"LONG-LIFE" ATOM-FREE RADICAL: GENERATION AND REACTIONS OF BROMINE ATOM-FREE RADICAL

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In order to study the damaging or beneficial properties of bromine atom-free radical, reaction of the free radical ($Br^\bullet$) with some biologically important compounds were investigated. $Br^\bullet$ was generated through electrochemical oxidation of bromide ion (Br^-). First the reactivity of $Br^\bullet$ atom-free radical *vis a vis* its dimerization to form Br_2, was studied using cyclic voltammetry and spectroelectrochemistry. Through these techniques it was ascertained that the substrates understudy and the under experimental conditions used, underwent reactions with $Br^\bullet$ and not with dibromine (Br_2). The monitoring of the reactions of $Br^\bullet$ with glycine and cytosine led us to conclude that whereas cytosine reacted with $Br^\bullet$ as simple chemical reaction (EC mechanism), glycine underwent a catalytic reaction (EC′ mechanism).
Keywords: Bromine; Atom-free radical; Glycine; Cytosine; Dibromine; Long-life free radical.

The role of free radicals in biology was recognized many decades ago[1–10]. Many biological reactions involve or proceed through free radical mechanism[1–10]. Free radicals have been postulated to take part in many biological/biochemical reactions[1–10].

Generally, free radicals have been regarded as villain. Among the villain free radicals are reduced oxygen species (ROS), i.e. superoxide and hydroxyl radical[11–12].

Kosower's celebrated pyridinyl radicals provided a useful link between chemistry and biology[13]. Another famous free radical of biochemical importance, rather a cation radical, methyl viologen cation radical ($MV^{\bullet+}$), is a widely studied free radical[13–19]. This cation radical, $MV^{\bullet+}$, also, provides a link between chemistry and biology, so do the ROS free radicals[13–19]. Yet

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another much studied and important free radicals are flavins[20–22] and quinones[23–26].

Free radicals are beneficial[1–10]. So there can be "friendly free radicals" [27]. In our earlier work[27], preliminary studies on iodine atom-free radical *vis a vis* its relevance to biochemical reaction was reported. The next step, obviously, is to repeat the same strategy for bromine atom-free radical.

There is nothing new about the production of halogen atom-free radicals and their chemistry. However, mostly, these atom-free radicals have been obtained through photochemical reaction or radiolysis process[28].

The main problem to study the chemistry of these halogen atom-free radicals is their dimerization/recombination reaction, and subsequent reaction of the dimer molecules

$$2\ X^{\bullet} = X_2$$

$$X_2 + Y = Z$$

where X is a halogen atom, Y is a reactant, and Z is a product.

Vetter[29] had proposed the production of $I^{\bullet}$ atom-free radical through electrooxidation of I^- ion, while Popov and Geske[30] reported recombination reaction of electro-generated $I^{\bullet}$ atom-free radical as quite slow compared to its reaction with (excess of) pyridine. These statements from Vetter, and Popov and Geske were confirmed in our earlier[27] work on $I^{\bullet}$. If the same is true for $Br^{\bullet}$ atom then the door of electrochemically generated $Br^{\bullet}$ atom-free radical chemistry is opened. This chemistry may very well be quite different from photochemically or radiolytically generated (hot) $Br^{\bullet}$ atom-free radical chemistry.

Report on the generation of $Br^{\bullet}$ atom-free radical and its chemical reactions, in solution, is scarce[31–35]. Bromine atom being reactive in nature, may react with substrate or may combine with other bromine atom-free radical to form dibromine (Br_2) and then react with the substrate as shown in Eqs (*1*)–(*4*) given below:

$$Br^- - e \rightarrow Br^{\bullet} \qquad (1)$$

$$Br^{\bullet} + \text{substrate} \rightarrow [\text{product}] \qquad (2)$$

or

$$Br^{\bullet} + Br^{\bullet} \rightarrow Br_2 \qquad (3)$$

$$Br_2 + \text{substrate} \rightarrow [\text{product}]\ . \qquad (4)$$

In most studies in the past, it has been suggested that when bromine atom-free radical ($Br^{\bullet}$) is generated it dimerizes to Br_2 (we will call Br_2 as dibromine) and then Br_2 reacts with the substrate[31–35].

