Chromatographic Separation of a Porphyrin Produced from Myoglobin by Gamma-Irradiation

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Methods were devised for studying the nature of the chemical changes that occur in the red pigment, myoglobin, when beef muscle or muscle extracts are irradiated with gamma rays. With the conditions devised, significant quantities of a green pigment were produced from myoglobin during irradiation with a total dosage approximating 1.3×10^6 rep. The irradiated preparations which contain the green pigment and unaltered myoglobin were partially purified and the heme moieties cleaved from the globin by acid-acetone treatment. The resultant heme derivatives were then successfully separated by paper chromatography. The data suggest that the altered heme compound is an oxidation product of the heme moiety of myoglobin. These and other studies will aid in obtaining a better understanding of the chemical changes that occur in foods treated with ionizing radiations.

Myoglobin in extracts of beef muscle tissue is changed to a green pigment when irradiated in a cobalt-60 source with a total dosage of 1.3×10^6 rep (*2*). The green pigment was detected by spectral analysis of the extracts after irradiation, and showed a peak of maximum absorption at 615 mμ with a reduction in the peak at 635 mμ. This latter peak is characteristic of metmyoglobin, the predominant heme pigment present in the nonirradiated control extracts. Preparations showing the green color after irradiation were cleaved by acid-acetone (*3*), and spectral data on the resultant hemin components showed that the porphyrin moiety had been chemically altered during the irradiation treatment (*2*).

Experimental

It was of importance to devise specific procedures for the production of the green compound and for the separation of the compounds (or the heme moieties) to provide a basis for subsequent identification studies. After a series of preliminary tests, a method for the production and isolation of the green pigment was developed. While some variation in the apparent quantity of the green pigment produced was observed from experiment to experiment, the following method was found to be the best of those examined.

A beef muscle cut (sirloin butt) was obtained from the local meat market. After all extraneous fat was removed the lean portion was ground and suspended in an equal amount of water and allowed to remain in the cold (approximately 4° C.) for about 3 hours. The extract was obtained by centrifugation and ammonium sulfate was added to a final concentration of 50%. This precipitated a considerable quantity of protein material which was removed by filtration. The extract thus obtained was exposed in test tubes to gamma rays from a cobalt-60 source to a dosage of 1.3×10^6 rep (at 4° C. over a 16-hour period). Appropriate nonirradiated preparations were retained for subsequent tests.

The irradiated extract was transferred to regenerated cellulose casings (Visking) and subjected to dialysis in running water overnight in the cold. This was effective in removing the ammonium sulfate which had been found to interfere in later steps of the isolation.

The contents of the cellulose casing were concentrated by means of "pervaporation."

A 2- or 3-ml. portion of the concentrated extract was added to 20 ml. of acid-acetone (0.5 ml. of concentrated hydrochloric acid in 500 ml. of acetone) which cleaved the heme portion from the globin of the myoglobin derivatives present.

Spectral curves were measured after each step of the preparation and the wave lengths of maximal absorption measured as shown in Table I. The peak at 410 mμ indicates the presence of heme pigments; the peak at 500 in combination with that at 635 mμ indicates the presence of metmyoglobin. The combination of peaks at 540 and 585 mμ indicates oxymyoglobin. Hemin chloride (protoporphyrin IX) in acid acetone gives peaks at 512 and 640 mμ, while the peaks at 615 and 605 to 615 mμ indicate the presence of the green compound, and the altered heme compound from the green compound, respectively. The latter peaks are present only in the preparations from the irradiated extracts.

