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Short communication

Primary structure of turkey myoglobin ☆

Poulson Joseph [a], Surendranath P. Suman [a,*], Shuting Li [a], James R. Claus [b], Michele Fontaine [c], Laurey Steinke [c]

[a] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA
[b] Meat Science and Muscle Biology Laboratory, University of Wisconsin-Madison, Madison, WI 53706, USA
[c] Protein Structure Core Facility, University of Nebraska Medical Center, Omaha, NE 68198, USA

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ABSTRACT

Our objective was to determine the amino acid sequence of turkey myoglobin. Turkey myoglobin was isolated from cardiac muscles via ammonium sulphate precipitation and gel-filtration chromatography. Purified turkey myoglobin, separated as a 17 kDa band in SDS–PAGE, was subjected to digestion with trypsin or aspartic acid endopeptidase. The resulting peptides were separated by reverse-phase HPLC, and then subjected to Edman degradation to obtain the amino acid sequence. The complete amino acid sequence of turkey myoglobin was determined and compared with that of poultry and red meat myoglobins. Turkey myoglobin has 153 amino acids and nine histidine residues. Proximal (position 93) and distal (position 64) histidine residues, responsible for maintaining the stability of haeme, are conserved in turkey myoglobin. Turkey myoglobin shares 100% sequence similarity with chicken myoglobin, whereas it shares 92.5% homology with ostrich, 76.5% with pig, and less than 73% with ruminant myoglobins.

1. Introduction

Pink colour defect is a quality problem in turkey which results in an uncooked pink appearance in fully cooked, uncured meat products, such as turkey rolls (Cornforth, Vahabzadeh, Carpenter, & Bartholomew, 1986) and intact turkey breasts (Sammel & Claus, 2003). Consumers often relate the pink appearance to an uncooked product, although microbiological safety of such products is assured. An estimated revenue loss of 32 million to 246 million dollars is incurred by the US poultry industry due to pink colour defect (Nalivka, 2003; National Turkey Federation, 2007; USDA-NASS, 2007). This defect has been attributed to different pre-harvest (genetics, feeding and stress) and post-harvest (irradiation, nitrates, cooking method and non-meat ingredients) factors. However, the interactions of myoglobin (the sarcoplasmic haeme protein responsible for meat colour) with ligands and small biomolecules were considered as major endogenous factors contributing to pink colour defect (Cornforth et al., 1986, 1998; Holownia, Chinnan, & Reynolds, 2003; Nam & Ahn, 2002).

Turkey skeletal muscle extracts containing myoglobin exhibited greater resistance to heat-induced denaturation than did beef or pork myoglobins (Trout, 1989). Recently, Joseph, Suman, Li, Beach, and Claus (2010a) determined the molecular mass of turkey myoglobin (17,295 Da), utilising mass spectrometry, and reported a greater thermostability for turkey myoglobin than beef myoglobin. In addition, these authors suggested that differences in thermostability of turkey and beef myoglobins could be attributed to a possible difference in their primary structures, which was indicated by an approximately 350 Da difference between their molecular masses (16,949 Da for beef vs. 17,295 Da for turkey).

Maheswarappa et al. (2009) reported that turkey and chicken myoglobins exhibited the same molecular mass (17,291 Da) and shared similar fragmentation patterns when subjected to electrospray ionisation tandem mass spectrometry. Furthermore, the redox stabilities of chicken and turkey myoglobins were similar when challenged with reactive lipid oxidation products. In their further investigations, Naveena et al. (2010) observed that 4-hydroxy-2-nonenal (HNE), a secondary lipid oxidation product, covalently adducted to chicken myoglobin at histidine residues 64 and 93, which are critical for maintaining the redox stability of haeme. However, these authors could not verify HNE adduction sites in turkey myoglobin because the primary structure of turkey myoglobin is yet to be characterised.

The primary structure of most of the poultry and red meat myoglobins has been characterised (www.expasy.org; www.ncbi.nlm.nih.gov). In the post-genomic era, the amino acid sequences of myoglobins from several mammalian (Dosi et al., 2006; Joseph et al., 2010b; Suman, Joseph, Li, Steinke, & Fontaine, 2009) and avian (Enoki, Ohga, Ishidate, & Morimoto, 2008; Suman et al., 2010) species have been determined, utilising mass spectrometry and Edman degradation. On the other hand, chicken myoglobin

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* Corresponding author. Tel.: +1 859 257 3248; fax: +1 859 257 7537.
E-mail address: spsuma2@email.uky.edu (S.P. Suman).

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was characterised almost three decades ago (Deconinck et al., 1975). However, turkey myoglobin's primary structure has not been characterised. Therefore, our objective was to determine the amino acid sequence of turkey myoglobin.

2. Materials and methods

2.1. Isolation of turkey myoglobin

Frozen turkey hearts were procured from a local processor and stored at −80 °C until used. Turkey myoglobin was isolated from cardiac muscles according to Joseph et al. (2010a) via ammonium sulphate precipitation and gel-filtration chromatography. The isolated myoglobin was further refined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) as described by Laemmli (1970) to ensure the purity. In SDS–PAGE, turkey myoglobin appeared as a 17 kDa band which was sliced and frozen at −80 °C for determination of amino acid sequence.

2.2. Determination of amino acid sequence

Purified turkey myoglobin, separated by SDS–PAGE, was subjected to digestion with trypsin (lyophilised, Worthington Biochemical Corporation, Lakewood, NJ, USA) or aspartic acid endopeptidase (Asp-N; lyophilised, VWR International, West Chester, PA, USA), followed by Edman degradation in a Procise 494 system (Applied Biosystems, Foster City, CA, USA).

