Enhancement of myoglobin autoxidation induced by phospholipids extracted from beef muscles of various metabolic types.

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SUMMARY : Upon addition of liposomes made of phospholipids (PL) extracted from beef muscles, an increase of MbO_2 oxidation rate proportionnal to PL/Mb ratio is observed. Some differences in MbO_2 oxidation enhancement seems to occur when lipids extracted from various muscles are added.

These results allow to relate color stability of muscles of different metabolic types to their phospholipid content and composition.

Increases of the turbidity of Mb-PL mixtures are observed simultaneously to oxidative phenomena demonstrating phospholipid-myoglobin interactions to take place.

INTRODUCTION : Color is one of the most important factors involved in consumer acceptance of meat. Meat color depends on oxidation state and on bound ligands on the main muscle pigment : myoglobin (Mb). Among the numerous biological and physicochemical parameters which determine oxymyoglobin (MbO_2) oxidation rate, the contractile and metabolic type of the muscles has been clearly demonstrated to be involved (RENERRE, 1984). However, the reason of the differences in color stability which are observed between various muscles is not yet clearly understood (RENERRE, 1990).

It has been recently established that phospholipid composition of muscles depends on their metabolic type (GANDEMER, 1990 ; LESEIGNEUR-MEYNIER and GANDEMER, 1991)) and that phospholipids are more sensitive to oxidation than neutral lipids during storage and processing of meat (GANDEMER, 1990). Otherwise, oxidation of lipid and oxidation of hemoglobin or of myoglobin are interdependant phenomena (LIN and HULTIN, 1977 ; SZEBENI et al., 1984 ; ANTON et al., 1991, for exemples). Moreover, it has been shown that interactions take place between myoglobin and fatty acids (STEWART, 1990) and between phospholipid and hemoglobin (SHVIRO et al., 1982 ; SZEBENI et al., 1988).

All these studies suggest that membrane lipids should be involved in myoglobin oxydation rate in meat. Few data are available concerning the influence of phospholipid upon myoglobin oxidation. Thus, in this work, the influence of phospholipids extracted from beef muscles showing different metabolic types, on myoglobin oxidation has been studied.

MATERIALS AND METHODS :

Muscles : Approximatively 100g of *longissimus dorsi*, *psoas major*, *diaphragma* muscles were taken off, 1 hour *post-mortem*, on Charolais cull cows, plunged in liquid nitrogen and stored at - 80°C until needed.

Myoglobin : Oxymyoglobin was extracted at 4°C from *longissimus dorsi* using 10 mM Tris-HCl buffer pH 8.4, and precipitated by ammonium sulfate as described by RENERRE et al. (1991). It was then purified by ion exchange chromatography (mono-Q column (Pharmacia) equilibrated with 10 mM Tris-HCl buffer, pH 8.4, 0-100mM NaCl linear gradient) (RENERRE et al., unpublished results). MbO_2 was divided in aliquots (1 ml) keeped a - 80°C until use.

Before oxidation measurements, Tris buffer was exchanged with 0.05 M phosphate buffer pH 5.8 ; 0.05 M NaCl, at 4°C, using a Sephadex G-25M desalting column (PD10 ; Pharmacia) (GENOT et al., 1990).

The concentration of myoglobin was determined by the use of its molar extinction coefficient at 280 nm : $E = 3\ 10^4\ cm^{-1}.mol^{-1}$ (LONGWORTH, 1971).

Phospholipids : Total intramuscular lipids were extracted from 10 g of minced samples using the method of FOLCH et al. (1957). The phospholipids were purified on silicic acid column, their composition in phospholipid classes was performed by HPLC and their quantification and fatty acid composition were achieved by gas liquid chromatography of fatty acid methyl esters using heptadecanoic acid as internal standard (LESEIGNEUR-MEYNIER and GANDEMER, 1991).

Liposomes preparation : Liposomes (0.1 mg/ml in 0.05 M phosphate buffer pH 5.8 ; 0.05M NaCl) were prepared by sonication as described by GENOT et al. (1990). Cares were taken to limit lipid oxidation during sonication by the use of degazed buffer, flushing a stream of nitrogen over the lipid suspension and controlling the temperature of the suspension by means of an ice bath.

