Hydroperoxyl Radical Generation by γ-Irradiation of Glassy Water at 77 K

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Received February 19, 1996[⊗]

Abstract: ESR spectroscopy has been used to identify the paramagnetic species trapped at 77 K upon γ-irradiation of glassy water prepared by hyperquenching of liquid water at cooling rates exceeding 10^5 K/s. Besides OH radicals, which are the only paramagnetic intermediates of water radiolysis stabilized at 77 K in hexagonal ice prepared by slow cooling of liquid water, comparable amounts of HO_2 radicals were found in glassy water. The ESR spectra for trapped OH radicals were typical of such radicals in a glass. Neither trapped electrons nor trapped hydrogen atoms were detected. The contributions of OH and HO_2 radicals to the ESR spectra of glassy water do not depend on irradiation dose in the range 0.8−18 kGy. We stress that HO_2 radicals are not detected in irradiated hexagonal ice under these conditions. They are formed as secondary products after annealing to destroy the OH radicals, but the yields are tiny relative to the remarkably high yields observed in the glassy water. Possible mechanisms for HO_2 radical formation in the early stages of water radiolysis in the glassy state are discussed.

Introduction

Radicals generated by high-energy irradiation of liquid water are one of the basic sources of radiation damage to biomolecules.[1] Since these radicals are short-lived at ambient temperature, they are often studied at cryogenic temperatures by electron spin resonance (ESR) spectroscopy after irradiating either crystalline ice[2] or highly concentrated aqueous electrolyte solution glasses.[3] In the latter case high electrolyte concentrations were chosen to slow down the crystallization rate of ice and to obtain a glass even on slow cooling. While these studies provided a wealth of information on e.g. the OH and hydroperoxy (HO_2) radicals, they also bear disadvantages in that further reactions of these radicals with biomolecules may not be those occurring in liquid water because of formation of other radicals from the solute in the case of the electrolyte solution glass[3] and/or perturbation of the water structure by the solute. Furthermore, in slow-cooled aqueous solutions where ice is formed and phase separation of the solute occurs, the OH radicals trapped in the ice compartments are unable to interact with solutes, such as DNA or nucleotide, because these are dissolved only in the "freeze-concentration" regions.[3,4] These problems can in principle be overcome by investigating water and dilute aqueous solutions in their glassy states which can be obtained by rapid quenching of the liquids.

To the best of our knowledge we report here the first ESR studies of paramagnetic species trapped in glassy water upon γ-irradiation at 77 K. Glassy water, known since 1980,[5] can now routinely be made in gram-quantities by so-called "hyperquenching" of micrometer-sized water droplets on a solid cryoplate.[6] A variant of this method has recently been reported.[7] The estimated[8] and calculated[9] cooling rates are $\approx 10^6$ to $\approx 10^7$ K s^{-1}, and these cooling rates are consistent with "freezing-in" of carbonylhemoglobin's CO conformer population by hyperquenching of its aqueous solution into the glassy state.[10] In this FTIR spectroscopic study, it was concluded by comparison of band area ratios of the CIV conformer band centered at $\approx$1970 cm^{-1} and the CIII conformer band centered at $\approx$1952 cm^{-1} that the highly dynamic conformer equilibrium has been immobilized close to ambient temperature by hyperquenching. Hyperquenched glassy water (HGW) has been studied by differential scanning calorimetry (DSC),[11−14] X-ray, neutron[15,16] and electron[17] diffraction, infrared spectroscopy,[7,18] dielectric

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[⊗] Abstract published in *Advance ACS Abstracts*, September 15, 1996.

