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Electrolytic reduction of heme proteins and an application of electrolytically reduced hemoglobin to sausage manufacture

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INTRODUCTION

It has been known that met-form of myoglobin and hemoglobin catalizes oxidation of lipids, resulting in the development of rancidity (Tarladgis, 1961). Because of such undesirable properties of heme proteins, the utilization of hemoglobin as protein resources or natural colorant has been one of the most difficult subjects in meat industry. It has been reported that oxidized form of hemin, cytochrome c, myoglobin and hemoglobin can be electrochemically transformed to reduced derivatives using polarographic techniques (Gygax and Jordan, 1968; Besto et al., 1972; Scheller et al., 1975). Recently, we have devised a simple apparatus for electrolysis of heme proteins, and have found that met-form of heme proteins can be electrolytically reduced to their reduced derivatives having ferrous ion. In the present study, therefore, we have investigated electrolytic reduction of met-form of myoglobin and hemoglobin in the presence or absence of sodium ascorbate and sodium nitrite. Study was also made on the effect of electrolytically reduced hemoglobin on the development of color of cooked sausages.

EXPERIMENTAL

Equine skeletal muscle myoglobin and bovine hemoglobin were purchased from Sigma Chemical Company. Pork meat for sausage manufacture was obtained from a private meat packing plant.

Myoglobin and hemoglobin were dissolved in 10 mM K-phosphate buffer (pH 5.5-7.5) with or without 5 mM sodium ascorbate and 5 mM sodium nitrite. The same solutions without heme proteins were used as electrolytic solutions. Electrolysis of heme proteins was made using an apparatus consisting of an electrolytic cell, anode and cathode, one layer of dialyzing tube which is enclosing heme protein solution and an electric power supply (Fig. 1). Upon electrolytic reduction of heme proteins, a cathode was placed in a sample solution enclosed by a dialyzing tube, while in the case of oxidation reaction an anode was placed in a sample solution. Electrolysis was done at the current of 50 mA for 5 min unless otherwise mentioned.

The spectra of non-treated and electrolytically reduced heme proteins were scanned with a Hitachi 557 Double Wavelength Double Beam Spectrophotometer at the speed of 120 nm/min.

SDS-polyacrylamide gel electrophoresis was performed on 7.5 - 17.5 % acrylamide gradient slab gels according to the procedure of Laemmli (1970). Gel electrofocusing of heme proteins was also made on 5 % polyacrylamide slab gels (crosslinking, 3 %; size, 110x110x0.5 mm) containing 20 % glycerol and 10 % ampholine (pH 5.0 - 8.0).

The susceptibility of electrolytically reduced heme proteins against chemical oxidation was examined as follows: the electrolytically reduced heme proteins were oxidized by the addition of 10-fold molar concentration of potassium ferricyanide at 25°C. The change of the spectrum of the mixtures was followed with the spectrophotometer.

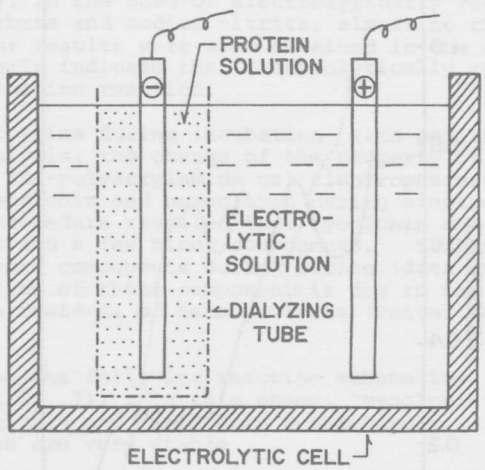

Fig. 1. Diagram of an apparatus for the electrolysis of heme proteins

Experimental sausages with or without the addition of electrolytically reduced hemoglobin were prepared. Coursely ground pork meat was cured either with a mixture of 2 % sodium chloride, 0.1 % sodium pyrophosphate, 150 ppm sodium nitrite and 550 ppm sodium ascorbate, or with an another mixture of 2 % sodium chloride and 0.1 % sodium pyrophosphate, for 24 hr at 3 - 5°C. To 100 g of the cured pork meat, cold 10 ml of non-treated or electrolytically reduced hemoglobin was added for the experiments. For controls cold 10 ml of non-treated or electrolysed buffer was added. After cooking and cooling, Hunter values of sausage slices (8 mm in thickness) were examined with a color and color difference meter (ND-K6B, Nihon Denshoku Ltd).

