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Trapped Hydrogen Atom Yields and Precursors in Frozen Solution Radiolysis

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Abstract: Trapped hydrogen atom yields from the radiolysis of γ-irradiated frozen solutions at 77°K have been measured for over 35 oxyanion salts; 15 of these were found to trap hydrogen atoms. Hydrogen atoms are trapped only by oxyanions in their highest oxidation states except for selenite. Hydrogen atom yields are decreased by added electron scavengers such as nitrate ion in ices containing mononegative and large dinegative oxyanions. The relative rates of decrease by nitrate and nitrite scavengers indicate that the mobile precursor of the trapped hydrogen atoms is an electron rather than a hydrogen atom. Optical bleaching on irradiated ices containing $H_2PO_4^-$ and ClO_4^- produces additional trapped hydrogen atoms. This bleaching effect is thought to be due to dissociation of trapped radical anions which have been formed by reaction of a mobile electron. Scavenging and bleaching experiments indicate that positive holes also react with the oxyanion to form trapped, bleachable centers.

Hydrogen atoms are trapped at 77°K in γ-irradiated frozen solutions of phosphoric, sulfuric, and perchloric acids[2] and the alkali metal salts of these acids.[3] The trapped hydrogen atom yields depend only on the nature of the anion and are independent of cation type and pH. For mononegative but not polynegative oxyanions which trap hydrogen atoms it has been postulated[3] that a mobile electron is the hydrogen atom precursor; this postulate is based primarily on the suppression of trapped hydrogen atom yields by added electron scavengers. In the present study, in order to learn what types of oxyanion systems trap hydrogen atoms, an extensive number of oxyanion frozen solutions were examined. Electron scavenger effects were studied and show that the above postulate must be modified to include large dinegative anions. Evidence is presented that points to the presence of stable hydrogen atom precursors. Presumably, these are dinegative species formed by reaction of an electron with bisulfate, dihydrogen phosphate, and perchlorate salts but not with sulfate or monohydrogen phosphate salts. It is also concluded that positive holes react with the oxyanion to form trapped, bleachable centers.

Experimental Section

Samples were prepared as previously described.[3] The selenates, tellurates, stannate, and arsenates were obtained from Alfa Inorganics; other salts were reagent grade. Radiolyses were performed at 77°K in a Co^{60} source at a dose rate of 0.7 Mrad/hr. The hydrogen atoms were detected by electron paramagnetic resonance with a Varian 4500 epr spectrometer. Optical bleaching was performed with a 1000-w tungsten lamp focused on the epr dewar which contained the ice sample immersed in liquid nitrogen.

The hydrogen atom yields reported were all determined in comparison with the hydrogen atom yield in 1.0 M $NaClO_4$ which was taken as $G(H) = 0.02$.[3] The yields are reported as G values, the number of hydrogen atoms trapped per 100 ev of radiation energy absorbed.

Results

A variety of oxyanions were examined for their ability to trap hydrogen atoms in irradiated frozen solutions at 77°K. The trapped hydrogen atom yields observed for the respective oxyanions are in Table I. Sodium and/or potassium salts were used in all cases.

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(1) University of Kansas.

(2) R. Livingston and A. J. Weinberger, *J. Chem. Phys.*, **33**, 499 (1960); R. Livingston, H. Zeldes, and E. H. Taylor, *Discussions Faraday Soc.*, **19**, 166 (1955).

(3) L. Kevan, P. N. Moorthy, and J. J. Weiss, *J. Am. Chem. Soc.*, **86**, 771 (1964).

In the absence of modulation and spin–spin broadening the line widths at maximum slope are 5 gauss for most salts, but in BO_2^-, SiO_3^{2-}, $Sn(OH)_6^{2-}$, SeO_3^{2-}, and $HAsO_4^{2-}$ the line widths are about 7 gauss, and in SO_4^{2-} the line width is 11 gauss. The relative yields were determined by calculating hw^2, where h is the height of the line and w its width.

