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The Effect of Ultraviolet Radiation on Sulfhydryl and Disulfide Containing
Amino Acids

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Radiation - Effect on compounds

Waves, Ultraviolet

Amino acids

Sulfur compounds

Atomic Energy (48)

Radioactivity (3)

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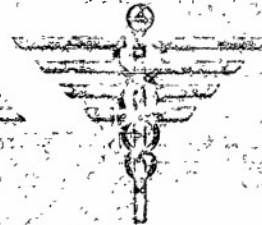
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**THE EFFECT OF ULTRAVIOLET RADIATION ON SULFHYDRYL
AND DISULFIDE CONTAINING AMINO ACIDS**

Subtask under Effect of Irradiation, AMRL Project No. 6-59-08-013,
Subtask, Quantitative Studies on the Effects of Non-Ionizing Radiation
on the Skin.



**MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY**

REPORT NO. 73

THE EFFECT OF ULTRAVIOLET RADIATION ON SULFHYDRYL
AND DISULFIDE CONTAINING AMINO ACIDS*

by

Dr. Klaus Schocken

from

Army Medical Research Laboratory
Fort Knox, Kentucky
2 January 1952

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Subtask, Quantitative studies on the Effects of Non-Ionizing Radiation
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Project No. 6-59-08-013
Subtask AMRL S-2

2 January 1952

ABSTRACT

THE EFFECT OF ULTRAVIOLET RADIATION ON SULFHYDRYL AND DISULFIDE CONTAINING AMINO ACIDS

OBJECT

According to Rothman and his associates pigmentogenic stimuli, such as ultraviolet radiation, cause pigmentation by oxidizing or destroying inhibitory sulfhydryl compounds, thus enabling an enzyme to act on the pigment precursor.

It was therefore of interest to study the effect of ultraviolet irradiation on the possible reduction of the -S-S- (disulfide) linkage of cystine and homocystine to -SH and possible oxidation of the -SH (sulfhydryl) group of cysteine to -S-S-. Methionine was also studied.

RESULTS AND CONCLUSIONS


It was shown that the following effects take place under ultraviolet irradiation of -SH and -S-S- containing amino acids. Cysteine is oxidized to cystine and cystine is reduced to cysteine. In addition, decomposition takes place which becomes more pronounced on prolonged irradiation. Methionine is destroyed. Homocystine is reduced to homocysteine. No evidence could be obtained to show that methionine is transformed into homocysteine.

RECOMMENDATIONS


None.

Submitted by:
Klaus Schocken, Biophysicist

Approved by:


RAY G. JAGGS
Director of Research

Approved by:


CARL F. TESSER
Lt. Col., MC
Commanding

THE EFFECT OF ULTRAVIOLET RADIATION ON SULFHYDRYL AND DISULFIDE CONTAINING AMINO ACIDS

I. INTRODUCTION

In previous studies quantitative measurements were made on the influence of ultraviolet irradiation on the rate of hydroxylation and of destruction of those amino acids which have been implicated in the process of skin pigmentation and erythema production (1). It was found that these effects were inhibited by the presence of sulfhydryl containing amino acids. The results obtained were compatible with the theory of Rothman and his associates, that pigmentogenic stimuli such as ultraviolet radiation cause pigmentation by oxidizing or destroying inhibitory sulfhydryl compounds, thus enabling an enzyme to act on the pigment precursor (2).

It thus became of interest to study the effect of ultraviolet irradiation on the possible reduction of the -S-S- (disulfide) linkage of cystine and homocystine to -SH and possible oxidation of the -SH (sulfhydryl) group of cysteine to -S-S-. Methionine was also studied.

II. EXPERIMENTAL

A. Apparatus and Methods

Solutions of either cystine or cysteine in 0.1 N hydrochloric acid and of methionine in 0.3 N hydrochloric acid were irradiated in test tubes 1 inch in diameter held in running water. The solutions were in layers from 0.5 to 2.5 cm in the test tubes. The unfiltered radiation of a Hanovia quartz mercury lamp was incident from above at 30 cm distance without any absorber (except air) between the lamp and the solution. After 30 minutes to 2 hours of irradiation the respective amino acid solutions were analyzed colorimetrically for cysteine and cystine (3) or methionine (4) with a recording spectrophotometer.

For irradiations with approximately monochromatic radiation, the Double Monochromator Model 300 DUV of the Farrand Optical Company was used in conjunction with the 1000 watt type A-H6 mercury lamp of General Electric. In this case, the solutions were contained in a quartz vessel with a quadratic cross section of 1 cm². The energy measurements, made with a calibrated thermopile, were accurate within $\pm 1\%$. The total energy incident on the solution was absorbed excepting small reflection losses amounting to about 4%. The reflection losses, which occur on the interfaces of the quartz cell, were not subtracted from the energies as they were measured.

