NITRIC OXIDE INHIBITS THE FORMATION OF NOT ONLY ZINC PROTOPORPHYRIN IX BUT ALSO PROTOPORPHYRIN IX

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Introduction

Parma ham, an Italian traditional dry-cured ham, is made only from the leg of a fattened pig and sea salt. Although nitrite or nitrate have not been added, the color of Parma ham is an extremely stable bright red and is not changed by exposure of the ham to light or heat (Morita et al., 1996). Wakamatsu et al. (2004a) reported that the red pigment extracted from Parma ham included zinc protoporphyrin IX (ZPP). ZPP is formed in dry-cured Parma ham and Iberian ham, while the use of nitrite as a curing agent was found to completely inhibit the formation of ZPP in meat products (Adamsen et al., 2006). Wakamatsu et al. (2004b) established a model experimental system to elucidate the mechanism by which ZPP is formed in nitrite-free dry-cured ham. Furthermore, Okui et al. (2006) established a new model system in which protoporphyrin IX (PPIX) accumulated instead of ZPP with the addition of EDTA, and they suggested that ZPP was derived not from heme but from PPIX and that nitrite suppressed the formation of not only ZPP but also PPIX. However, the mechanism by which nitrite inhibits the formation of PPIX is not known. The aim of this study was to elucidate the inhibition mechanism of nitrite by using model experimental systems.

Materials and Methods

Model experiment Model solutions consisted of pork loin homogenates (final meat concentration of 20%), antibiotics (100 units/ml of penicillin G potassium, 0.1 mg/ml of streptomycin sulfate and 0.05 mg/ml of gentamicin sulfate), and EDTA (0.5 mM). Sodium nitrite, sodium hydrosulfite, potassium ferricyanide or S-Nitroso-N- acetyl-DL-penicillamine (SNAP) was mixed into each model solution. After adjusting the pH of the solution to 5.5, the solution was put into a gas-impermeable bag with an oxygen absorber and incubated in the dark at 25 ºC for 5 days.

HPLC analysis The contents of PPIX, ZPP and heme were determined by HPLC. The solutions were mixed well with nine volumes of acetic acid/ethyl acetate (4:1 v/v) and held on ice in the dark for 15 min. After centrifuging at 3,000 rpm for 15 min at 4ºC, the supernatant of each solution was mixed with an equal amount of a mobile phase consisting of methanol/1 M ammonium acetate (84:16, v/v, pH 5.16). An STR ODS-2 column (4.6 × 150 mm) was used at a flow rate of 0.6 ml/min at 34 ºC. PPIX was detected by fluorescence from excitation of 400 nm and emission of 630 nm. ZPP was detected by fluorescence from excitation of 420 nm and emission of 590 nm. Heme and PPIX were detected by adsorption at 400 nm.

Determination of ORP Each of the model solutions before incubation was diluted with an equal amount of distilled water, and oxidation-reduction potential (ORP) was determined using an OPR meter.

Results and Discussion

First, we examined the effect of nitrite on the formation of PPIX. As shown in Fig. 1, the formation of PPIX was almost completely inhibited by the addition of more than 25 μM of nitrite. The amount of heme did not vary

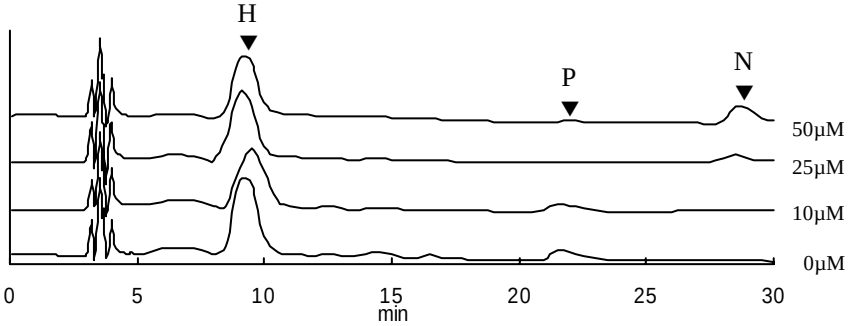

Figure 1. HPLC chromatogram of solutions to which sodium nitrite (below 0, 10, 25, 50 μM) had been added. H: heme, P: PPIX, N: nitrosyl heme. λ=400 nm

with different nitrite concentrations. These results agree with the result of a previous study (Okui et al., 2006). On the other hand, the peak at 29 min was enlarged with addition of nitrite. Analysis of absorption suggested that this peak was nitrosyl heme. The addition of more than 25 μM nitrite is thought to result in the formation of nitrosyl myoglobin.