Oxidation rate measurements : The liposomes suspension was reoxygenated just before measurement by a gentle bubbling of filtered air for 5 min. Aliquots (0 to 1 ml) were poured in optical quartz cells (volume : 1.5 ml ; optical pathway : 1mm) and completed to 1ml with phosphate buffer. After temperature equilibration (25°C) of the samples in the thermostated cell chamber of the spectrophotometer (KONTRON UVIKON 940 with sample transport accessory), 0.2 ml of MbO_2 solution was put in each cell. Final concentration in MbO_2 in each cell was about 0.05 mg/ml and phospholipid (PL) concentration varied between 0 to 0.1 mg/ml. After efficient agitation, absorbances at 280 (A^{280}), 295 (A^{295}) , 580 (A^{580}) and 750 nm (A^{750}) were recorded for 3 hours at 25°C. Measurements were spaced by 2 min during the first 30 minutes and then by 15 min.

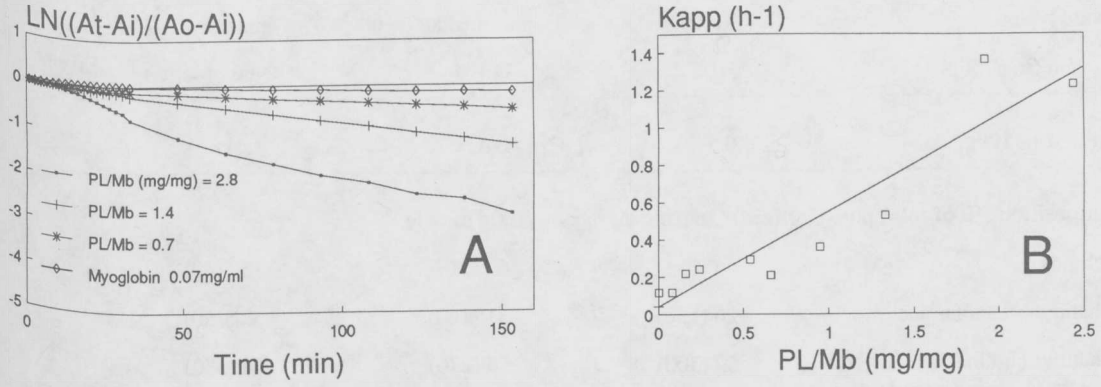
Assuming A^{750} to be only due to liposome turbidity, A^{295} of myoglobin solution to be constant versus time and turbidity (T^{λ}) to fit the equation : $T\lambda = C/\lambda^n$, (λ : wavelength ; C and n : calculated parameters) ; turbidity at 580 nm could be calculated for PL+Mb mixtures at any time. It was then substracted to the total absorbance of mixtures in order to obtain myoglobin absorbance at 580 nm (A_t).

$Ln((A_t-A_i)/A_0-A_i))$ was plotted versus time and global slopes of resulting curves were taken as apparent rate constants (Kapp) of MbO_2 oxidation ($A_0=A^{580}$ for t=0 ; $A_i=A^{580}$ at infinite time, determined by adding potassium ferrycyanide) (STEWART, 1990).

RESULTS AND DISCUSSION :

Addition of phospholipid to oxymyoglobin leads to an increase of oxidation rate of MbO_2 proportional to PL/Mb ratio (fig. 1).

Figure 1. Influence of phospholipids extracted from beef *longissimus dorsi*, on myoglobin oxidation.
1-A : Effect of PL/Mb ratio on the kinetic of MbO_2 oxidation. 1-B : Effect of PL/ Mb ratio on Kapp.



Reaction monitored at 580 nm; experimental conditions : see text.

In presence of phospholipids extracted from various muscles an important dispersion of the Kapp values versus PL/Mb ratio was observed (fig. 2). Calculating the slopes (a) of the linear regressions shows a greater oxidative effect of *diaphragma* (a=1.47±0.37 ; r=0.784) compared with *longissimus dorsi* (a=0.51±0.068 ; r=0.935). With *psoas major* phospholipids the oxidative effect seems to be intermediate (a=0.64±0.12 ; r=0.867).

