THE DIFFERENCE BETWEEN THE CHEMICAL STRUCTURE OF NITROSOHEME AND NITROSOMYOGLOBIN

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Abstract – In this study, the role of the globin moiety in the structure of meat 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 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, is 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.

Key Words – Myoglobin; Hemin; Nitric oxide.

I. INTRODUCTION

A significant characteristic of meat, whether raw or cured, is its color. The distinguished 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 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] or a six-coordination dinitrosylheme [3,4].

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.

II. MATERIALS AND METHODS

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. The nitrite (200mM) and ascorbic acid (123mM) 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 was then capped and shaken vigorously for 30 s. The preformed, cured-meat pigment was stored in the dark until further application.

Myoglobin was extracted according to Joseph et al. (2010) [6] with few modifications and the concentration of heme was then measured in extracted myoglobin according to procedure of Hornsey (1956) [7]. Fifty ml of extracted myoglobin was transferred to a 3-neck flask and diluted with 95 ml of 0.2 M phosphate buffer with pH=6. Then, the contents of the flask were agitated. Nitrite and ascorbic acid were subsequently added to the mixture using a syringe. Upon completion of the nitrosating treatment, the temperature of solution was increased to 70ºC for denaturation of globin moiety and production of nitrosyl hemochromogen. Finally, the solution was centrifuged for 15 minutes at 5000 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 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. The absorption spectra of nitrosoheme, extracted from cured myoglobin, was directly recorded without dilution.

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

The mass spectral data were obtained in the positive ion mode on an LCQ Advantage (Thermo Finnigan Co., Illinois, USA), which was equipped with an electrospray ionization source. The electrospray ionization source and capillary were operated at 3.06 kV and 5.41 V, respectively. The capillary temperature was set to 200ºC. 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.

III. RESULTS AND DISCUSSION

The most important property of nitrosoheme pigment for judging its quality is its ability to reproduce the typical nitrite-cured color in meat. 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) reported maximum absorbance for synthesized nitrosoheme at 540 and 563 nm [8]. 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. Miller, Pedraza & Chance (1997) 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 [9]. 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.

The FTIR spectra of heme and the synthesized pigments are depicted in Figures 1. The hemin showed a significant band at ν=1622 and 1703 cm^{-1}, which are the dominant signal 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 [10]. After reaction of myoglobin with NO three peaks at 1083, 1415, 1658 cm^{-1} appeared. The peak at 1658 is related to stretching frequency of mononitrosyl heme, so according to FTIR spectrum it seems that nitrosoheme extracted from nitrosomyoglobin is a mononitrosyl heme. Sun, Zhou, Xu & Peng (2009) studied the IR spectra of extracted cured pigment and related the band at 1653 cm^{-1} to the Fe-NO complex [5]. 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}. It appears 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 spectra, 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, 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 ring changed and signals at 600-1500 cm^{-1}, corresponding to porphyrin ring, which became sharper. Evidences obtained from IR pattern indicated that the other NO group attached to -COOH group of the propionic acid of heme ring.

The ESI mass spectra obtained for heme is shown in Figure 2. 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 3); that is not consistent with the m/z of mononitrosylheme (m/z~646) or dinitrosylheme (m/z~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 centre 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; this is related to mononitrosylheme and non-covalent bound between the compound with m/z 658.8 and acetone-H_2O, respectively.