RESULTS

Figure 2 shows absorption spectra of myoglobin before and after electrolysis at pH 7.0. The spectrum of metmyoglobin having its absorption maximum at 505 nm before electrolysis was changed to the one having two absorption maxima, which is quite similar to that of oxymyoglobin having its absorption maxima at 544 and 582 nm, after the electrolysis at 50 mA for 5 min. This result indicates that ferri-state of the ion of metmyoglobin was reduced to ferrous-state by the electrolysis.

Figure 3 shows the effect of electrolysis on the spectrum of myoglobin in the presence of sodium ascorabte. In this experiment metmyoglobin was pre-incubated with 50-fold molar concentration of sodium ascorabte for 1 hr at 25°C prior to electrolysis. The spectrum of metmyoglobin was almost changed to that of oxymyoglobin during incubation with sodium ascorbate. Then, the spectrum of myoglobin was further changed to that of reduced one having single absorption maximum at 555 nm after the electrolysis. This result indicates that in the presence of sodium ascorbate oxymyoglobin is transformed to reduced myoglobin by the electrolysis.

Figure 4 shows the effect of electrolysis on the spectrum of metmyoglobin in the presence of sodium ascorbate and sodium nitrite. The spectrum of metmyoglobin was changed to the one having two absorption maxima, which is similar to that of nitrosyl myoglobin having two absorption maxima at 545 and 575 nm, after the incubation of metmyoglobin with 5 mM sodium ascorbate and sodium nitrite for 1 hr at 25°C. However, the extinction coefficients at these two peaks were considerably lower than those of nitrosyl myoglobin (Fox and Thomson, 1963). This indicates that metmyoglobin is partially transformed to nitrosyl derivative during the incubation. After a limited electrolysis (50 mA, 5 min) of the incubated mixture of metmyoglobin and ascorbate and nitrite, the spectrum of the mixture was further changed to the one having single absorption maximum at around 546 nm and a shoulder at around 570 nm. Finally, the spectrum of myoglobin was changed to the one having its absorption maximum at about 555 nm after a prolonged electrolysis (50 mA, 10 min; 100 mA, 5 min). This result indicates that prolonged electrolysis of metmyoglobin following the incubation with sodium ascorbate and sodium nitrite produces reduced form-like myoglobin. In the case of methemoglobin, quite similar results were obtained after electrolysis both with and without sodium ascorbate and sodium nitrite(Fig. 5).

Figure 6 shows the effect of potassium ferricyanide on the stability of chromophore of electrolytically reduced myoglobin. Since potassium ferricyanide has a strong oxidizing ability, the addition of 10-fold molar concentration excess of this reagent to nitrosyl myoglobin markedly changes its spectrum. On the other hand, electrolytically reduced myoglobin in the absence of sodium nitrite and sodium ascorbate was not readily oxidized by

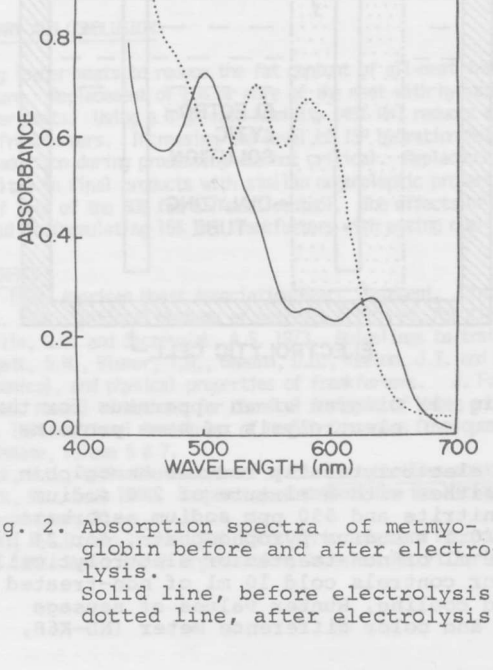

Fig. 2. Absorption spectra of metmyoglobin before and after electrolysis.
Solid line, before electrolysis; dotted line, after electrolysis.

