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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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was then capped and shaken vigorously for 30 s. The preformed, cured meat pigment was stored in the dark until further application.

Extraction of Myoglobin. Myoglobin was extracted according to Joseph et al. (2010) [14] with few modifications. The upper parts of a cow leg were provided from retail market within 24 h postslaughter and used for extraction of myoglobin. First the visible extraneous fat and connective tissue were trimmed off. Then, muscle was homogenized in buffer (5 mM Tris−HCl, 1 mM EDTA, pH 8.0, 4 °C) and centrifuged at 5 000*g* for 10 min at 4 °C. Afterward, the volume of supernatant was measured, brought to 50% (w/v) ammonium sulfate saturation, and centrifuged at 18 000*g* for 20 min at 4 °C. Finally, the resulting supernatant was saturated with adding ammonium sulfate until it precipitated (100%) and was centrifuged at 20 000*g* for 1 h at 4 °C.

Preparation of Nitrosomyoglobin. Because the amount of nitrite and ascorbic acid was determined based on heme concentration, for production of nitrosomyoglobin, the concentration of heme was measured in extracted myoglobin according to the procedure of Hornsey (1956).[15] Myoglobin, nitrite, and ascorbic acid were weighed as the mM ratio of hemin/nitrite/ascorbic acid was 1:123:200. Fifty mL of extracted myoglobin was transferred to a 250-mL 3-neck flask and diluted with 95 mL of 0.2 M phosphate buffer with pH = 6. To this solution, 4 or 5 drops of Tween-80 food-grade antifoam was added. Then, the contents of the flask were agitated by magnetic stirrer on medium speed to maintain a uniform suspension. Before reaction of the solution with nitrite, it was necessary to purge the reaction vesse1 of air by passing nitrogen gas through the stirred solution and exhaust for 5 min. Nitrite and ascorbic acid were dissolved in 5 mL of phosphate buffer and added to the mixture using a syringe. Upon completion of the nitrosating treatment, the temperature of the solution was increased to 70 °C for denaturation of globin moiety and production of nitrosyl hemochromogen. Finally, the solution was centrifuged for 15 min at 5 000*g*. The supernatant was discarded while the nitrosyl hemochromogen was recovered. The nitrosoheme was extracted from the pellet with 80% acetone and used for further analysis. The extracted nitrosoheme has a concentration of 1 mM and was used directly without dilution or concentration.

Preparation of Light-Faded Pigments. Two mL of synthesized and extracted nitrosoheme was separately transferred to a 5-mL transparent container that was covered with an oxygen-permeable polyethylene film (oxygen transmission rate > 1 000 $cm^3/m^2/atm/24$ h), and then placed in air under fluorescence light (D65 lamps) at room temperature until the redness was entirely faded and green color appeared. This faded pigment was used for further analysis.

Absorption Spectra of Pigments. The pigment from hemin−nitrite synthesis was diluted 12.5-fold with a 4:1 (v/v) acetone/water solution, and the absorption spectra were recorded using a Camspect spectrophotometer (M350, Leeds, U.K.). The absorption spectrum of nitrosoheme, extracted from cured myoglobin, was directly recorded without dilution. All absorption spectrum in the visible range were recorded.

FTIR Analysis. The FTIR from 4000−600 cm^{-1} were recorded on a Bruker TENSOR27 IR spectrometer (Bruker Instruments, Germany).

Mass Spectroscopy. The mass spectral data were obtained in the positive ion mode on an LCQ Advantage (Thermo Finnigan Co., IL), which was equipped with an electrospray ionization source. The instrument was calibrated with a solution of caffeine, MRFA (L-methionylarginylphenylalanylalanineacetate·H_2O), and Ultramark 1621, according to manufacturers' instructions. The electrospray ionization source and capillary were operated at 3.06 kV and 5.41 V, respectively. The capillary temperature was set to 200 °C. The accuracy and resolution of mass spectroscopy were 10 pg reserpine (10:1 signal-to-noise) and 0.7 fwhm across the mass range, respectively. First-order ESI mass spectra were recorded in the mass range m/z = 100−1000. The heme, nitrosoheme, and oxidized nitrosoheme solutions were introduced into the mass spectrometer at a constant flow rate of 5 μL/min by a syringe pump employing a 100 μL syringe.

