RADIATION — INDUCED DECOMPOSITION OF RADIOIODINE — LABELLED 3 — IODO — L - TYROSINE [1].

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SUMMARY

In investigating the radiolytic decomposition of 3-iodo-L-tyrosine we find :

1. *that, by studying the effects of dissolved argon, nitrous oxide and hydrogen, e^-_{aq} is the reactive intermediate of greatest interest in choosing a protector of the labelled compound. This technique should have general application.*
2. *that O_2/ ethanol and N_2O/ethanol are both good combinations for the purpose of protecting the substrate.*
3. *that in dilute solutions of MIT, with adequate protector present, high specific activity compound should be no more prone to radiation-induced self-decomposition than is compound labelled at a lower specific activity.*

Items (2) and (3) should be noted particularly, in view of the widespread belief that high specific activity and the presence of oxygen are always deleterious to the stability of labelled compounds.

INTRODUCTION

Radiation-induced self-decomposition of radioactive compounds in aqueous solution presents a real source of difficulty in the preparation and, particularly, storage of these substances. Despite the fact that ^{131}I labelled thyroid metabolites are especially susceptible to this effect (2), the number of systematic studies involving the stability of such compounds is surprisingly small, and published observations are frequently conflicting. In view of the importance of radiochemical purity to the clinical tests in which thyroid metabolites are used, further investigation of these compounds seemed justified.

We have noted in the literature of self-decomposition the prevalence of two generalizations. They are, first, that high specific activities in a labelled compound lead inevitably to high rates of self-decomposition (expressed as percent decomposed in unit time) (3), and second, that the presence of air or oxygen should be avoided if self-decomposition is to be minimized (4). Nevertheless very high specific activity (600 Ci. $mMole^{-1}$) ^{131}I-iodotyrosines appear to be surprisingly stable (5), and removal of oxygen has a deleterious effect on the stability of 3:5 diiodo-L-tyrosine, ^{131}I (DIT) (6). We therefore felt it would be of interest to investigate more closely the validity of these generalizations when applied to labelled thyroid metabolites. 3-iodo-L-tyrosine (MIT) was chosen as a model compound, being chemically the simplest of the organic thyroid metabolites and since its response should be representative of this class of compound.

Frequently attempts are made to minimize self-decomposition by the use of dissolved solutes which will hopefully act as free-radical "scavengers", thereby "protecting" the labelled compound. The major attacking radical species must first be identified, so that a protector can be chosen based on its reactivity towards this species. Despite the fact that tables of bimolecular rate constants are available for many compounds reacting with the radicals produced in water radiolysis (7) little use has been made of them in choosing protecting agents. In this work, we have identified the major attacking species by using solute

systems to adjust the relative proportions of the three primary radicals $\cdot H$, $\cdot OH$ and e^-_{aq}. Protectors have been chosen for their reactivity towards the species so identified.

Unfortunately a major disadvantage of all chemical protectors is that they constitute a chemical impurity. We have studied a number of gases as chemical protectors, since gaseous solutes would seem to have the advantage that they can be easily stripped from solution if desired.

Experimental

Solutions of 3-iodo-L-tyrosine labelled with ^{125}I at trace levels were irradiated with ^{60}Co γ-radiation. Although the primary processes in gamma radiolysis and self-radiolysis are different, chemical decomposition proceeds in both cases through the ionizing effect of energetic electrons. In gamma radiolysis these are mainly Compton scattered electrons. By using an external gamma source it was therefore possible to simulate self-radiolysis of this compound labelled at high specific activity with the beta-emitting isotope ^{131}I. From the standpoint of our experiments, the concentration of the substrate can be changed while maintaining the absorbed dose constant - this is equivalent to altering the specific activity in a self-radiolysis experiment.

This technique avoids a number of difficulties inherent in self-irradiation investigations. For example, in such experiments the dose rates are usually very low as compared to external gamma radiolysis, and rapid decay of the isotope makesnecessary frequent preparations with the danger of batch-to-batch inconsistencies.

Dosimetry problems are also avoided, including those arising from changing sample geometry when sequential analyses are performed. High specific activity preparations are also plagued by chemical impurities, and the chemical concentrations are usually known only approximately by calculation from preparation conditions. It has been shown that the same qualitative and quantitative results are obtained in radiation-induced decomposition studies of iodotyrosines, whether the absorbed dose was administered by self-radiolysis or by an external radiation source (6).