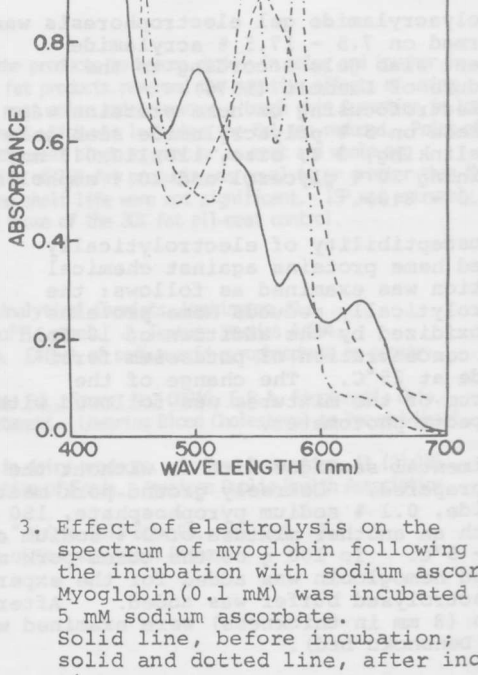

Fig. 3. Effect of electrolysis on the spectrum of myoglobin following the incubation with sodium ascorbate. Myoglobin(0.1 mM) was incubated with 5 mM sodium ascorbate.
Solid line, before incubation; solid and dotted line, after incubation;
broken line, after electrolysis.

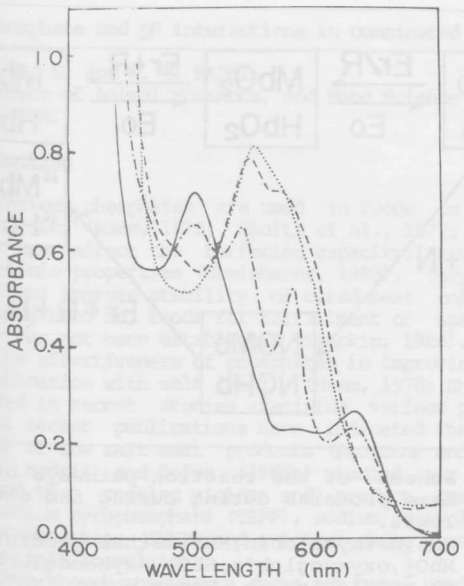

Fig. 4. Effect of electrolysis on the spectrum of myoglobin following the incubation with sodium ascorbate and sodium nitrite.
Myoglobin (0.1 mM) was incubated with 5 mM sodium ascorbate and 5 mM sodium nitrite, followed by electrolysis. Solid line, before incubation; solid and dotted line, after incubation; broken line, after a limited electrolysis; dotted line, after a prolonged electrolysis.

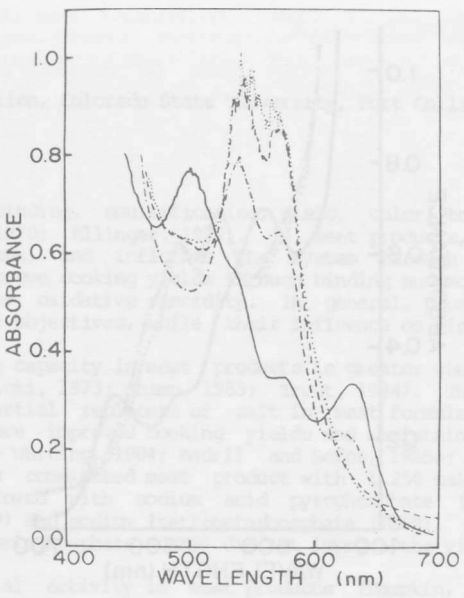

Fig. 5. Effect of electrolysis on the spectrum of hemoglobin following the incubation with sodium ascorbate and sodium nitrite.
All indications are the same as in Fig. 4.

the addition of potassium ferricyanide. Especially, in the case of electrolytically reduced myoglobin following the incubation with sodium ascorbate and sodium nitrite, almost no change was observed in its spectrum(Fig. 6). Quite similar results were also obtained in the case of hemoglobin (data not shown). These results clearly indicate that electrolytically reduced heme proteins are highly resistant against oxidation reaction.

In addition to the change of the spectrum of heme proteins during incubation with sodium ascorbate and sodium nitrite and also during electrolysis, the change of the property of globin moiety of heme proteins was also examined by SDS-polyacrylamide gel electrophoresis. There was no change in the electrophoretograms of myoglobin and hemoglobin during electrolysis (data not shown). However, electrofocusing procedure revealed that myoglobin and hemoglobin are separated into three major components and a few minor components. It also demonstrated a little change in the proportion of those components during curing (data not shown). It is likely that the change in the proportion of those components is due to the deamination of amide group of aspargine or glutamine residues of heme proteins (Satterlee and Snyder, 1969).