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S0002-7863(96)00518-5 CCC: $12.00

relaxation,[19] and hole burning.[20] The structure of HGW differs from that of liquid water at ambient temperature in that liquid water on supercooling undergoes structural changes giving a more open, fully hydrogen-bonded tetrahedral network.[15,16,21−24] Even so, glassy water is the best "model" for liquid water where the effects of thermal excitation can be separated from those of positional and orientational disorder.[25] On heating at 30 K min^{-1}, the onset temperature of the glass → liquid transition (T_g) is at 136 K for glassy H_2O water and at 137 K for D_2O water.[12−14] The isothermal kinetics of their subsequent crystallization to cubic ice has been studied by FT-IR spectroscopy.[26]

We show in this work that, besides OH radicals, which are the only paramagnetic intermediate of water radiolysis stabilized in irradiated hexagonal ice at 77 K, we have found in glassy water comparable amounts of HO_2 radicals. Surprisingly, the HO_2 radicals are produced in much earlier stages of water radiolysis than expected. Irradiation of HGW is compared with that of hexagonal ice and the experimental spectra are compared with simulated curves.

Experimental Section

Glassy water (HGW) was prepared by so-called hyperquenching of aerosol droplets on a copper substrate held at 77 K. Briefly, water droplets of ≈5 μm diameter size made by means of an ultrasonic nebulizer (HICO ULTRASONAT, model 706E, operating at 1.7 MHz, droplet size according to company specifications) were suspended in gaseous nitrogen (99.999%) and passed into a high-vacuum cryostat through a 300-μm aperture. Once inside, the droplets moved at supersonic speed toward the substrate and deposited on it. Droplet size follows from its inverse relationship with ultrasonic frequency which has been tested for 3-MHz frequency.[27] One-hour deposition produced a 2−3 mm thick, opaque layer of glassy solid water with a porcelain-like appearance and texture. According to X-ray diffractograms it contained at most 5% crystalline, mainly cubic, ice.[6,11−14] DSC studies of HGW deposited on a copper plate, on a brass plate, or on IR transmitting materials gave identical results. All transfers of the sample from one container to the other were made when immersed in liquid N_2. The γ-irradiations were performed at 77 K at a dose rate of about 5 kGy/h.

The ESR spectra were recorded with an X-band ER 200D-SRC spectrometer, on line with ESP 3220−200SH data acquisition and processing system (BRUKER, Analyt. Messtechnik GmbH). The spectrometer was provided with ESR 900 (Oxford Instruments Ltd.) continuous helium gas flow or a home made cold nitrogen gas flow cryostat driven by a ITC-4 temperature controller (Oxford Instruments Ltd.). For irradiation and measurements at 77 K liquid nitrogen was used.

The recorded spectra were compared with those simulated.[28] Rhombic **g** and **A** tensors were assumed. The mutual orientation of their principal axes was taken to be identical with that deduced from our earlier studies on HO_2 radicals trapped in an irradiated single crystal of hexagonal ice.[29] The positions of hyperfine lines were calculated to the first-order approximation and correction for the transition probability in the field-swept spectrum was introduced. The line shapes were simulated from Gaussian and Lorentzian components, whose relative contributions were chosen to obtain the best visual fit with the experimental spectrum. The rise of the high field line was particularly sensitive criterion for that.

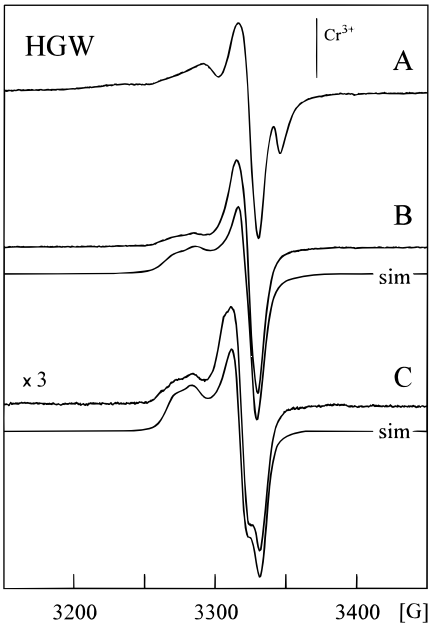

Figure 1. X-band ESR spectra recorded at 77 K for HGW γ-irradiated (20 kGy) at 77 K: (A) just after the irradiation; (B) after subsequent annealing for 5 min at 100 K; (C) after further annealing for 5 min at 140 K. The simulated spectra for HO_2 radicals with rhombic **g** and **A** tensors are also shown, cf. Table 1.