A 500 Ci. (nominal) ^{60}Co "cave-type" source housed in a shielded greenhouse was used to externally irradiate the samples. Dose rates were about 2×10^4 rads $hr.^{-1}$ and were determined accurately by Fricke dosimetry, assuming $G(Fe^{3+}) = 15.5$, where G = molecules chemically changed per 100 e.V. absorbed (8). Total doses up to 2×10^5 rads were given.

The irradiation vessels were glass "Microflex" tubes, made by Kontes Glass Co., Vineland, N.J., of cylindrical outside shape. The inner geometry was a cone-shaped space in which 0.15 ml. samples were held for exposure to radiation. The vessels were capped with a screw-on plastic cap with a replaceable "Teflon" insert. Before use, vessels were cleaned by soaking in permanganic acid and rinsed with triply distilled water, with which they were then filled and pre-irradiated to a dose of about 10^6 rads.

^{125}I labelled MIT was supplied by E.R. Squibb & Sons, Inc. at 1.82×10^{-4}M. and 2.6×10^5 Ci. $mole^{-1}$. It contained no added chemical protector, and was diluted on receipt with MIT carrier

to 2.0×10^{-4}M. and 25 ci. $mole^{-1}$ to serve as a stock solution. Ethanol was "Rossville Gold Shield" by Commercial Solvents Corporation; hydrogen, carbon dioxide, oxygen, nitrous oxide and argon were "High Purity" from Matheson Gas Co.

Samples were saturated with appropriate gases by bubbling through the solution for 20-25 minutes using a hypodermic needle that passed through the "Teflon" insert of the irradiation vessel and reached the inverted apex of the conically-shaped interior. A second needle permitted the gas to bleed off. In all experiments, an unirradiated blank was treated and analyzed the same way as the irradiated samples.

Analysis for extent of decomposition was by one-dimensional descending paper chromatography using n-butanol:acetic acid:water = 4:1:5 as solvent and Whatman No. 1 paper. The major radiochemical impurity detected was iodide (R_f = 0.35) together with much smaller amounts of diiodotyrosine (DIT) (R_f = 0.70). The unchanged MIT had an R_f of 0.58. To keep the iodide in the reduced form, thiosulfate was spotted and dried on the origin before applying the samples. We investigated the possibility of loss of activity during chromatography, which has been reported (9), and found none.

Developed chromatograms were either scanned automatically using a Nuclear Chicago Actigraph II gas flow ionization chamber, or cut into thin strips which were individually counted using a

Tracerlab Multimatic gas flow ionization chamber. Both techniques were checked periodically by liquid scintillation counting of the same strips in a Beckman LS-133 system, using "Aquasol" or "Aquaflor" solvent (New England Nuclear Corporation).

The difference between percentage of total activity attributable to MIT in the blank and that in an irradiated sample was taken to be radiation decomposition due to aqueous free radicals, and G-values for decomposition were calculated from

$$G(-MIT) = \frac{[MIT] \times \% \text{ Decomposition} \times N}{\text{Dose Rate (Rads hr}^{-1}) \times \text{Exposure Time (hr)} \times 6.24 \times 10^{16}} \quad A$$

where the factor 6.24×10^{16} converts rads to e.V. $l.^{-1}$.

Results and Discussion

In an effort to identify the major reactive species attacking MIT, we have studied separately the effects of dissolved nitrous oxide, argon and hydrogen. In nitrous oxide solutions, the major reducing radical (the hydrated electron, e^-_{aq}) is quantitatively converted to the oxidizing .OH radical, providing a system in which .OH is by far the dominant reactive species:

$$e^-_{aq} + N_2O \longrightarrow N_2 + O^- \quad , k = 5.6 \times 10^9 M^{-1} \text{ sec.}^{-1} \quad (7) \qquad (1)$$

$$O^- + H_2O \rightleftharpoons OH^- + .OH \qquad (2)$$

A portion of the small ($G \approx 0.6$) yield of hydrogen atoms may also be converted to .OH,

$$N_2O + .H \longrightarrow N_2 + .OH, \; k = 2.2 \times 10^5 \; (7) \qquad (3)$$

the amount depending on the relative reactivities of .H towards N_2O and MIT. In argon-saturated solutions, oxidizing and reducing

radicals are both present. Table I shows the effects of these solutes on the radiolytic decomposition of MIT (2×10^{-4}M.) and the amount of each primary radical expected to be present. (No rate constants for radical reactions with MIT are available in the literature, so only upper and lower limits can be given for these amounts).

