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Capacity of Skeletal Muscle for the Formation of Nitrosylhaemoproteins During the Curing of Meat*

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ABSTRACT

The effects of total haem pigment content of pork muscle on the potential for the formation of nitric oxide pigment during curing are reported. A linear relationship between the amount of endogenous pigment present and yield of nitric oxide pigment was found. Although the conversion to nitric oxide pigment was far from complete, the reducing capacity of muscle was more than adequate to convert all the haemoprotein present to nitric oxide pigment. The role of myoglobin and haemoglobin in these conversions is discussed.

INTRODUCTION

It has been shown that fresh muscle is able to reduce nitrite ion anaerobically to nitric oxide, part of which exists in combination with the muscle pigment myglobin (Mb) as nitric oxide myglobin (NOMb) (Walters & Casselden, 1973). Skeletal muscle mitochondria which survive curing are implicated in this process (Walters *et al.*, 1975). The natural pigment content of muscle is thought to be positively correlated with the presence of enzymes which are normally involved in the respiratory activities of the tissues. Differences in the ability of different muscles to reduce metmyoglobin (MetMb) have also been reported (Renerre, 1978). It is likely, therefore, that the tendency for the formation of cured meat colour is a function of the type of muscle used.

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This study was undertaken to examine possible differences between muscles with regard to their ability to form cured meat colour from endogenous and added haem pigment and nitrite. Muscles with considerably different pigment contents were used with a view to examining if the capacity of the muscle tissue to form nitric oxide pigment is related to its natural pigment content. The aim was to establish whether exhaustion of the reduction enzyme systems of the tissue is responsible for the incomplete conversion of endogenous pigments to the nitric oxide form, during the curing process.

EXPERIMENTAL

The biceps femoris (BF), rectus femoris (RF), vastus lateralis (VL) and adductor (AD) muscles used in this study were dissected from the leg of 24 h post-mortem pig carcasses, wrapped in aluminium foil and refrigerated at 4°C until required. Immediately before use they were trimmed of external fat and finely minced by means of a surgical scalpel.

To assess the reactivity of the meat sample towards nitrite ion, fresh mince (3 g), phosphate buffer (2·25 ml, 0·2 M) and sodium nitrite solution (0·5 ml, 0·5 mg $NaNO_2$/ml) were incubated in the dark, under nitrogen, at $25{\cdot}0 \pm 0{\cdot}02$°C, with shaking, for 2 h. To examine the nitric oxide pigment-forming activity (NPFA) of the various pork muscles, 0·5, 1·0 or 1·5 ml of the buffer was replaced by an equal volume of a standard solution of methaemoglobin (MetHb) in the same buffer and the mixture incubated as before.

In all experiments, nitric oxide pigments were extracted by addition of acetone (20 ml). After filtration, concentrated hydrochloric acid (2 drops) was added to convert the pigments to acid haematin and the extracts made up to 25 ml with 80% acetone in water. The mixture was held at 4°C overnight to ensure complete precipitation of any remaining protein. After centrifuging, the absorbance of the clear supernatants was measured at 512, 540 and 640 nm and concentrations of acid haematin determined from calibration plots obtained by measuring the absorbance of standard acid haematin (prepared from haemin chloride) at the same wavelengths (Hornsey, 1956). Total haem pigments were also determined using the method described by Hornsey. For convenience, both total and nitric oxide pigments were expressed as mg of Mb instead of weight of haematin, assuming a molecular mass of 17 000 daltons for Mb and 657 daltons for haematin. All experiments were carried out in duplicate and appropriate blank determinations were made for each set of analyses.

RESULTS AND DISCUSSION

Assuming that meat contains 75% water, reaction mixtures were composed of 5 ml water, 0·75 g dry matter and 43·5 mg/kg $NaNO_2$. No attempt was made to eliminate inter-muscle pH variability, and instead the pH of the buffers was adjusted so as to maintain the original pH of the meat sample.

