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Effect on pH of the Coordination of Nitric Oxide Complex of Iron(II) Swine Myoglobin

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Background and Objective: The ferrous complex of myoglobin containing nitric oxide (NO) is called NO complex of Fe(II) myoglobin (nitrosylmyoglobin). NO complex of Fe(II) myoglobin has two states, namely pentacoordinate and hexacoordinate. In either sperm whale or horse heart myoglobin, binding of NO and lowering of solution pH work together to weaken, and ultimately break, the bond between iron and the proximal histidine. Several spectral properties have been used to investigate the coordination state of iron of various hemoprotein complexes with NO at different pH's. Little is known of the coordination of swine nitrosylmyoglobin, and we found no reports of the coordination of nitrosylmyoglobin in cured pork.

Our objective is to examine the effect of pH on the coordination of nitrosylmyoglobin in pork by ESR spectroscopy.

Methods: Each sample (1.0 g chopped pork) was directly transferred to an ESR tube. ESR spectra were recorded on an ESR spectrometer (JES-TE3X, JEOL Co., Ltd.) under the following conditions: microwave power, 4 mW; modulation frequency and width, 100 kHz and 0.5 or 1.0 mT; temperature, 77 K; measurement time, 8 min. Nitrite or 3-[2hydroxy-1-(1-methylethyl)-2-nitrosohydrazino]-1-propanamine (NOC 5, Fig. 1) were used as NO donors.

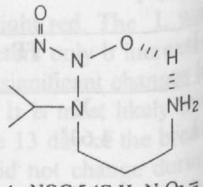

Fig. 1 NOC 5 ($C_6H_{16}N_4O_2$ = 176.2) 3-[2-Hydroxy-1-(1-methylethyl)-2-nitrosohydrazino]-1-propanamine

Results and Discussion: When the pH of horse nitrosylmyoglobin changed from 7.0 to 4.0, it affected the conversion of myoglobin from hexacoordinate (Fig. 2a) to pentacoordinate (Fig. 2b) (Duprat et al., 1995; Morita et al., 1998). Lowered pH produced pentacoordinated NO complex of Fe(II) myoglobin. Several spectral properties have been used to investigate the coordination state of iron of various hemoprotein complexes with NO at different pH's. When the pH of nitrosylmyoglobin changed from 7.0 to 4.0, it affected the conversion of myoglobin from hexacoordinate to pentacoordinate. Furthermore, Duprat et al.[8] demonstrated that lowered pH produced pentacoordinated NO complex of Fe(II) myoglobin.

Fig. 3 shows that characteristic ESR spectra of cured pork (nitrite addition) at pH 5.6, 4.5 and 7.0. In the cured pork at pH 7.0, hexacooredinate and pentacoordinate NO complexes of Fe(II) myoglobin are both present (Fig. 3b). The cured pork was changed from pH 5.6 to pH 4.5 using lactic acid. In a similar way, the pH of the cured pork was increased to 7.0 from 5.6 using sodium hydroxide. When the pH of the cured pork was decreased to pH 4.0 with lactic acid, the immediate conversion of hexacoordinate NO complex of Fe(II) myoglobin to pentacoordinate caused the hyperfine structure (Fig. 3a).

On the other hand, characteristic ESR spectra of pork added NOC 5 as a NO donor at pH 5.6, 4.5 and 7.0 are shown in Fig. 4. The effect of pH on the coordination state was the same as that of nitrite addition.

As sown (Fig. 5), the ESR spectrum of a pork at pH 7.0, changed from pH 5.6 by sodium hydroxide, indicates hexacoordination because of the absence of hyperfine structure around g = 2.

Conclusion: Raw pork generally shows pH around 5.5. The raw pork (pH 5.6) used in this experiments supplemented with either nitrite or NOC 5 as NO donor. At pH 5.6, both hexacoordinate and pentacoordinate NO complexes of Fe(II) myoglobin in raw pork existed. Pentacoordinate NO complex of Fe(II) myoglobin is generally nitrosylmyoglobin formed in cured meat. When the pH of the cured pork changed from 5.6 to 4.5 with lactic acid, it completely affected the conversion to pentacoordinate NO complex of Fe(II) myoglobin. When the pH of the pork shifted from 5.6 to 7.0 by sodium hydroxide and NOC 5 was supplemented, the nitrosylmyoglobin was pentacoordinate NO complex of Fe(II) myoglobin. This conversion process by pH was reversible.