2.2.1. Tryptic digestion

Turkey myoglobin, separated in SDS–PAGE gel, was treated according to Rosenfeld, Capdevielle, Guillemot, and Ferrara (1992) with the following modifications prior to tryptic digestion. After the initial wash of the gel piece in 200 mM ammonium carbonate in 50% acetonitrile for 1 h at 37 °C to remove the SDS and Coomassie blue dye, the gel band was completely dried utilising a speed-vac centrifugation system (Thermo Electron, Waltham, MA, USA). For in-gel tryptic digestion, the protein sample was incubated at an enzyme: protein final ratio of 1:20 (w/w) for 18 h at 37 °C, and the reaction was stopped by addition of 2 μl of trifluoroacetic acid (Rosenfeld et al., 1992). The resulting peptide extract was dissolved in 0.3 ml of 0.1% trifluoroacetic acid containing 2% acetonitrile. The tryptic peptides were separated on a Vydac (Hesperia, CA, USA) C-18 column (2.1 × 250 mm, 5 μm particle size) and eluted with 0.1% trifluoroacetic acid in acetonitrile, using a Magic Microbore HPLC system (Michrom BioResources, Auburn, CA, USA). Peptides were detected at 215 nm and collected using a peak activated fraction collector (Isco, Lincoln, NE). Fractions containing peptides were concentrated, using a speed-vac centrifugation system (Thermo Electron, Waltham, MA, USA), before subjecting them to Edman degradation. Edman degradation is the process of sequentially cleaving amino acids from the N-terminal of a protein by the application of trifluoroacetic acid after derivatisation of the amino terminus with phenyl isothiocyanate (Edman, 1949). The process has been automated (Niall, 1973).

2.2.2. Asp-N digestion

Turkey myoglobin, separated by SDS–PAGE gel, was washed twice with 200 μl of ammonium carbonate (200 mM) in 50% acetonitrile for 60 min at 37 °C to remove the SDS and Coomassie blue dye. The gel bands were then completely dried with a speed-vac centrifugation system. Dried gel pieces were partially rehydrated with 3 μl of 200 mM ammonium carbonate in 0.02% Tween 20. The gel pieces were further rehydrated with 5 μl of Asp-N at an enzyme: protein ratio of between 1:20 and 1:200 (w/w) and incubated for 18 h at 37 °C. A minimal volume (100–200 μl) of 200 mM ammonium carbonate was added to keep the gel pieces immersed. Two microlitres of trifluoroacetic acid were added to stop the digestion, and the fluid was removed to a fresh tube. The gel pieces were then washed twice with 200 μl of 60% acetonitrile in 0.1% trifluoroacetic acid for 20 min at 37 °C, and all supernatants were combined and concentrated to approximately 20 μl using a speed-vac centrifugation system (Thermo Electron, Waltham, MA, USA). The peptides were then dissolved, separated, and sequenced as described earlier.

2.2.3. Determination of sequence similarity

The amino acid sequence of turkey myoglobin was used to determine the homology with myoglobins from other avian (chicken, ostrich) and livestock (beef, buffalo, pig, sheep and goat) species, using the Basic Local Alignment Search Tool (Ye, McGinnis, & Madden, 2006).

3. Results and discussion

In the present study, turkey myoglobin, separated on SDS–PAGE as a 17 kDa band, was digested with trypsin or Asp-N, and the resulting peptides were subjected to Edman degradation to determine the complete amino acid sequence. The sequence of peptides obtained after enzymatic digestion is presented in Table 1. Tryptic digestion produced peptides which revealed most of the primary structure of turkey myoglobin, and the rest of the sequence was determined utilising Asp-N-generated peptides. The primary structure of turkey myoglobin was derived by overlapping the peptide sequences and using homology with other well-characterised avian myoglobins.

The amino acid sequence of turkey myoglobin, in comparison with other poultry and red meat myoglobins, is presented in Fig. 1. Like poultry and red meat myoglobins, turkey myoglobin has 153 amino acids. In addition, the distal (position 64) and proximal (position 93) histidine residues responsible for coordinating the haeme group are conserved in turkey myoglobin. The percentage of sequence similarity between turkey and other poultry and red meat myoglobins is provided in Table 2. Interestingly, turkey myoglobin shares 100% sequence similarity with chicken myoglobin. Previously, Cornish and Froning (1974) suggested high similarity in amino acid residues in turkey and chicken myoglobins, based

Table 1
Sequences of peptides generated after enzymatic digestion of turkey myoglobin with trypsin and aspartic acid endopeptidase (Asp-N).

No.	Peptide sequence	Position of amino acids	Enzyme
1	GLSDQEWQQVLTIWGKV[a]	1–17	Trypsin
2	VEADIAGHGHEVLMR	17–31	Trypsin
3	LFHDHPETLDRFDK	32–45	Trypsin
4	DKFKG[a]	44–48	Asp-N
5	GLKTPDQM[a]	48–55	Trypsin
6	TPDQMKGSED[a]	51–60	Trypsin
7	GSEDLK	57–62	Trypsin
8	HGATVLTQLGK	64–74	Trypsin
9	ILK	75–77	Trypsin
10	QK	78–79	Trypsin
11	GNHESELKPLAQTHATK	80–96	Trypsin
12	HKIPVK	97–102	Trypsin
13	YLEFISEVIIK	103–113	Trypsin
14	VIAEK	114–118	Trypsin
15	HAADFGADSQAAMKK	119–133	Trypsin
16	ALELFR	134–139	Trypsin
17	DSQAAMKKALELFRN	126–140	Asp-N
18	DMASKYKEFG	141–150	Asp-N
19	YKEFGFQG	146–153	Trypsin

[a] There was not enough peptide isolated to sequence to the end of the proteolytic product.