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Amino acid sequence of Japanese quail (*Coturnix japonica*) and northern bobwhite (*Colinus virginianus*) myoglobin

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ABSTRACT

Myoglobin has an important physiological role in vertebrates, and as the primary sarcoplasmic pigment in meat, influences quality perception and consumer acceptability. In this study, the amino acid sequences of Japanese quail and northern bobwhite myoglobin were deduced by cDNA cloning of the coding sequence from mRNA. Japanese quail myoglobin was isolated from quail cardiac muscles, purified using ammonium sulphate precipitation and gel-filtration, and subjected to multiple enzymatic digestions. Mass spectrometry corroborated the deduced protein amino acid sequence at the protein level. Sequence analysis revealed both species' myoglobin structures consist of 153 amino acids, differing at only three positions. When compared with chicken myoglobin, Japanese quail showed 98% sequence identity, and northern bobwhite 97% sequence identity. The myoglobin in both quail species contained eight histidine residues instead of the nine present in chicken and turkey.

1. Introduction

Myoglobin (Mb) is a sarcoplasmic oxygen-binding heme protein primarily responsible for poultry meat color and visual appearance. Depending on the species, Mb consists of a polypeptide chain of 145–154 amino acids folded into a globular structure with a heme group situated toward the center (Livingston & Brown, 1981). Pigment is influenced by the presence of bound oxygen and the degree of oxidation of the heme group, which can assume various distinct redox states (Young & West, 2001). Differences in the primary structure of Mb may influence the mechanistic properties of the protein and the overall stability of the various redox states (Suman, Joseph, Li, Steinke, & Fontaine, 2009). Additionally, changes in the amino acid sequence of Mb can influence molecular interactions with ligands and small biomolecules like lactate, which may alter the oxygen binding affinity of the protein (Suman & Joseph, 2013).

Japanese quail (*Coturnix japonica*) is a species of Old World (Phasianidae) migratory quail originating in East Asia and originally domesticated in Japan (Wakasugi, 1984). Northern bobwhite quail (*Colinus virginianus*) is another species of quail similar in appearance to Japanese quail; they are, however, non-migratory and native to most of North America, including southern Canada, eastern and central United States, Mexico, and the Caribbean (Dimmick, 1992). Despite their similar appearance, bobwhite quail are not particularly closely related to Old World quail, and as such are grouped in a separate family, commonly known as New World quail (Odontophoridae) (Cox, Kimball, & Braun, 2007). Commercially, within the United States, northern bobwhite quail are considered game birds and are, therefore, mostly bred for hunting (Judd, 1905). In the consumer market, quail is generally regarded as a gourmet or ethnic food, but consumer interest is growing in quail meat as an alternative to chicken and turkey. Genchev, Mihaylova, Ribarski, Pavlov, and Kabakchiev (2008) stated that quail meat composition and quality has nutritional advantages over other poultry birds.

Chicken and turkey belong to the same order as Japanese and bobwhite quail (Galliformes), and recent research has shown the primary structure of turkey Mb is identical to chicken (Joseph et al., 2011). The Mb of many domesticated meat and poultry animals have been characterized and studied (www.expasy.org; www.ncbi.nlm.nih.gov). To date, however, the amino acid sequences of Japanese and bobwhite quail Mb have not been determined. Therefore, the objective of this study was to characterize the amino acid sequence of Japanese and northern bobwhite quail Mb.

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2. Materials and methods

2.1. RNA isolation and cDNA synthesis

Live Japanese quail (Quail International, Greensboro, GA, USA) and northern bobwhite quail (University of Georgia, Athens, GA, USA) specimens were sacrificed and 50–100 g of breast muscle tissue samples were collected. Total RNA was extracted using TRIzol (Life Technologies, Carlsbad, CA, USA) in accordance with the manufacturer's protocol. A 20 μL aliquot of total RNA was used with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) to synthesize *c*DNA. The reaction was carried out at 37 °C for 2 h and terminated by 5 min of heat inactivation at 85 °C.

2.2. cDNA cloning of Japanese quail Mb

Table 1 shows nucleotide sequences of primers used for polymerase chain reaction (PCR). Primers JQF and JQR were designed using the nucleotide sequence of the conserved internal regions of chicken Mb to amplify the coding regions of Japanese quail Mb. PCR was run with the designed primers for 35 cycles using a Veriti (Applied Biosystems) 96-well thermal cycler. Initial denaturation occurred for 30 s at 98 °C. Each thermal cycle consisted of denaturation for 5 s at 98 °C, annealing for 5 s at 55 °C, extension for 10 s at 72 °C, and a final extension for 1 min at 72 °C. PCR amplification products were validated by analysis of amplicon molecular weight on 2% agarose gel electrophoresis.

PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. The purified DNA was then ligated using the CloneJET PCR Cloning Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) in accordance with the manufacturer's instructions.