Pertinent literatures: 1) Duprat, A.F., Traylor, T.G., Wu, G.-Z., Coletta, M., Sharma, V.S., Walda, K.N. and Magde, D. 1995. *Biochemistry* 34: 2634-2644. 2) Morita, H., Sakata, R. and Nagata, Y. 1998. *J. Food Sci.* 63: 352-355. 3) Hori, H., Ikeda-Saito, M. and Yonetani, T. 1981. *J. Biol. Chem.* 256: 7849-7855. 4) Pegg, R.B., Shahidi, F., Gogan, N.J. and DeSilva, S.I. 1996. *J. Agric. Food Chem.* 44: 416-421.

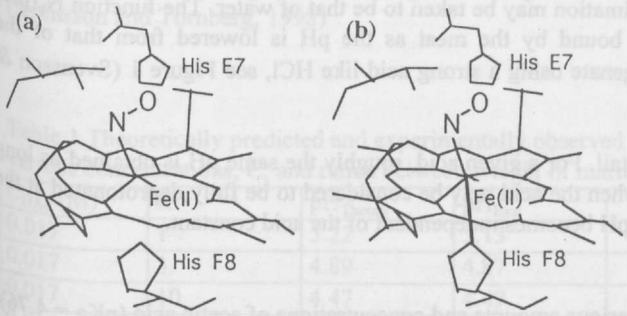

Fig. 2 Illustrations of pentacoordinate (a) and hexacoordinate (b) NO complex of Fe(II) myoglobin.

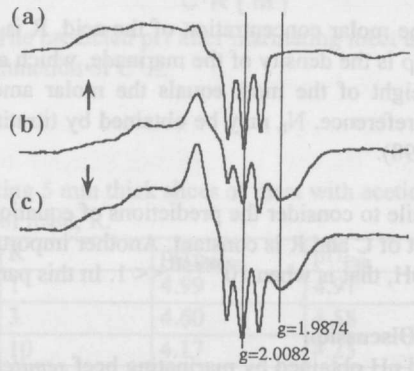

Fig. 3 Characteristic ESR spectra (a) cured pork changed from pH 5.6 to pH 4.5 by lactic acid, after added nitrite ; (b) cured pork at pH 5.6 ; (c) cured pork changed from pH 5.6 to pH 4.5 by NaOH, after added nitrite. NO complex of Fe (II) myoglobin in pork formed by reaction of myoglobin with NO from nitrite.

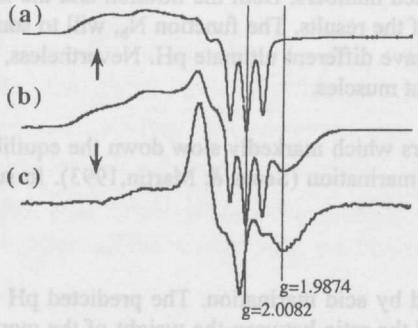

Fig. 4 Characteristic ESR spectra (a) pork changed from pH 5.6 to pH 4.5 by lactic acid, after added NOC 5 ; (b) pork at pH 5.6 added NOC 5 ; (c) pork changed from pH 5.6 to pH 4.5 by NaOH, after added NOC 5. NO complex of Fe (II) myoglobin in pork formed by reaction of myoglobin with NO from NOC 5.

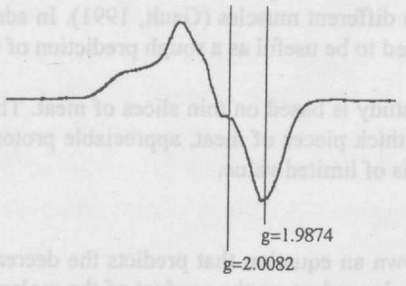

Fig. 5 Characteristic ESR spectrum of pork changed from pH 5.6 to pH 7.0 by NaOH, then added NOC 5.