In summerizing the results of Figures 2-6, we propose the following reaction scheme for electrolytic reduction of myoglobin and hemoglobin (Fig. 7). In this sheme, "myoglobin" and "hemoglobin" indicate electrolytically modified myoglobin and hemoglobin respectively. As described above, chemical nature of these derivatives are very stable.

In order to examine the availability of electrolytically reduced hemoglobin in meat processing, experimental sausages were prepared with or without the addition of electrolytically reduced hemoglobin. Table 1 represents Hunter values of those sausages. As is clearly shown in this table, redness (a value) of sausages considerably increased after the addition of electrolytically reduced hemoglobin in the presence of sodium ascorbate and sodium nitrite. This indicates that the addition of electrolytically reduced hemoglobin in the presence of sodium nitrite and sodium ascorbate is certainly effective in increasing red color of cooked sausages.

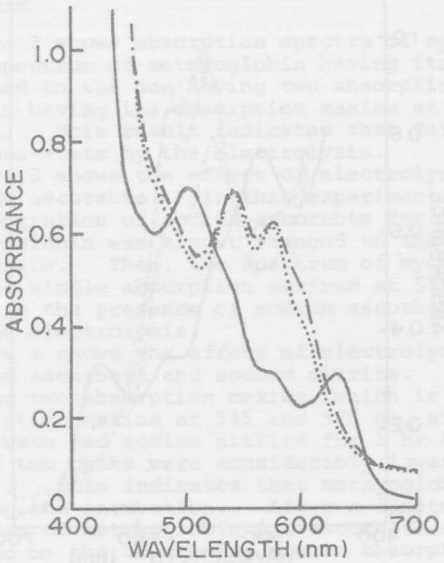

Fig. 6. Effect of potassium ferricyanide on the spectrum of myoglobin.
Solid line, spectrum of non-treated myoglobin after the addition of ferricyanide;
solid and dotted line, spectrum of electrolytically reduced myoglobin following the incubation with ascorbate and nitrite;
dotted line, spectrum of the electrolytically reduced myoglobin after the addition of ferricyanide.

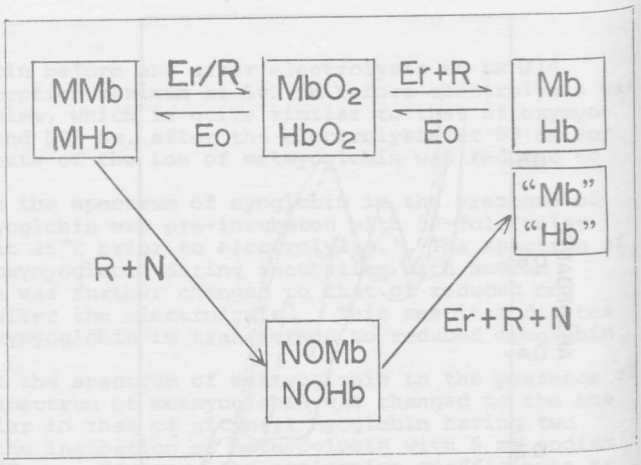

Fig. 7. Scheme of the reaction pathways of heme proteins during curing and electrolysis.
MMb, metmyoglobin; MHb, methemoglobin; MbO_2, oxymyoglobin; HbO_2, oxyhemoglobin; Mb, myoglobin; Hb, hemoglobin; "Mb", electrolytically modified myoglobin; "Hb", electrolytically modified hemoglobin; NOMb, nitrosyl myoglobin; NOHb, nitrosyl hemoglobin; Er, electrolytic reduction; Eo, electrolytic oxidation; R, reducer (sodium ascorbate); N, nitrite (sodium nitrite).

Table 1. Hunter values (L, a and b) of experimental sausages with or without the addition of electrolytically reduced hemoglobin*

Hunter values \ Sample	I	II	III	IV
L	61.4	58.9	64.5	60.1
a	10.1	12.5	8.9	10.3
b	7.1	7.6	7.0	6.4

*The numbers listed in this table are the mean of five determinations.

Sample I, cured meat (in the presence of ascorbate and nitrite) + 1/10 vol of electrolysed buffer containing ascorbate and nitrite;
Sample II, cured meat (in the presence of ascorbate and nitrite) + 1/10 vol of electrolysed hemoglobin containing ascorbate and nitrite;
Sample III, cured meat (in the absence of ascorbate and nitrite) + 1/10 vol of non-electrolysed phosphate buffer;
Sample IV, cured meat (in the absence of ascorbate and nitrite) + 1/10 vol of non-electrolysed hemoglobin.

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