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MASTER

TECHNICAL PROGRESS REPORT

AEC Contract No. AT(11-1)1728 "Chemical Consequences of the Auger Effect in Thyroxine and its Analogues"

1. Personnel:

a. Principal Investigator: Dr. Bodo Diehn, Assistant Professor.

Time devoted to project: 1/2 time during summer

1/4 time during academic year 1968/69

b. Postdoctoral Associate: V. George Thomas, devoting full time to the project since joining it September 15, 1968.

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2. Work done on the project as of May 1, 1969:

In our studies of the iodine yield as a function of photon energy in the iodothyronine systems, we have now demonstrated that there is a distinct and discontinuous increase of this yield, by a factor of about 8, at the iodine K_{α} absorption edge. This shows that there is indeed a chemical consequence of resonance absorption in condensed phases. The effect is even stronger than would be expected from calculations using published values of the K-to-L-shell Auger yield. One might speculate that here we could possibly be observing an influence of chemical environment on Auger resp. fluorescence yields. A manuscript describing this work and our studies of the dose dependence of the iodine yield has been submitted for publication and is attached as part of this report.

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Gas chromatographic studies of the product spectrum resulting from irradiation of thyroxine indicate that aside from iodine and 3,5 3' triiodothyronine (T_3), at least two other products are formed. We are now testing the assumption that these are isomers of T_3 , or of pentaiodothyronine. Monoiodotyrosine, although it is the simplest of the thyroid hormone metabolites, exhibits a more complex radiation chemistry than the iodothyronines. Iodine, iodide, and tyrosine were found as products, along with 3 compounds which have not been identified as yet, but are definitely neither phenylalanine, alanine, or phenol. All 3 products are amino acids, probably condensed systems, and contain a reducing functional group (phenolic OH, iodine, or both).

We are also now investigating the Auger chemistry of iodobenzene, which can be considered a simple model system for the thyroid hormones. Among 8 products detected by gas chromatography, we have identified iodine, benzene, biphenyl, and o-, m- and p-diiodobenzene, while 2 others might be iodobiphenyls. No gaseous products are formed. These observations lead us to conclude that in this system, the Auger effect simply results in ejection of the affected atom with considerable kinetic energy. No fragmentation of the aromatic ring occurs, probably both because of the high degree of resonance stabilization and because of the ease with which collisional deactivation is possible in condensed phases. Using this hypothesis, straight-forward mechanisms can be postulated for the formation of the observed products. In a very interesting experiment, we found that no detectable products were formed when a solution of iodine in benzene was irradiated. This indicates that the iodine must be bound to the ring for chemical reaction to occur.

We have also irradiated iodocyclohexane and detected a variety of products, but have not yet attempted identification. In contrast to the iodobenzene case, a solution of iodine in cyclohexane yielded exactly the same product spectrum upon irradiation as did iodocyclohexane.

It is planned to purchase an interface for our gas chromatograph and the Nuclide Mass Spectrometer available in our department. This, together with ^{131}I labeling experiments, will greatly facilitate detection, identification, and quantitative measurement of reaction products.

CHEMICAL CONSEQUENCES OF THE AUGER EFFECT

I. IODINE YIELD FROM IODOAMINO ACIDS AS A FUNCTION OF X-RAY DOSE AND ENERGY^{*}

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ABSTRACT. Absorption of X-radiation in the iodothyronines results mainly in deiodination. G-values and dose effect curves were determined from 0.5 to 3.0 Mrads total dose for 3,5-diiodo-DL-thyronine, 3,5,3'-triiodo-DL-thyronine, and thyroxine. I_2 yield measurements as a function of maximum X-ray energy indicate after correction for radiation dose, that there is a distinct effect of resonance absorption upon the ensuing chemical transformation. This effect is stronger than would be predicted from published values of the K-to L-shell Auger yields in iodine.

I. INTRODUCTION

Radiation acts upon atoms and molecules, in essence, by delivering energy to single atomic particles. The primary process resulting from the interaction of a single X-ray photon and an atom is the ejection of an inner-shell electron. The electronic readjustment required to fill the vacancy created may result in an Auger cascade imparting a large positive charge to the atom (1). If the Auger effect is induced in iodine incorporated in simple molecules such as CH_3I , electron redistribution within the molecule occurs within a very short time after the Auger cascade. The result is a large positive charge on each of the constituent atoms. In the gas phase, this immediately leads to complete disintegration of the molecule because of Coulomb repulsion (2). Much less is understood about the events occurring in condensed media, although the importance of Auger Effect chemical consequences under these conditions has been pointed out and discussed by several workers (3-5).

