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学位論文題名

Biochemical study on denaturation mode of myosin and actin in frozen fillet of catfish *Clarias macrocephalus x Clarias gariepinus*

（ナマズ凍結フィレ中でのミオシンおよびアクチン変性様式に関する生化学的研究）

学位論文内容の要旨

Introduction

As protein resource, fish have been playing a very important role traditionally for peoples in Asian countries. Aquacultures of marine fish and culture freshwater fish have been very important in these areas. In Thailand, there are various fish have been cultured in inland traditionally for a domestic and local consumption. Hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*), rather than domestic one is the most abundantly cultured. Economically the fish culture is important because frozen fillet and frozen mince are exported to Western countries especially to United State of America. However, there is little information on the properties of muscle protein, myosin, especially on its biochemical functions and stability. The information on the stability and denaturation of myosin of the fish would be valuable in the quality evaluation of the filet. Tilapia is another important fish species cultured in Thailand, and properties of its myosin is well characterized, especially its stability. In the dissertation myofibrils were mainly used as a model material for studying the properties of catfish myosin. After investigation on the thermal denaturation of catfish myosin, myosin denaturation furing the frozen storage was also studied. Moreover, myofibrils were replaced by meat itself for obtaining the information on myosin denaturation in frozen fillet.

Materials and Methods

Hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*), Hybrid tilapia (*Oreochromis niloticus x Oreochromis mossambicus*) and American catfish (*Clarias gariepinus*) were the samples for this work. A majority of the research was conducted with myofibrils suspended in 0.1 M NaCl, pH 7.5 because the material is considered as a model material of meat, and because the preparation of myofibrils is easy to prepare. Heating and frozen storage at various temperatures are the method to induce denaturation. Meat itself was also used as the last stage of the research. Myosin denaturation was monitored by employing established several indices, Ca^{2+}-ATPase inactivation, loss of salt-solubility, aggregate formation as studied by ammonium sulfate fractionation, and myosin head (S-1) and tail (rod) denaturation mode as studied by chymotryptic digestion.

Results and Discussions

Chapter 1

Thermal stability of catfish myosin in isolated form and in myofibrils (stabilized form by F-actin binding) was indistinguishable from corresponding preparations of tilapia. This is reasonable because catfish and tilapia are cultured under the same conditions especially high water temperature (25- 29°C throughout a year). It was also found that both myosin preparations are equally stabilized by F-actin. However, chymotryptic digestion revealed different denaturation pattern between two species of fish myofibrils. A quick denaturation of rod than S-1 was the pattern of catfish, while the pattern of tilapia myofibrils was opposite. It was concluded that catfish myosin in myofibrils was characterized by a quick denaturation of tail region.

Chapter 2

To understand the different patterns obtained, myofibrils were heated by changing the conditions. The conditions were arranged by considering myosin filament structure because the rod denaturation detected by chymotyrptic digestion was detecting myosin filament structural change. The most effective and easy factor that can change filament structure was pH, namely myosin forms rigid and strong filament at acidic conditions, and oppositely forms fragile and weak filaments at alkaline pH. As expected, pH change for heating significantly affected rod denaturation without affecting on S-1 denaturation. Consequently, S-1 and rod denaturation pattern was pH dependent in addition to fish species dependent. Comparing the patterns as affected by pH, catfish myofibrils required lower pH by 1 unit to have a similar pattern given for tilapia myofibrils. Thus, fragile filament structure for catfish myosin filament was proved. The conclusion was confirmed by the chymotryptic digestion of the unheated myofibrils at varied pH. Cleavage at HMM/LMM junction became obvious at pH 8 for catfish but the site was well protected for tilapia.

Chapter 3

Structural difference of myosin tail was compared with isolated rod and its inner subfragments (Subfragment-2, S-2, and light meromyosin, LMM). Unfolding profiles of rod upon raising temperature for two were very similar to each other. LMM from two species of fish showed the single unfolding peak at the same temperature. Meanwhile, S-2 preparations from both two fishes gave two unfolding peaks. The peak found at low temperature was the same as found with LMM, additionally another unfolding peak was found at much higher temperature. It was demonstrated that S-2 region consisted of two regions with a slight different stability.

Chapter 4

Myosin and actin denaturation during the frozen storage of myofibrils were compared between catfish and tilapia. Myosin denaturation rate in myofibrils was found to be storage temperature dependent, the lower the temperature the slower the denaturation. Salt-solubility was kept high for both species. A very slow denaturation of rod portion relative to S-1 portion was the common pattern obtained. These were completely different from the patterns obtained in heating myofibrils. A surprising finding was that actin denatures very quickly for both preparations. The rate was greater than that for myosin.

Myosin and actin denaturation in frozen stored meat was finally investigated. For analyzing myosin and actin denaturation, meat had to be converted into quantitative material. The procedures to convert frozen Surimi into homogenate were successfully applied for the purpose. As established by the study with myofibrils, ATPase inactivation proceeded slowly when stored at lower temperature such as -40°C than at -10°C. However, ATPase inactivation in meat was very slow compared with one in myofibrils. Moreover, no actin denaturation was detected at any storage temperatures. These two showed that myofibrils cannot be used as a model material for meat, especially prediction of myosin denaturation extent in meat from the study with myofibrils is impossible. From the results obtained, the most sensitive index to detect myosin denaturation was ATPase inactivation, which was not the fastest in thermal denaturation process.

Actin denaturation was not detected in the heated myofibrils, which was the same as found with heated meat. Myofibrils can be used as model material for thermal denaturation.