Results and Discussion

A typical ESR spectrum, recorded at the irradiation temperature of 77 K for glassy water, is presented in Figure 1A. It is dramatically different from that recorded for hexagonal ice under similar conditions of irradiation and measurement, cf. Figure 2A, attributed to an anisotropic, powder spectrum of OH radicals.[29] Besides the difference in line shapes shown in Figures 1A and 2A we have found that the total radiation yield of trapped paramagnetic species in HGW is approximately twice that of OH in I_h. No traces of trapped hydrogen atoms or trapped electrons were found in HGW irradiated at 77 K.

Thermal annealing reveals that the spectrum of HGW γ-irradiated at 77 K, Figure 1A, consists of two components of about equal contributions. One of them decays completely at about 100 K leaving the spectrum presented in Figure 1B. The decaying component, denoted as B in Figure 3, obtained by subtraction of the spectrum of the sample annealed at 100 K from that just after the irradiation at 77, is typical for OH radicals trapped in glassy matrices.[3]

In glassy water, the OH radicals are less stable than those trapped in hexagonal ice. They decay completely at about 100 K, i.e. some 30 K below the temperature of their disappearance upon stepwise annealing of hexagonal ice.[30] This is consistent with recent findings[31] on enhanced mobility in HGW even below its glass transition temperature. The more stable component

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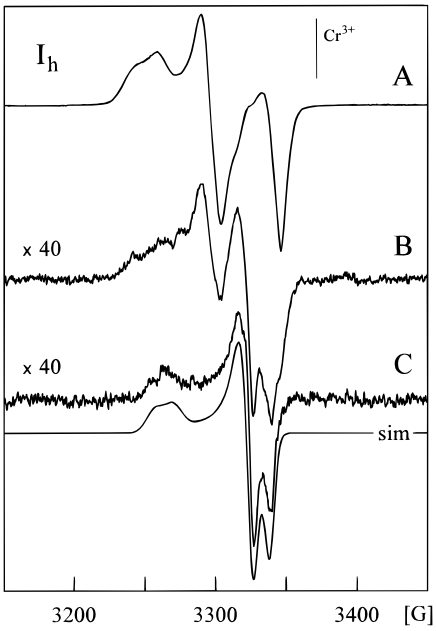

Figure 2. X-band ESR spectra, recorded at 77 K for I_h ice γ-irradiated (20 kGy) at 77 K: (A) just after the irradiation; (B) after subsequent annealing for 5 min at 120 K; (C) after further annealing for 5 min at 150 K. The simulated spectrum for HO_2 radicals with rhombic **g** and **A** tensors is also shown, cf. Table 1.

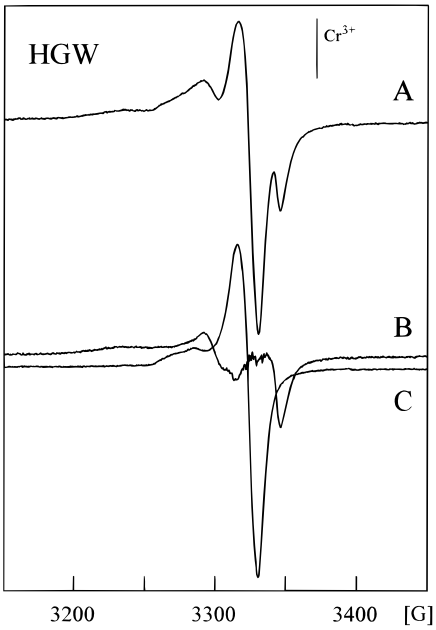

Figure 3. Analysis of the X-band ESR spectrum recorded for HGW γ-irradiated at 77 K. The spectrum A recorded just after the irradiation is the superposition of the signal B decaying upon annealing at 100 K (difference of spectra A and B in Figure 1) attributed to OH radicals in glassy environment and the signal C (cf. Figure 1B) assigned to HO_2 radicals.