A condensed system which is of particular theoretical and practical interest is that of the iodothyronines. The thyroid hormones are the most important iodine-containing compounds of biological significance. Moreover, X-irradiation has been the preferred treatment of diseases such as thyroid cancer and hyperthyroidism (6). An understanding of what happens under these conditions is clearly desirable. A study of the X-radiolysis of thyroxine and 3,5,3' triiodothyronine has been published (7), but it was concerned mainly with the identification of the products formed upon irradiation in aqueous propylene glycol solution, and did not consider the effects of radiation dose and energy. We decided to irradiate the compounds under investigation in the solid state. In solution, chemical effects are generally determined by the interaction of radicals from radiolysis of the solvent. This would unnecessarily complicate a study designed to show whether there is any chemical effect due to resonance absorption of X-rays in the iodine.

II. EXPERIMENTAL

Pure DL-thyroxine (T_4), 3,5,3'-triiodo-DL-thyronine (T_3), and 3,5-diiodo-DL-thyronine (T_2), were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, and used without further purification.

For X-irradiations, the continuous energy spectrum generated in a copper target (General Electric CA-7 X-ray tube with a 20 mil thickness beryllium window) at selected tube currents and high voltages was used. A piece of polyacetate polymer (surface density 0.033 g/cm^2) was placed above the tube window to completely filter out any X-ray energies below 5 KeV. Iodothyronine samples (10.0 mg) uniformly distributed on the

scotch tape base of a lucite sample holder (0.636 cm^2 internal cross section) were X-irradiated for periods of several hours. The sample was water-cooled during irradiation to maintain a temperature of 25°C .

Fricke's ferrous sulfate dosimeter was used for measurement of the X-ray doses (8). The dosimetry solution was oxygen saturated and irradiated in the same sample holder and under the same conditions as the actual samples. The Fe(III) ion concentration was measured spectrophotometrically at 305 nm by means of a Cary 14 recording spectrophotometer, using the unirradiated sample as the blank solution. The total dose absorbed by each iodothyronine sample was then calculated by using appropriate correction factors.

The irradiation products were identified by paper chromatography on Whatman No. 1 using tert. amyl alcohol saturated with 6N ammonia as the ascending phase. The I_2 yield in a given irradiation was determined as follows: As soon as possible after irradiation, each sample was dissolved in 1:1 water/ ethanol mixture acidified with HCl. The acid facilitates dissolution and prevents decomposition of the parent molecule during the time required for analysis. The molecular iodine in this solution was determined spectrophotometrically as the triiodide in the presence of excess KI at 352 nm. The UV absorption spectrum was recorded on the Cary 14 against a solution blank. The UV absorption of unirradiated samples of thyronine and of the iodinated thyronines showed no interference with the iodine absorption band at 352 nm under the experimental conditions employed.

Where desired, the total amount of iodine liberated, i.e. the sum of iodide, elemental and molecular iodine, as well as higher oxidation states

of the element, was determined gas chromatographically (9) by measuring the amount of thyronine produced containing one less iodine atom than the parent compound. Liberation of more than one iodine atom per molecule, caused by absorption of more than one photon per molecule, is unlikely at the radiation doses employed.

III. RESULTS AND DISCUSSION

Irradiation of the systems under investigation results in energy deposition mainly in iodine atoms in the molecule. The total X-ray absorption cross section of the compounds approximates that of the weighted absorption cross section for iodine above about 5 KeV, and the contribution due to other elements present in the molecule is practically negligible (10,11). Since X-ray energies below 5 KeV were effectively filtered out, the absorbed photons created electron vacancies mainly in the L-shell of iodine. At photon energies above 33.16 KeV (iodine K-absorption edge), electrons were mainly ejected from the K-shell.

The principal products resulting from irradiation of the systems under study were found to be iodine, iodide, and defodinated amino acids. The absence of significant fragmentation is reasonable in the context of the constraint imposed by the solid matrix. On the other hand, in the gas phase a molecule such as $\text{Pb}(\text{CH}_3)_4$, with 17 atoms, still disintegrates completely (12). It is possible that the iodine is formed in solution from excited iodine species trapped in the solid. Claridge and Willard (13), for example, indicated that I_2^- is one of the main trapped metastable intermediates formed in the radiolysis of alkyl iodides in the glassy state.

The yield of molecular iodine found after dissolution of the irradiated samples exhibited the same dose dependence as the total iodine yield in the three compounds studied. Since the spectrophotometric method for determination of molecular iodine is about twice as accurate ($\pm 15\%$) as the gas chromatographic measurement of total iodine liberated, the former was chosen as a suitable index of the chemical effect of the X-radiation.

Figure 1 shows the dependence of I_2 -yield on radiation dose at 45 KV and 12 mA tube current. Evidently, T_2 is much more radiosensitive than T_3 and T_4 , which are of almost equal sensitivity. One is lead to conclude that a T_2 molecule is less easily de-excited in its crystal lattice than a T_3 or T_4 molecule, or, alternatively, that re-entry of an ejected I atom into its original position is less likely in the case of T_2 than in the case of T_3 or T_4 . The $G(I_2)$ values vary from 0.6 to 0.4 over the dose range studied for T_3 and T_4 , but exhibit a decline from 2.3 at 0.47 Mrads to 0.75 at 2.74 Mrads in the case of T_2 . This is most probably not the result of overlap between neighboring events during sample irradiation, but is due to radiation annealing, and thus again points to differences in the crystal structures of T_2 and T_3/T_4 .

