

PHOTOCHEMISTRY OF THE THIAZOLE COMPONENT OF VITAMIN B₁

BY FRED M. UBER AND FRANK VERBRUGGE

(From the Biophysical Laboratory of the Department of Physics, University of Missouri, Columbia)

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Photolysis of the pyrimidine component of vitamin B₁ by ultra-violet radiation has been reported in a recent paper by the present writers (1). It was shown that inactivation resulted not only from the breakdown of the pyrimidine ring structure but also from the loss or alteration of those side groups or radicals known to be essential for the biological functioning of the vitamin. Nevertheless, only 1 quantum out of more than 50 was found to be effective in bringing about inactivation. With the thiazole component an analogous quantitative analysis can be made according to the same method, for it has been shown (2, 3) that the fungus, *Phycomyces blakesleeanus*, can develop quite as efficiently when administered equimolecular quantities of the two components of vitamin B₁ as when given the thiamine itself, and hence an assay for either component can be carried out when the concentration of the other is known. In the case of the "thiazole" molecule, it is clear from the work of both Robbins and Kavanagh (4) and Bonner and Erickson (3) that inactivation may mean either some change in the hydrogen of the 2 position or in the 5-hydroxyethyl group rather than a breakdown of the ring itself, as each of the two groups mentioned has been shown to be essential for growth. Since decomposition of the ring structure is reflected in the loss of selective absorption, these two types of photochemical inactivation may be distinguished experimentally.

EXPERIMENTAL

A sample of 4-methyl-5- β -hydroxyethylthiazole, obtained from Merck and Company through the courtesy of George W. Lewis,

and referred to hereafter as simply "thiazole," has been used for all the reported measurements. Absorption data have been obtained with a medium Hilger spectrograph and Spekker photometer, a tungsten steel spark source, Eastman No. 33 plates, and 1 cm. absorption cells. The single broad absorption band around 2510 Å., as shown in the top curve of Fig. 1 for a concentration of 12.5×10^{-5} M, is in agreement with the published results of Ruehle (5) on the basic cleavage product of vitamin B₁ and related thiazole derivatives. Beer's law was found to be obeyed within the limits of error of the measurements for the

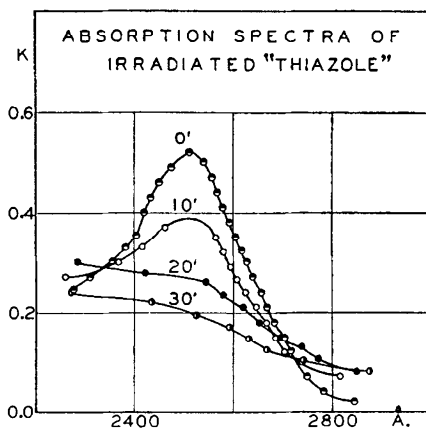


FIG. 1. Absorption spectra of the irradiated "thiazole" component of vitamin B₁. See Table I for values of absorbed energy corresponding to the various exposures.

concentrations upon which the photochemical yields are based.

In our investigation, each liter of nutrient solution contained 100 gm. of dextrose (cerelose), 4.0 gm. of *l*-asparagine, 1.5 gm. of KH₂PO₄, and 0.5 gm. of MgSO₄·7H₂O in redistilled water, together with 2-methyl-5-ethoxymethyl-6-aminopyrimidine at a resultant concentration of 1.0×10^{-7} M.

For the control growth curves, the "thiazole" concentration in the nutrient medium was varied from 0.5×10^{-7} M down to zero. After 25 cc. of nutrient solution were added to each of ten 125 cc. Erlenmeyer flasks and sterilized for 12 minutes at 15 pounds pressure, each flask was inoculated with 2 drops of a sterile spore

suspension from cultures of *Phycomyces blakesleeanus*, plus strain, which had been growing for 10 or more days on potato-dextrose-agar slants. The mature cultures in the flasks, having grown for 10 days in a dark oven at 23–24°, were autoclaved and the mycelial mats removed, washed, dried for 24 hours at 95–97°, and weighed.

The control growth curves showing the average dry weight in mg. per culture flask as a function of the "thiazole" concentration for two different determinations are shown in Fig. 2. In the concentration range of 0.025 to 0.250×10^{-7} M, the dry weight is

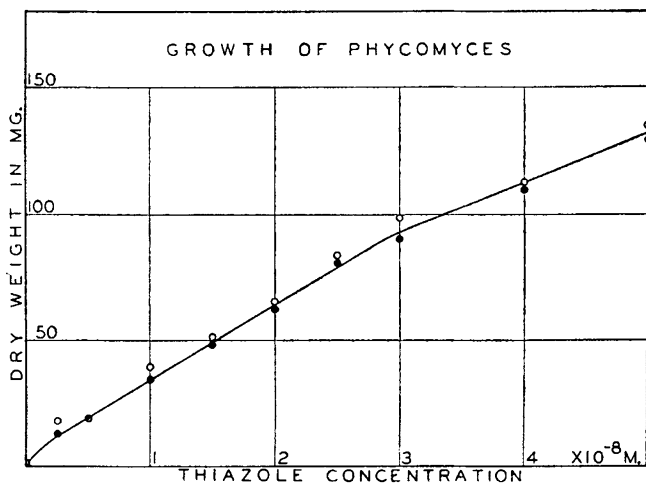


FIG. 2. Increase in dry weight of *Phycomyces* cultures as a function of "thiazole" concentration, with "pyrimidine" concentration constant at 1.0×10^{-7} M (two separate trials).

seen to be roughly proportional to the concentration. At still higher concentrations of "thiazole," the growth became limited by the "pyrimidine," whose concentration was maintained constant at 1.0×10^{-7} M.

