Free Rad. Res. Comms., Vol. 1, No. 4, pp. 235–241
8755-0199/86/0104-0235 $25-00/0

Printed in Great Britain

FREE RADICAL INDUCED DEGRADATION OF 1,2-DIBROMOETHANE. GENERATION OF FREE Br· ATOMS

MANOHAR LAL, JÖRG MÖNIG, and KLAUS-DIETER ASMUS†

Hahn-Meitner-Institut für Kernforschung Berlin
Bereich Strahlenchemie, D-1000 Berlin 39
Federal Republic of Germany

(Received July 1, 1985)

Free Br· atoms have been found to be generated upon e_{aq}^- and ·OH radical induced degradation of 1,2-dibromoethane in aqueous solution. The relevant process is β-bromine cleavage from CH_2BrCH_2 and $CH_2BrCHBr$ radicals, respectively. An absolute rate constant of $k = 2.8 \times 10^6\ s^{-1}$ has been determined for the reaction $CH_2BrCH_2 \rightarrow Br^- + CH_2=CH_2$, while an estimate of $k \sim 10^6\ s^{-1}$ can be given for $CH_2BrCHBr \rightarrow Br^- + CH_2=CHBr$. The Br· atoms have been identified through their reaction with Br^- to $Br_2^{\cdot-}$ ($k = 7.7 \times 10^9\ mol^{-1}dm^3s^{-1}$) and their reaction with 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonate) ($k = 6.8 \times 10^9\ mol^{-1}dm^3s^{-1}$). Ethylene and vinyl bromide have been identified via GC. The results substantiate earlier findings that free radical induced mechanisms can serve as informative probe for metabolic processes.

Key words: free radicals; 1,2-dibromoethane; Br· atoms, β-bromine cleavage, absolute rate constants, vinyl bromide

INTRODUCTION

Halogenated organic compounds are known to be quite hazardous if metabolized in living species. From several studies on carbon tetrachloride (CCl_4) and halothane ($CF_3CHClBr$), for example, it has become evident that the toxic action of these compounds can be associated with the generation of free radicals both under normoxic and hypoxic conditions[1,2,3,4,5,6,7,8,9,10,11]. The initial chemical step is considered to be a dissociative electron capture

$$RX + e^- \longrightarrow R^{\cdot} + X^- \qquad (1)$$

with the electron being provided by, for example, the enzymatic cytochrome P-450 system of the endoplasmic reticulum of the liver.

In several recent studies on the radiation chemically induced degradation of CCl_4 and $CF_3CHClBr$ a striking agreement has been found between the product patterns arising from metabolic and free radical induced degradation of these compounds. It

†Correspondence to K.-D. Asmus

was therefore suggested that radiation chemical studies provide an insight into the chemistry which occurs in the biological systems[12,13,14].

The radical R· formed in reaction (1), e.g. $\dot{C}Cl_3$ or $CF_3\dot{C}HCl$ from CCl_4 or halothane, respectively, can be expected to react with the immediate surroundings. Primarily this could be a hydrogen atom abstraction reaction or addition (binding) to the lipid. In the presence of oxygen the carbon centered radicals usually suffer fast O_2 addition

$$R\cdot + O_2 \longrightarrow ROO\cdot \qquad (2)$$

to yield peroxyl radicals. It has been demonstrated that halogenated peroxyl radicals are reasonably good oxidants[13,14,15,16,17,18,19,20]. In an environment which enables hydrolysis they also are a source for organic acids[14], e.g. CF_3COOH from halothane.

One of the halogenated compounds which has been classified as particularly hazardous is 1,2-dibromoethane, CH_2BrCH_2Br (1,2-DBE)[21]. Metabolic products include ethylene[22], bromide, bromoacetaldehyde[23] and mercapturic acids[24,25]. 1,2-DBE is believed to be metabolized either via conjugation to glutathione or via oxidative dehalogenation[26,27]. Recently, however, still another pathway of metabolic activation was discovered, namely the reduction of 1,2-DBE to a free radical intermediate[28]. In view of the possible free radical involvement in the toxic action of 1,2-DBE we have now undertaken a radiation chemical study on aqueous solutions of this compound.