RESULTS AND DISCUSSION

Absorption Spectra. The most important property of nitrosoheme pigment for judging its quality is its ability to

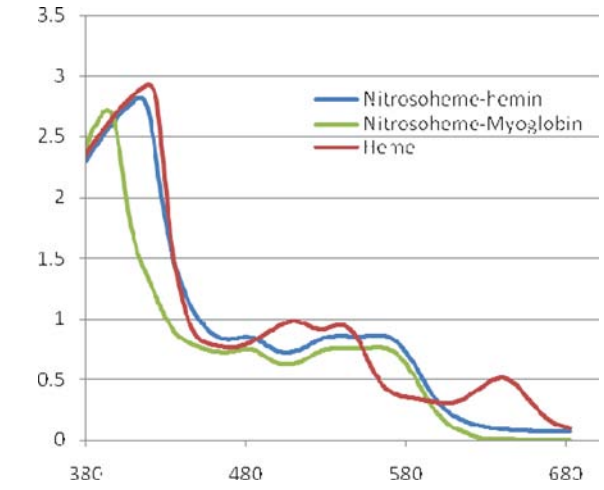

Figure 1. Absorption spectra of heme, nitrosoheme synthesized from hemin, and nitrosoheme extracted from nitrosomyoglobin.

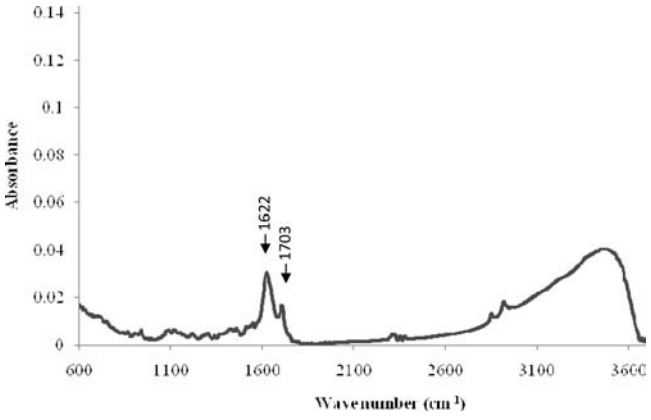

Figure 2. Fourier transform infrared spectroscopy spectrum of heme.

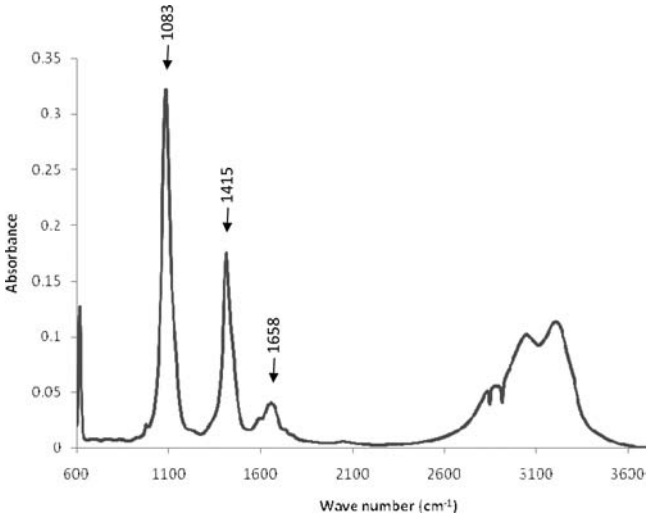

Figure 3. Fourier transform infrared spectroscopy spectrum of nitrosoheme extracted from nitrosomyoglobin.

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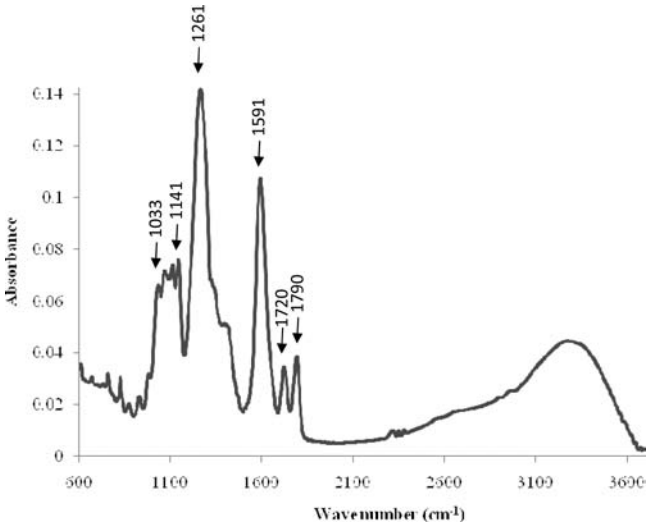

Figure 4. Fourier transform infrared spectroscopy spectrum of nitrosoheme synthesized from hemin.