In those experiments in which the isolation of homocysteine was attempted, solutions of homocystine and methionine in 0.1 N hydrochloric acid were irradiated by the Hanovia lamp, as described above, in layers of 4 cm for 2-1/2 hours. The procedure used in the identification of homocysteine from homocystine was as follows:

The dry residues of 4 irradiations of 400 mg homocystine in 200 ml of 0.1 N HCl, were dissolved in 12 ml of water, filtered and neutralized with NaOH. A precipitate (I) formed at pH 5-6, which was filtered, washed with water and then dissolved in 1 N HCl and reprecipitated by neutralization with NH_4OH . This procedure was repeated twice. Then the precipitate was filtered, washed with water and dried in the desiccator in vacuo. To the filtrate of precipitate I, 600 mg of NaOH were added, followed by 0.5 ml of benzylchloride, and the mixture was shaken for 100 minutes in a small separatory funnel. The solution was extracted with 5 ml of ether four times to remove the uncombined benzylchloride. The alkaline solution was freed from ether in vacuo, filtered, and acetic acid was added until the solution was slightly acid to litmus. After standing 48 hours in the refrigerator the precipitate was filtered off, washed with ice cold water and recrystallized from boiling water. This procedure was repeated twice. Altogether 39.9 mg of a benzylated product were obtained.

The procedure used for attempts to identify the irradiation product of methionine was in principle the same.

B. Results

1. Irradiation of cystine and cysteine

The results obtained on irradiation of cystine and cysteine with the Hanovia lamp are illustrated in Table 1. It can be seen that with increasing exposures of cystine, increasing amounts of this substance were reduced to cysteine, and simultaneously increasing amounts of it were destroyed. With irradiation of cysteine, at first only oxidation to cystine took place without destruction; then, with increasing irradiation, increasing destruction of both cysteine and cystine occurred. During the irradiations the formation of hydrogen sulfide was noticeable by odor.

Similar results were obtained with monochromatic radiation of wavelength 225 m μ and wavelength width 10 m μ . With increasing irradiation of cystine, increasing amounts of cysteine were found in the range of energies from 0.33 to 2.50 joule/mg. With increasing irradiation of cysteine, the amount of cystine found remained approximately

constant in the range of relatively weak energies from 0.63 to 5.28 joule/mg. No destruction of either cystine or cysteine could be detected, probably because the radiant energy was considerably less than that produced by the Hanovia lamp. No odor of hydrogen sulfide could be detected.

2. Irradiation of homocystine

After irradiation of homocystine a positive test for -SH (sulfhydryl) was obtained. From the irradiation mixture S- benzyl-homocysteine could be isolated. The mixed melting point with an authentic sample of S- benzylhomocysteine gave no depression.

Analysis:

Theor. for S- benzylhomocysteine
 $C_{11}H_{15}NO_2S$:

	C (%)	H (%)	N (%)	S (%)
Theor. for S- benzylhomocysteine	58.64	6.71	6.22	14.23
Found for isolated product :*	57.95	7.00	6.78	13.35
	58.31	7.04	6.58	13.56

The finding of the conversion of homocystine to homocysteine agrees with the observation that cystine is reduced to cysteine by ultraviolet irradiation (5). The precipitate I was identified as unchanged homocystine.

3. Irradiation of Methionine

The results obtained on irradiation of methionine with the Hanovia lamp are illustrated in Table 2. The destruction increased with the incident energy.

Using monochromatic radiation of a wavelength 225 m μ and wavelength width 10 m μ , destruction was found within a range of energies from 0.42 joule/mg to 1.12 joule/mg. Since these energies are very small, no quantitative results concerning the degree of decomposition were obtained.

* The analyses were performed by Dr. Carl Tiedcke of the Laboratory of Microchemistry, Teaneck, N. J.

It was found that a solution of methionine gave a positive color reaction with sodium nitroferricyanide after irradiation for one hour with the Hanovia lamp, but not before and not if the radiation was first passed through an acetone filter which absorbs the wavelengths shorter than 300 m μ . Because of this finding the isolation of homocysteine was attempted from an irradiated methionine solution. No S-benzylhomocysteine could be isolated, only unchanged methionine could be identified. It is possible that methyl mercaptan CH₃SH was formed.

III. CONCLUSIONS

It was shown that the following effects take place under ultraviolet irradiation of -SH and -S-S- containing amino acids. Cysteine is oxidized to cystine and cystine is reduced to cysteine. In addition decomposition takes place which becomes more pronounced on prolonged irradiation. Methionine is destroyed. Homocystine is reduced to homocysteine. No evidence could be obtained to show that methionine is transformed to homocysteine.

IV. RECOMMENDATIONS

None

Thanks are expressed to the Biochemical Department for its support of this paper.

V. BIBLIOGRAPHY

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TABLE 1
EFFECT OF ULTRAVIOLET RADIATION ON
CYSTINE AND CYSTEINE IN 0.1 N HCl.

I	II	III	IV	V	VI	VII
Amino Acid Irradiated (1 mg)	Time of Irradiation (minutes)	Cystine & Cysteine after Irradiation (mg)	Cysteine after Irradiation (mg)	Cystine after Irradiation Calculated by Difference of Columns III & IV	Amount Destroyed (mg)	Degree of Decomposition (%)
Cystine	30	0.821	0.283	0.538	0.179	17.9
Cystine	60	0.702	0.396	0.306	0.298	29.8
Cystine	120	0.587	0.449	0.138	0.413	41.3
Cysteine	30	1.090	0.740	0.350	0	0
Cysteine	60	0.890	0.591	0.299	0.110	11.0
Cysteine	120	0.560	0.469	0.091	0.440	44.0

Each value is an average of 5 experiments made simultaneously.

TABLE 2
EFFECT OF ULTRAVIOLET RADIATION
ON METHIONINE IN 0.3 N HCl.

Time of Irradiation (minutes)	Degree of Decomposition (Per Cent)
30	30.0
60	40.5
120	76.0

Each value is an average of 2 experiments made simultaneously.