Studies on some compounds with dibromine (Br_2) are available in literature[36,37]. Here we present the result of our studies on the generation of $Br^{\bullet}$ atom-free radicals electrochemically, monitoring the primary electrochemical product (PEOP) (Eqs (*1*) and (*2*)), ascertaining the identity of PEOP, followed by the reaction of cytosine and glycine with the PEOP ($Br^{\bullet}$) atom-free radical *vis a vis* their reactions with dibromine (Br_2). Reactions of cytosine and glycine with iodine atom-free radical had been studied and reported previously[27]. Cyclic voltammetry, controlled potential electrolysis and spectroelectrochemistry were used to generate $Br^{\bullet}$ and monitor its reaction with the substrates. For comparison the reaction of the substrates with dibromine were studied spectrophotometrically.

The term long-life is used for the comparative lifetime of $Br^{\bullet}$ for the reaction with the substrate as compared to dimerization under the same condition.

EXPERIMENTAL

Instruments

Cyclic voltammetry experiments were performed using Analytical Electrochemical Work Station, Model AEW2-10mA (Sycopel Scientific, Ltd.) controlled by IBM compatible computer with software EC Prog V3. Saturated calomel electrode was used as reference electrode, while Pt (BAS) and graphite (PAR) electrodes were used as working and counter electrodes, respectively. The bulk electrolysis was performed using Ministat Precision Potentiostat, rating 25V @ 1A (Sycopel Scientific, Ltd.). The UV-Vis spectra were recorded on GENESYS 10 UV spectrophotometer (Thermo, USA). Spectroelectrochemistry was performed using a custom made spectroelectrochemical cell[19].

Chemicals

Glycine (Merck, 99.7%), KBr (Merck, 99.5%), potassium perchlorate (Panreac, 99%), Br_2 (Aldrich, 99.5%) were used without further purification. Tetra-*n*-butylamminum perchlorate was prepared from (mixing solution of) tetra-*n*-butyl bromide (Acros Organics, 99%) and magnesium perchlorate (Fisher Scientific).

5 mM KBr solution was prepared in water. 0.1 M $KClO_4$ was used as supporting electrolyte. 1, 5 and 50 mM solutions of glycine, cytosine were used to react with bromine atom-free radical.

Spectroelectrochemistry studies were carried out by carrying out *in situ* electrolysis at the pertinent potential and recording spectra simultaneously.

In chloroform (Fisher Scientific, HPLC grade), saturated solution of LiBr (~1 mM) was prepared. 0.1 M TBAP was used as supporting electrolyte in this solution.

RESULTS AND DISCUSSION

Generation of $Br^{\bullet}$: Cyclic Voltammetry of Br^- in Water

Cyclic voltammogram (CV) of Br^- in water (5 mM aqueous solution, 0.1 M $KClO_4$ supporting electrolyte, Pt electrode as working electrode), is given in Fig. 1. In water the CV shows an anodic peak ($(E_p)_a$ = 979 mV vs SCE) and a corresponding cathodic peak ($(E_p)_c$ = 834 mV vs SCE). The CV could be interpreted through processes given in Eqs (*1*) and (*2*) (a little quasireversible process followed by a slow chemical reaction), or Eqs (*1*) and (*3*) (quasireversible process followed by (slow) dimerization).

$$Br^- - e = Br^{\bullet} \qquad (1)$$

or (dimerization)

$$Br^- - e = Br^{\bullet} \qquad (1)$$

$$Br^{\bullet} + Br^{\bullet} \overset{k_{(\mathrm{Dimer})}}{\rightleftarrows} Br_2 \qquad (3)$$

could be followed by

$$Br_2 + e = Br_2^- \qquad (5)$$

($E_{1/2}$ around the same as for the process in Eq. (*1*)).

From the analysis of the CV as per stationary electrode polarography theory for a dimerization following a charge transfer[37] (Eq. (*3*)), the rate of dimerization can be calculated which comes out to be [O] × 10^3 M^{-1} s^{-1} (see below).

The theory of dimerization[38], process Eq. (*3*), states that in linear scan voltammetry the peak potential shifts with the increase in concentration of the electroactive species and the scan rate[38] as

$$n(E_p - E^o) = RT/3F\ (\ln \psi - 3.12) \qquad (6)$$

where $\psi = k_{(\mathrm{Dimer})}C^*/a$; C^* is the concentration of Br^- in the bulk and $a = nFv/RT$, where v is the scan rate (V/s), n is taken as 1.

In the present case, it was found to be true: the peak potential in the CV's shifted as the theory predicted.

According to this theory[38], the rate of dimerization can be obtained from concentration versus E_p plot. The dimerization rate constant ($k_{(\mathrm{Dimer})}$) was calculated from the expression (Eq. (*6*)) and from the graph (Fig. 2), it came

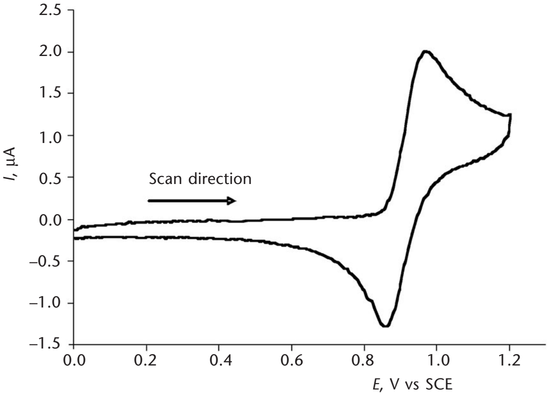

FIG. 1

Cyclic voltammogram of 5 mM Br^-. Scan rate 50 mV/s, supporting electrolyte 0.1 M $KClO_4$, working electrode Pt, reference electrode SCE, secondary electrode graphite, solvent H_2O

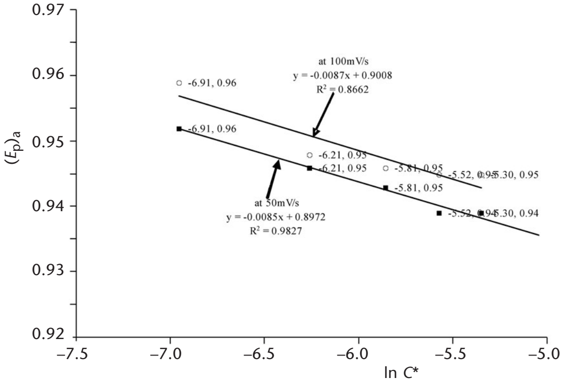

FIG. 2

E_p vs ln C^* graph (see Eq. (*5*))

out to be around 1.0 × 10^3 M^{-1} s^{-1}. This also means, for a concentration of [O] ≤ 1 mM of $Br^\bullet$, $t_{1/2}$ is estimated to be of the order of [O] 1 s. It must be noted that Eq. (*6*) is valid for the system where there is no follow-up electrochemical process after dimerization[38]. Nevertheless it is a useful estimate of $t_{1/2}$ for dimerization of electrochemically generated $Br^\bullet$ under the present experimental condition It may also be noted that this $k_{(\text{Dimer})}$ seems to be on lower side as compared to radiolytically and photochemically generated $Br^\bullet$ [21]. But it is believed that it is a reasonably good estimate under the present experimental condition (see below).

Generation of $Br^\bullet$: Cyclic Voltammetry of Br^- in Chloroform

The cyclic voltammogram of Br^- in chloroform is given in Fig. 3. From the figure – no reduction peak corresponding to the anodic peak – it is evident that a rapid reaction of the PEOP with chloroform is occurring (Eqs (*1*) and (*2*)). There is no reason to believe that PEOP is the dimer product of $Br^\bullet$ atom-free radical – the dibromine Br_2 – which reacted with chloroform: it is known and well established that dibromine is stable in chloroform (see also later spectroscopic studies). It is also evident that the reaction of PEOP (proposed as) $Br^\bullet$ with chloroform is fast – may be as fast as the rate of dimerization, or even faster. From the CV (Fig. 3) and if it is assumed that by the time the scan is reversed and reached the position where $(E_p)_c$ should have been located, 99% of PEOP ($Br^\bullet$) is used up, then a (pseudo-)

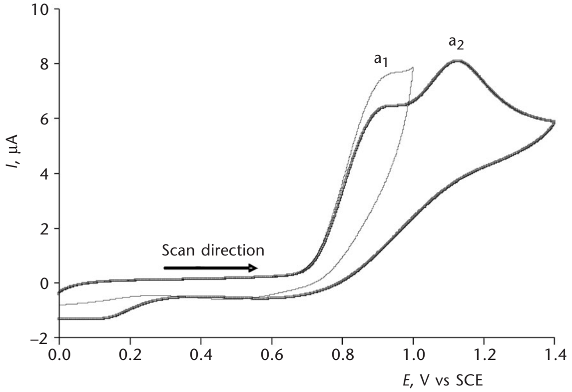

FIG. 3
Cyclic voltammogram of 5 mM Br^-. Scan rate 50 mV/s, supporting electrolyte 0.1 M TBAP, working electrode Pt, reference electrode SCE, secondary electrode graphite, solvent $CHCl_3$

first order rate constant (chloroform is in large excess) could be estimated as about 0.5 s^{-1} (or even higher) which would give its half-life about 1 s (or lower).

Spectroelectrochemistry of Br^- and Spectrophotometry of Br_2

The production of (PEOP) $Br^\bullet$ atom was further confirmed through spectroelectrochemistry. The UV-Vis spectrum of electrolyzed product of Br^- aqueous solution, produced through controlled potential oxidation of aqueous solution of Br^-, on gold electrode is quite different from the aqueous solution of Br_2 and Br_3^- (Fig. 4): λ_{max} of electrochemically generated PEOP ($Br^\bullet$) or its reaction product in H_2O is 254 nm, that of Br_2 and Br_3^- in H_2O is 265 nm. Also the spectrum of dibromine Br_2 in chloroform is given in Fig. 5, λ_{max} = 412 nm. This establishes the stability of Br_2 in chloroform and at the same time the instability of the PEOP of Br^- (that is $Br^\bullet$) in this solvent. These spectra clearly establish that $Br^\bullet$, instead of reacting with itself to form Br_2, reacts with (or attaches itself to) the environment, here solvent, as given in Eqs (*1*) and (*7*).

$$Br^- - e \rightarrow Br^\bullet \qquad (1)$$

$$Br^\bullet + \text{solvent} \rightarrow \text{product} \qquad (7)$$

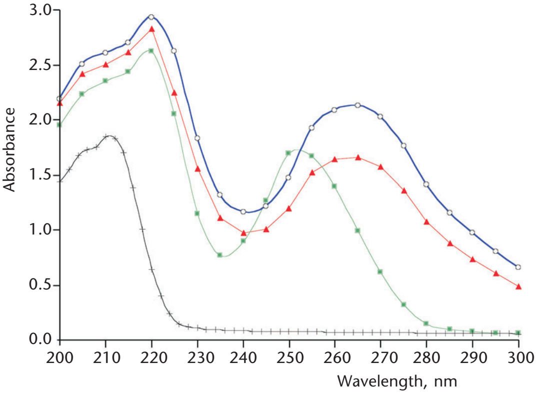

FIG. 4
UV spectra of electrolyzed product of 5 mM bromide ion (Br^-) (green), 3 mM dibromine (Br_2) (red), 3 mM tribromide ion (blue) and 5 mM KBr (black). Solvent H_2O. A gold (Au) foil was used as a working electrode for bulk electrolysis of Br^-

Reaction of Br$^\bullet$ and Br$_2$ with Cytosine

After establishing the formation of PEOP as Br$^\bullet$, on the electrooxidation of Br$^-$, one can embark upon studying its reaction with some biological compounds.

Cytosine as a substrate was selected as a representative of DNA component bases. The reaction of cytosine with iodine atom had been studied and the reaction was found quite fast[27].

The cyclic voltammogram of Br$^-$ in the presence of cytosine is given in Fig. 6 (curve a). From the figure one can easily conclude that there is a reaction between cytosine and Br$^\bullet$. However, it may be a little modified EC mechanism. It is observed that though cathodic peak corresponding to the anodic peak of Br$^-$ was gone, there was neither significant increase in $(i_p)_a$ nor there is much shift in $(E_p)_a$. The mechanism of the reaction between cytosine and Br$^\bullet$ may be proposed as

$$\mathrm{Br^- - e = Br^\bullet}$$

$$\mathrm{Br^\bullet + Cyt} \underset{k_b}{\overset{K}{\rightleftharpoons}} \mathrm{Z}$$

$$\text{Z is a product} \tag{8}$$

or

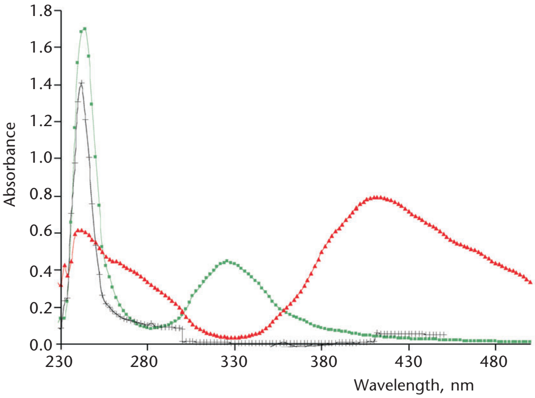

FIG. 5
UV spectra of electrolyzed product of 5 mM bromide ion (Br$^-$) (green), 7.8 mM dibromine (Br$_2$) (red) and saturated solution of 1 mM LiBr (black). Solvent CHCl$_3$. A platinum (Pt) foil was used as a working electrode for bulk electrolysis of Br$^-$

$$Z \xrightarrow{\text{slow}} \text{another product .} \tag{8'}$$

The simulated CV is given in Fig. 6 (curve b), while the CV of 5 mM Br^- (in the absence of cytosine) is given in Fig. 6 (curve c). Again from the time taken for sweeping the potential from anodic peak potential to the location of would be cathodic peak potential and assuming 99% of $Br^\bullet$ consumed during this time a $t_{1/2}$ can be estimated as 1 s. So the reaction is quite fast.

Spectroelectrochemical studies also confirmed that cytosine reacted with bromine atom-free radical and not with dibromine. This observation was obtained during CPE of Br^- in the presence of cytosine.

The reaction of cytosine with Br_2 was also studied. The UV spectra of the product obtained from the reaction of dibromine with cytosine is quite different from the UV spectra of the product (Z) obtained during electrolysis of Br^- in the presence of cytosine (Fig. 7).

Reaction of $Br^\bullet$ and Br_2 with Glycine

Glycine, while reportedly reacting with iodine atom-free radical quite slow[27], seems to react with bromine atom-free radical faster and catalytically. The

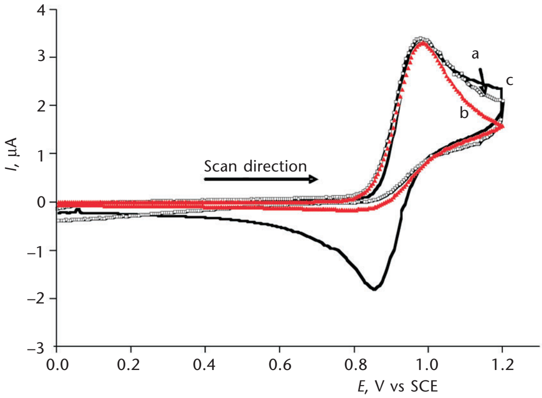

FIG. 6

Cyclic voltammogram of 5 mM Br^- in the presence of 5 mM cytosine (a), simulated CV (b), experimental CV of 5 mM Br^- (c). Mechanism in Digi-Sim Program, A + e = B, B + C = D, $K_{eq} = 1 \times 10^4$, $k_b = 10\ s^{-1}$, $k_s = 0.0035$ cm/s, $E_{1/2} = 0.91$ V; $[C]_A = [C]_C = 5$ mM, $[D]_A = [D]_B = [D]_C = [D]_D = 1 \times 10^{-5}\ cm^2/s$, $k_s = 0.5$ cm/s. It may be noted that Digi-Sim is in reduction process mode, but in simulation of CV it does not make any difference whether it is reduction or oxidation process shape of cyclic voltammogram of the oxidation of Br^- as shown in Fig. 8 strongly suggests that reaction follows the mechanism of a catalytic reaction, Eq. (*9*)[39]. The mechanism being

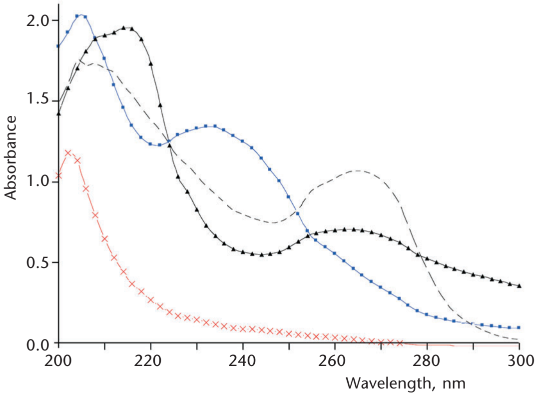

FIG. 7
Optical spectra of 0.2 mM cytosine (dashed line), 1.5 mM dibromine (black), 0.2 mM cytosine + 1.2 mM dibromine (blue) and electrolysis (product) of 5 mM KBr in the presence of 5 mM cytosine (red). Solvent H_2O

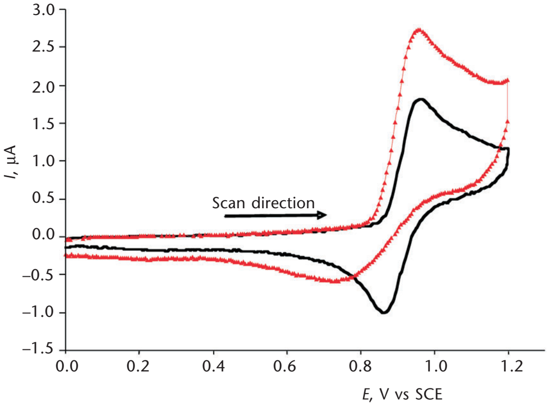

FIG. 8
Cyclic voltammogram representing reaction of electrooxidation product of Br^- with glycine on platinum (Pt) electrode. CV of 5 mM Br^- (black) and 5 mM Br^- in the presence of 50 mM glycine (red). Solvent H_2O

$$\mathrm{Br}^- - \mathrm{e} \rightarrow \mathrm{Br}^\bullet \tag{1}$$

$$\mathrm{Br}^\bullet + [\mathrm{Gly}] \quad [\mathrm{Gly\text{-}Br}]^\bullet \rightarrow [\mathrm{product}] + \mathrm{Br}^- . \tag{9}$$

When Br^- was electrolyzed in the presence of large excess of glycine (Gly), a UV spectrum similar to the spectrum recorded during the electrolysis of bromide ion alone was obtained. From this observation also, it is inferred that the mechanism is catalytic. Comparing the catalytic shape of the curve with those of case VI of ref.[39], the pseudo-first order rate constant k' is estimated to be $k' > 0.53\ \mathrm{s}^{-1}$. The bimolecular rate constant $k_2 = k'/[\mathrm{Gly}] \approx 10.6\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$, when concentration of Gly was taken as 50 mM.

Glycine does not have absorption in uv-vis range of interest. Dibromine has the absorption at $\lambda_{max} = 265$ nm (in water). So the reaction can be followed in this region. When glycine was added to dibromine, an absorption peak appeared at 240 nm (as shown in Fig. 9a) which gradually decayed with time (Fig. 9b). This new peak indicates that there is an intermediate formed during the reaction of dibromine with glycine:

$$\mathrm{Br}_2 + \text{glycine} \xrightarrow{\text{fast}} [\text{intermediate}] \xrightarrow{\text{slow}} \text{product} + \mathrm{Br}^- . \tag{10}$$

The kinetics of this reaction under the pseudo-first order condition monitored at 240 nm (λ_{max}, at which the intermediate is formed), gave an exponential decay curve (b in Fig. 6) with pseudo-first order rate constant $k' \approx 0.0049\ \mathrm{s}^{-1}$ for $t_{1/2} = 143$ s while bimolecular rate constant, $k_2 = k'/[\mathrm{Gly}] \approx 0.0649\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ (75 mM glycine).