Quality evaluation of frozen fillet has been an important subject from scientifically and economically. There are several established indices to access the quality such as drip content, color of dark meat, texture, and bad smell. An attempt was made to establish the method to evaluate the quality by studying myosin denaturation with frozen fillet. The proposed procedures to evaluate the quality of frozen meat are the preparation of homogenate from the meat and its Ca-ATPase measurement. Combination of other well established indices and ATPase activity measurement is strongly recommended. Fundamental experiments are always important for the information on the prediction of shelf-life of fish fillet. The present results clearly showed that its quality of frozen fillet can be kept high when stored at -20°C. Trimming catfish meat to shape the fillet produces small pieces of meat. The meat can be stored frozen for the production of value-added meat-based-products such as fish ball, fish sausage, fish hams, fish burgers and extruded products.

学位論文審査の要旨

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アジアの国々では伝統的に魚肉はタンパク質源として古くから重要な地位を占めてきた。さらに安定な供給のための養殖も盛んであり、大陸内部ではナマズやティラピアなどの淡水魚養殖が重要とされてきた。現在のタイ国では、その成長が速いこと、肉質が好ましいこと、魚肉の回収率が高いなどから、品種改良がされた交雑種が中心として養殖されている。本論文では国内消費ばかりでなく、凍結フィレに加工され米国などに輸出されているナマズに注目した。このように重要魚種ではあるが、魚肉の主要成分である魚肉タンパク質に関する研究報告がほとんどない。そこで、研究成果がある程度蓄積されていて、同じように重要魚種である養殖ティラピアを対照にしながら、ナマズのミオシンの特性、特に変性特性を明らかにしようとした。最終的には、凍結フィレ中のミオシンおよびアクチン変性の解析から品質評価の方法を提案することを目的とした。

調製したナマズミオシンの ATPase 失活の比較からティラピアからのものと同じ熱安定性を示すことを見出した。また、魚肉中の状況に想定されるアクチン結合による安定化も 100 倍程度で同じであった。しかし、筋肉のモデル試料である筋原線維を加熱したときのミオシン変性の解析から、ナマズではミオシン尾部での変性がティラピアに比べ顕著であることを見出した。この原因がナマズミオシンの形成するフィラメントが脆弱であることを、フィラメントの条件を変えて加熱する実験（溶解試料、pH を変えた試料）などから明らかにした。ナマズミオシン尾部を単離してその原因を探ろうとしたが、安定性は同じで両者に差は認められなかった。

加熱変性の結果に基づき、凍結貯蔵中の変性を筋原線維をモデルに貯蔵温度を変えて行った。すると、ミオシン変性様式は加熱とは大きく異なり、しかも、ティラピアとの違いも検出されないように変化し

た。すなわち、両者でミオシン尾部の変性が頭部に比べ非常に緩やかに進行し、ナマズで認められた急激な尾部の変性は認められなくなった。さらに、加熱では認められない現象としてアクチンの著しい変性が進行する事実を見出した。筋原線維は筋肉中の構造を維持しているため、そのモデルとして広く使用され、多くの成果が蓄積されている。そこで、この筋原線維で得られた凍結変性に関する成果が魚肉の凍結にそのまま適応されるのかについて検討した。魚肉中のミオシン、アクチン変性を検出する確立された方法がなかったので、変性ミオシンをもすべて回収できるよう魚肉を均一にホモジナイズした懸濁液をそのまま用いる試料の調製法を確立した。最も変性が進行しやすい - 10℃で魚肉を貯蔵しても、ATPase 失活は 6 か月にかけて緩やかに進行し、2 週間で大きな変性が検出された筋原線維と異なった。それより低温での貯蔵ではその半年間でほとんど変性が認められなかった。これはナマズでもティラピアでも同じであり、 - 20℃以下で貯蔵すれば、少なくとも半年程度はミオシン変性なしに貯蔵できることを明らかにした。この結果は現実的に用いられているフィレの貯蔵条件と矛盾せず、その理由を科学的に証明したことになる。さらに、変性が検出された - 10℃での魚肉および筋原線維の変性を比較すると、フィレではアクチン変性が全く起こらない相違点が見出された。すなわち、筋原線維を凍結変性のモデルとして用いてには限界があることを示した。しかし、塩溶解性が筋原線維でもほとんど認められないことは魚肉の凍結でも同じであり、その理由はミオシン尾部の変性が進行しないからであることを明らかにした。筋原線維と魚肉で大きな違いがあることが示されたので、加熱の場合にも心配となった。そこで、魚肉そのものと筋原線維を同じ条件で加熱し、ミオシン、アクチン変性の様子を確認した。ATPase 失活速度に両者で大きな違いは認められなかったので、凍結とは異なった。しかし、魚肉で加熱した場合は、ナマズ筋原線維で特徴的に見出された急激なミオシン尾部の変性は起こらず、魚肉ではミオシン尾部は加熱でも安定に保たれていると推定した。また、ティラピア肉の加熱も同じであり、筋原線維で両者の変性様式の違いは消失した。なお、加熱では筋原線維でもミオシン変性が起きる範囲では全くアクチン変性が認められなかったので、筋原線維中のアクチンが特徴的に不安定になると考えられた。最終的にフィレを用いた加熱・凍結実験から、古くから用いられているミオシン変性指標である ATPase 失活が最も先に低下し、魚肉の品質評価には感度の良い品質評価の指標であると結論した。

これらの研究成果は、アジアで重要な資源である淡水魚、特にナマズ肉の凍結保蔵、品質維持のために大きな貢献をすることが期待される。よって、審査員一同は申請者が博士（水産科学）の学位を授与される資格があるものと判定した。