EXPERIMENTAL

The 1,2-dibromoethane (1,2-DBE) had a purity of >99% (Aldrich) and was used as received. All other chemicals were also of the purest commercially available grade and used without further treatment. The solvent was deionized, "Millipore" filtered water the quality of which corresponded to triply quartz distilled water. Deoxygenation of the solution, if required, was achieved by bubbling the solution with N_2 prior to the addition of 1,2-dibromoethane (bubbling time ca. 1 h per dm^3 solution). For the study of ·OH radical induced processes N_2O or N_2O/O_2 (4:1) mixtures were used instead of N_2. The function of the N_2O is to convert hydrated electrons into ·OH radicals ($e_{aq}^- + N_2O \rightarrow \cdot OH + N_2 + OH^-$). Both e_{aq}^- and ·OH are formed as major primary species upon radiolysis of water with about equal yield of ca. 0.3 μmol per Gy (G = 2.8 species per 100 eV absorbed energy; 1 Gy = 1 J kg^{-1} = 100 rad). Reactions of e_{aq}^- were investigated in the N_2 saturated solutions to which tert-butanol was added as ·OH scavenger.

γ-Irradiations were carried out by exposing the solutions (in rubber sealed glass vessels) to the field of a 7000 Ci ^{60}Co source at an absorbed dose rate of 350 Gy/h. The time resolved experiments were done by subjecting the solutions to short pulses of high energy electrons from a 1.55 MeV or a 3.8 MeV Van de Graaff accelerator. Typical doses per pulse were ca. 5 Gy. Analysis of ionic end products was carried out with high performance ion chromatography using a DIONEX 2010*i* machine. Olefinic products were identified by GC/MS. Details on irradiation procedures, including dosimetry, and on the analysis of compounds have already been described[18,29]. All experiments were carried out at room temperature.

RESULTS AND DISCUSSION

One-Electron Induced Degradation of 1,2-DBE

The one-electron reduction of 1,2-dibromoethane (1,2-DBE) as induced by hydrated electrons was studied by pulse radiolysis. The first step, i.e. dissociative electron capture

$$CH_2BrCH_2Br + e_{aq}^- \longrightarrow CH_2BrC\dot{H}_2 + Br^- \quad (3)$$

has kinetically been characterized by following the decay of the e_{aq}^- absorption (at 700 nm) in pulse irradiated N_2 sat. solutions (10% v/v tert-butanol) in the presence of various concentrations of 1,2-DBE (45 to 120 μmol dm^{-3} solutions). The bimolecular rate constant thus derived amounts to $k_3 = (1.2 \pm 0.2) \times 10^{10}$ mol^{-1}dm^{3}s^{-1} which indicates an essentially diffusion controlled process.

Addition of small amounts of bromide ions resulted in the formation of the well known $Br_2^{\cdot-}$ absorption with λ_{max} at 365 nm. The first order rate constant $k = \ln 2/t_{½}$ is plotted *vs.* the Br^- concentration in Figure 1. It is seen to increase with [Br^-] at low bromide concentrations and to level off above 2 mmol dm^{-3} Br^-. From the initial slope a bimolecular rate constant of $(7.7 \pm 1.0) \times 10^9$ mol^{-1}dm^{3}s^{-1} is derived. Within experimental limits of error this value agrees with that for the forward reaction in the equilibrium[30]

$$Br^{\cdot} + Br^- \rightleftharpoons Br_2^{\cdot-} \quad (4)$$

The fact that the $Br_2^{\cdot-}$ formation becomes independent of [Br^-] at higher bromide concentrations means that a preceding reaction takes over as rate determining step. At the 1,2-DBE concentration of 10 mmol dm^{-3} used in these experiments the halflife for the initial dissociative electron capture amounts to only 5.8 ns. This clearly eliminates reaction (3) as rate limiting step. In addition, the results also unequivocally demonstrate that the $Br_2^{\cdot-}$ formation cannot be the result of a direct bromine atom transfer from $CH_2BrC\dot{H}_2$ to Br^- but must involve a free bromine atom as an intermediate. The plateau value of $k = 2.8 \times 10^6$ s^{-1}, corresponding to $t_{½} = 250$ ns, is consequently attributed to the β-bromine cleavage.