It is concluded from the above data that as is the case with iodine atom-free radical[27], $Br^\bullet$ atom-free radical can be generated through electro-oxidation of Br^-. This electrochemically generated $Br^\bullet$ atom-free radical, under certain experimental conditions, instead of dimerizing to dibromine (Br_2, bromine molecule), can be made to react with its environment: water and chloroform or with substrates cytosine (Cyt) (EC mechanism) and glycine (Gly) (EC′ mechanism). The reaction with glycine is catalytic. The statement of Vetter[29] and experiments of Popov and Geski[30] for the formation and reaction of iodine atom-free radical, are also valid for the formation of bromine atom-free radical.

$$\mathrm{Br}^- - \mathrm{e} = \mathrm{Br}^\bullet$$

$$\mathrm{Br}^\bullet + \mathrm{Cyt} = \mathrm{Z}\ (\text{product(s)}) \tag{11}$$

$$\mathrm{Br}^\bullet + \mathrm{Gly} = [\mathrm{Br\text{-}Gly}]^\bullet = \mathrm{Br}^- + \text{product(s)} \tag{12}$$

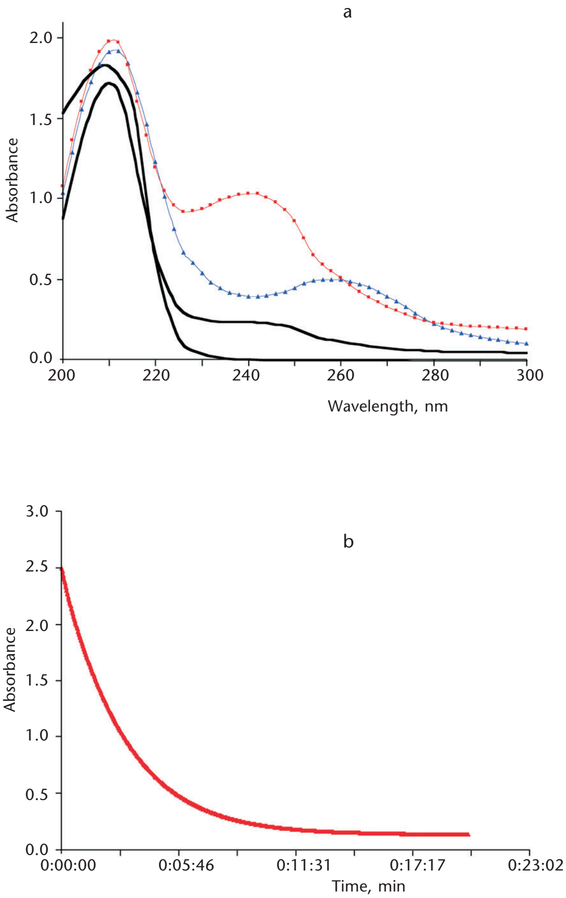

FIG. 9
Optical spectra of the 1 mM Br_2 + 30 mM glycine "mixture" A = Br_2, B, C, D, reaction profiles at various time (a). Decay of the reaction intermediate formed by the reaction of Br_2 with glycine (b)