Table 1 demonstrates the efficiency of minces from 24 h post-mortem pork muscle to convert endogenous haem pigments to the nitric oxide form, when incubated with 43·5 mg/kg $NaNO_2$ under anaerobic conditions. The experimental data show that the actual amount of pigment converted to the nitric oxide form increased with increasing concentration of total pigments, not only between muscles of the same leg but also when data from a different leg are included in the comparison. A good linear correlation between the yield of nitric oxide pigment and the total amount of haemoprotein present (expressed as Mb) is shown In Fig. 1. When extrapolated, the line shown intersects the y-axis at an NOMb content of 0·2 mg, which is sufficiently close to the origin to suggest linear behaviour below 5·4 mg Mb. The yield of the nitric oxide pigment, given by the slope of the line, is 45% based on the total amount of haemoprotein present. Two reasons for the observed correlation may be advanced. Firstly, it is possible that the more highly pigmented muscles may also contain higher amounts of enzymes and cofactors which are normally involved in the reduction of nitrite ion and, hence, the formation of nitric oxide pigments or that the activity of these enzymes systems may be greater. Secondly, nitrite ion is known to react rapidly with a

TABLE 1

Formation of Nitric Oxide Pigment during Anaerobic Incubation of Fresh Pork Minces (3 g) with $NaNO_2$ (43·5 mg/kg). All Amounts of Haemoprotein expressed as Weight of Myoglobin (Mb) or Nitrosylmyoglobin (NOMb)

Original muscle				*Reaction mixtures*	
Leg	*Muscle type*	*pH*	*Total pigment content (mg Mb/g mince)*	*Total pigment content (mg Mb)*	*Amount of nitric oxide pigment formed (mg NOMb)*
1	RF	6·10	2·90, 2·95	8·7	3·98, 4·22
	VL	5·90	2·30, 2·32	6·9	3·30, 3·42
	BF	5·85	1·85, 2·00	5·7	2·72, 2·72
	AD	5·80	1·83, 1·80	5·4	2·59, 2·55
2	RF	6·10	3·05, 3·10	9·3	4·58, 4·02
	VL	6·01	2·60, 2·64	7·8	3·67, 3·95
	BF	5·89	1·98, 2·15	6·3	2·95, 3·05
	AD	5·93	1·75, 1·85	5·4	2·45, 2·51

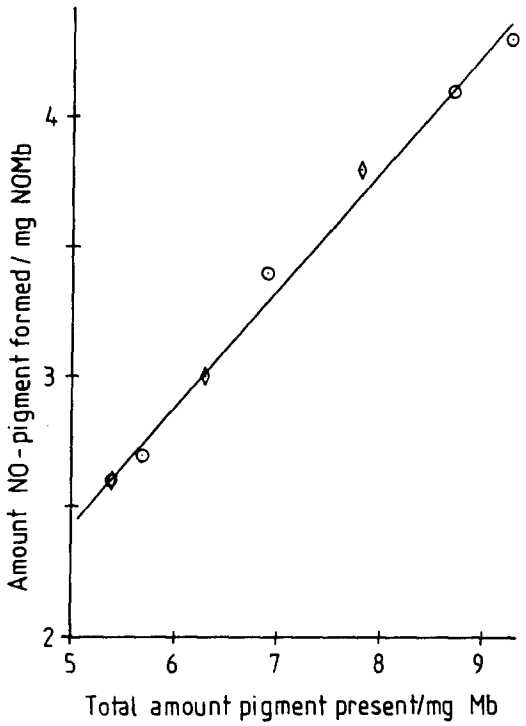

Fig. 1. Relationship between the mean amount of nitric oxide pigment formed during anaerobic incubation of fresh pork minces (3 g) with $NaNO_2$ (43·5 mg/kg) and the total amount of haem-pigment present. The data plotted are shown in Table 1. Leg 1: ⊙ Leg 2: ◇.

number of cured meat components including protein thiol and amino groups, lipids as well as haem pigments. If the concentration of haemoprotein is increased relative to these non-haem fractions, a corresponding increase in the nitric oxide pigment level is likely to result simply because of more effective competition for the available nitrite.