Table I. Trapped Hydrogen Atom Yields per 100 Ev in γ-Irradiated 1 *M* Frozen Solutions of Various Oxyanions at 77°K

	Oxyanion	100-ev yield
Borate	BO_2^-	0.010[a]
	$B_4O_7^{2-}$	0.010[a]
Carbonate	HCO_3^-	0.010
	CO_3^{2-}	0.006
Silicate	SiO_3^{2-}	0.012
Phosphate	$H_2PO_4^-$	0.27
	HPO_4^{2-}	0.07[a]
	PO_4^{3-}	0.03[a]
Sulfate	HSO_4^-	0.14
	SO_4^{2-}	0.04
Perchlorate	ClO_4^-	0.02
Arsenate	$HAsO_4^{2-}$	0.007
Selenate	SeO_4^{2-}	~0.001
Selenite	SeO_3^{2-}	0.012
Stannate	$Sn(OH)_6^{2-}$	0.014

[a] Yields were extrapolated linearly to 1 *M*.

The hydrogen atom yields from borates, carbonates, silicate, arsenate, stannate, and selenite are all near $G = 0.01$. The yields from the phosphates, sulfates, and perchlorate, all of which are salts of strong acids, are considerably larger than this. Selenate gives a very small hydrogen atom signal which is about at our limit of detection for a 1 *M* solution at a 4-Mrad dose.

Some 20 other oxyanion salts have been irradiated as saturated or 1 *M* solutions up to a dose of 4 Mrads; they show no detectable trapped hydrogen atoms ($G < 0.001$). Because they are significant to the discussion these salts are given in Table II.

Table II. Oxyanion Types Not Yielding Trapped Hydrogen Atoms in γ-Irradiated Frozen Solutions

Central atom in maximum oxidation state		Central atom in lower oxidation state	
Nitrate	NO_3^-	Nitrite	NO_2^-
Thiosulfate	$S_2O_3^{2-}$	Phosphite	$H_2PO_3^-$
Tellurate	TeO_4^{2-}		HPO_3^{2-}
Periodate	IO_4^-	Hypophosphite	$H_2PO_2^-$
		Sulfite	HSO_3^-
Dichromate	$Cr_2O_7^{2-}$		SO_3^{2-}
Permanganate	MnO_4^-	Pyrosulfite	$S_2O_5^{2-}$
		Hyposulfite	$S_2O_4^{2-}$
		Chlorate	ClO_3^-
		Arsenite	AsO_2^-
		Bromate	BrO_3^-
		Tellurite	TeO_3^{2-}
		Iodate	IO_3^-

Added nitrate and nitrite ions decrease the yield of trapped hydrogen atoms in certain of the oxyanion–ice systems. Table III illustrates these effects for those oxyanions not previously reported to trap H atoms.

Table III. Effect of Added Electron Scavenger on Trapped H Atom Intensity in γ-Irradiated Oxyanion Ices

		Scavenger concn to give 50% reduction in H atom intensity	
Oxyanion	Concn	$NaNO_3$	$NaNO_2$
SeO_3^{2-}	1 *M*	0.06 *M*	0.10 *M*
SnO_3^{2-}	1 *M*	0.12 *M*	0.19 *M*
SiO_3^{2-}	1 *M*	0.48 *M*	0.82 *M*
BO_2^-	Satd	H atom intensity decreased[a]	
$B_4O_7^{2-}$	Satd	H atom intensity decreased[a]	
$HAsO_4^{2-}$	1 *M*	H atom intensity not decreased	

[a] Signal was too small to obtain quantitative results.

Upon γ-irradiation several of the oxyanion ices develop visible colors; phosphates and perchlorates become pink and sulfates become yellow. These colors are thought to be associated with a paramagnetic intermediate, the spectrum of which is superimposed upon a doublet from trapped hydroxyl radicals. In phosphates the paramagnetic intermediate is a doublet split by about 32 gauss, in sulfates a singlet is seen, and in perchlorate a weak quartet is discerned.

In order to determine whether these color centers are associated with the trapped H atom precursor, the effects of optical bleaching and of electron scavengers were investigated. Table IV shows that optical bleaching of the color centers increases the H atom yield in certain systems but not in others. Bleaching for times greater than 1 min does not cause further increase. Since the ices are opaque it is believed that only the color centers in the surface layers are bleached. The effect of nitrate ion on the color center intensity and on the H atom yields in $H_2PO_4^-$ ices is shown in Table V.