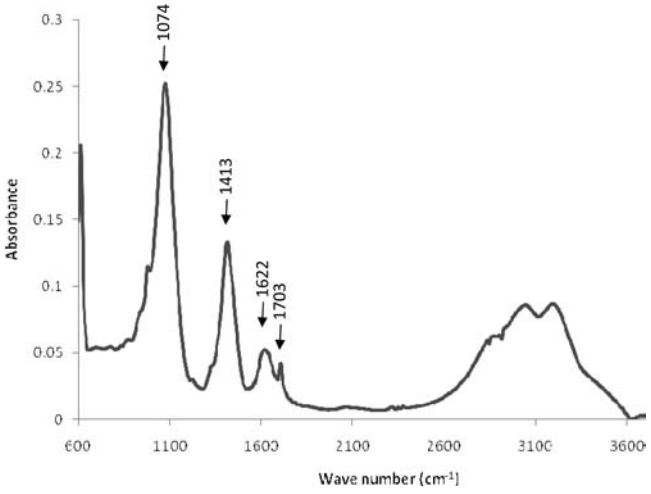

Figure 5. Fourier transform infrared spectroscopy spectrum of nitrosoheme extracted from nitrosomyoglobin after photooxidation.

reproduce the typical nitrite-cured color in meat. Figure 1 indicates the absorption patterns of the visible region of hemin

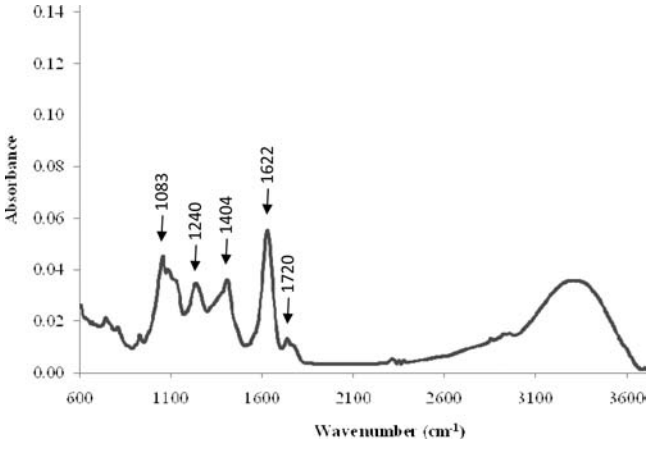

Figure 6. Fourier transform infrared spectroscopy spectrum of nitrosoheme synthesized from hemin after photooxidation.

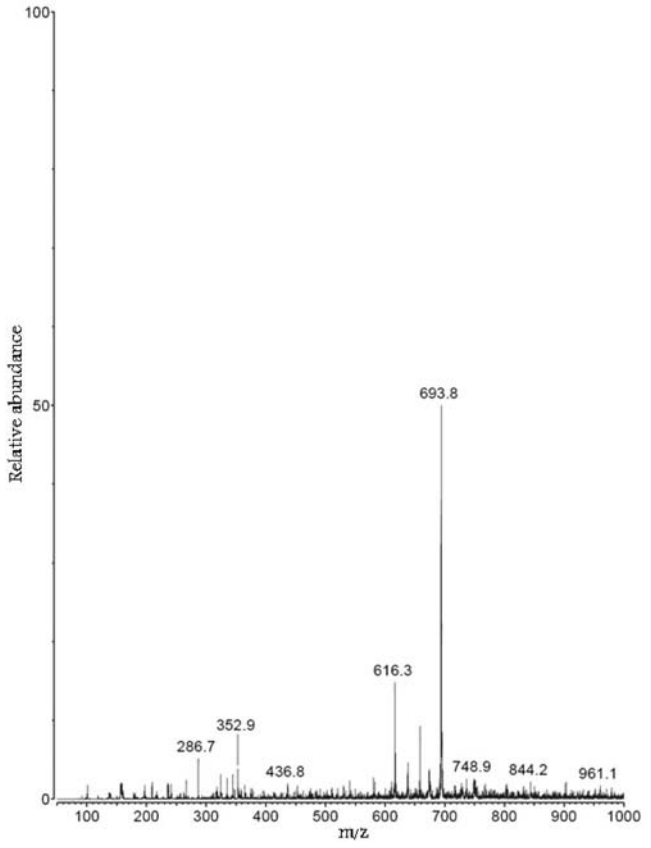

Figure 7. Electrospray ionization mass spectrum of heme.

and two synthesized nitrosoheme pigments. All pigments depicted the characteristic absorption pattern of the iron–porphyrin compound with a red color and had maxima at 454–481, 508–540, and 537–561 nm. Shahidi and Pegg (1991) [16] reported maximum absorbance for synthesized nitrosoheme at 540 and 563 nm. Evaluating the absorption spectra, Fox and Thompson (1963) [17] followed the conversion of metmyoglobin to nitrosylmyoglobin and reported that the absorption maximum of nitrosylmetmyoglobin was at 535 nm and that a slight shift toward longer wavelengths would be expected if nitrosylmetmyoglobin converted to nitrosylmyoglobin. The same trend was observed in this study after nitrosoheme production from hemin.