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REFERENCES AND NOTES

1. a) Pryor W. A.: *Free Radical in Biology* (W. A. Pryor, Eds), pp. 1–53. Academic Press Inc., New York 1976; see also b) Pryor W. A.: *Introduction to Free Radical Chemistry*. Prentice Hall, New York 1966.
2. Kochi J. K.: (Ed.): *Free Radicals*, Vols 1 and 2. Wiley, New York 1973.
3. Hay J. M.: *Reactive Free Radicals*. Academic Press, New York 1974.
4. Minsci F.: *Free Radicals in Biology and Environment*. NATO ASI Series 3, High Technology 27.
5. Halliwell B.: *Encyclopedia of Molecular Cell Biology and Molecular Medicine*. Wiley, New York 2006.
6. Cheeseman K. H., Slater T. F.: *Br. Med. Bull.* **1993**, *49*, 481.
7. Riley P. A.: *Int. J. Radiat. Biol.* **1994**, *65*, 27.
8. Frey P. A., Hegman A. D., Reed G. H.: *Chem. Rev.* **2006**, *106*, 3302.
9. Chatgilialoglu C., Ferreri C.: *Acc. Chem. Res.* **2005**, *38*, 441.
10. Book of Abstracts of the *International Conference on Free Radicals in Biosystems, Kuwait University, March 18–21, 2007*.
11. Autor A. P. (Ed.): *Pathology of Oxygen*. Academic Press Inc., New York 1982.
12. McCord J. E., Roy R. S.: *Can. J. Physiol. Pharmacol.* **1982**, *60*, 1346.
13. a) Kosower E. M. in: *Free Radical in Biology* (W. A. Pryor, Ed.), Vol. II. Academic Press Inc., New York 1976, and references therein; b) Kosower E. M. in: *Topics in Current Chemistry* (F. L. Boschke, Ed.), Vol. 112, p. 117–162. Springer-Verlag, Berlin Heidelberg 1983, and references therein.
14. Mohammad M.: *J. Org. Chem.* **1987**, *52*, 2779; and references therein.
15. Asada K., Heber C. N., Shrieber U.: *Plant Cell Physiol.* **1990**, *31*, 557.
16. Monk P. C.: *The Viologens: Physicochemical Properties, Synthesis and Applications of the Salts of 4,4'-Bipyridine*. Wiley, New York 1998.
17. Fridovich I.: *Science* **1978**, *201*, 875; see also refs[11,12] above.
18. Mohammad M., Khan A. Y., Subhani M. S., Ahmed S., Bibi N., Saleemi S: *React. Intermed.* **2001**, *17*, 219; and references therein.
19. Siddiq A.: *M.S. Thesis*. California State University, Northridge (CA) 2004.
20. Walker W. H., Ehrenberg A., Lhoste J. M.: *Biochim. Biophys. Acta* **1970**, *215*, 166.
21. Yano Y.: *Antioxid. Redox Signal.* **2001**, *3*, 899; and references therein.
22. Müller F., Brüstlein M., Hemmerich P., Massey V., Walker W. H: *Eur. J. Biochem.* **1972**, *25*, 573.
23. Mans D. R., Retel J., van Maanen J. M., Lafleur M. V., van Schaik M. A., Pinedo H. M., Lanlelma J.: *Br. J. Cancer* **1990**, *62*, 54.
24. Zhang J., Schulz W. A., Li Y., Wang R., Zotz R., Wen D., Siegel D., Ross D., Gabbert H. E., Sarbia M.: *Carcinogenesis* **2003**, *24*, 905.
25. Ma B., Carr B. A., Krolikowski P., Chang F. N: *Chem. Res. Toxicol.* **2007**, *20*, 72.
26. Perchellet E. M., Sperfslage B. J., Qabaja G. J., Graham B., Perchellet J.-P.: *Anti-Cancer Drugs* **2001**, *12*, 401.
27. a) Mohammad M., Dar A., Jahangir S., Tahiri I. A.: Presented at *9th Eurasia, Conf., Chem., Sci., Antalya, Turkey, September 9–13, 2006*; also in b) Mohammad M., Dar A., Jahangir S., Tahiri I. A.: *Innovations in Chemical Biology* (B. Sener, Ed.), Chap. 27. Springer Science + Business media B. V., 2009.
28. Merenyi G., Lind J.: *J. Am. Chem. Soc.* **1994**, *116*, 7872.

29. a) Vetter K. J.: *Z. Electrochem. C* **1951**, *55*, 121; b) Vetter K. J.: *Z. Phys. Chem.* **1952** *199*, 22, as quoted in refs[19,20] below.
30. a) Popov A. I., Geski D. H.: *J. Am. Chem. Soc.* **1958**, *80*, 1340; b) Popov A. I., Geski D. H.: *J. Am. Chem. Soc.* **1958**, *80*, 5346.
31. WEBSITE search: There was practically no reference on the generation of bromine atom-free radical in aqueous media.
32. Allen G. D., Buzzo M. C., Davies I. G., Villagram C., Hardcare C., Compton R. G.: *J. Phys. Chem. B* **2004**, *108*, 16322.
33. Allen G. D., Buzzo M. C., Villagram C., Hardcare C., Compton R. G.: *J. Electroanal. Chem.* **2005**, *575*, 311.
34. Sato K., Takekoshi Y., Kanno S., Kawase S., Jin J-Y., Takeuchi T., Miwa T.: *Anal. Sci.* **1999**, *15*, 947.
35. Nematollahi D., Akaberi N.: *Molecules* **2000**, *6*, 639.
36. Moore A. M., Anderson S. M.: *Can. J. Chem.* **1959**, *37*, 590.
37. Streitwieser A., Heatcook C. H., Kosower E. M.: *Introduction to Organic Chemistry*, 4th ed., p. 430. MacMillan Publishing Company, Inc., New York 1992.
38. Olmstead M. L., Hamilton R. G., Nicholson R. S.: *Anal. Chem.* **1969**, *41*, 260.
39. Nicholson R. S., Shain I.: *Anal. Chem.* **1964**, *36*, 706.