（学位第３号様式）

学 位 論 文 要 旨

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題　目	The study on the conformational structure of fish myoglobin and its suppressive effect in autoxidation of myoglobin （魚類ミオグロビンの分子状態とメト化抑制に関する研究）

Frozen fish products are generally transported and stored at -20˚C. This storage condition, however, causes rapid progression of autoxidation of myoglobin and browning of dark muscle, reducing the commercial value of fish for sashimi. Because the discoloration of tuna meat also proceeds during frozen storage at around -20˚C, tuna meat is stored at the ultra-low temperature (-60˚C). On the other hand, the discoloration of highly fresh tuna meat could effectively be suppressed even if stored at -20˚C. However, the suppressive mechanism of the discoloration is not well understood. In this study, I forcused on a function of ATP contained with high concentration in highly fresh meat. The effects of ATP on the autoxidation rate and molecular state of southern bluefin tuna myoglobin were investigated. The autoxidation rate of tuna myoglobin at 25˚C was suppressed in the presence of ATP especially in acidic pH range. Mixing ATP with myoglobin induced a spectral perturbation in the Soret region of myoglobin. Quenching of myoglobin fluorescence was also caused by ATP. According to dynamic light-scattering measurements the molecular weights of tuna myoglobin changed from 15.5 kDa to 11.3 kDa with ATP and zeta-potential measurements gave also a negative surface charge without ATP and a positive one with ATP, respectively. The above results indicate that ATP-induces changes in the conformational structure of myoglobin. The effects of ATP on myoglobin could thus provide a possible mechanism to regulate the autoxidation of myoglobin.　Then, I had investigated the effect of ATP in muscle on the progression of autoxidation of myoglobin in dark muscle of amberjack during frozen storage -20˚C. In the early stage of this study, I found that the method of Bito for measuring the ratio of generated metMb in tuna myoglobin was not applicable for amberjack myoglobin. This suggested that for measuring the ratio of generated metMb, an individual method for each fish myoglobin should be developed. Therefore I tried to develope a method for measuring the ratio of generated metmyoglobin in myoglobin for each fish. I prepared purified myoglobin from 12 kinds of fish and then prepared deoxymyoglobin, oxymyoglobin and metmyoglobin. The wavelength of isosbestic point among visible spectra of deoxymyoglobin, oxymyoglobin and metmyoglobin was measured.　From these data, I developed a method for measuring of the ratio of generated metmyoglobin in myoglobin for each fish and then measured the discoloration rate constant of each myoglobin.

Then I investigated the effect of ATP in muscle on the progression of myoglobin in dark muscle of cultured amberjack during frozen storage at -20˚C. Amberjack fillets　quick-frozen at -50°C containing various concentrations of ATP were prepared and then stored at -20°C for four months. The progress of autoxidation of myoglobin was suppressed in the fillets containing high concentrations of ATP.

The above results indicate that fish meat contained ATP with high concentration could be possible to store at -20˚C with good quality as sashimi. It is expected that frozen fish products for sashimi with the good quality are produced and distributed globally by using this technical idea.