The Soret band was observed at 415 nm for nitrosoheme prepared from hemin and 395 nm for pigment prepared from myoglobin (Figure 1). Miller, Pedraza, and Chance (1997) [18] observed a Soret band for nitrosylmyoglobin at 422 nm before it shifted to 393 nm, which is indicative of the formation of the corresponding five-coordinate gas complexes. It seems that, in cured hemin, one nitric oxide molecule is bound to iron and forms a five-coordination complex, but in cured myoglobin, two nitric oxide molecules are attached to heme and form a six-coordination complex.

After exposure of the pigments to light and oxygen, the absorption spectra changed and resembled heme. Thus, the product of light fading was metheme with maximum absorption at 511, 540, and 640 nm. Anderson and Skibsed (1992), [19] who evaluated thermal oxidation and photooxidation of nitrosylmyoglobin in aqueous solution, also observed the absorption spectrum of metmyoglobin after the light fading of nitrosomyoglobin.

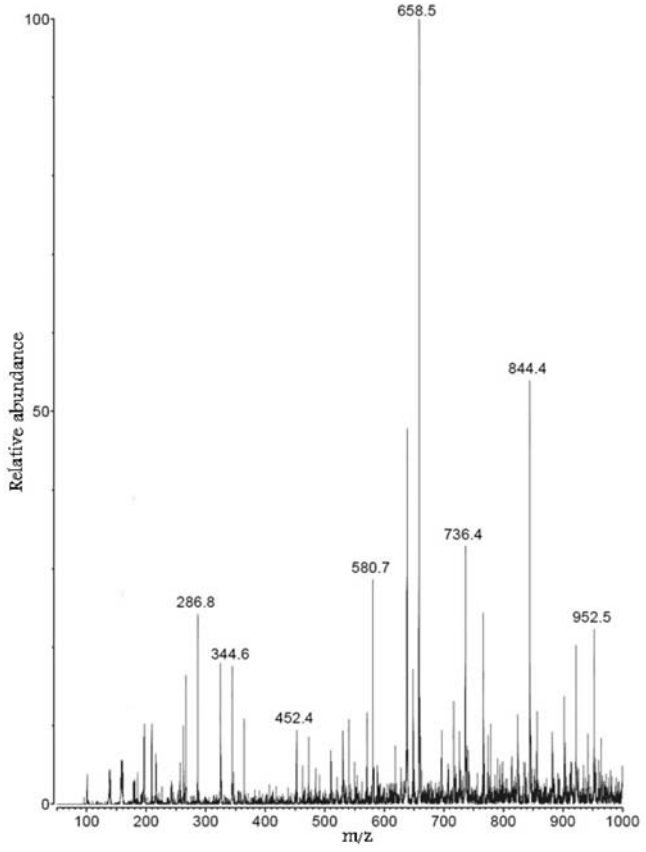

Figure 8. Electrospray ionization mass spectrum of nitrosoheme synthesized from hemin.

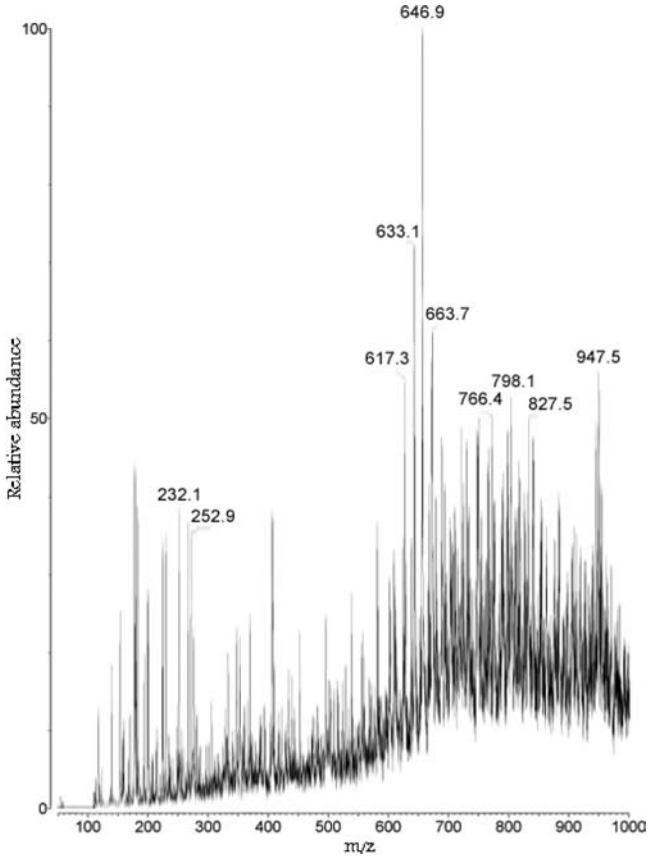

Figure 9. Electrospray ionization mass spectrum of nitrosoheme extracted from nitrosomyoglobin.

