Mode of Action of High-voltage Cathode Rays on Aqueous Solutions of Amino-acids

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Previous reports by the authors (Bhatia & Proctor, 1951; Proctor & Bhatia, 1950, 1952) have shown that irradiation of haddock fillets by cathode rays resulted in no significant loss of amino-acids in fish muscle, but that cathode-ray irradiation of aqueous solutions of histidine monohydrochloride, trypto-

that, in air, tyrosine was converted partly into 3:4-dihydroxyphenylalanine (DOPA). Schocken (1950) has reported on the effect of ultraviolet irradiation on certain amino-acids. The degree of conversion of phenylalanine to tyrosine and of tyrosine to DOPA has been determined.

Table 1. *Yields of ammonia from amino-acids in 0.1M solution in 0.1N-HCl upon irradiation with a cathode-ray dose of 250 000 r.e.p.*

Amino-acid	NH ₃ -N (μg./ml.)					NH ₃ (μg./ml.)
	Replicates				Average	
Histidine monohydrochloride*	11.24	11.22	11.22		11.23	13.63
Cystine	5.61	5.49	6.09	6.03	5.81	7.05
Phenylalanine	3.87	3.84	3.36		3.69	4.48
Tyrosine	3.33	3.27	3.48	3.18	3.32	4.03
Tryptophan	1.29	1.29	1.38		1.32	1.60

* In a previous report (Bhatia & Proctor, 1951), deamination studies were conducted on solutions containing 100 μg. histidine monohydrochloride monohydrate/ml.

phan, tyrosine, phenylalanine, and cystine resulted in the decomposition of these amino-acids. Evidence has been presented that the histidine molecule is deaminated and that the glyoxaline ring of histidine is broken by irradiation with cathode rays. Evolution of hydrogen sulphide upon irradiation of aqueous solutions of cystine indicated that the molecule of cystine is decomposed at the disulphide linkage.

Allen, Steiger, Magill & Franklin (1937) reported that several amino-acids and their derivatives undergo change when exposed to the influence of cathode rays and ultraviolet radiations. Ammonia was liberated from all compounds upon irradiation. Deamination of amino-acids upon irradiation with X-radiation has been observed also by Stein & Weiss (1948) and by Dale, Davies & Gilbert (1949).

Arnow (1942) and Spiegel-Adolf (1937) noted that phenylalanine solutions became pigmented when exposed to ultraviolet rays. Irradiated neutral, acid, or alkaline solutions of phenylalanine were found by Arnow to give a positive Millon test, indicating the formation of phenol. Tyrosine has also been investigated by Arnow (1937), who found

The present investigation was conducted to elucidate the mode of chemical action of cathode rays on certain amino-acids.

Table 2. *Effect of irradiation with high-voltage cathode rays on pH of solutions of amino-acids*

Dose (r.e.p.)	pH of solution		
	Phenylalanine (200 μg./ml.)	Tryptophan (50 μg./ml.)	Tyrosine (100 μg./ml.)
Control (non-irrad.)	2.66	2.94	2.96
100 000	2.64	2.94	2.80
200 000			2.76
250 000	2.64	2.94	
400 000			2.77
500 000	2.65	2.94	
800 000			2.77
1 000 000	2.65	2.93	
1 500 000			2.85

EXPERIMENTAL AND RESULTS

The amino-acids used were L-histidine monohydrochloride monohydrate, DL-phenylalanine, DL-tryptophan, L-tyrosine, and L-cystine.

Solutions (0.1M) of these amino-acids were prepared in 0.1N-HCl; 3 ml. samples of the solutions were sealed into

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Fig. 1. Ultraviolet absorption spectra of irradiated and control solutions of DL-phenylalanine (concn. 200 $\mu\text{g./ml.}$). Cathode-ray dose: curve A, non-irradiated; curve B, 100 000 r.e.p.; curve C, 250 000 r.e.p.; curve D, 500 000 r.e.p.; curve E, 1 000 000 r.e.p.

Fig. 3. Ultraviolet absorption spectra of irradiated and control solutions of L-tyrosine (concn. 100 $\mu\text{g./ml.}$). Cathode-ray dose: curve A, non-irradiated; curve B, 100 000 r.e.p.; curve C, 200 000 r.e.p.; curve D, 400 000 r.e.p.; curve E, 800 000 r.e.p.; curve F, 1 500 000 r.e.p.