These results with argon and N_2O suggest that hydrated electrons are much more important than .OH radicals in destroying MIT, since removing the e^-_{aq} results in a twofold decrease in G(-MIT), even though the number of .OH radicals available has been at least doubled. To check this further, the effect of dissolved hydrogen was studied. Hydrogen can convert the hydroxyl radical to a reducing species:-

$$H_2 + .OH \longrightarrow .H + H_2O \quad , \; k = 4.5 \times 10^7 \; (7) \qquad (4)$$

Reaction (4) would be in competition with any reaction of .OH with MIT; the fraction of .OH radicals converted would therefore depend on this competition. The effect of hydrogen on 2×10^{-4}M.MIT is shown in Table I, where G(-MIT) is almost double the value in an argon atmosphere.

The results with argon, nitrous oxide and hydrogen clearly show that the reducing radicals are much more important than the oxidizing radical in destroying MIT.

We next irradiated an air-saturated solution of MIT (2×10^{-4}M.), and found G(-MIT) = 0.45. Clearly, some component of pure air is a better protector than either N_2O, H_2 or Ar. Since nitrogen is inert, it is indeed an unlikely candidate. The only other

molecules found in air in any significant quantity are O_2 and CO_2. Oxygen has been cited frequently for its deleterious effects with respect to radiation-induced decomposition (4, 10, 11, 12). So, O_2 and CO_2 were investigated for their effects on the system.

TABLE I

Effect of some dissolved gases (Pressure = 1 Atm.) on radiolytic decomposition of 3-iodotyrosine (2×10^{-4}M).

Atmosphere	Primary Radicals Present (G-value)					G(-MIT)
	e^-_{aq}	·H	·OH	$\cdot HO_2$	$\cdot CO_2^-$	
Ar	2.76[a]	0.55[a]	2.74[a]			1.17
N_2O		⩽0.55	5.5-6.1			0.565
H_2	2.76	0.55-3.3	⩽2.74			2.0
Air	⩽2.76	⩽0.55	2.74	⩽3.3		0.45
O_2	⩽2.76	⩽0.55	2.74	⩽3.3		0.369
CO_2	⩽2.76	0.55	2.74		⩽2.76	0.716

a. B.H.J. Bielski and A.O Allen, Int. J. radiat. Phys. Chem., 1, 153 (1969)

The results also appear in Table I, and indicate the protective property of oxygen and the relatively harmful effect of CO_2. In view of our findings previously discussed that the reducing radicals are most important in destroying the substrate, it is not too

surprising that oxygen affords protection in view of its reactivity towards .H and e^-_{aq}:-

$$.H + O_2 \longrightarrow .HO_2 \quad , k = 1.9 \times 10^{10} \ (7) \qquad (5)$$

$$.HO_2 \rightleftharpoons H^+ + .O_2^- \quad , pK = 4.4 \ (13) \qquad (6)$$

$$e^-_{aq} + O_2 \longrightarrow .O_2^- \quad , k = 1.9 \times 10^{10} \ (7) \qquad (7)$$

Again, the fraction of .H and e^-_{aq} removed will be a function of their relative reactivities towards O_2 and MIT.

Carbon dioxide was thought to have good possibilities as a protector in view of its great solubility (about 25x that of oxygen) and reactivity towards the hydrated electron.

$$e^-_{aq} + CO_2 \longrightarrow .CO_2^- \quad , k = 7.7 \times 10^9 \ (7) \qquad (8)$$

That CO_2 is not as good as oxygen or N_2O could be due to some degree of reactivity of the $.CO_2^-$ species with the labelled compound. Alternatively, the deleterious effect of $.CO_2^-$ might be associated with its known reactivity towards organic radicals (14)

$$.R + .CO_2^- \longrightarrow RCO_2^- \rightleftharpoons RCO_2H \qquad (9)$$

It can be seen in Table I that the G(-MIT) values are always smaller than the total yield of available radicals. This could indicate that extensive back-reactions involving re-formation of the MIT are possible. The attack of $.CO_2^-$ on a MIT radical forming a carboxylic acid would block the possibility of MIT being reconstituted. However, the greatest significance of the CO_2 result is related to the reactivity of CO_2 to e^-_{aq} - inasmuch as CO_2 removes some of the e^-_{aq} from solution it is a better protector than H_2 or Ar, which do not.