FTIR of the Synthesized Pigment. The FTIR spectra of heme and the synthesized pigments are depicted in Figures 2−4. The hemin showed a significant band at ν = 1622 and 1703 cm^{-1}, which are the dominant signals in the middle of the infrared (IR) range. The signal at 1703 cm^{-1} can be clearly attributed to the carbonyl stretching mode of the protonated heme propionates.[20] After reaction of myoglobin with NO, three peaks at 1083, 1415, and 1658 cm^{-1} appeared (Figure 3). The peak at 1658 is related to the stretching frequency of mononitrosyl heme. So according to the FTIR spectrum, it seems that nitrosoheme extracted from nitrosomyoglobin is a mononitrosyl heme. Sun, Zhou, Xu, and Peng (2009) [5] studied the IR spectrum of extracted cured pigment and related the band at 1653 cm^{-1} to the Fe−NO complex. Maxwell and Caughey (1976) [21] stated that the stretching frequency of the bent Fe−NO moiety of preformed cured pigment was in the range of 1600−1700 cm^{-1}. Killday, Tempesta, Bailey, and Metral (1988) [1] came up with the idea that the infrared spectrum of extracted curing pigment had a nitrosyl stretch at 1656 cm^{-1}. Pegg and Shahidi (1996) [2] and Jankiewicz, Kwany, Wasylik, and Graczyk (1994) [6] reported that the stretching frequency (m = 1659 cm^{-1}) of preformed cured pigment was consistent with the first nitrosyl group bound with the ferrous atom of the heme molecule.

After the reaction of hemin with nitric oxide, the propionic acid band disappeared and strong signals appeared at 1031, 1066, 1111, 1141, 1261, 1591, 1720, and 1790 cm^{-1} (Figure 4). It seems that the reaction of the −COOH groups of hemin propionate with nitric oxide is the main reason for the disappearance of the carbonyl band in the IR spectrum, whereas the two bands obtained at 1720 and 1790 cm^{-1} may be related to the interaction of nitric oxide with the −COOH group of propionic acid. The peak at 1591 cm^{-1} corresponds to the presence of a bent Fe−NO moiety and a pentacoordinate complex. Miller, Pedraza, and Chance (1997) [18] indicated that the primary ligand-bound state falls at 1613 cm^{-1} for $Mb^{14}NO$ and shifts to 1587 cm^{-1} for $Mb^{15}NO$. According to Killday et al. (1988),[1] the second nitrosyl group in cured pigment has the stretching band at m = ca. 1900 cm^{-1}, which was not observed in this study; however, it seems that two nitrosyl groups are bound to hemin, one to propionic acid and the other to Fe. Also, after attachment of NO to porphyrin, the symmetry of the ring changed and signals at 600−1500 cm^{-1}, corresponding to the porphyrin ring, became sharper. Andersen et al. (1990),[22] using labeled ^{15}NO in aqueous model systems, reported that only one NO molecule coordinated to Fe(II) of purified equine myoglobin, and heating the reaction mixture to temperatures in excess of 70 °C increased the ^{15}NO content of the product without resulting in any significant changes in the visible absorption spectrum. However, the authors stated that the increased labeling probably indicated nitrosation of the globin chain, because such a reaction is expected to have little influence on the spectral properties of the iron(II) chromophore, in contrast to the coordination of two NO molecules directly to iron(II). Evidence obtained from the IR pattern indicated that the other NO group attached to the −COOH group of the propionic acid of the heme ring.

After photooxidation of nitrosoheme synthesized from myoglobin, the signal at 1658 cm^{-1} disappeared. This peak, resulting from the attachment of the NO group to the central