Table IV. Effect of Optical Bleaching on H Atom Intensity in γ-Irradiated Oxyanion Ices

	Relative H atom intensity[a]		
Oxyanion	Before bleach	After bleach[b]	Net change, %
$H_2PO_4^-$	100	113 ± 4	+13
HSO_4^-	100	111 ± 2	+11
ClO_4^-	100	110 ± 3	+10
HPO_4^{2-}	100	100 ± 2	0
SO_4^{2-}	100	100 ± 2	0

[a] Only values given in each row are comparable. [b] Averages of five or more samples.

Table V. Effect of NO_3^- on Color Centers and H Atoms in γ-Irradiated $H_2PO_4^-$ Ice

$NO_3^-/H_2PO_4^-$	Color intensity	Relative H atom yield
0.0	Strong pink	1.00
0.1	Strong pink	0.05
0.3	Medium pink	0.01
0.5	Weak pink	0.00
0.7	Weak pink	0.00
1.0	White	0.00

Much higher concentrations of nitrate ion are needed to scavenge the color centers than are needed to scavenge the H atom precursors.

Discussion

Trapped Hydrogen Atom Yields. The only trend that appears from the H atom yields in Tables I and II is a trend with oxidation state; those oxyanions in their highest oxidation states trap hydrogen atoms. Phosphites, sulfites, chlorates, and arsenites do not trap hydrogen atoms. The sole exception is selenite, SeO_3^{2-}, which traps hydrogen atoms in comparable yield to many of the other salts. Furthermore, hydrogen atom trapping with selenate, SeO_4^{2-}, is just detectable.

The transition metal oxyanions do not trap hydrogen atoms; and whereas stannate does, the other oxyanions in the same row—tellurates, tellurites, periodates, and iodates—do not trap.

It appears that oxyanions in their maximum oxidation state that are relatively poor oxidizing agents are most likely to trap H atoms. Another factor may be the nonexistence of a stable oxidation state one unit lower than the maximum. It is postulated that a mobile electron, represented here by e_m^-, reacts with certain oxyanions to give a radical-anion intermediate

$$e_m^- + MO_x^{-n} \longrightarrow [MO_x \cdot H_2O]^{-(n-1)} \longrightarrow MO_x^{-n} + OH^- + H \quad (1a)$$

$$e_m^- + MO_x^{-n} \longrightarrow [MO_x \cdot H_2O]^{-(n-1)} \longrightarrow MO_{x-1}^{-n} + OH^- + OH \quad (1b)$$

as in eq 1a. The H atom formed is stabilized in the field of the oxyanion. If the oxyanion is eaily reduced, reaction 1b may occur and produce OH instead of H. Reaction 1b could be a factor contributing to the small H atom yields associated with some oxyanions, but no definite evidence for this reaction has been found. We now examine the evidence for the mobile electron precursor and the radical-anion precursor.

Mobile Precursor of Hydrogen Atoms. We have previously demonstrated that electron scavengers such as nitrate ion, nitrite ion, acetone, etc., decrease the yield of trapped hydrogen atoms associated with the mononegative oxyanions, HCO_3^-, $H_2PO_4^-$, HSO_4^-, and ClO_4^-, in accordance with simple competition kinetics.[3] The hydrogen atom yields associated with the polynegative oxyanions, CO_3^{2-}, HPO_4^{2-}, PO_4^{2-}, and SO_4^{2-}, are unaffected by electron scavengers. We suggested that two mechanisms are operative: one involving a mobile electron and one that does not. From Table III it is seen that borate, BO_2^-, and monohydrogen arsenate, $HAsO_4^{2-}$, fit into the previously postulated correlation but that $B_4O_7^{2-}$, SiO_3^{2-}, $Sn(OH)_6^{2-}$, and SeO_3^{2-} do not. With added electron scavenger these exceptions all show a decrease in trapped H atom yield, but about 20% of their H atom yield is unscavengeable. This unscavengeable yield is thought to be formed by a mechanism not involving a mobile electron precursor. Both mechanisms are thus operative in these latter four dinegative ions. This observation is probably related to the relatively larger size of these four ions (SiO_3^{2-} does not exist as a discrete ion). The negative charge on these ions is more delocalized than on smaller ions and it does not constitute as much of a coulomb barrier to the approach of an additional electron as does the charge on smaller polynegative ions.