of the ESR spectrum of irradiated glassy water, Figure 1B, gains resolution for samples annealed at about 140 K, cf. Figure 1C. It decays at about 150 K. Its shape is characteristic of radicals with axial or almost axial **g** tensors, $g_x \approx g_y < g_z$, and g_x quite close to the free spin value. The hyperfine splitting along g_z is about 12 G, cf. Table 1. These features are characteristic of HO_2 radicals.[30]

In ice I_h only small amounts of HO_2 radicals are formed by secondary processes, cf. Figure 2C, following decay of OH radicals upon annealing of the irradiated sample above 130 K. In marked contrast, HO_2 is present in very high relative yields

Table 1. ESR Parameters from Simulations of Powder Spectra of HO_2 Radicals in HGW and I_h[a]

matrix	g_x	g_y	g_z	A_x	A_y	A_z
HGW, Figure 1B	2.0040	2.0075	2.0355	5.0	15.0	6.0
HGW, Figure 1C	2.0055	2.0075	2.0350	12.0	12.5	6.0
I_h, Figure 2C	2.0033	2.0080	2.0450	12.5	13.0	6.5

[a] The components of the **A** tensor are given in G.

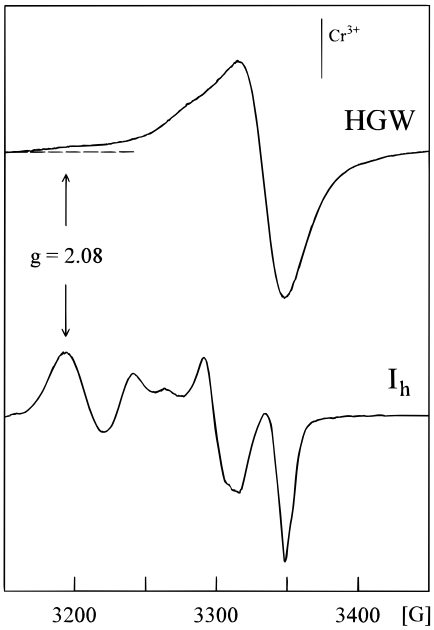

Figure 4. X-band ESR spectra, recorded at 4 K, for HGW and ice I_h γ-irradiated (20 kGy) at 77 K. The position of $g_\perp$ for O^- anion radicals is indicated. They are formed reversibly from OH radicals in I_h ice. Excessive line broadening obscures this transformation in HGW.

in the glassy ice matrix at 77 K. The quantitative analysis of ESR spectra shows that the yields of OH and HO_2 radicals are almost equal at 77 K, cf. Figure 3.

The proton hyperfine splittings in HGW are slightly smaller than those in ice I_h and the anisotropy of the **g** tensor is significantly decreased. All this suggests some libratory motion of hydrogen-bonded HO_2 radicals in HGW even at 77 K. It is not possible to check the suggestion directly by analysis of spectra recorded at lower temperatures, because of extensive broadening of lines. This effect is seen in Figure 4 presenting the spectra recorded at 4 K for HGW and I_h γ-irradiated at 77 K. The broadening is probably due to the distributions of components of **g** and **A** tensors reflecting the distribution of trapping sites in glassy environment.

The shape of the ESR spectrum of glassy water does not change at 77 K with the dose of γ-irradiation in the range 0.8−18 kGy. This means that the relative contribution of OH and HO_2 radicals remains constant and independent of dose. For hexagonal ice, the weak signal of HO_2 radicals starts to build up on the top of the spectrum of OH radicals at the doses of hundreds of kGy. The results clearly indicate that HO_2 radicals in glassy water are formed in much earlier stages of γ-radiolysis than those in γ-radiolysis of crystalline or polycrystalline ices. It is almost certain that the OH radicals in ice I_h are trapped in the original sites,[29,30,32] cf. left-hand side of Scheme 1 showing the ionization event of the H_2O molecule in a local configuration of C_{2v} pentamer symmetry.