The fact that only a proportion of the natural pigment of the minces was converted to nitric oxide pigment, under the experimental conditions used, therefore does not necessarily mean that the NPFA of the muscles had been exhausted. Indeed, the amount of pigment converted to the nitric oxide form was always greater in the presence of added MetHb (Table 2). When sufficient added haemoprotein was present in the incubations, the amount of NOMb/Hb formed exceeded the amount of pigment present naturally in the mince, establishing, therefore, the potential of the systems within the fresh tissue to convert, not only endogenous, but also added haem pigment, to the nitric oxide form. Within the range of concentrations used the increase in the amount of converted pigment was proportional to the amount of MetHb added in the incubations. It is interesting to note that the yield of nitric oxide pigment at the highest concentrations is in the region of 50% and close

TABLE 2

Nitric Oxide Pigment-forming Activity of Pork Muscles in the Presence of added Methaemoglobin (MetHb). Where both Haemoglobin and Myoglobin are present. Amounts are Expressed as Weight of Myoglobin (Mb) or Nitrosylmyoglobin (NOMb) as appropriate

Muscle	*pH*	*Concentration of MetHb solution added (mg/ml)*	*Volume of MetHb solution added (ml)*	*Reaction mixtures*	
				Total pigment content (mg Mb)	*Amount of nitric oxide pigment formed (mg NOMb)*
RF	6·10 ± 0·01	8·5	0	9·3	4·58, 4·02
			0·5	13·6	6·73, 6·79
			1·0	17·8	9·26, 9·01
			1·5	22·1	11·48, 12·28
VL	6·01 ± 0·01	9·3	0	7·8	3·67, 3·95
			0·5	12·5	5·68, 5·33
			1·0	17·1	8·21, 7·41
			1·5	21·8	10·25, 11·60
BF	5·89 ± 0·01	9·1	0	6·3	2·95, 3·05
			0·5	10·9	5·27, 5·49
			1·0	15·4	8·09, 8·44
			1·5	20·0	10·00, 9·75
AD	5·93 ± 0·01	8·8	0	5·4	2·51, 2·45
			0·5	9·8	4·44, 4·27
			1·0	14·2	6·48, 6·30
			1·5	18·7	8·27, 8·64

to the value of 45% obtained from Fig. 1. The highest concentrations of haemoprotein are, however, comparable with those of nitrite ion, and the continuing linear increase of amount of nitric oxide pigment formed with increase in amount of haemoprotein indicates that the potential for nitric oxide pigment formation is considerably larger than observed in these experiments.

MetHb does not reduce nitrite ion directly (Uchida & Klapper, 1970). This essential stage in the formation of the cured meat pigment must be accomplished by reducing systems of the muscle tissue. Yet these same reducing systems, which, in the presence of added haemoprotein can form amounts of nitric oxide pigment far in excess of the natural pigment content of the meat, fail to achieve complete conversion of the endogenous haemoprotein. It appears, therefore, that Mb and Hb are not simply receptors of nitric oxide as far as formation of nitric oxide pigment is concerned, but they may catalyse the reduction of nitrite by shifting the redox equilibrium in the direction of nitric oxide, probably because of their great affinity for this ligand.

Since the mechanism of formation of cured meat colour is believed to involve nitric oxide intermediates (Fox & Nicholas, 1974; Walters *et al.*, 1975) from which nitric oxide may be released to complex with MetMb/Hb, it is possible that the addition of haemoprotein to the system may well enhance the formation of these intermediates and/or facilitate the release of nitric oxide from them. Furthermore, it may provide some control over the amount of nitrite ion available for side reactions with non-haem constituents, some of which may represent a potential for the formation of N-nitroso-compounds.

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