Since nitrite has a strong oxidizing property, we examined the effect of the oxidizing property of nitrite on the formation of PPIX. The addition of more than 5 mM of potassium ferricyanide as an oxidizing reagent inhibited the formation of PPIX, but the addition of less than 5 mM of potassium ferricyanide promoted the formation of PPIX (data not shown). Therefore, we investigated the effect of ORP on the formation of PPIX (Fig. 2). ORPs of the original model solutions were about 100 mV. With addition of a reducing reagent, both ORP and the amount of PPIX were decreased. ORP was increased with addition of an oxidizing reagent, whereas the amount of PPIX increased with increase of ORP up to about 300 mV and further increase in ORP significantly inhibited the formation of PPIX. However, ORP of the model solutions with added sodium nitrite (25 μM) in which the formation of PPIX was almost completely inhibited was about 100 mV. Thus, it is thought that the oxidizing property of nitrite did not inhibit the formation of PPIX.

In cured meat products, nitric oxide is produced from nitrite and binds to myoglobin, resulting in the formation of nitrosyl myoglobin. We therefore examined the effect of nitric oxide on the formation of PPIX. The formation of PPIX was significantly decreased when SNAP, a nitric oxide donor, was added at concentrations of 30 μM and 300 μM (Fig. 3). This indicated that nitrite oxide inhibits the formation of PPIX. In addition, the formation of ZPP was also significantly inhibited in a model system with no addition of EDTA when more than 30 μM of SNAP was added (data not shown). These results suggest that the mechanism by which nitrite inhibits the formation of PPIX is not the oxidizing property of nitrite but the action of nitric oxide produced by nitrite. We speculate that the significant decrease in ZPP formation by nitrite results from the inhibition of PPIX production by nitric oxide. However, the inhibition mechanism of nitric oxide is still not clear and further investigation is needed.

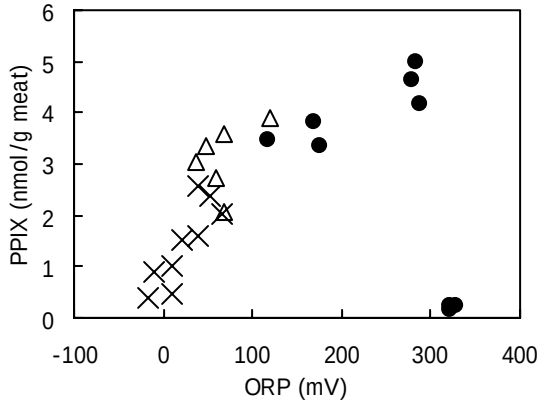

Figure 2. Effect of ORP on the formation of PPIX. ● (solutions with added oxidizing reagent), Δ (original solutions), × (solutions with added reducing agent)

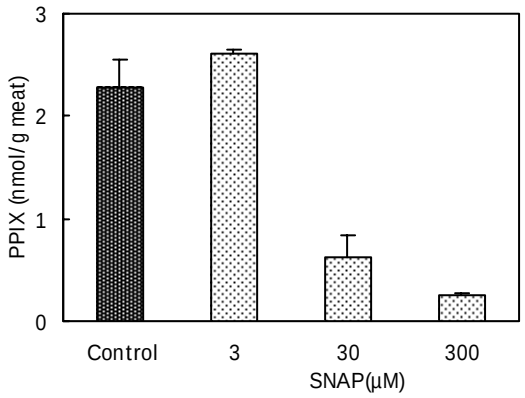

Figure 3. Effect of SNAP (nitric oxide donor) on the formation of PPIX.

Conclusions

Not the oxidizing property of nitrite but nitric oxide produced from nitrite inhibited the formation of PPIX, and ZPP was therefore not formed in cured meat products with the addition of nitrite or nitrate.

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