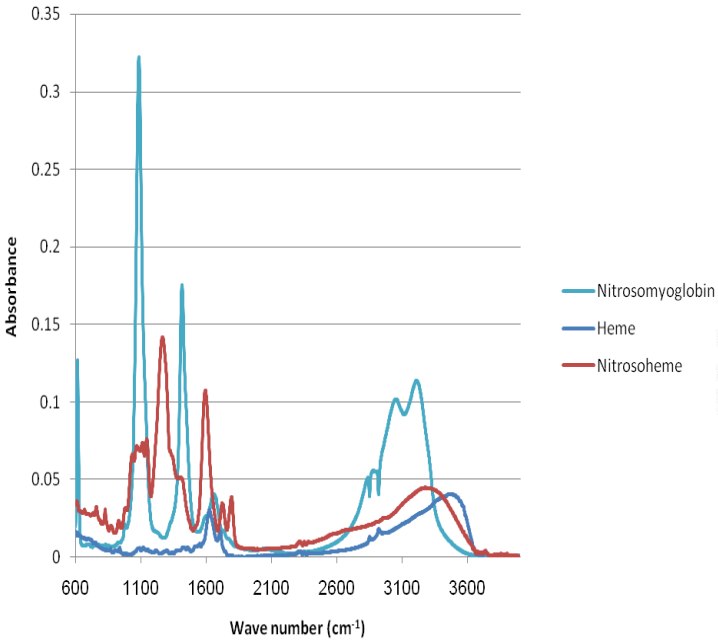

Figure 1. The FTIR spectrum of heme, Nitrosoheme and Nitrosomyoglobin

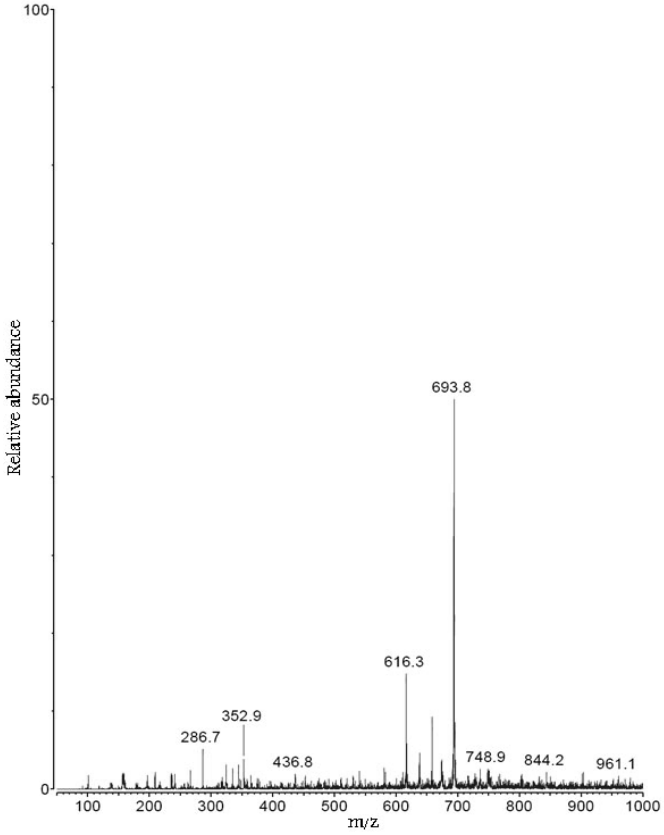

Figure 2. The ESI mass spectrum of heme.

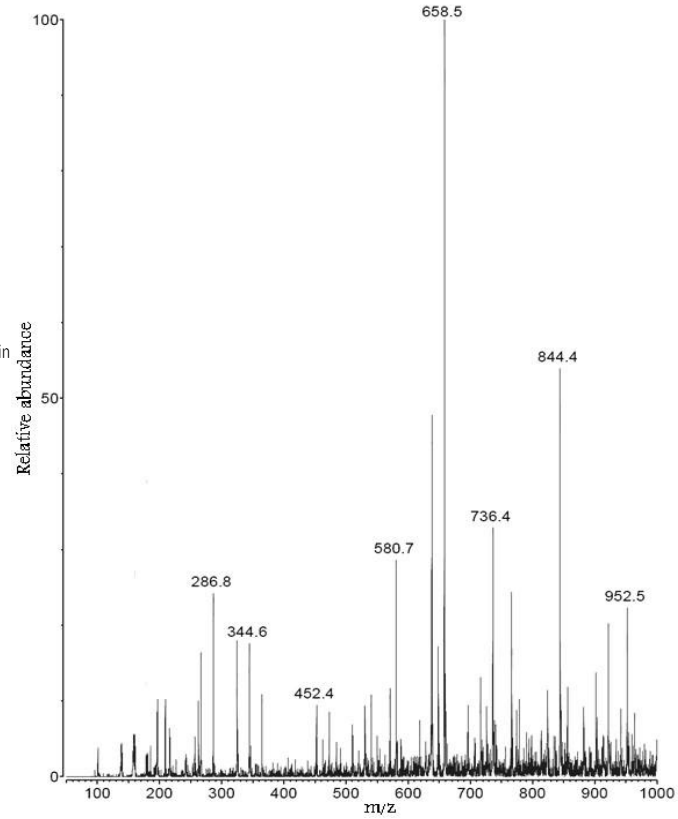

Figure 3. The ESI mass spectrum of nitrosoheme synthesized from hemin.

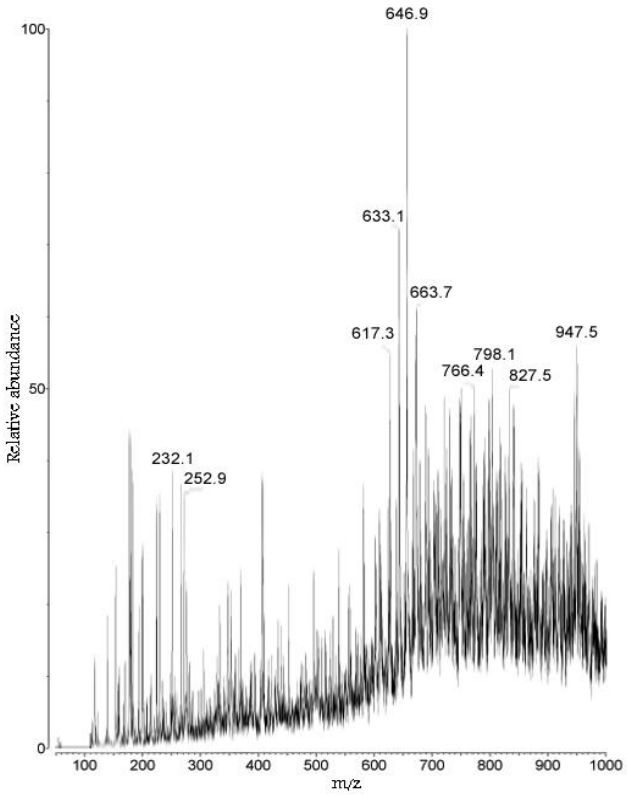

Figure 4. The ESI mass spectrum of nitrosoheme extracted from nitrosomyoglobin.

In the ESI-MS of nitrosoheme extracted from cured myoglobin, an intense peak was produced by mononitrosyl heme at m/z=646.9 (Figure 4) that showed a ~31 Da shift from 616.3 m/z (heme) in Figure 2 to 646.9 m/z (mononitrosyl heme) in Figure 4. This peak related to attachment of NO group to 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 second NO to central Fe) or 659.3 m/z (reaction of second NO with propionate side chain) appeared. It seems that in nitrosomyoglobin, only one NO group binds to the central iron and mononitrosyl heme produces.

IV. CONCLUSION

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, and dinitrosylheme has been identified in the counterparts without the globin group. The results of this study, using myoglobin and hemin as model systems, show 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 which protects the heme from the second NO group. It was also found that the second NO reacts with propionate side chain of hemin and did not form dinitrosylheme.

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