The ratio of nitrate to nitrite ion concentration necessary to give a 50% reduction in the H atom intensity is the reciprocal of the relative reaction rate of the H atom precursor with nitrate and nitrite ions. The observed rate ratio is 1.7 as determined from the result in Table III and from previous results.[3] We have previously postulated[3] that a mobile electron, somewhat analogous to the solvated electron formed in water radiolysis, is the precursor of the scavengeable trapped H atoms. From aqueous radiolysis studies[4] $k(e^-_{aq} + NO_3^-)/k(e^-_{aq} + NO_2^-) = 1.7$ to 2.4. In contrast, the rate ratio for H atoms in aqueous solutions is $k(H + NO_3^-)/k(H + NO_2^-) = 0.012$.[5] The close correspondence of the rate ratio found for solvated electrons in aqueous solutions with that for the postulated mobile electrons in frozen solutions may be partially fortuitous, but since the rate ratio for H atoms is 170-fold smaller we consider it to be excellent evidence that a mobile electron rather than a hydrogen atom is the mobile precursor of scavengeable,[6] trapped hydrogen atoms found in frozen solutions.

Color Centers and Trapped Precursors of Hydrogen Atoms. The results in Table IV of optical bleaching of the color centers demonstrate that the hydrogen atom yield increases about 10% upon bleaching in $H_2PO_4^-$, HSO_4^-, and ClO_4^- ices, while it shows no change in HPO_4^{2-} and SO_4^{2-} ices. In other words, only those oxyanions that react with mobile electrons to ultimately produce trapped hydrogen atoms show increases after optical bleaching. This implies either that bleaching releases mobile electrons, or that it causes dissociation of a radical-anion intermediate as shown in eq 2. We consider the second alternative more likely.

$$H_2PO_4 \cdot H_2O^{2-} \xrightarrow{h\nu} H_2PO_4^- + OH^- + H \quad (2)$$

The effect of nitrate ion on the color centers in Table V does not bear out this interpretation at first glance. Low concentrations of nitrate ion sufficient to eliminate all of the H atoms and consequently the postulated radical-anion intermediate do not significantly decrease the color-center intensity. However, the color centers are scavenged at higher nitrate concentrations. Nitrate ion seems to scavenge two different species. This paradox is resolved if we recognize that nitrate ion can react with *both* electrons and positive holes. Previously[7] we have given independent evidence for the reaction of NO_3^- with H_2O^+.

From scavenging and optical bleaching results, we must conclude that the observed color centers are due to two different types of species. In the $H_2PO_4^-$ system we may represent these species by $[H_2PO_4 \cdot H_2O]$ and $[H_2PO_4 \cdot H_2O]^{2-}$ which are formed respectively by reactions of H_2O^+ and e_m^-. A rough comparison of the number of color centers bleached with the number of additional H atoms trapped shows that the former is in excess by about an order of magnitude; consequently, $[H_2PO_4 \cdot H_2O]$ contributes at least 90% of the intensity of the color center in the $H_2PO_4^-$ ice. This is consistent with the fact that a low concentration of NO_3^-

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(5) G. Navon and G. Stein, *ibid.*, **69**, 1384 (1965).

(6) "Scavengeable" is used loosely here to designate those systems in which the H atom yield is decreased when another species is added; the H atom itself does not react with the additive.

(7) L. Kevan, *J. Phys. Chem.*, **68**, 2590 (1964).

scavenges most of the H atoms with little change in color-center intensity.

Similarly we conclude that both $[ClO_4 \cdot H_2O]$ and $[ClO_4 \cdot H_2O]^{2-}$ and both $[HSO_4 \cdot H_2O]$ and $[HSO_4 \cdot H_2O]^{2-}$ contribute to the color centers in ClO_4^- and HSO_4^- ices. But in SO_4^{2-} and HPO_4^{2-} ices only the $[SO_4 \cdot H_2O]^-$ and $[HPO_4 \cdot H_2O]^-$ centers are formed. Bleaching of these centers does not produce any additional trapped H atoms. The $[SO_4 \cdot H_2O]^-$ and $[HSO_4 \cdot H_2O]$ centers are presumably interconvertible.