The ligation mixture was used directly for transformation of GC10 competent cells (Sigma–Aldrich, St. Louis, MO, USA). A 1 μL amount of the ligation mixture was added to a 50 μL volume of competent cells on ice for 15 min. The mixture was heated at 37 °C for 45 s and placed on ice for 2 min to heat shock the cells. Cells were plated to lysogeny broth (LB) agar (ampicillin 100 μg/mL) plates and incubated at 37 °C for 16 h. Colonies were picked from the plates and analyzed using colony PCR/restriction analysis. Validated colonies were transferred to LB tubes and of 1 μL/mL ampicillin was added. The tubes were incubated for 16 h, and plasmids were isolated from the liquid cultures using the QIAGEN Plasmid Mini Kit (Qiagen, Valencia, CA, USA) consistent with the manufacturer's protocol. The purified DNA from the picked colonies was sequenced at the Georgia Genomics Facility (University of Georgia, Athens, GA, USA), using the BigDye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on a 96-capillary 3730 × l DNA Analyzer (Applied Biosystems).

2.3. cDNA cloning of northern bobwhite Mb

Primers (JQF and JQR) previously created for Japanese quail unfortunately failed to amplify the *c*DNA of northern bobwhite quail. Therefore two sets of primers (BWX1 and BWX3) were designed using the nucleotide sequence for chicken Mb to amplify the areas of the sequence directly surrounding the 5′ and 3′ ends of the northern bobwhite Mb coding region, which are highly conserved. Amplicons were then purified, ligated, cloned, and sequenced as previously described. The sequences of the amplicons were used to design sequence-specific primers (BWWF and BWWR) to PCR amplify the entire coding region of northern bobwhite Mb. PCR settings were identical to what was previously stated, although annealing temperature was raised to 57 °C and extension time prolonged to 15 s. PCR purification, ligation, cloning, and sequencing were carried out as previously described.

Table 1
Sequence of primers used for cDNA cloning.

Primer	Sequence
JQF	5′-AACCATGGGGCTCAGCGACCA-3′
JQR	5′-GCATACATGAAGCCAGGAAAGG-3′
BWX1F	5′-AGGCAACAGCCGTAGGCAGCA-3′
BWX1R	5′-CTCATCAGAACCTCATGTCCAT-3′
BWX3F	5′-TCATTTCTGAAGTCAAGGTCAT-3′
BWX3R	5′-GGTGACAGATAACCCCTTATATTATTTT-3′

2.4. Sequence alignment and analysis

DNA sequences from Japanese and northern bobwhite quail were aligned using ApE software (http://biologylabs.utah.edu/jorgensen/wayned/ape/) to ensure fidelity of the sequence, because reverse transcriptase lacks proofreading capabilities and is therefore error-prone (Varela-Echavarría, Garvey, Preston, & Dougherty, 1992). The translated and deduced protein sequences were aligned using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). Molecular masses and isoelectric points of Mb proteins were calculated using Compute pI/Mw (http://web.expasy.org/compute_pi/).

2.5. Isolation of Japanese quail Mb

Mb was purified according to the methods used by Faustman and Phillips (2001) with minor modifications. Briefly, fresh Japanese quail hearts were collected from Quail International, Inc. (Greensboro, GA, USA) and frozen at −40 °C until further analysis was performed. The frozen hearts were thawed at 4 °C and trimmed of visible fat and connective tissue. The cardiac muscle was homogenized in homogenization buffer (20 mM ammonium bicarbonate, pH 9.0, 4 °C) at a 1:1 (w/w) ratio and centrifuged at 5000*g* for 10 min. The supernatant was brought to 50% ammonium sulphate saturation and further centrifuged for 20 min at 18,000*g*. The resulting supernatant was brought to complete ammonium sulphate saturation and further centrifuged for 1 h at 20,000*g*. The precipitate was resuspended in the homogenization buffer and dialyzed for 24 h against the same buffer at 4 °C. The dialyzed sample was loaded into a Sephacryl S-200 HR gel-filtration column (2.5 × 100 cm, GE Healthcare, Piscataway, NJ, USA) equilibrated with the homogenization buffer using a peristaltic pump (NE-9000, New Era Pump Systems, Inc., Farmingdale, NY, USA) at a flow rate of 60 mL/h. Mb containing fractions were collected, pooled, and concentrated by saturation with ammonium sulphate, centrifugation, and dialysis.

To further isolate and determine the quality of the prepared protein, the resulting samples were subjected to SDS–PAGE. Samples were prepared with 4× Laemmlli sample buffer (277.8 mM Tris–HCl, pH 6.8, 4.4% LDS, 44.4% (w/v) glycerol, 0.02% bromophenol blue) at a 3:1 ratio (v/v) and heated for 5 min at 95 °C. Multiple 10 μL aliquots and a commercially available protein standard ladder were loaded onto an 18% polyacrylamide Tris–HCl precast gel and run in a Criterion cell unit (Bio-Rad, Hercules, CA, USA) held at 200 V for 1 h. Gels were stained with Biosafe Coomassie (Bio-Rad), and bands were sliced for in-gel digestion.

2.6. Japanese quail Mb mass spectrometric analysis

Gel isolated Japanese quail Mb was subjected to enzymatic digests using trypsin, endoproteinase Asp-N, or endoproteinase Glu-C. Enzymes were selected based on probable sequence coverage, peptide length, site-specific cleavage, and degree of overlap