That it is reasonable to postulate a competition between the substrate and oxygen for reducing radicals can be seen when the effect of varying the MIT concentration in oxygen-saturated solutions is studied. When two solutes compete for the same radical it can be shown (15) that

$$G(-A) = \frac{G_R}{1 + k_B [B] / k_A [A]}$$

where A and B are the competing solutes, k_A and k_B the bimolecular rate constants for their reactions with the radical, and G_R the initial radical yield. When $G(-A) \ll G_R$, $k_A[A] \ll k_B[B]$, so we can rearrange the above expression and make the approximation

$$G(-A) = \frac{G_R \cdot k_A [A]}{k_A [A] + k_B [B]} \approx \frac{G_R \cdot k_A [A]}{k_B [B]}$$

A plot of log G(-A) versus $\log \frac{[A]}{[B]}$ should therefore give a line whose slope approaches one as G(-A) decreases. Figure I shows a plot of log G(-MIT) versus $\log \frac{[MIT]}{[O_2]}$ for γ-irradiated, oxygen-saturated solutions (this work) and for X-irradiated, air-saturated solutions (16). A line of unit slope is shown for reference. We conclude therefore that there is a competition between the iodotyrosine and dissolved oxygen for a reducing radical, and that this radical is almost certainly e^-_{aq}. H-atom is unlikely to be the major attacking species because (a) it represents only about 20% of the reducing radicals, (b) ·H is an electrophile (17) and would be less reactive than e^-_{aq} (a nucleo-

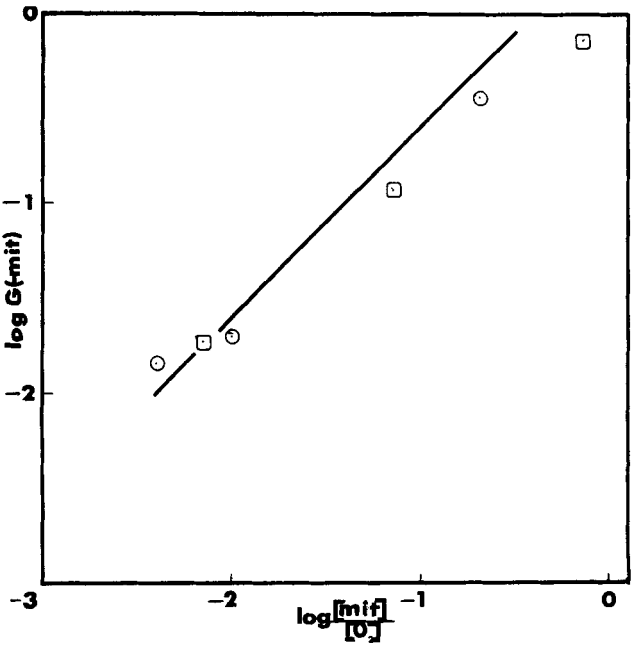

Figure 1.

Dependence of G(-MIT) on $\frac{[MIT]}{[O_2]}$ for 3-iodo-L-tyrosine (MIT) solutions irradiated ⊙ in oxygen-saturated solution with γ-rays (this work) and ⊡ in air-saturated solution with X-rays (Ref. 15).

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phile) towards the electron-deficient aromatic nucleus of MIT,

(c) it has been observed that lowering the pH to 2.6, which would have the effect of transforming e^-_{aq} into .H

$$e^-_{aq} + H_3O^+ \longrightarrow .H + H_2O \qquad (10)$$

has almost no effect on the radiolytic decomposition of 3:5

diiodo-L-tyrosine (6), (d) G-values much greater than $G_H = 0.6$ have been observed for radiation-induced deiodination of both MIT (16) and DIT (6, 16).