Since the main objective of this study was to determine whether resonance absorption of the radiation has a detectable chemical consequence, the energy of the X-ray quanta was varied in the succeeding experiments. In tables 1-3, I_2 yields and $G(I_2)$ values are given as a function of tube voltage (corresponding to E_{max} of the X-ray photons) and tube current in the three systems investigated. The experimental excitation functions were calculated from the difference in I_2 yields found between successive tube voltages. Standard functions describing the proton spectrum generated in a copper target operated at different high voltages were used in the calculations.(14)

The results of these calculations, after correction for dose effects, are given in Fig. 2. The solid line shows the calculated X-ray resonance absorption curve for T_3 , while the broken line represents the same curve corrected for K- to L-shell Auger yield. In performing the calculation, use has been made of tabulations of X-ray attenuation coefficients (10,11), and of the values 0.85 and 0.13 for the respective K- and L-shell fluorescence yields (15). The corresponding absorption curves for T_2 and T_4 differ from the T_3 curve by less than the spread in experimental data and for this reason are not shown.

It is evident that the observed radiation chemical effect exhibits a break at the iodine K-absorption edge, and that its course is similar to that of absorption of radiation. Thus, the extent of damage is shown to depend on photon energy. Moreover, the chemical effect of resonance absorption is not only demonstrable, but in excess of that predicted from the Auger yield of the K-shell in relation to the Auger yield of the L-shell.

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LEGENDS TO FIGURES AND TABLES

Fig. 1. Percent of total iodine atoms liberated by X-irradiation of crystalline iodoamino acids (45 KV, 12 mA).

Δ : 3,5-diiodo - DL - thyromine

$\square$: 3,5,5'-triiodo - DL - thyronine

O : DL - thyroxine

Fig. 2. Experimental Iodine yield from 10.0 mg samples of crystalline iodoamino acids as a function of photon energy. (Solid line: calculated photon absorption cross section for 3,5,5'-triiodo - DL - thyronine).

Δ : 3,5-diiodo - DL - thyromine

$\square$: 3,5,5'-triiodo - DL - thyronine

O : DL - thyroxine

Table 1. Iodine yield from ^{10.0mg} 3.5 diiodo-DL-thyronine at selected tube voltages and currents.

Table 2. Iodine yields from ^{10.0mg} 3.3'5-triiodo-DL-thyronine at selected tube voltages and currents.

Table 3. Iodine yields from ^{10.0mg} DL-throxine at selected tube voltages and currents.

TUBE VOLTAGE KV	TUBE CURRENT mA	RADIATION DOSE Mrads	IODINE YIELD moles $\times 10^{-6}$	G(I ₂)
15	37	0.09	0.56	6.01
20	28	0.19	0.96	4.78
25	22	0.30	1.00	3.25
30	19	0.42	1.15	2.65
32	18	0.47	1.16	2.37
35	16	0.53	1.17	2.13
38	15	0.64	1.18	1.78
40	14	0.70	1.12	1.53
42.5	13	0.81	1.33	1.57
45	12	0.91	1.36	1.44
47.5	11.5	1.04	1.28	1.19
50	11	1.17	1.38	1.14

TABLE 1. Iodine yield from 3.5 diiodo-DL-thyronine at selected tube voltages and currents.

TUBE VOLTAGE KV	TUBE CURRENT mA	RADIATION DOSE Mrads	IODINE YIELD moles $\times 10^{-6}$	G(I ₂)
15	37	0.098	0.25	2.45
20	28	0.214	0.31	1.39
25	22	0.330	0.37	1.08
30	19	0.483	0.46	0.92
32	18	0.536	0.54	0.97
35	16	0.607	0.50	0.79
38	15	0.725	0.64	0.85
40	14	0.813	0.56	0.66
42.5	13	0.928	0.64	0.66
45	12	1.107	0.56	0.49
47.5	11.5	1.203	0.60	0.48
50	10	1.239	0.55	0.43

TABLE 2. Iodine yields from 3,3',5-triiodo-DL-thyronine at selected tube voltages and currents.

TUBE VOLTAGE KV	TUBE CURRENT mA	RADIATION DOSE Mrads	IODINE YIELD moles x 10 ⁻⁶	G(I ₂)
15	37	0.102	0.34	3.12
20	28	0.226	0.37	1.58
25	22	0.36	0.46	1.23
30	19	0.52	0.45	0.83
32	18	0.58	0.51	0.84
35	16	0.65	0.51	0.75
38	15	0.80	0.60	0.72
40	14	0.89	0.60	0.65
42.5	13	1.02	0.61	0.58
45	12	1.15	0.62	0.52
47.5	11.5	1.31	0.68	0.50
50	10	1.35	0.64	0.46

TABLE 3. Iodine yields from DL-throxine at selected tube voltages and currents.

% CONVERSION