Evidence for formation of similar hole centers has been reported for H_2SO_4, $HClO_4$, and H_3PO_4 acid glasses at 77°K,[8] in sodium metaphosphate glass at room temperature,[9] and in a single crystal of KH_2PO_4 at 77°K.[10] In the acid glasses bleaching of the hole species gave rise to a new epr spectrum which was attributed to H_2O^+. Warming to 120°K reversed the process to re-form the color center. In the polycrystalline ices studied here, no epr spectrum attributable to H_2O^+ is seen although a small spectrum would have been obscured by the OH spectrum. More importantly, the color centers are not re-formed on warming. Although the original color centers are undoubtedly the same in the acid glasses and the polycrystalline ices the reactions they undergo upon warming or bleaching depend on the media.

In summary, our results indicate that H atoms are trapped in a variety of frozen oxyanion solutions, that a part of these H atoms arise from the reaction of a mobile electron with an oxyanion to form a radical anion which is trapped or which dissociates to give a trapped H atom, and that a positive hole may also react with the oxyanion to form a trapped color center. Both electrons and holes seem to react relatively readily with oxyanions in frozen solutions.

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[10] W. E. Hughes and W. G. Moulton, *ibid.*, 39, 1359 (1963).

Free-Radical Distribution in the γ-Radiolysis of Dry Ribonuclease[1]

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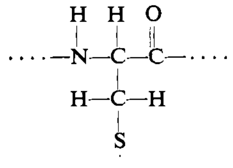
Contribution from the National Cancer Institute, National Heart Institute, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland. *Received September 7, 1965*

Abstract: A new method for determining the distribution of free radicals on carbon in irradiated dry proteins has been developed. γ-Radiolysis of ribonuclease in the absence of air and subsequent exposure to tritiated hydrogen sulfide leads to the formation of carbon–tritium bonds. The amount of such tritium incorporated per amino acid residue is very much greater for certain amino acids (lysine > methionine > proline > histidine) than for others (phenylalanine > isoleucine > valine); the maximum difference exceeds a factor of 70. Contrary to past interpretations of epr spectra, glycine radicals do not play a dominant role under these conditions. Analogous experiments with carboxymethylated reduced ribonuclease suggest that free-radical distribution depends markedly on molecular conformation of the protein.

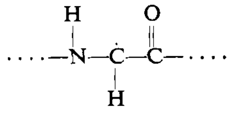
When dry proteins are irradiated with γ-rays in the absence of air, trapped free radicals are generated. The question may be asked whether these radicals are distributed randomly along the polypeptide chair or whether they are preferentially located on certain amino acid residues.

The electron paramagnetic resonance (epr) patterns of proteins irradiated at room temperature have been found[2-5] to be mainly of two kinds. One of these has been interpreted[2] as due to a cysteine radical (I) and the other as predominantly due to a glycine radical (II). However, the assignment of the free radical II as the species responsible for the doublet observed with irradiated proteins is not without ambiguity because of the difficulties of unraveling superimposed spectra.



$$\cdots\text{—}\overset{H}{\overset{|}{N}}\text{—}\overset{H}{\overset{|}{\underset{\underset{\underset{\dot{S}}{|}}{H\text{—}C\text{—}H}}{\underset{|}{C}}}}\text{—}\overset{O}{\overset{\|}{C}}\text{—}\cdots$$

I



$$\cdots\text{—}\overset{H}{\overset{|}{N}}\text{—}\underset{\underset{H}{|}}{\dot{C}}\text{—}\overset{O}{\overset{\|}{C}}\text{—}\cdots$$

II

To identify the free radicals on carbon in irradiated proteins, a highly reactive scavenging agent is required which will tritium label the radicals produced by radiation without creating a new radical population by secondary reactions. Since hydrogen sulfide is known[6] to have a high rate of reaction with alkyl radicals in the gas phase and since sulfhydryl radicals will not abstract hydrogen from primary, secondary, and tertiary carbon–hydrogen bonds of hydrocarbons,[7] we have investigated the reaction of irradiated ribonuclease

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[1] Presented at the 13th Annual Meeting of the Radiation Research Society, Philadelphia, Pa., May 23, 1965.

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