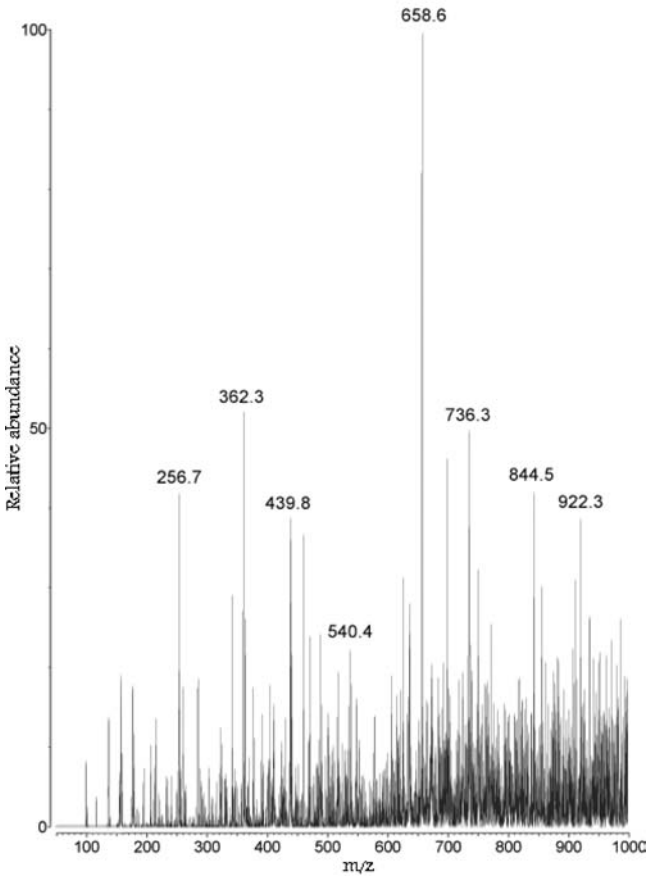

Figure 10. Electrospray ionization mass spectrum of nitrosoheme synthesized from hemin after photooxidation.

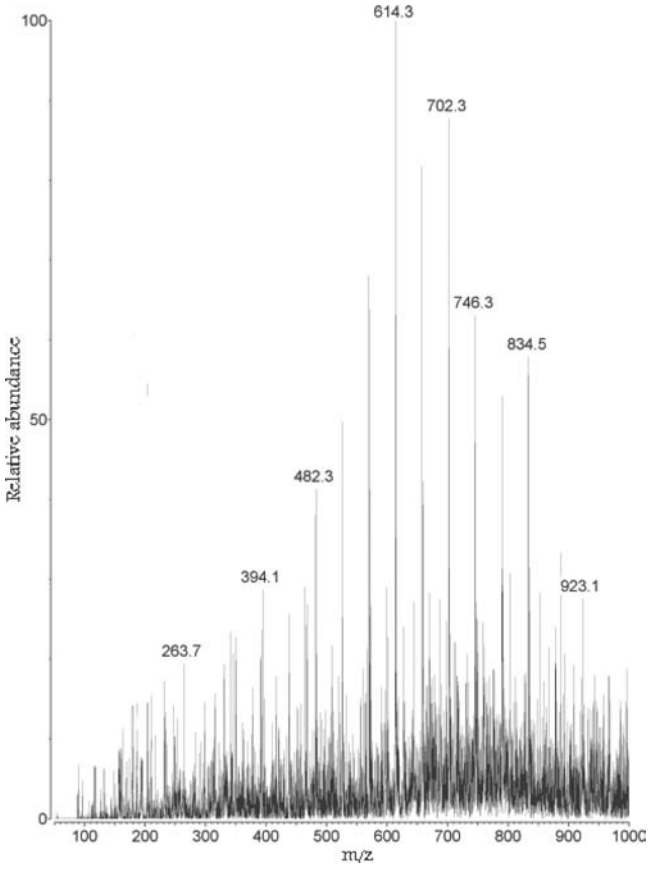

Figure 11. Electrospray ionization mass spectrum of nitrosoheme extracted from nitrosomyoglobin after photooxidation.

iron, disappeared after dissociation of this group from Fe (Figure 5). In light-faded nitrosoheme synthesized from hemin, the peak at 1790 cm^{-1} completely disappeared and just a small peak at 1720 cm^{-1} can be observed (Figure 6) that may be related to detachment of NO from the −COOH group of the propionic acid side-chain. However, it seems that light fading has not completely proceeded because attachment of the NO group to propionate (which is related to the peak at 1720 cm^{-1} of the FTIR spectrum in Figure 6) still existed, although its content has been considerably decreased. After this reaction, the carboxylic acid of propionic acid converted to carboxylate and a signal for ν −C═O at 1404 cm^{-1} appeared. Dissociation of NO from Fe reduced the stretching strength at 1591 cm^{-1}. Sun et al. (2009) [5] demonstrated that the band at ν = 1653.31 cm^{-1} in the FTIR spectra of extracted cooked cured meat pigment disappeared after light fading. However, they reported that infrared laser Raman spectra of cooked cured meat pigment oxidized in air and light did not change at ν = 1656.69 cm^{-1}, supplying evidence that the NO group might not detach itself from the heme moiety.

