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# Role of Globin Moiety in the Chemical Structure of Curing Pigment

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**ABSTRACT:** In this study, the role of the globin moiety in the structure of this pigment has been evaluated, using myoglobin and hemin as model systems. After the synthesis of the cured pigment from the compounds used in this study, the absorption spectra, Fourier transform infrared spectroscopy (FTIR), and electrospray ionization (ESI)/MS spectroscopy were used to evaluate the chemical structure. Results indicated that the UV/visible, IR absorption, and mass spectroscopy of the cured pigment produced from myoglobin and its counterpart without the globin moiety, hemin, are different. Whereas myoglobin produced mononitrosylheme, hemin converted to dinitrosylheme, but probably the second nitric oxide group attached to the propionate side chain of the heme ring. It seems that the globin moiety protected heme ring against the second nitric oxide group.

**KEYWORDS:** *myoglobin, hemin, nitric oxide, globin, light fading, FTIR, ESI/Mass spectroscopy*

## INTRODUCTION

A significant property of meat, whether raw or cured, is its color. It has a major influence on the consumer's decision to purchase because it is usually associated with the quality of the product. Consumers prefer a bright pink color of cured pigment appearing after reaction of nitrosating species produced from nitrite with myoglobin. The characteristic color of raw cured meat (i.e., before thermal processing) is due to nitrosomyoglobin.[1] During thermal processing, globin denatures and detaches itself from the iron atom and surrounds the heme moiety. Nitrosylmyochromogen or nitrosylprotoheme is the pigment formed after cooking, and it confers the characteristic pink color to cooked cured meat.[2] From 1956 until now, many attempts have been done to characterize the chemical structure of cooked cured meat pigment.[1−5] Some researchers identified this pigment as a five-coordination mononitrosylheme[1,2,6] or a six-coordination dinitrosylheme.[3,4,7] Lee and Cassens (1976) [7] used 2-fold consumption of labeled $^{15}NO$ in solution relative to myoglobin as evidence to the formation of dinitrosyl ligation. Yet the possibility that NO may bind with other constituents of the hemoprotein was not considered. In a series of papers, Bonnett and co-workers reported that the reaction of sodium nitrite with hemoproteins under mildly acidic conditions can occur at the ferrous ion to give the nitrosylheme pigment,[8] in the porphyrin ligand,[9,10] or in the protein.[11] However, the role of the globin moiety in the chemical structure of nitrosoheme has not been evaluated yet.

On the other hand, nitrosylmyochromogen is susceptible to photodissociation and, in the presence of light and oxygen, its color alters to dull gray. Although the pathway of photooxidation still remains unknown, some mechanisms have been proposed. Pexara et al. (2002) [12] suggest that light can catalyze dissociation of nitric oxide (NO) from the cured pigment and cause discoloration in the presence of oxygen. Therefore, the probable mechanism is light-accelerated dissociation of NO from the heme followed by oxidation of both the NO moiety and the ferrous heme iron.[13] As a result of these reactions, a brownish-gray color develops on the exposed meat surface during color fading; this pigment, sometimes called a hemichrome, has its heme group in the ferric state. The effective way to prevent light fading is to exclude oxygen or light contact with cured meat surfaces.

Many articles dealt with the chemical structure of cooked cured meat pigment extracted from meat or synthesized in vitro. Myoglobin, hemin, or enzymes with similar structures to these compounds have been used as a model system to study the cured meat pigment. In this study, myoglobin and hemin (counterpart of myoglobin without the protein moiety) have been used as model systems to evaluate the role of the globin in the reaction of nitric oxide with the porphyrin ring and subsequent oxidation and discoloration.

## MATERIALS AND METHODS

**Materials.** All chemicals and solvents used in this study were analytical-grade commercial products. Sodium nitrite, hydrochloric acid (37%), acetone (pro analysis grade, 99.8%), and Tween-80 were purchased from the Merck Chemical Company, Germany. L-Ascorbic acid sodium salt was obtained from Alfa Aesar Chemical Company, Germany. Hemin (98% pure, high-performance liquid chromatography (HPLC) grade) was purchased from the Fluka Company, Switzerland.

**Preparation of Nitrosoheme.** In the previous study, the concentration of HCl (1, 2, and 3%), ascorbate (100, 150, and 200 mM), and nitrite (50, 100, and 150 mM) for production of cured meat pigment were optimized using the response-surface method. It was determined that the optimal conditions for production of nitrosoheme from hemin and nitrite are as follows: HCl percentage = 1.19%, ascorbic acid concentration = 123.08 mM, and nitrite concentration = 200 mM. Bovine hemin (6.52 mg) was dissolved in 1.88 mL of a 0.1 N NaOH solution to prepare nitrosoheme pigment from hemin and nitrite. This solution was diluted with 8 mL of acetone and then 0.12 mL of concentrated hydrochloric acid was added, giving a solution of acid hematin in 80% acetone. The nitrite and ascorbic acid were weighed and added directly to acid hematin solution. The container was gently shaken until nitric oxide slowly bubbled into the mixture and emitted into the air from the top of the container. The container

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