$$\begin{array}{l} Br \\ | \\ CH_2\text{—}\dot{C}H_2 \end{array} \longrightarrow Br^{\cdot} + CH_2{=}CH_2 \quad (5)$$

Our result provides the first absolute measure for the rate of this process, and agrees well with estimates of $t_{½} < 1$ μs which can be drawn from the fact that β-bromoethyl radicals could not be detected by ESR in solutions at room temperature.

The formation of a strongly oxidizing species upon the one-electron reduction of 1,2-DBE is also evident when small amounts of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonate), ABTS, instead of Br^- is present in the pulse irradiated solutions. In this case $ABTS^+$ radical cations which show a strong absorption band at 415 nm[31] are formed with a bimolecular rate constant of $(6.8 \pm 0.7) \times 10^9$ mol^{-1}dm^{3}s^{-1}. The latter is attributed to the reaction

$$Br^{\cdot} + ABTS \longrightarrow ABTS^+ + Br^- \quad (6)$$

Corresponding γ-radiolysis experiments with N_2 sat. (pH 5.7) solutions of 2.9 mmol dm^{-3} 1,2-DBE and 100 mmol dm^{-3} tert-butanol gave a total bromide ion

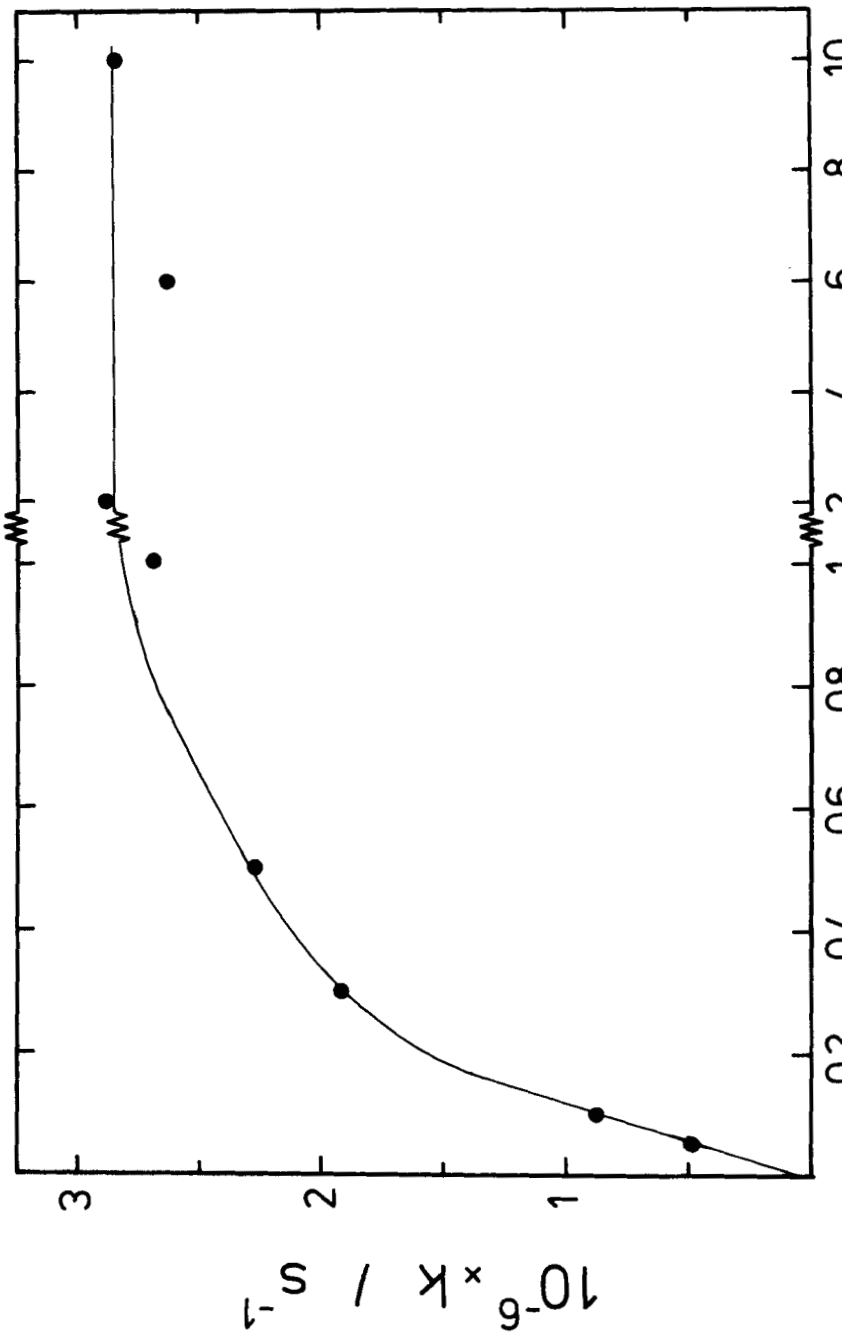

FIGURE 1 First order rate constant $k = \ln 2/t_{½}$ *vs.* $[Br^-]$ for the formation of Br_2 in pulse irradiated, N_2 sat. aqueous solutions of 10 mmol dm^{-3} 1,2-dibromoethane and 10% (v/v) tert-butanol.

yield of $G(Br^-) = 6.4$. This value is slightly higher than twice the yield of hydrated electrons (*ca* $2 \times 2.75 = 5.5$) suggesting that indeed both bromine atoms have been released from 1,2-DBE. The conversion of Br· into Br^- can be envisaged to result from a hydrogen atom abstraction reaction from the tert-butanol and possibly via reaction with the solvent water. To some extent $\dot{C}H_2C(CH_3)_2OH$ radicals also contribute to the Br^- formation. This can be concluded from $G(Br^-) = 2.5$ obtained upon irradiation of N_2O saturated but otherwise identical solutions. Since $G(\dot{C}H_2C(CH_3)_2OH)$ is about 6 under the latter conditions, however, only a fraction ($\approx 40\%$) of these radicals leads to the formation of bromide ions. Considering that in N_2 saturated solutions tert-butanol radicals are formed with $G \approx 2.8$ the total Br^- expected on this basis in the N_2 sat. solutions would amount to $5.5 + 1.1 = 6.6$ which agrees well with the experimental value of 6.4 within the general radiation chemical error limit of $\pm 10\%$. (The underlying mechanism for the Br^- formation through $\dot{C}H_2C(CH_3)_2OH$ still remains to be evaluated).

The other stable product expected from reaction (5), namely ethylene has been identified by headspace gas chromatography.

·*OH-Radical Induced Degradation of 1,2-DBE*

Hydroxyl radicals also react with 1,2-DBE as is evidenced by a high yield of Br^- ions ($G = 5.6$) measured in irradiated N_2O saturated, pH 5.7 solutions of 2.9 mmol dm^{-3} 1,2-DBE. Numerically this corresponds to a $Br^-/{\cdot}OH$ ratio of about 1:1. Since ethylene is practically not formed the underlying mechanism is suggested to be hydrogen atom abstraction

$$\cdot OH + CH_2BrCH_2Br \longrightarrow CH_2Br\dot{C}HBr + H_2O \qquad (7)$$

followed by β-bromine cleavage

$$\begin{array}{l} \quad Br \\ \quad | \\ CH_2\text{---}\dot{C}HBr \end{array} \longrightarrow Br\cdot + CH_2{=}CHBr \qquad (8)$$

Both Br· and $CH_2 = CHBr$ have positively been identified as $Br_2^{\cdot-}$ by pulse radiolysis in Br^- containing solutions (as described above) and by headspace gaschromatography (comparison with an authentic sample), respectively. An absolute rate constant for the β-bromine cleavage could not be evaluated in this case. At the required high Br^- concentrations (see Figure 1) Br^- would be oxidized to $Br_2^{\cdot-}$ by ·OH directly. From the kinetic data on the $Br_2^{\cdot-}$ formation at low Br^- concentrations ($\leqslant 0.5$ mmol dm^{-3}) an estimate of $k \sim 10^6\ s^{-1}$ can, however, be derived for reaction (8).

Further evidence for $CH_2Br\dot{C}HBr$ radicals as intermediates is provided by appreciable yields of $CH_2BrCOOH$ ($G \sim 1.4$) upon γ-irradiation of air saturated solutions. In analogy to the corresponding acid formation from various chlorinated ethanes[14,32] the generation of bromoacetic acid requires O_2 addition to a carbon centered radical which still carries the bromine atom. $CH_2BrCOOH$ would then be a direct degradation product of the $CH_2BrCHBrOO\cdot$ peroxyl radical. Although a detailed quantitative study still remains to be done it appears that O_2 addition to $CH_2Br\dot{C}HBr$ and β-bromine cleavage according to reaction (8) are competing processes occurring with about equal rates in air sat. solutions. Taking a typical rate constant of $(1\text{–}4) \times 10^9\ mol^{-1}dm^3s^{-1}$ for the O_2 addition to various chloroalkyl radicals[13,17,33] again a rate constant in the order of $10^6\ s^{-1}$ or slightly less can be

estimated for the β-bromine cleavage from the $CH_2BrĊHBr$ radical.

The formation of bromide ions is not as straight forward as in the case of the electron induced degradation of 1,2-DBE. Possible sources are hydrolysis of the vinyl bromide and Br· atom interaction with the solvent water. Whether this includes also a short chain reaction (e.g. due to regeneration of ·OH in the $Br· + H_2O$ reaction) is still under investigation.

CONCLUSION

Our results have clearly demonstrated that free bromine atoms are generated upon both a reductive (e_{aq}^-) and oxidative (·OH) radical attack on 1,2-dibromoethane. The Br· atoms in both cases result from β-cleavage in the initial radicals $CH_2BrĊH_2$ and $CH_2BrĊHBr$, respectively. Absolute rate constants for these latter processes have now been measured for the first time and indicate that Br· atoms are available for consecutive reactions within less than 1 μs. In oxygenated solutions a competetive route, namely reaction of the above radicals with O_2, is nevertheless still possible. This could positively be proven for the oxidative route where the $CH_2BrCHBrOO·$ peroxyl radical in aqueous solution is converted to bromo acetic acid. It can be anticipated that both the peroxyl radicals $CH_2BrCHBrOO·$ and $CH_2BrCH_2OO·$ are also a source of bromoacetaldehyde which has been suggested to be among the most toxic of all products derived from oxidative metabolic degradation of 1,2-DBE[23].

Another compound of significance with respect to its potential toxicity is the vinyl bromide generated as a result of an initial hydrogen atom abstraction from 1,2-DBE.

Acknowledgement

This work has been performed pursuant to a contract with the National Foundation for Cancer Research (NFCR).

References

1. T.C. Butler, *J. Pharmacol. Exp. Ther.*, **134**, 311, (1961).
2. R.O. Recknagel, *Pharmacol. Rev.*, **19**, 145, (1967).
3. T.F. Slater, in "*Free Radical Mechanisms in Tissue Injury*" (Pion Ltd.: London, 1972).
4. R.O. Recknagel, E.A. Glende Jr., and A.M. Hruszkevycz, in "*Free Radicals in Biology*", ed. W.A. Pryor (Academic Press: New York, 1977) vol. III, p. 97.
5. E.S. Reynolds and M.T. Moslen, in "*Free Radicals in Biology*", ed. W.A. Pryor (Academic Press: New York, 1980) vol. IV, p. 49.
6. R.C. Jee, I.G. Sipes, A.J. Gandolfi, and B.R. Brown Jr., *Toxicol. Appl. Pharmacol.*, **52**, 267, (1980).
7. R.P. Hanzlik, *Biochem. Pharmacol.*, **30**, 3027, (1981).
8. J. Lee Poyer, P.B. McCay, C.C. Weedle, and P.E. Downs, *Biochem. Pharmacol.*, **30**, 1517, (1981).
9. J.R. Trudell, B. Bösterling, and A. Trevor, *Biochem. Biophys. Res. Commun.*, **102**, 372, (1981).
10. T.F. Slater, in "*Free Radicals, Lipid Peroxidation and Cancer*", ed. D.C.H. McBrien and T.F. Slater (Academic Press: London, 1982) p. 243.
11. K. Fujii, N. Miki, M. Kanashiro, R. Miura, T. Sugiyama, M. Morio, T. Yamano, and Y. Miyake, *J. Biochem.*, **91**, 415, (1982).
12. J. Mönig, K. Krischer, and K.-D. Asmus, *Chem. Biol. Interact.*, **45**, 43, (1983).
13. J. Mönig, D. Bahnemann, and K.-D. Asmus, *Chem. Biol. Interact.*, **47**, 15, (1983).
14. K.-D. Asmus, D. Bahnemann, K. Krischer, M. Lal, and J. Mönig, *Life Chem. Reports*, 1985, vol. 3, p. 1.
15. J.E. Packer, T.F. Slater, and R.L. Willson, *Life Sci.*, **23**, 2617, (1978).
16. J.E. Packer, R.L. Willson, D. Bahnemann, and K.-D. Asmus, *J. Chem. Soc. Perkin II*, 296, (1980).
17. J. Mönig, K.-D. Asmus, M. Schaeffer, T.F. Slater, and R.L. Willson, *J. Chem. Soc. Perkin II*, 1133, (1983).

18. J. Mönig and K.-D. Asmus, *J. Chem. Soc. Perkin II*, 2057, (1984).
19. J. Mönig and K.-D. Asmus, in "*Oxygen Radicals in Chemistry and Biology*", ed. W. Bors, M. Saran and D. Tait (W. de Gruyter and Co.: Berlin, 1984) p. 57.
20. J. Mönig, M. Göbl, and K.-D. Asmus, *J. Chem. Soc. Perkin II*, 647, (1985).
21. U. Rannug, *Mut. Res.*, **76**, 269, (1980), and references cited therein.
22. J.C. Livesey and M.W. Anders, *Drug Metab. Dispos.*, **7**, 199, (1979).
23. D.L. Hill, T.W. Shih, T.P. Johnston, and R.F. Struck, *Cancer Res.*, **38**, 2438, (1978).
24. E. Nachtomi, *Biochem. Pharmacol.* **19**, 2853, (1970).
25. P.J. van Bladeren, D.D. Breimer, G.M.T. Rotteveel-Smijs, P. de Knijff, G.R. Mohn, B. van Meeteren-Wälchli, W. Buijs, and A. van der Gen, *Carcinogenesis*, **2**, 499, (1981).
26. M.W. Anders and J.C. Livesey, *Banbury Rep.*, **5**, 331, (1980).
27. R.D. White, A.J. Gandolfi, G.T. Bowden, and I.G. Sipes, *Toxicol. Appl. Pharmacol.* **69**, 170, (1983).
28. A. Tomasi, E. Albano, M.U. Dianzani, T.F. Slater, and V. Vannini, *FEBS Let.*, **160**, 191, (1983).
29. K.-D. Asmus, in "*Methods in Enzymology*", ed. L. Packer (Academic Press: Orlando, 1984) vol. 105, p. 167.
30. M.S. Matheson, W.A. Mulac, J.L. Weeks, and J. Rabani, *J. Phys. Chem.*, **70**, 2092, (1966).
31. B.S. Wolfenden and R.L. Willson, *J. Chem. Soc. Perkin II*, 805, (1982).
32. M. Lal, J. Mönig, and K.-D. Asmus, *Proc. Int. Symp. on Artificial Radioactivity*, (Pune, India 1985) RC-3[1]-RC-3[7].
33. M. Lal, J. Mönig, and K.-D. Asmus to be published.

Accepted by Dr. P. Wardman