Figure 2. Influence of muscle origin of the PL on myoglobin oxidation

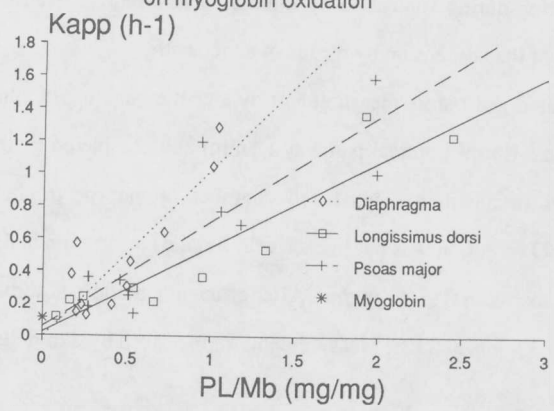
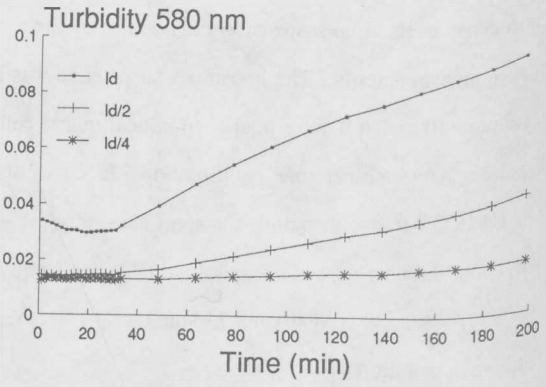


Figure 3. Evolution of calculated turbidity at 580nm of PL+Mb mixtures versus time (PL from LD)



Further experiments are needed to confirm if PL extracted from muscles of various metabolic types led to different oxidation rate of myoglobin. If that is the case, phospholipid extracted from a muscle of oxydative type, which have a poor color stability (*diaphragma*) would enhance MbO_2 oxidation more than PL extracted from a glycolytic muscle whose color stability is better (*longissimus dorsi*). According to this assumption, ANTON et al. (1991) have recently shown, by fluorescence measurements, that in presence of MbO_2, microsomal fractions of *psoas major* muscles may produce more oxygen radicals than *longissimus dorsi* muscles and led to a greater MbO_2 oxydation rate.

These results can be related to the content in phospholipids of muscles and to the composition of phospholipid extracts (table 1).

TABLE 1 : Phospholipid content and composition of *longissimus dorsi, psoas major* and *diaphragma.*

Muscles	*longissimus dorsi*	*psoas major*	*diaphragma*
metabolic type	glycolytic	intermediate	oxidative
Mb content (mg/100g)(1)	510	540	900
PL content (g/100g)	0.5	0.5	1.1
PL composition (% of total phospholipid) ; in parenthesis : mg/100g muscle			
Cardiolipids	6 (30)	6 (30)	13 (140)
Phosphatidyl-ethanolamine	26 (130)	31 (160)	32 (350)
Phosphatidyl-choline	59 (300)	54 (270)	48 (530)
Polyunsaturated fatty acids			
% of total fatty acids	26 (86)	28 (92)	32 (230)
Polyunsaturated/Saturated	0.88	0.95	0.97

(1) : RENERRE, personnal communication

Phospholipids containing the highest content in polyunsaturated fatty acid, cardiolipids and phosphatidyl-ethanolamine would led to the greatest increase of oxidation rate. These results allow not to know if the prooxidative effect of PL versus myoglobin is due to lipid oxidation (which is favoured by high content of polyunsaturated fatty acids), or to interaction phenomena as described by SHVIRO et al., 1982 ; SZEBENI et al., 1988 ; and STEWART, 1990.

In our experiments, increases of turbidity of Mb+PL mixtures were observed simultaneously to MbO_2 oxidation (fig. 3). This demonstrates aggregation phenomena to take place simultaneously to oxidation phenomena. Thus PL-Mb interactions occur in our systems. Their nature, and their influence on MbO_2 as well as on lipid oxidation has to be studied.

However that may be, if the composition of phospholipids takes a part in the oxidative effect induced by muscle PL on MbO_2 oxidation, the PL/Mb ratio in muscles (0.98 ; 0.93 and 1.2 for *longissimus dorsi, psoas major* and *diaphragma* respectively) should be taken into account to explain stability of meat color. Nevertheless, the oxidation rates measured in our model systems were high as compared to those observed on whole stored muscles in which cellular structure and natural preserving agents can reduce the extent of oxidative phenomena. The influence of those parameters on MbO_2 oxidation should be studied in order to better understand the mystery of meat color stability.

CONCLUSIONS :

These results demonstrate the importance of phospholipid/myoglobin ratio and of phospholipid composition in enhancing MbO_2 oxidation rate. The nature of involved phenomena has to be studied.

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