For the photochemical inactivation of the "thiazole," a cylindrical, fused quartz irradiation cell with plane-parallel faces was filled with approximately 5 cc. of a 12.5×10^{-5} M solution, buffered with KH_2PO_4 at pH 4.4, and placed at a distance of 10.5 cm. from a low pressure mercury discharge tube (Hanovia Sc-2537), whose ultraviolet emission was almost solely the λ 2537 Å. line. The incident intensity of the radiation was measured with a vacuum

type thermopile, and the absorbing layer of solution, thickness 1.0057 cm., was stirred continuously.

At appropriate intervals during irradiation, a 0.1 cc. sample of the solution was withdrawn from the cell, and its "thiazole" concentration determined by a *Phycomyces* assay based on the culture procedure just outlined and on a comparison with the control growth curves. For this comparison, the growth for any given dose of radiation in the series was computed as a percentage of the growth by a non-irradiated control for a concentration of 0.5×10^{-7} M, made during the same series; a similar calculation was made for the control series data plotted in Fig. 2, and the comparisons were then made on this percentage basis. This procedure seemed justified by the observation that for the concentrations used in the assays, the end-products of irradiation did not inhibit the growth of *Phycomyces*.

Results

During ultraviolet irradiation of the 12.5×10^{-5} M solution of the 4-methyl-5- β -hydroxyethylthiazole, its absorption spectrum undergoes the changes shown in Fig. 1, where the extinction coefficient, K , represents the product of the molecular extinction coefficient and the molar concentration. The energy absorbed by the active "thiazole" solution for the various time intervals indicated on the curves is given in joules per cc. at the top of Column 5 of Table I. A steady decline in the absorption maximum can be noted until finally all selective absorption in this wave-length region disappears, thus signifying the decomposition of the unsaturated thiazole ring structure. But when the extinction coefficients for the inactive decomposition products were plotted as a function of wave-length for various times of irradiation, it was found that they exhibited selective characteristics. This indicates that inactivation has resulted also from alterations in essential radicals as well as from a breakdown of the ring structure. The best guess would seem to be a change in the hydroxyl radical or the entire hydroxyethyl group.

Quantum yields for the inactivation of the essential "thiazole" have been calculated from the four different irradiation trials summarized in Table I. The dry weight in each case is the average for ten culture flasks, with the probable errors as indicated. Column 4 represents the average in per cent of the incident energy

absorbed by the active "thiazole" during the respective irradiation intervals, while Columns 5 and 6 give the total energy absorbed in joules per cc. for various irradiation times and the quanta absorbed per molecule of "thiazole" initially present, respectively. The last column shows the quantum yield at 2537 Å. as determined for each interval separately. The average value is seen to be

TABLE I
Quantum Yield Data for "Thiazole" Inactivation

Exposure time	Dry weight of cultures	Pyrimidine inactivated	Average energy absorbed per interval	Total energy absorbed by active pyrimidine in		Quantum yield
				Joules per cc.	Quanta per original molecule	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
<i>min.</i>	<i>mg. per flask</i>	<i>per cent</i>	<i>per cent</i>			
0	125.4 ± 1.9	00.0		0.000	0.00	
10	77.2 ± 1.1	50.0	53.3	0.079	1.35	0.370
20	46.0 ± 1.0	72.0	31.2	0.125	2.13	0.338
30	19.5 ± 0.4	89.0	17.2	0.151	2.57	0.346
0	125.2 ± 1.3	00.0		0.000	0.00	
10	69.4 ± 1.1	54.2	51.8	0.083	1.41	0.384
20	37.3 ± 0.9	77.5	27.5	0.127	2.16	0.359
30	19.0 ± 0.3	91.5	14.0	0.149	2.54	0.360
0	125.2 ± 1.3	00.0		0.000	0.00	
11	65.0 ± 0.7	57.5	50.8	0.089	1.52	0.378
20	44.2 ± 1.3	72.4	28.7	0.131	2.22	0.326
30	20.2 ± 0.4	90.0	16.8	0.158	2.68	0.336
0	121.8 ± 1.0	00.0		0.000	0.00	
5	97.2 ± 1.1	28.0	60.1	0.048	0.82	0.342
15	59.7 ± 1.1	60.0	42.4	0.116	1.96	0.306
30	21.1 ± 0.8	89.5	21.5	0.167	2.84	0.315
Average.....						0.347

0.347, which means that on the average only 1 molecule is inactivated for every 3 quanta absorbed. This may be compared with the value of 0.0184 obtained earlier (1) for the effect of ultra-violet radiation upon the vitamin B₁ component containing the pyrimidine ring. Thus the relative ease with which these two thiamine components are broken down in metabolic processes is reflected in the relative quantum yields for photochemical inactivation.

The above quantum yield has been calculated on the assumption that all the *ultraviolet* radiation from the low pressure mercury discharge tube consists of the single wave-length 2537 Å. As these tubes are known to radiate a few per cent of their energy at λ 1849 Å., where the quantum yield might conceivably be much higher, a check with monochromatic radiation seemed very desirable. An orientation experiment of this kind was performed at λ 2537 Å. with a water-cooled mercury arc and monochromator, but the same yield value was obtained within the limits of experimental error. With the yield so nearly unity, this agreement was anticipated.

SUMMARY

The photochemical decomposition at λ 2537 Å. of the "thiazole" component (4-methyl-5- β -hydroxyethylthiazole) of vitamin B₁ has been demonstrated by its loss of selective absorption and its inability to support the growth of *Phycomyces* cultures.

The quantum yield for inactivation, when inactivation results from changes in side groups as well as from a breakdown of the ring structure, has been found to be 0.347. The corresponding value previously found for the pyrimidine component was 0.0184.

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