Fig. 2. Ultraviolet absorption spectra of irradiated and control solutions of DL-tryptophan (concn. 50 $\mu\text{g./ml.}$). Cathode-ray dose: curve A, non-irradiated; curve B, 100 000 r.e.p.; curve C, 250 000 r.e.p.; curve D, 500 000 r.e.p.; curve E, 1 000 000 r.e.p.

Fig. 4. Ultraviolet absorption spectra of irradiated and control solutions of benzene in water (37.2 mg./100 ml.). Cathode-ray dose: curve A, non-irradiated; curve B, 1 500 000 r.e.p.

5 ml. glass ampoules, and the ampoules were subjected to electron irradiation.

Replicate determinations of free NH_3 by the micro-diffusion method (acidimetric) as described by Conway (1947) were made on solutions of these amino-acids after exposure to a cathode-ray dose of 250 000 r.e.p. (Table 1).

Spectrophotometric determinations of the absorption spectra of three of the amino-acids were made (Figs. 1-3). For these determinations, suitable concentrations of the amino-acids were chosen, and the solutions were exposed to graded doses of cathode rays.

Determinations of pH were made on solutions of the same three amino-acids (Table 2). No significant change was noted in the pH of the solutions of phenylalanine and tryptophan upon irradiation with increasingly greater doses of cathode rays, but a slight drop was noted in the pH of solutions of tyrosine.

The techniques used were the same as described previously (Bhatia & Proctor, 1951; Proctor & Bhatia, 1952).

DISCUSSION

It has been demonstrated that amino-acids are deaminated upon irradiation with high-voltage cathode rays. As shown in Table 1, histidine was deaminated to the greatest extent and the order of deamination runs as follows: histidine > cystine > phenylalanine > tyrosine > tryptophan. As reported previously (Bhatia & Proctor, 1951), ammonia nitrogen evolved upon irradiation of histidine is contributed both by the nitrogen in the imidazole ring and the α -amino group. In their experiments with X-rays, Dale *et al.* (1949) observed the phenomenon of the specificity of the deamination of amino-acids and other nitrogen-containing compounds.

The relative radiosensitivities, based on microbiological assay data and expressed in terms of the specific inactivation dose (D/C) in r.e.p./g./ml., of these amino-acids have been calculated. They are as follows: histidine, 1.25×10^6 ; phenylalanine, 1.7×10^6 ; cystine, 2.40×10^6 ; tyrosine, 2.60×10^6 ; tryptophan, 10.00×10^6 .

In the studies on ultraviolet absorption spectra, phenylalanine showed absorption minima at 233, 254 and 261 m μ . and maxima at 252, 257 and 263 m μ . The selective absorption changed with increasing doses of radiation, and at the doses of 500 000 and 1 000 000 r.e.p. the peaks essentially

disappeared, indicating that the benzene ring had been split open. There was a considerable increase in the absorption, which probably was due to the molecular fragments obtained upon irradiation of the molecule of phenylalanine.

Tryptophan was characterized by absorption minima at 242 and 286 m μ . and maxima at 280 and 287 m μ . Upon irradiation, both the maxima and the minima disappeared, which is evidence that the indole ring of tryptophan was broken.

Tyrosine was characterized by an absorption minimum at 245 m μ . and a maximum at 274-275 m μ . When tyrosine solutions were irradiated at 1 500 000 r.e.p., the peaks disappeared and a curve with a general absorption was obtained.

Irradiation of the three aromatic amino-acids, tryptophan, phenylalanine, and tyrosine has indicated that cathode rays are capable of splitting the benzene ring. To confirm this observation further, an aqueous solution of benzene was exposed to a cathode-ray dose of 1 500 000 r.e.p. and ultraviolet absorption spectra were determined with irradiated and non-irradiated solutions. Fig. 4 demonstrates clearly that the selective absorption characteristic of benzene disappears upon irradiation. This indicates beyond any doubt that the benzene ring is broken.

SUMMARY

1. Exposure of amino-acids to high-voltage cathode rays results in deamination.
2. Determinations of the ammonia yields from 0.1 M solutions of certain amino-acids upon irradiation with a cathode-ray dose of 250 000 r.e.p. indicate that the order of deamination of the amino-acids studied was: histidine > cystine > phenylalanine > tyrosine > tryptophan.
3. Spectroscopic evidence is presented that irradiation with high-voltage cathode rays causes the fission of the ring structure of the aromatic amino-acids.

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