ESI-MS of Nitrosoheme. The ESI mass spectrum obtained for heme is shown in Figure 7. In the heme spectrum, the following ions are abundant: heme (m/z = 616.3) and [heme/acetone/H_2O] (m/z = 693.8). After conversion of hemin to nitrosoheme, the most intense peak is m/z = 658.5 (Figure 8); that is not consistent with the m/z of mononitrosylheme ($m/z \approx 646$) or dinitrosylheme ($m/z \approx 676$). As mentioned in the discussion of the FTIR spectrum of nitrosoheme, we propose that two nitric oxide molecules have been bound to heme. One of them coordinates with iron at the center of heme, and the other reacts with the propionic acid side-chain of heme that is accompanied by loss of one molecule of H_2O. Therefore, a compound with a molecular weight of 658.5 is produced. Other abundant peaks have m/z of 646 and 736.4; they are related to attachment of a NO group to the central iron of hemin and noncovalent bound between the compound with m/z = 658.8 and acetone−H_2O, respectively. Sun et al. (2009) [5] also obtained a peak at m/z = 657.3 for a cooked cured pigment that had been extracted from cured meat. However, they used HPLC/ESI-HR-MS with acetonitrile as a mobile phase for analysis of the extracted pigment and related the molecular ion peak to coordination of iron porphyrin with an acetonitrile.

In the ESI-MS of nitrosoheme extracted from cured myoglobin, an intense peak was produced by mononitrosyl heme at m/z = 646.9 (Figure 9) that showed a ∼31 Da shift from 616.3 m/z (heme) in Figure 7 to 646.9 m/z (mononitrosyl heme) in Figure 9. This peak related to attachment of the NO group to the central iron of heme. In this spectrum, there was no evidence to show attachment of a second NO group to the heme and no signal at m/z = 677.3 (attachment of the second NO to the central Fe) or 659.3 m/z (reaction of the second NO with the propionate side chain) appeared. It seems that in nitrosomyoglobin, only one NO group binds to the central iron and mononitrosyl heme is produced. Upmacis, Hajjar, Chait, and Mirza (1997) [23] evaluated the ESI-MS of myoglobin before and after nitrosylation and found a mass shift equal to 31 Da that is related to attachment of one nitrosyl group to the heme.

Upon exposure of cured pigments to light and oxygen until the green color appeared, a mass shift for nitrosoheme synthesized from hemin and extracted from nitrosomyoglobin occurred (Figures 10 and 11). However, the major molecular ion peak of photooxidised nitrosoheme synthesized from hemin possessed the same m/z (658.6 m/z) as that of the fresh pigment (see Figures 8 and 10); the enhancement of other peaks's intensity shows the dissociation of NO from propionic acid side-chain that has reduced the mother peak (658.6 m/z) intensity. As can be seen in the FTIR spectrum (Figure 6), the detachment of NO from nitrosoheme has not completely occurred, so the peak at 658.5 still is observable. For nitrosoheme extracted from nitrosomyoglobin, the major peak with m/z of 646.9 (Figure 9) disappeared and a new peak at m/z of 614.3 sharpened (Figure 11) that is probably related to the heme. Although Sun et al. (2009) [5] reported that the structure of oxidized cured pigment in air and light might be a changed conjugated system and the molecular weight was not changed compared with that of fresh one, Pexara et al. (2002) [12] found that light can catalyze the dissociation of nitric oxide from cured meat pigments and cause discoloration especially when oxygen is present. Andersen et al. (1992) [19] showed that, on admission of air into a solution of nitrosomyoglobin, the initial spectrum is converted to the spectrum of metmyoglobin, and 95% of the coordinated NO was detected as NO_3^- by a nitrate-selective electrode. However, other changes in the molecule, besides the change in the oxidation state of the iron from Fe(II) to Fe(III), are evident, because the product could be only partly converted (~86%) back to nitrosomyoglobin.

In the studies that investigated the structure of nitrosoheme, mononitrosyl, or dinitrosyl heme, the role of globin in the number of NO groups that can be bound to heme has not been considered. It was found that mononitrosylheme has been recognized in studies where myoglobin or cured meat was used as a model system,[1,2,6] and dinitrosylheme has been identified in the counterparts without the globin group.[3,4,7] The results of this study, using myoglobin and hemin as model systems, shows that mononitrosylheme is formed with myoglobin whereas two NO groups were bound to the porphyrin ring in hemin. It was concluded that nitrosoheme was physically trapped within a matrix of denatured globin that protects the heme from the second NO group. It was also found that the second NO reacts with the propionate side-chain of hemin and did not form dinitrosylheme.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Killday, K. B.; Tempesta, M. S.; Bailey, M. E.; Metral, C. J. Structural characterization of nitrosylhemochromogen of cooked cured meat: Implications in the meat-curing reaction. *J. Agric. Food Chem.* **1988**, *36* (5), 909−914.

(2) Pegg, R. B.; Shahidi, F. A novel titration methodology for elucidation of the structure of preformed cooked cured-meat pigment by visible spectroscopy. *Food Chem.* **1996**, *56* (2), 105−110.

(3) Shahidi, F.; Rubin, L. J.; Diosady, L. L.; Chew, V. Preparation of dinitrosyl ferrohemochrome from hemin and sodium nitrite. *Can. Inst. Food Sci. Technol. J.* **1984**, *17* (1), 33−37.

