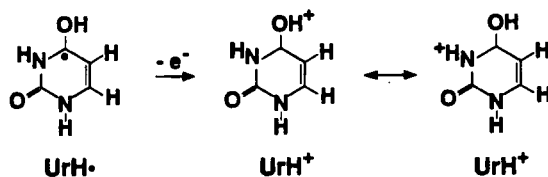
Solutes ⁽²⁾	pH	G	pH	G
1 mM BrdU	6.8	2.67	10.0	2.69
1 mM BrdU, 2 mM dU	6.5	2.51	10.1	2.54
1 mM BrdU, 5 mM dU	6.4	2.26	10.2	2.22
1 mM BrUr, 10 mM dU	6.3	2.00	9.8	1.98
1 mM BrUr, 20 mM dU	6.5	1.80	10.1	1.77

1. Yields were averaged from at least three experiments.
2. All solutions contained 0.4 M t-BuOH and were saturated with N₂.

of uracil (Ur⁻) and its derivatives are readily protonated by H⁺, most likely at O(4) (reaction 8), the rate constants ranging from 3 x 10¹⁰ to 6 x 10¹⁰ M⁻¹ s⁻¹. (17), (18)



The rate constant for the protonation of the thymine radical anion by water is $< 2 \times 10^4$ ⁽¹⁹⁾, which is in agreement with the pKa 7.2 of the protonated electron adducts of uracil and thymine. ⁽²⁰⁾ The bromide yields at pH 5 and 10 are similar (Figure 1, Tables II and III), suggesting that both Ur^\cdot and UrH^\cdot must be strong one electron reductants, as found in the case of the adenine radical anion and its protonation form. ⁽³⁾ The reducing power is not surprising since UrH^+ (TH^+ , dUH^+ and dUMPH^+), produced on removal of an electron from UrH^\cdot (TH^\cdot , dUH^\cdot and dUMPH^\cdot), is expected to be highly stabilized by the nitrogens in α -position:



Besides the fast protonation at oxygen, there is a slow protonation at C(6) (reaction 9), which leads to a more stable radical at C(5). ⁽³⁾

CONCLUSIONS

The study indicates that intermolecular electron transfer is very efficient in aqueous solutions of nucleobases, nucleosides, and nucleotides containing 5-bromouracil and its derivatives at room temperature, in which base stacking is not expected. The radical anion and its protonated form of thymine, uracil and their derivatives are very strong reducing species.

ACKNOWLEDGMENTS

Work was supported by the Office of Health and Environmental Research (OHER), U.S. Department of Energy, under contract DE-AC06-76RLO 1830.

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