At about 130 K the OH radicals become mobile and are lost, presumably via the formation of H_2O_2. As the concentration

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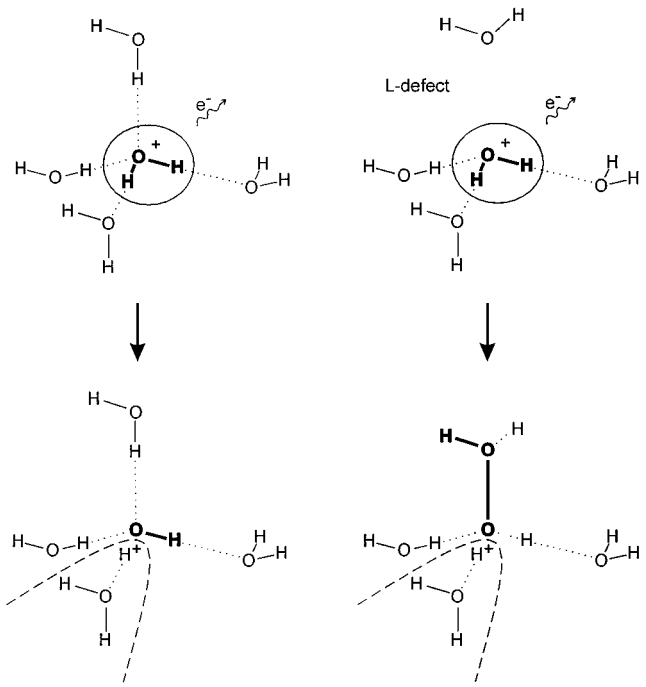
Scheme 1



of H_2O_2 builds up, then there is a probability[33] that reaction 1 also occurs, with the formation of HO_2 radicals in low concentration

$$H_2O_2 + OH \rightarrow HO_2 + H_2O \quad (1)$$

It is very difficult to understand[33] how such a process could become so efficient in the glass that very high yields of HO_2 radicals could be formed. We have therefore sought an alternative mechanism such as that[34] shown in reaction 2

$$OH + H_2O \rightarrow HO_2 + H_2 \quad (2)$$

However, as it is written, reaction 2 also seems unlikely. Hence we envisage a reaction that occurs at Bjerrum-like L-defects, present in a relatively high concentration in glassy water.[35] The required oxygen−oxygen bond can be formed incipiently as a weak 3-electron bond.[36] Because it may be necessary to invoke the participation of excited species for this reaction we postulate a concerted process of the type depicted in Scheme 1 (right-hand side), with O−O bond formation, loss of H_2, and formation of H_3O^+ occurring at about the same time. In hexagonal ice, because of much lower concentration of Bjerrum-like L-defects, the above postulated mechanism would be of minor importance.

As with normal ice, we were unable to detect either trapped hydrogen atoms or trapped electrons in hyperquenched glassy water γ-irradiated at 77 K. This means that the hyperquenched glassy water does not provide good trapping sites for hydrogen atoms and this is in contrast with a number of aqueous binary systems that freeze to give glasses.[3] The same is true for trapped electrons. They were highly expected[37] but there was complete absence of any color or ESR signal attributable to trapped electrons.

Conclusions

Our results show that, compared with crystalline ice, there is a remarkably high yield of trapped HO_2 radicals in hyperquenched glassy water γ-irradiated at 77 K. This yield of HO_2 radicals is comparable with that of OH radicals, which had ESR spectra characteristic of OH radicals in a glassy medium. The complete absence of trapped electrons and trapped hydrogen atoms is evidence that this glassy medium, contrary to expectation, is not providing at 77 K the regions in which electrons or hydrogen atoms may be trapped. It contains a relatively high amount of L-defects which seem to be involved in HO_2 formation.

Acknowledgment. This work was supported by KBN grant No. 3 T09A 025 10 (J.B. and A.P.) and by Forschungsforderungsfonds of Austria, project P10404-PHY (A.H. and E.M.). The authors are also grateful to the British Council for the travel grant (MCRS) and to the participants of the workshop on structure of oxygen radicals in irradiated solids, SORIS'96 (Nieborow, Poland), for many helpful comments.

JA960518W

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