With the preceding established, it is clear that the common practice of employing only an .OH scavenger is inadequate for the iodotyrosines. Ethanol, which reacts with .H and .OH but not at all with e^-_{aq} has been recommended recently (18) as a general chemical protector. A combination of ethanol with a good electron scavenger would seem more appropriate. We chose as the electron scavengers O_2 and N_2O because (a) they react rapidly with e^-_{aq} (b) they are quite soluble (N_2O especially so), (c) they are gases and hence could be readily removed if necessary from solutions of high specific activity labelled compounds, (d) they are non-toxic and hence would be acceptable for clinical applications. Table II shows the results for various combinations of ethanol with air, O_2 or N_2O.

The most striking feature of Table II is the great stability of the MIT, as measured by % decomposition per unit dose, during a change in its concentration of over 100. The exception to this is seen at 3.2×10^{-5}M.MIT and 10^{-3}M. ethanol - an anomalous result which seems to be suppressed at higher concentrations of ethanol. This anomaly could be the result of impurity in the sample used for the 3.2×10^{-5}M.MIT concentration, which under irradiation is activated towards MIT but is, itself, a scavengable entity and therefore its effect is reduced at the higher ethanol scavenger concentrations. On the whole, though, it is seen that where $[EtOH] \geq 10^{-2}$M. there

TABLE II

Effect of ethanol concentration in presence of N_2O or O_2 (1 Atm) on radiolytic decomposition of 3-iodotyrosine (MIT).

Ethanol Concentration, M	MIT Concentration, M	G(-MIT)	% Decomp/10^5 rads
	N_2O SATURATED		
10^{-1}	1.6×10^{-4}	.207	13.0
	3.2×10^{-5}	.039	12.5
	2.8×10^{-6}	.003	11.0
10^{-2}	1.6×10^{-4}	.291	18.8
	3.2×10^{-5}	.064	20.8
	2.8×10^{-6}	.005	17.3
10^{-3}	2.24×10^{-5}	.122	56.0
	6.48×10^{-6}	.045	72.0
	2.0×10^{-6}	.023	12.0
1.45×10^{-3}	1.2×10^{-4}	.406	35.0
	O_2 SATURATED		
10^{-1}	1.6×10^{-4}	.238	15.4
	3.2×10^{-5}	.053	17.1
	2.8×10^{-6}	.004	14.0
10^{-2}	1.6×10^{-4}	.300	19.4
	3.2×10^{-5}	.078	25.0
	2.8×10^{-6}	.007	24.0
10^{-3}	2.24×10^{-5}	.094	43.4
	6.48×10^{-6}	.030	48.0
1.32×10^{-3}	1.3×10^{-4}	.346	27.6

is significant stability regardless of the gas chosen as the solvated electron scavenger.

That % decomposition is almost independent of $[MIT]$ can also be concluded from Fig. I, since a direct proportionality between G(-MIT) and $[MIT]$ requires % decomposition per absorbed dose to be

constant (expression A above). This condition exists when scavenging of the reactive intermediates by protectors predominates over their reaction with the substrate.

Based on this rationale, the stability of high specific activity iodotyrosines stored in very dilute solution with dissolved air present becomes understandable.

It is also clear from Table II that in terms of protection:

10^{-1}M. EtOH $>$ 10^{-2}M. EtOH $>$ 10^{-3}M. EtOH

and that:

$N_2O > O_2 >$ Air

The increased protection furnished by increasing the concentration of ethanol leads one to wonder at what point is the protective effect maximized with respect to ethanol? Hoye and Steinnes (18) found that 60-70% ethanol (13-17M.) offered some real protection! This concentration is 130-170 times the maximum $[EtOH]$ used in this work, and under those conditions molecules of ethanol would outnumber molevules of MIT by 10^5 to 10^6 -- in truth, the study then would not be of an aqueous solution.

N_2O is about 12 times as soluble in water as is pure oxygen. The fact that it is less reactive than oxygen towards the solvated electron ($k_{N_2O + e} / k_{O_2 + e} \approx 0.3$) (7) would still lead one to predict a greater protective capability for solutions saturated with N_2O than with O_2, and this is what is seen.

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