(4) Shahidi, F.; Rubin, L. J.; Diosady, L. L.; Wood, D. F. Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome, from hemin and nitric oxide. *J. Food Sci.* **1985**, *50* (1), 272−273.

(5) Sun, W. Q.; Zhou, G. H.; Xu, X. L.; Peng, Z. Q. Studies on the structure and oxidation properties of extracted cooked cured meat pigment by four spectra. *Food Chem.* **2009**, *115* (2), 596−601.

(6) Jankiewicz, L.; Kwany, M.; Wasylik, K.; Graczyk, A. Structure studies on the nitrosyl derivative of heme. *J. Food Sci.* **1994**, *59* (1), 57−59.

(7) Lee, S. H.; Cassens, R. G. Nitrite binding sites on myoglobin. *J. Food Sci.* **1976**, *41* (4), 969−970.

(8) Bonnett, R.; Chandra, S.; Charalambides, A. A.; Sales, K. D.; Scourides, P. A. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 4. Pentaco-ordinate nitrosylprotohaem as the pigment of cooked cured meat. Direct evidence from e.s.r. spectroscopy. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1706−1710.

(9) Bonnett, R.; Charalambides, A. A.; Martin, R. A. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 1. Porphyrins and metalloporphyrins. *J. Chem. Soc., Perkin Trans. 1* **1978**, No. 9, 974−980.

(10) Bonnett, R.; Hursthouse, M. B.; Scourides, P. A.; Trotter, J. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 3. Attack at the vinyl groups of protoporphyrin dimethyl ester. X-ray analysis of 81 (E)-82-nitroprotoporphyrin dimethyl ester. *J. Chem. Soc., Perkin Trans. 1* **1980**, 490−494.

(11) Bonnett, R.; Nicolaidou, P. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 2. The reaction of nitrous acid with the side chains of *R*-acyl-amino-acid esters. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1969−1974.

(12) Pexara, E. S.; Metaxopoulos, J.; Drosinos, E. H. Evaluation of shelf life of cured, cooked, sliced turkey fillets and cooked pork sausages-"piroski"-stored under vacuum and modified atmospheres at +4 and +10 C. *Meat Sci.* **2002**, *62* (1), 33−43.

(13) Pegg, R. B.; Shahidi, F.; Fox, J. B. Unraveling the chemical identity of meat pigments. *Crit. Rev. Food Sci. Nutr.* **1997**, *37* (6), 561−589.

(14) Joseph, P.; Suman, S. P.; Li, S.; Beach, C. M.; Steinke, L.; Fontaine, M. Characterization of bison (Bison bison) myoglobin. *Meat Sci.* **2010**, *84* (1), 71−78.

(15) Hornsey, H. C. The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigment. *J. Sci. Food Agric.* **1956**, *7*, 534−540.

(16) Shahidi, F.; Pegg, R. Encapsulation of the preformed cooked cured meat pigment. *J. Food Sci.* **1991**, *56* (6), 1500−1504.

(17) Fox, J. B.; Thomson, J. S. Formation of bovine nitrosylmyoglobin. I. pH 4.5−6.5. *Biochemistry* **1963**, *2* (3), 465−470.

(18) Miller, L. M.; Pedraza, A. J.; Chance, M. R. Identification of conformational substrates involved in nitric oxide binding to ferric and ferrous myoglobin through difference Fourier transform infrared spectroscopy (FTIR). *Biochemistry* **1997**, *36* (40), 12199−12207.

(19) Andersen, H. J.; Skibsted, L. H. Kinetics and mechanism of thermal oxidation and photooxidation of nitrosylmyoglobin in aqueous solution. *J. Agric. Food Chem.* **1992**, *40* (10), 1741−1750.

(20) Dörr, S.; Schade, U.; Hellwig, P. Far infrared spectroscopy on hemoproteins: A model compound study from 1800−100 cm^{-1}. *Vib. Spectrosc.* **2008**, *47* (1), 59−65.

(21) Maxwell, J. C.; Caughey, W. S. An infrared study of NO bonding to heme B and hemoglobin A. Evidence for inositol hexaphosphate induced cleavage of proximal histidine to iron bonds. *Biochemistry* **1976**, *15* (2), 388−396.

(22) Andersen, H. J.; Johansen, H. S.; Shek, C. K.; Skibsted, L. H. Nitric oxide exchange in nitrosylmyoglobin. *Z. Lebensm. Unters. F., A* **1990**, *191* (4), 293−298.

(23) Upmacis, R. K.; Hajjar, D. P.; Chait, B. T.; Mirza, U. A. Direct observation of nitrosylated heme in myoglobin and hemoglobin by electrospray ionization mass spectrometry. *J. Am. Chem. Soc.* **1997**, *119* (43), 10424−10429.