Paper chromatographic techniques for separating the heme compounds present after acid-acetone cleavage were tested and the method of Nicholas and Remington (*4*) as modified by Chu and Chu (*1*) was adopted. This system uses 2,6-lutidine–water solvent system in a water-saturated atmosphere and is reported to separate porphyrins on the basis of the number of carboxyl groups present in the molecule. After development of the chromatograms, the cleaved heme compounds from irradiated and nonirradiated extracts and control compounds were located on the paper by observing

Table I. Wave Length of Maximum Absorption Observed for Myoglobin Derivatives and Cleaved Hemins from Irradiated and Nonirradiated Myoglobin Preparations

Step in Procedure	Wave Length, mμ	
	Nonirradiated preparation	Irradiated preparation
Before dialysis	410, 500, 545, 585, 635	410, 500, 615
After dialysis and concentration	410, 500, 545, 585, 635	410, 500, 615, 635
After acid acetone cleavage	512, 640	512, 605, 615, 640

Table II. R_f Values of Heme Compounds Derived from Irradiated and Nonirradiated Myoglobin Preparations

Sample Treatment	R_f Values[a] Experiment 1	Experiment 2
Nonirradiated meat extract	0.77	0.65
Hemin chloride	0.80	0.66
Irradiated meat extract	0.82	0.70
	0.62	0.47
Nonirradiated meat extract plus irradiated meat extract	0.79	0.70
	0.60	0.47
Hemin chloride plus irradiated meat extract	0.84	0.74
	0.64	0.53

[a] Two duplicate series of experiments. Separation of altered heme porphyrin was achieved in both experiments.

the chromatogram under a Mineralite lamp of 3600 A. wave length.

Results

It is apparent from Table I that the method outlined is successful for the production and detection of the altered heme compound in the irradiated extracts. The R_f values presented in Table II show that two heme compounds can be detected in the irradiated extracts, and the presence of the altered heme compound can be demonstrated in the presence of added nonirradiated control samples or hemin chloride. One of the compounds has an R_f corresponding to protoporphyrin IX derived from unreacted myoglobin and the second compound has a lower R_f. On the basis of earlier studies with this chromatographic system (*1*, *4*), the new heme compound (derived from the green pigment) probably has four carboxyl groups, as compared to two for unchanged hemin.

These techniques have permitted the detection and separation of the altered heme compound arising from myoglobin during irradiation. Studies to produce larger quantities of the isolated heme compounds for further chemical characterization are now in progress. The exact quantity of green pigment produced cannot be precisely calculated, until the compound has been isolated and the extinction coefficient determined. On the basis of the quantity of unaltered myoglobin present in the irradiated extracts as compared to nonirradiated extracts, the maximal quantity of green pigment produced would approximate 20% of the total pigment concentration.

Literature Cited

(1) Chu, T. C., Chu, E., *J. Biol. Chem.* **212,** 1 (1955).
(2) Ginger, I. D., Lewis, U. J., Schweigert, B. S., J. AGR. FOOD CHEM. **3,** 156 (1955).
(3) Lewis, U. J., *J. Biol. Chem.* **206,** 109 (1954).
(4) Nicholas, R. E. H., Remington, C., *Scand. J. Clin. Lab. Invest.* **1,** 12 (1949).

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PESTICIDES LITERATURE

A Multi-Indexed Machine-Sorted, Punch Card System for Pesticide Metabolism Data

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IBM punch card methods have been perfected by the Chemical-Biological Coordination Center for coding information concerning the biochemical transformations undergone by pesticides in the course of their metabolism. A code based upon a comprehensive classification of enzymes permits the recording of the effects of pesticides upon these all-important biochemical catalysts. This information can be retrieved by searching for the type of reaction, the organ, the species, the names of the pesticides, and their products.

RESEARCH IN THE PESTICIDE FIELD during the past decade has resulted in the accumulation of an enormous body of information, and there is no indication that activity in this area of chemical and biological endeavor will level off in the near future. With the ever-increasing number of publications in this field, the investigator is faced with the almost insoluble problem of keeping up with the literature.

The situation is aggravated by the fact that pesticide research cuts across traditional lines of academic disciplines with abandon. It is not unusual to find pertinent information concerning a chemical compound, of interest as a pesticide, in journals devoted to organic chemistry, entomology, biochemistry, pharmacology and toxicology, industrial medicine, and public health. For example, the *Biochemical Journal*, for July 1955, contains two excellent papers on insecticides (*1*, *7*), and the December