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Characterisation of myoglobin from sardine (*Sardinella gibbosa*) dark muscle

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Abstract

Myoglobin from the dark muscle of sardine (*Sardinella gibbosa*) with the molecular weight of 15.3 kDa was isolated and characterised. The different myoglobin derivatives exhibited varying thermal unfolding characteristics. Deoxymyoglobin showed a single distinct endothermic peak at 74.5 °C, whereas two transition temperatures were noticeable for oxymyoglobin (64.5 and 78.4 °C) and metmyoglobin (59.0 and 76.0 °C). The spectrum of deoxymyoglobin and oxymyoglobin had absorption bands at 739, 630, 575, 500 and 405 nm, while the disappearance of the peak at 575 nm was found in the spectrum of metmyoglobin. The soret peak of all derivatives was noticeable at 405 nm. The autoxidation of myoglobin became greater at very acidic or alkaline conditions as evidenced by the formation of metmyoglobin, the changes in tryptophan fluorescence intensity as well as the disappearance of soret absorption. The higher temperature, particularly above 40 °C, and the longer incubation time induced the higher metmyoglobin formation as well as the conformational changes.

Keywords: Myoglobin; Sardine; Dark muscle; pH; Temperature; Oxidation

1. Introduction

Myoglobin is a globular heme protein localised in red muscle fibers. Myoglobin concentration generally depends on species, breed, sex and age of animal, training and nature of nutrition, muscular activity, oxygen availability, blood circulation and muscle type as well as the way the meat is treated (Giddings, 1974; Livingston & Brown, 1981; Postnikova, Tselikova, Kolaeva, & Solomonov, 1999). Myoglobin has been known to be a major contributor to the colour of muscle, depending upon its derivatives and concentration (Faustman, Yin, & Nadeau, 1992; Postnikova et al., 1999). The stability of myoglobin also affects the colour of meat (Chanthai, Neida, Ogawa, Tamiya, & Tsuchiya, 1998; Chen, 2003; Suzuki & Kisamori, 1984).

The use of under-utilised small pelagic fish species, such as sardine and mackerel, for surimi production is limited, mainly due to the large quantity of lipids and myoglobin in the muscle tissue. Chaijan, Benjakul, Visessanguan, and Faustman (2004) reported that lipid and myoglobin contents were higher in dark muscle than in ordinary muscle of both sardine and mackerel, and higher contents of both constituents were found in sardine muscle than mackerel muscle. Myoglobin and hemoglobin play an essential role in the whiteness of surimi (Chen, 2002). Hemoglobin is lost rather easily during handling and storage, while myoglobin is retained by the muscle intracellular structure (Livingston & Brown, 1981). Therefore, colour changes in meat are most likely due to the reaction of myoglobin with other muscle components, especially myofibrillar proteins (Hanan & Shaklai, 1995). Since the

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abundant dark muscle is difficult to remove with a meat separator for red-fleshed fish (Ochiai, Ochiai, Hashimoto, & Watabe, 2001), changes in myoglobin may be associated with the discolouration of resulting surimi. During the handling and storage of fish, a number of biochemical, chemical and microbiological changes also occur and are associated with the discolouration (Faustman et al., 1992; O'Grady, Monahan, & Brunton, 2001; Pacheco-Aguilar, Lugo-Sanchez, & Robles-Burgueno, 2000). Discolouration of tuna during frozen storage is caused by the formation of metmyoglobin (Haard, 1992). This phenomenon can be influenced by many factors such as pH, temperature, ionic strength and oxygen consumption reaction (Renerre & Labas, 1987). Metmyoglobin formation is positively correlated with lipid oxidation (Chan, Faustman, Yin, & Decker, 1997; Lee, Phillips, Liebler, & Faustman, 2003). Recently, Chaijan, Benjakul, Visessanguan, and Faustman (2005) reported that prolonged iced storage decreased the myoglobin extracting efficiency in sardine and mackerel muscle due to the insolubility of myoglobin caused by the oxidation of myoglobin to form metmyoglobin. This change might be related to the intrinsic and extrinsic factors determining the myoglobin molecules. However, information regarding the characteristics and properties of myoglobin from sardine, which has been paid increasing attention as raw material for surimi processing, is scarce. The objective of this investigation was to partially purify and characterise the myoglobin from sardine dark muscle.

2. Materials and methods

2.1. Chemicals

Sodium dodecyl sulfate (SDS), dithiothreitol (DTT), β-mercaptoethanol (βME) and Triton X-100 were purchased from Sigma (St. Louis, MO, USA). Acrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), bis-acrylamide, disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Fluka (Buchs, Switzerland). Sodium dithionite was obtained from Riedel (Seeize, Germany). Sodium chloride and Tris(hydroxymethyl)–aminomethane were procured from Merck (Damstadt, Germany).

2.2. Fish samples

Sardine (*Sardinella gibbosa*) with an average weight of 55–60 g was caught from Songkhla-Pattani Coast along the Gulf of Thailand in March, 2005. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1–2 h. The fish were immediately washed, filleted and manually excised. The dark muscles were collected and used for myoglobin extraction.

2.3. Extraction and purification of myoglobin from sardine muscle

Extraction and purification of myoglobin was performed according to the methods of Trout and Gutzke (1996) with a slight modification. The dark muscle (100 g) was coarsely minced and mixed with 300 ml of cold extracting medium (10 mM Tris–HCl, pH 8.0 containing 1 mM EDTA and 25 g/l Triton X-100). The mixture was homogenised for 1 min using an IKA Labortechnik homogeniser (Selangor, Malaysia). After centrifugation at 9600*g* for 10 min at 4 °C using the RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA), the supernatant was filtered through a Whatman No. 4 filter paper. The filtrate was then subjected to ammonium sulfate fractionation. The precipitate obtained with 65–100% saturation was dissolved in a minimal volume of cold 5 mM Tris–HCl buffer, pH 8.5, which was referred to as starting buffer. The mixture was then dialysed against 10 volumes of same buffer with 20 changes at 4 °C. The dialysate was immediately applied to a Sephadex G-75 column (0.26 × 70 cm; Amersham Bioscience, Uppsala, Sweden) which was equilibrated with the starting buffer. The separation was conducted using the starting buffer at a flow rate of 0.5 ml/min and 3-ml fractions were collected. The fractions containing myoglobin were combined and further purified by ion-exchange chromatography on a HiTrap DEAE FF column (prepacked 5 ml; Amersham Biosciences, Uppsala, Sweden). After the column was washed with starting buffer, the elution was performed with a linear gradient of 0–0.5 M NaCl in starting buffer at a flow rate of 1 ml/min. Fractions of 3 ml were collected and those with high content of myoglobin were pooled.

During purification, the fractions obtained were measured at 280 and 540 nm using a UV-1601 spectrophotometer (Shimadzu, Japan). The fractions with the high absorbance at 540 nm were also subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE).

2.3.1. Determination of molecular weight

The molecular weight of myoglobin was determined by SDS–PAGE using a 4% stacking gel and 17.5% separating gel according to the method of Laemmli (1970). The electric current for each gel was maintained at 15 mA using a Mini Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie brilliant blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid. The myoglobin separated on SDS–PAGE was estimated for its molecular weight by plotting the logarithm of molecular weight of the protein standards against relative mobility. The low-molecular-weight-protein standards [albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase